



**University of
Zurich**^{UZH}

**Zurich Open Repository and
Archive**

University of Zurich
University Library
Strickhofstrasse 39
CH-8057 Zurich
www.zora.uzh.ch

Year: 2017

Tree diversity increases levels of herbivore damage in a subtropical forest canopy: evidence for dietary mixing by arthropods?

Brezzi, Matteo ; Schmid, Bernhard ; Niklaus, Pascal A ; Schuldt, Andreas

Abstract: Aims Plant diversity has been linked to both increasing and decreasing levels of arthropod herbivore damage in different plant communities. So far, these links have mainly been studied in grasslands or in artificial tree plantations with low species richness. Furthermore, most studies provide results from newly established experimental plant communities where trophic links are not fully established or from stands of tree saplings that have not yet developed a canopy. Here, we test how tree diversity in a species-rich subtropical forest in China with fully developed tree canopy affects levels of herbivore damage caused by different arthropod feeding guilds. **Methods** We established 27 plots of 30×30 m area. The plots were selected randomly but with the constraint that they had to span a large range of tree diversity as required for comparative studies in contrast to sample surveys. We recorded herbivore damage caused by arthropod feeding guilds (leaf chewers, leaf skeletonizers and sap feeders) on canopy leaves of all major tree species. **Important Findings** Levels of herbivore damage increased with tree species richness and tree phylogenetic diversity. These effects were most pronounced for damage caused by leaf chewers. Although the two diversity measures were highly correlated, we additionally found a significant interaction between them, whereby species richness increased herbivory mostly at low levels of phylogenetic diversity. Tree species with the lowest proportion of canopy leaf biomass in a plot tended to suffer the highest levels of herbivore damage, which is in contrast to expectations based on the resource concentration hypothesis. Our results are in agreement with expectations of the dietary mixing hypothesis where generalist herbivores with a broad spectrum of food plants benefit from increased resource diversity in tree species-rich forest patches.

DOI: <https://doi.org/10.1093/jpe/rtw038>

Posted at the Zurich Open Repository and Archive, University of Zurich

ZORA URL: <https://doi.org/10.5167/uzh-145124>

Journal Article

Published Version

Originally published at:

Brezzi, Matteo; Schmid, Bernhard; Niklaus, Pascal A; Schuldt, Andreas (2017). Tree diversity increases levels of herbivore damage in a subtropical forest canopy: evidence for dietary mixing by arthropods? *Journal of Plant Ecology*, 10(1):13-27.

DOI: <https://doi.org/10.1093/jpe/rtw038>

Tree diversity increases levels of herbivore damage in a subtropical forest canopy: evidence for dietary mixing by arthropods?

Matteo Brezzi^{1,*}, Bernhard Schmid¹, Pascal A. Niklaus¹ and
Andreas Schuldt²

¹ Department of Evolutionary Biology and Environmental Studies and Zürich-Basel Plant Science Center, University of Zürich, Winterthurerstr. 190, CH-8057 Zürich, Switzerland

² Institute of Ecology, Animal Ecology Group, Leuphana University Lüneburg, Scharnhorststr. 1, D-21335 Lüneburg, Germany

*Correspondence address. Department of Evolutionary Biology and Environmental Studies, University of Zürich, Winterthurerstr. 190, CH-8057 Zürich, Switzerland. Tel: +41 (0)76 822 2169; E-mail: matteo.brezzi@ieu.uzh.ch

Abstract

Aims

Plant diversity has been linked to both increasing and decreasing levels of arthropod herbivore damage in different plant communities. So far, these links have mainly been studied in grasslands or in artificial tree plantations with low species richness. Furthermore, most studies provide results from newly established experimental plant communities where trophic links are not fully established or from stands of tree saplings that have not yet developed a canopy. Here, we test how tree diversity in a species-rich subtropical forest in China with fully developed tree canopy affects levels of herbivore damage caused by different arthropod feeding guilds.

Methods

We established 27 plots of 30 × 30 m area. The plots were selected randomly but with the constraint that they had to span a large range of tree diversity as required for comparative studies in contrast to sample surveys. We recorded herbivore damage caused by arthropod feeding guilds (leaf chewers, leaf skeletonizers and sap feeders) on canopy leaves of all major tree species.

Important Findings

Levels of herbivore damage increased with tree species richness and tree phylogenetic diversity. These effects were most pronounced for damage caused by leaf chewers. Although the two diversity measures were highly correlated, we additionally found a significant interaction between them, whereby species richness increased herbivory mostly at low levels of phylogenetic diversity. Tree species with the lowest proportion of canopy leaf biomass in a plot tended to suffer the highest levels of herbivore damage, which is in contrast to expectations based on the resource concentration hypothesis. Our results are in agreement with expectations of the dietary mixing hypothesis where generalist herbivores with a broad spectrum of food plants benefit from increased resource diversity in tree species-rich forest patches.

Keywords: arthropod herbivore damage, feeding guilds, forest canopy, generalist herbivores, leaf biomass, resource dilution, specialist herbivores, tree species richness, tree phylogenetic diversity

Received: 23 August 2015, Revised: 24 April 2016, Accepted: 25 April 2016

INTRODUCTION

In the context of biodiversity–ecosystem functioning (BEF) studies, foliar herbivory is thought to be important because it affects ecosystem processes, plant diversity and plant species composition (Bagchi *et al.* 2014; Coley and Barone 1996; Mulder *et al.* 1999; Stein *et al.* 2010; Whiles and Charlton

2006). Plant diversity, in turn, can affect levels of arthropod herbivory on plant leaves. However, our knowledge on this topic is incomplete and comes mostly from experiments conducted in grasslands; less information is available on more complex systems such as forests (Cardinale *et al.* 2011). Most studies on plant diversity–herbivory relationships in forests were carried out in species-poor forest stands (mostly two or

three species mixtures, reviewed by Jactel and Brockerhoff 2007) or only saplings were sampled (Schuldt *et al.* 2010). Little is known about processes occurring in forest canopies of established species-rich forests, although this uppermost layer of forest vegetation is crucial for biomass production (Lowman 2009), contains a great amount of resources and consumers (Basset *et al.* 2003) and hosts a large proportion of all arthropod species occurring in these forests (Floren *et al.* 2014; Stork and Grimbacher 2006). Many herbivores occur exclusively in the forest canopy (Stork and Grimbacher 2006), limiting our ability to predict canopy processes from patterns observed at the ground layer of forest vegetation.

Studies linking tree diversity to foliar herbivory so far did not reveal a consistent pattern. Higher tree species richness, a basic metric of diversity, has been linked with lower (Castagneyrol *et al.* 2014; Jactel and Brockerhoff 2007), higher (Schuldt *et al.* 2010) or unchanged levels of herbivore damage (Vehviläinen *et al.* 2007). Recent studies highlighted the complexity of herbivory-driving factors, and other types of diversity effects may complement or even overrule effects of plant species richness. For example, herbivory may be related to plant species composition (Jactel and Brockerhoff 2007) or to the presence or absence of particular plant functional groups (Loranger *et al.* 2014). Particularly important among these other aspects of plant diversity may be plant phylogenetic and functional diversity, which can strongly influence levels of herbivory (Castagneyrol *et al.* 2014; Dinnage 2013; Schuldt *et al.* 2014a). Furthermore, variation among the herbivores themselves, in particular their way of feeding (feeding guild) or preference for many or particular plant species (degree of specialization), affects the severity and quality of herbivore damage (Castagneyrol *et al.* 2014).

Plant diversity can act on levels of herbivore damage by affecting the abundance and diversity of herbivores. The different hypotheses we review below make predictions on how this may affect total herbivory in a plant community or average levels of herbivore damage on single leaves, the variable of interest in the present study. Increasing plant diversity is thought to increase the difficulty for specialized herbivores to find suitable hosts because these are more diluted (resource concentration hypothesis; Root 1973). In contrast, generalist herbivores should not be (or be less) sensitive to resource dilution and might profit directly from more plant species available through resource complementarity (dietary mixing hypothesis; Bernays *et al.* 1994), or indirectly by an increased amount of biomass available (Loranger *et al.* 2014). Given that many specialized arthropods are not strictly monophagous but rather feed on a number of related plant species (Forister *et al.* 2015), the phylogenetic or functional diversity of a plot might be of primary importance by determining the availability of suitable hosts.

Effects of plant species richness on herbivory could be enhanced if the plant species in a community differ substantially in trait values (i.e. functional diversity) or are distant in their evolutionary linkage (i.e. phylogenetic diversity, as potential proxy for phytochemistry diversity) (Castagneyrol *et al.* 2014; Dinnage 2013; Schuldt *et al.* 2014a). Indeed, as Castagneyrol

et al. (2014) showed vegetation patches with high plant phylogenetic diversity may even exceed generalist herbivores feeding capacities, which then also experience a resource dilution effect. Such generalists, according to the dietary mixing hypothesis, may mainly benefit from increasing species richness at low phylogenetic diversity. In this case, effects of plant species richness would actually be stronger at low than at high phylogenetic diversity, i.e. species richness is more important when species are more similar. In summary, total herbivory in a plant community or levels of herbivore damage on individual trees can depend on complex interactions between taxonomic and phylogenetic diversity at the producer level and the consumer level.

Plant diversity may reduce overall herbivore abundance indirectly through an increase in predator abundance (enemy hypothesis; Root 1973). Alternatively, abundance may increase because of an increase of plant biomass (more individuals hypothesis (Srivastava and Lawton 1998; Wright 1983)). Although the enemy hypothesis was demonstrated in simple crop fields (Root 1973; Russel 1989; Siemann *et al.* 1998), evidence particularly for more complex species-rich ecosystems is less clear (Riihimäki *et al.* 2005; Schuldt *et al.* 2011; Staab *et al.* 2014; Vehviläinen *et al.* 2006; Zhang and Adams 2011). Positive effects of plant species richness on plant biomass have been shown at our study site (Barrufol *et al.* 2013), and such increased biomass could affect the abundance of herbivores and therefore the amount of total plant community biomass consumed (Loranger *et al.* 2014). However, if insect abundance increases proportionally with plant community biomass, the levels of herbivore damage should remain constant at the level of single trees.

Here, we investigate which mechanisms occur most likely in the canopy of a species-rich subtropical forest, taking advantage of 27 comparative study plots deliberately selected to represent a broad and regular distribution of tree diversities (Barrufol *et al.* 2013; Chi *et al.* 2017; Peng *et al.* 2017), which were set up within the so-called BEF China project (<http://www.bef-china.de>, Bruelheide *et al.* 2011, 2014). We attempt to disentangle plant species richness effects from plant species identity and composition effects. We additionally focus on herbivore damage levels caused by different feeding guilds because these guilds may damage plants to different extents (see Vehviläinen *et al.* 2007) and respond in different ways to variation in plant diversity (Castagneyrol *et al.* 2013). Only few studies so far discriminated among feeding guilds in their herbivory assessment (Andrew *et al.* 2012; Garibaldi *et al.* 2011). We also considered tree phylogenetic diversity in order to test if this aspect of diversity and the interaction of species richness with phylogenetic diversity increased explanatory power in the statistical analysis of levels of herbivore damage. We additionally tested for potential effects of the total community leaf biomass and of the relative leaf biomass of the affected tree species on levels of herbivore damage.

We hypothesized that increasing tree species richness decreases the overall levels of herbivore damage at both the overall plot level and the level of individual tree species within plots. However, as outlined above, we expected that

phylogenetic diversity or total leaf biomass might change the magnitude of the species richness effects. Tree species within plots are likely to differ in their levels of herbivore damage, in part because of differences in their relative abundances (resource concentration hypothesis) and in part because of differences in their leaf traits. Moreover, because different herbivore feeding guilds might differ in their degree of host specialization and thus respond in different ways to plot-level tree species richness and the relative abundance or leaf quality of the tree species, we expected to find varying levels of damage caused by different herbivore feeding guilds.

METHODS

Study site and plot selection

The study was conducted between the end of June and mid-August 2010 in the Gutianshan National Nature Reserve in Kaihua County, Zhejiang Province, China (29°8'18"–29°17'29"N, 118°2'14"–118°11'12"E). This reserve is situated in mountainous terrain ranging in altitude from 250 to 1260 m above sea level. With a subtropical climate subjected to monsoon, the rain falls mainly in May and June, reaching a yearly total of 2000 mm (Geißler *et al.* 2012). The average annual temperature is 15°C (Yu *et al.* 2001). The reserve is covered by a forest containing a majority of evergreen broadleaved woody plant species, followed in abundance by deciduous broadleaved and some coniferous species (Yu *et al.* 2001). Past anthropogenic disturbances created areas of different stand age and successional stages.

In summer 2008, members of the BEF-China project selected 27 comparative study plots of 30 × 30 m in a stratified random sampling scheme (see Barrufol *et al.* 2013). With this stratification, we attempted to have nine plots each of low (3–8 species of trees with diameter at breast height (DBH) > 10 cm), medium (8–12 species with DBH > 10 cm) and high tree species richness (12–20 species with DBH > 10 cm) and nine plots each of young (fifth largest tree in plot 22–52 years old, see Bruelheide *et al.* 2011), middle-aged (fifth largest tree in plot 53–82 years old) and older tree stands (fifth largest tree in plot 82–116 years old). Because these two stratification factors were correlated to some extent ($R^2 = 0.41$), the design was not fully factorial and missed the combination of young tree stands with high species richness. The stratification allowed us to treat the two stratification factors as independent variables in the statistical analysis (comparative study, see Snedecor and Cochran 1989). In the present study, we used the species richness per plot of trees with DBH > 10 cm and the estimated stand age in five successional classes as defined in Bruelheide *et al.* (2011) as independent variables in all statistical analysis. In the rest of this study, tree species richness always refers to species richness of trees with DBH > 10 cm. All trees with DBH > 10 cm were measured and identified to the species level (Barrufol *et al.* 2013). According to this definition, 67 species were identified, 36 evergreen broadleaved, 28 deciduous broadleaved and 3 evergreen coniferous

tree species (for list of tree species with a DBH > 10 cm, see supplementary Appendix S1).

Sampling

As our study focused on the canopy, we specifically selected plant individuals from tree species reaching the canopy. A DBH of 10 cm was used as threshold and only trees with a greater DBH were considered for herbivory sampling.

To best represent the plot as a community and to have sufficient replication, we sampled 20 trees per plot except for plots with fewer individuals. We chose four individuals of the five most abundant tree species within a plot. In most of the plots, species with fewer individuals were included in order to reach 20 sampled trees; these additional species were the most abundant ones among the remaining species. When two species had the same abundance, we chose the species with the greater total basal area. Within tree species, we selected individuals at random. With this strategy, a sample of trees representing on average 40% of the total basal area in each plot (range: 13–85%) was obtained.

Within each tree, we cut three branches located at different height (bottom, middle and top) using a tree pruner mounted on interlocked 2-m poles. Branches located up to 15–16 m above ground could be reached; branch height was estimated using the length of the tree pruner. Because some tree crowns were compact (therefore, having branches very close to one another), we decided after a visual inspection to take only one to two branches in such cases. Less than three branches were also obtained when the tree was too high such that only the lower parts of the crown could be accessed.

Herbivory scoring

Shortly after collection (maximum delay 24 hours), leaves were scanned and herbivore damage assessed later on the digital images. The herbivore damage was estimated visually using damage classes of 0, 1–5%, 5–10%, 10–25%, 25–50%, 50–75% and 75–100%. Visual damage estimation has been shown to be reliable and previously used in other herbivory studies (Castagneyrol *et al.* 2013; Hahn *et al.* 2017; Schuldts *et al.* 2011; Vehviläinen *et al.* 2006). Different types of damage were distinguished and assigned to the following feeding guilds: leaf chewers, leaf skeletonizers, gall makers, leaf rollers and leaf miners (damage and abundance). Sap-feeder damage was scored by estimating the depigmented leaf areas caused by the piercing-sucking activity of these herbivores, which appeared as little white dots. Arthropod damage with unclear origin was marked as 'undefined'. A separate herbivory score for each feeding guild and a total herbivory score were estimated for each leaf. This scoring process was repeated on several twigs with about 5–10 leaves for each branch to account for within-branch variability, generally leading to 20–30 scored leaves per branch. Samples from species with needle leaves (*Pinus massoniana* Lamb., *P. taiwanensis* Hayata) that could not be scanned were scored visually shortly after collection (3–4 twigs per branch). Although less visible, cut

needles (counted as chewers) and little white dots (counted as sap feeders) could be scored. Twigs of the coniferous species *Cunninghamia lanceolata* (Lamb.) Hook. are flat; they were digitally scanned and processed similarly to the broad leaves of angiosperm trees. In order to prevent biases, a single person (M.B.) performed the whole herbivory assessment. Furthermore, after completion, the scoring for a single branch was repeated on the leaves that were first scored to correct for the evolution of leaf damage perception by the observer. This procedure was iterated until less than 10% of the leaf damage scores had to be changed.

Leaf biomass and tree phylogenetic diversity

In order to test the dilution effect hypotheses, effects of tree phylogenetic diversity and effects of biomass availability, several covariables were included in the analyses (see Table 1 for variable definition, details of variables hierarchy can be seen in supplementary Appendix S2).

Leaf biomass was estimated with three separate allometric models for broadleaved evergreen, broadleaved deciduous and coniferous tree species. These models scaled leaf biomass with DBH and were obtained from another allometric study conducted in the same area: 154 trees ranging from 1 to 37.5 cm DBH were felled and their leaf biomass accurately estimated according to standard methodologies (Brezzi 2015 adapted from Snowdon *et al.* 2002). The measured trees belonged to eight species available at that time for harvesting: *Cunninghamia lanceolata* and *P. massoniana* (coniferous, 36 individuals); *Castanopsis fargesii* Franchet, *Castanopsis sclerophylla* (Lindl. et Pax.) Schott. and *Schima superba* Gardn. & Champ. (broadleaved evergreen, 62 individuals); *Alniphyllum fortunei* (Hemsl.) Makino, *Liquidambar formosana* Hance and *Sassafras tzumu* Hemsl. (broadleaved deciduous, 56 individuals). The DBH range of the trees used to produce the allometric models was close to the DBH range of the trees found in the study plots (only 3.6% of the study plot tree stems had a DBH >40 cm).

Total plot leaf biomass was calculated as the sum of the predicted leaf biomass of all the trees with a DBH >10 cm within the plot. The coefficients of variation of each plot—calculated according to Chave *et al.* (2014)—of the total plot leaf biomass ranged from 16% to 43%. These uncertainties are relatively high because leaf biomass was very variable, even among individuals of the same species. The sum of all the individual leaf biomasses belonging to one species was divided by the total plot leaf biomass and this proportion of the total leaf biomass per plot was used as a measure of relative leaf biomass per tree species within each plot. This procedure was applied to the nine most common tree species (see below).

Tree species phylogenetic diversity and mean phylogenetic distance of each plot were calculated using all the trees with a DBH >10 cm. The phylogenetic data were acquired from an ultrametric phylogenetic tree of the tree species found in the 27 study plots (Michalski and Durka 2013). The coniferous species were excluded from the calculation first because

Table 1: explanatory variables used in statistical analyses of level of herbivory damage

Short name	Definition
Date	Sampling date
SR	Species richness: number of tree species with stem >10 DBH present in the plot
Age	Stand age
Elevation	Plot elevation (m above sea level)
Lf_bio	Total leaf biomass of the trees with DBH >10 cm in the plot (kg)
Con	Contrast between the coniferous <i>Pinus massoniana</i> and the other eight broadleaved species
Spec	Identity of the nine most common species (or 8 if Con is applied before Spec in statistical models)
SLA	Specific leaf area of the tree species within the plot (cm ² /g)
Tough	Leaf toughness of the tree species within the plot
Rel_Lf	Relative percentage of leaf biomass (proportion of total leaf biomass) of a species within a plot
Height	Height (m) at which the sampled branch was located
Guild	Identity of herbivore feeding guild responsible for the damage (chewers, skeletonizers, sap feeders, others)
PD	Tree phylogenetic diversity of tree species with stem >10 DBH present in the plot
MPD	Mean phylogenetic diversity between tree species with stem >10 DBH present in the plot

they were only abundant in very few plots and thus would have had a disproportionate impact on phylogenetic distance measurements at the community level (see e.g. Schuldt *et al.* 2014b) and second because we wanted to focus on the phylogenetic diversity and distance among angiosperms, which attracted the majority of herbivore species in the present study. Phylogenetic diversity (PD) was calculated by summing the phylogenetic tree branch lengths according to the method of Petchey and Gaston (2006). PD was closely related to tree species richness ($R^2 = 0.92$). Nevertheless, there was sufficient independent variation between the two variables such that their interaction could be tested in some of the statistical models as explained below. Mean phylogenetic distance (MPD) was calculated as the mean divergence in phylogenetic distance among all canopy tree individuals within each plot (abundance-weighted MPD) (Webb *et al.* 2002). MPD was used to explore differences between plots of given species richness. Because MPD is an average measure, it by definition reaches maximum values at species richness 2 and should not be used to compare plots of different species richness (Allan *et al.* 2013). In the present study, MPD was, as expected, significantly negatively related to tree species richness ($R^2 = 0.54$).

In order to investigate the effect of two important morphological leaf traits, specific leaf area (SLA) and leaf toughness (Eichhorn *et al.* 2007; Kitajima and Poorter 2010; Pérez-Harguindeguy *et al.* 2003) were measured for each species in each plot on 10 randomly chosen healthy leaves. Leaf toughness was defined as the force required to pierce the leaf with a standard needle (mN, measured with Holec spring scale

50CN); the average of the 10 leaves was taken as the value for this tree species within the plot. The SLA was obtained by scanning and measuring the area of the 10 fresh leaves and dividing the sum of their area by the sum of their dry mass (cm^2/g).

Statistical analysis

The mid-values of each herbivore damage class (per leaf; 0, 2.5%, 7.5%, 17.5%, 37.5%, 62.5% and 87.5%) were averaged at branch level (Scherber et al. 2010; Schuldt et al. 2012) because no variables were measured at leaf level. Heteroscedasticity of the residuals was removed by applying a log transformation. The different models (see below) incorporated variables that reflected the questions we were asking. We deliberately did not include a large number of covariates or high-order interactions because they would have reduced the chance to detect effects of the main variables of interest, in particular the independent design variable tree species richness, and would have increased the type-I error probability. Models always included sampling date (treated as a block effect), stand age (Age), tree species richness (SR) and branch height from the ground (Height). Plot elevation (m above sea level, ranging from 251 to 903 m) was also tested in each model given its potential influence on temperature-dependent herbivore physiology and performance (Garibaldi et al. 2011), however, this covariate was never significant. Depending on the model, several further covariates were added (see below). We retained in minimal models those additional terms, which were related to the question asked or which were significant (i.e. successively deleting non-significant terms in a stepwise backward selection procedure until only significant terms and the design variables were retained in the minimal models). The order of explanatory terms in the models was based on the given hierarchy: first plot-level, then species-level, then population-level (species within plot), then individual-level and finally branch-level variables (see also supplementary Appendix S2). Plot identity and tree individual identity were used as random-effects terms, that is, as error terms for testing fixed effects at the corresponding level of the given hierarchy. Branch identity was incorporated as a random-effects term for models including the herbivore feeding guilds (several measures on each branch) and tree species identity was incorporated as a random-effects term for the models including species contrasts. Random-effects terms are listed with their variance components in tables after the fixed-effects terms with their *F*-tests. The statistical program R 3.2.0 was used for all statistical analyses (R Development Core Team 2012). Mixed-effect models were run with ASReml for R (Gilmour et al. 2009; Schmid et al. 2017) and multivariate analyses were done with the package ‘vegan’ for R (Oksanen et al. 2012). ASReml uses restricted (=residual) maximum likelihood estimation of variance components and also allows negative variance components to be estimated (variance components, in contrast to variances, can be negative). Denominator degrees of freedom for the *F*-tests in the mixed-effects models were

calculated with the Kenward and Roger adjustment method (Kenward and Roger 1997).

Plot-level analysis

Average levels of herbivore damage at the plot level were investigated with the following Model 1:

$$1) \text{ Log(percent damage + 1) } \sim \text{Date} + \text{Age} * \text{SR} * \text{Height}$$

Here the * sign indicates factorial multiplication, i.e. $A * B = A + B + A \times B$, where $A \times B$ is the interaction between A and B (see Table 1 for additional explanations).

Model 2 was used to investigate the average levels of feeding guild damage at the plot level:

$$2) \text{ Log(percent damage + 1) } \sim \text{Date} + \text{Age} * \text{SR} * \text{Guild} * \text{Height}$$

Except for the three-way interaction ‘SR \times Age \times Guild’ higher than two-way interactions were excluded from the analysis. In order to test the potential effect of total plot leaf biomass, we additionally run models where this term was inserted in the minimal Models 1 and 2 before the Age and SR terms. We assumed stand age and tree species richness to cause total community leaf biomass variation and this assumption was reflected by fitting this covariate before the Age and SR terms. The effect of phylogenetic diversity was tested by rerunning Models 1 and 2 successively with PD fitted before or after tree species richness and stand age. The other phylogenetic measurement, mean phylogenetic diversity, was also tested by rerunning Models 1 and 2 but always fitting MPD after tree species richness. This allows to test MPD after adjusting for tree species richness, or in other words by holding tree species richness constant (Schmid et al. 2002).

Tree-level analysis

In order to test the influence of tree species richness on the differences in levels of herbivore damage among tree species, we built a reduced dataset including only species with enough replication; less replicated species would be confounded with plot effects. Nine species were retained as they matched with the replication threshold that we set: to have at least 40 branches sampled and to be present in at least 5 plots. These species were *Castanopsis eyrei* (Champ. ex Benth.) Tutch., *Castanopsis fargesii*, *Castanea henryi* (Skan) Rehd. et Wils., *Daphniphyllum oldhamii* Hemsley, *Lithocarpus glaber* (Thunb.) Nakai, *Myrica rubra* Sieb. et Zucc., *P. massoniana*, *Quercus serrata* Murray and *S. superba*. The sum of their basal area represented 41–100% of the total plot basal area (except for an outlier plot where they represented only 10% of the total plot basal area).

Model 3 was used to investigate the importance of tree species identity on levels of herbivore damage:

$$3) \text{ Log(percent damage + 1) } \sim \text{Date} + (\text{Age} * \text{SR}) * (\text{Con} + \text{Spec}) * (\text{Tough} + \text{SLA}) * \text{Height}$$

We considered two-way interactions and the two three-way interactions ‘Age \times SR \times Con’ and ‘Age \times SR \times Spec’.

Model 4 was used to test if the levels of feeding guild-specific herbivore damage were different among tree species and responded to the tree diversity gradient:

$$4) \text{Log}(\text{percent damage} + 1) \sim \text{Date} + (\text{Age} * \text{SR}) * (\text{Con} + \text{Spec}) * (\text{Tough} + \text{SLA}) * \text{Height} * \text{Guild}$$

Only interactions up to third order were considered. In order to test for a potential dilution effect, for instance, if the relative leaf biomass of the tree species could explain variation in levels of herbivore damage, final Models 3 and 4 were rerun inserting the relative biomass before the Age and SR terms. As for the total community leaf biomass, we assumed a causal relationship between tree species identity and relative leaf biomass.

Multivariate analysis

In order to test for a potential effect of tree species composition on levels of herbivore damage, the same tree species that we used to calculate the phylogenetic diversity were ordinated through Nonmetric Multidimensional Scaling (NMDS, package 'vegan' for R (Oksanen *et al.* 2012)). This procedure first performs a two-dimensional ordination that automatically chooses the best data transformation for the species scores and axes scaling. The Jaccard dissimilarity index was used for the distance among plots. Second, the explanatory variables were fitted in turn according to their most rapid change and their highest possible correlation in the ordination space. A permutation procedure (10^6 times) was used to calculate an empirical *P*-value (Oksanen *et al.* 2012). Average levels of herbivore damage and the average damage of the different feeding guilds at the plot level were tested with this method.

RESULTS

General statistics

A total of 31 249 leaves (or twigs concerning the three coniferous species, see Methods) were measured on a total of 1291 branches and 510 trees. The average level of herbivore damage (amount of photosynthetic tissue removed by herbivores

per leaf) at the plot level was 7.0%, ranging from 4.3% to 11.2%.

The different feeding guilds caused different levels of herbivore damage ($F_{3,76.4} = 139.3$, $P < 0.001$). The chewers caused the majority of the damage (66.3%) followed by the sap feeders (15.4%) and the skeletonizers (9.0%). About 9% of the damage could not be linked to a feeding guild (undefined). Leaf rollers and leaf miners caused negligible levels of damage (together $<0.4\%$); for the sake of simplicity, they were excluded from the analyses of feeding guild herbivory (but their contribution is included in the total level of herbivore damage and in the multivariate analysis).

Tree species richness and herbivore damage at plot level

The minimal version of Model 1 indicated a clear positive link between tree species richness and average level of herbivore damage per plot (Fig. 1A; Table 2). This relationship was independent of stand age: the variability explained by tree species richness remained virtually the same controlled or not for stand age. Branch height had a clear effect: with increasing height the leaves were less damaged (Table 2).

Adding the feeding guilds as predictor showed that they caused significantly different levels of herbivore damage (Table 3). Stand age and tree species richness were independent (as in Model 1) but not their interactions with feeding guilds (see Model 2a versus Model 2b). This is because these two interactions were partially confounded. Hence, the first interaction term removes both the variation that the two interactions explain together and the variation that this first interaction explains exclusively by itself, so that the second interaction can only remove the variation that it explains exclusively by itself. Nevertheless, the interaction of tree species richness with feeding guilds remained significant even when fitted after the interaction of stand age with feeding guilds. Different feeding guild responses to tree species richness were mainly due to a strong positive response of chewers (Fig. 1B).

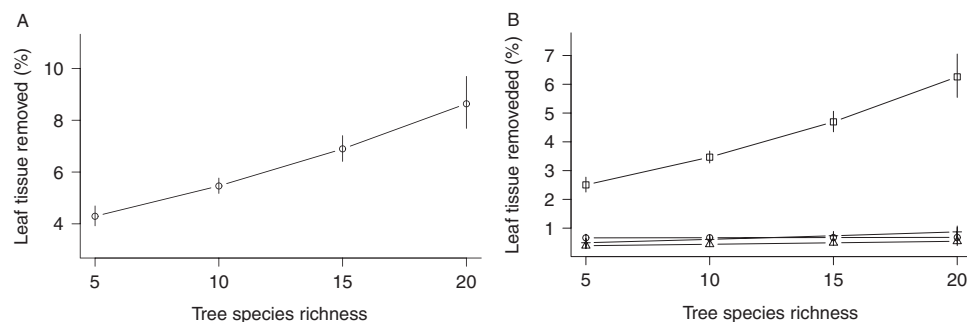


Figure 1: **A.** Percentage of photosynthetic leaf tissue removed by herbivores (\pm SE) predicted by Model 1 (Table 2). **B.** Percentage of photosynthetic leaf tissue removed by the different feeding guilds (\pm SE) predicted by Model 2 (Table 3). Square symbols are for chewer damage, circles for leaves damaged by undefined feeding guilds (excluding all others, i.e. also excluding leaf roller and gall makers), triangles for skeletonizer damage and crosses for sap-feeder damage. Predictions were calculated for the different levels of species richness by keeping the other explanatory variables at their average value. Means and standard errors are back-transformed from log+1-transformed percentages.

Stand age and tree species richness explained together 62.8% of the community leaf biomass variation among plots. A large part of the explanatory power was confounded

Table 2: effects of tree species richness and stand age on average levels of herbivore damage of branches in the tree canopy per plot (Model 1 in text)

	df	den df	<i>F</i>	<i>P</i>	Direction of effect
Date	1	21.0	4.85	0.039	Positive
Age	1	21.9	2.17	0.155	None
SR	1	21.1	13.56	0.001	Positive
Age × SR	1	21.1	3.71	0.068	None
Height	1	1037	23.20	<0.001	Negative
Stand age/tree species richness reversed					
SR	1	21.8	14.40	0.001	Positive
Age	1	21.4	1.33	0.261	None
Random terms	VC		SE		Z ratio
Plot	0.0222		0.0102		2.1784
Individual	0.1454		0.0138		10.547
Residuals	0.1500		0.0076		19.725

Stand age/tree species richness reversed show the results of tree species richness and stand age when their position was reversed in the model. The dependent variable was log+1-transformed level of herbivore damage per branch ($n = 1291$), calculated as mean damage per leaf. For variable names, see Table 1. Abbreviations: den df = denominator degree of freedom, df = degree of freedom, SE = standard error, VC = variance components.

Table 3: effects of tree species richness and stand age on average feeding guild-specific levels of herbivore damage of branches in the tree canopy per plot (Model 2 in text)

Model 2a	df	den df	<i>F</i>	<i>P</i>	Model 2b	<i>P</i>
Date	1	21.2	15.16	<0.001	Date	<0.001
Age	1	21.8	1.61	0.2175	SR	0.0054
SR	1	21.4	8.68	0.0076	Age	0.3991
Age × SR	1	21.2	1.72	0.2042	SR × Age	0.2042
Height	1	965.6	15.66	<0.001	Height	<0.001
Guild	3	67.8	200.6	<0.001	Guild	<0.001
Age × Guild	3	69.2	7.64	<0.001	SR × Guild	<0.001
SR × Guild	3	68.4	4.58	0.0055	Age × Guild	0.0782
Age × SR × Guild	3	67.4	3.27	0.0263	Age × SR × Guild	0.0263
Height × Guild	3	2887.0	20.00	<0.001	Height × Guild	<0.001
Random terms	VC		SE		Z ratio	
Plot	0.0026		0.0036		0.7211	
Individual	0.0011		0.0037		0.2926	
Branch	0.0017		0.0014		1.2001	
Damage:plot	0.0221		0.0056		3.9701	
Damage:individual	0.1529		0.0072		21.102	
Residuals	0.0916		0.0027		34.120	

Model 2b is equal to Model 2a but with the terms for tree species richness and stand age reversed. The dependent variable was log+1-transformed level of feeding guild-specific herbivore damage per branch ($n = 5164$, four guild-specific values per branch), calculated as mean of feeding guild-specific damage per leaf. For further explanations, see Table 1. Abbreviations: den df = denominator degree of freedom, df = degree of freedom, SE = standard error, VC = variance components.

between the two variables (36.5%): species richness contained only 6.2% of independent information, whereas stand age contained 20.1% of independent information. When introduced before stand age and tree species richness in the final model for average level of herbivore damage per plot (Model 1, Table 2), the amount of leaf biomass explained only a marginal amount of variation ($F_{1,21.0} = 3.5$, $P = 0.08$). In addition, the amount of variance explained by stand age and species richness was only marginally altered when they were fitted after leaf biomass, indicating that their link with herbivore damage was independent of leaf biomass.

In the models where PD was fitted after SR and Age, SR + Age removed all the variance that PD could explain and the results were similar to the previous results of Models 1 and 2 (see supplementary Appendix S3). In the models where PD was fitted first, it was highly significant. Because PD and SR were correlated, their effects in the models were to a large extent exchangeable. Still, there was enough independence between PD and SR that when they were fitted before stand age they produced a significant interaction affecting average levels of herbivore damage per plot ($F_{1,18.1} = 5.6$, $P < 0.05$, Fig. 2). The positive effect of tree species richness on level of herbivore damage was stronger at low levels of phylogenetic diversity and disappeared at higher levels of phylogenetic diversity (Fig. 2). Regarding the levels of herbivore damage caused by the different feeding guilds per plot, PD was never significant when fitted after SR. On the other hand, SR was still explaining a significant amount of variance when fitted

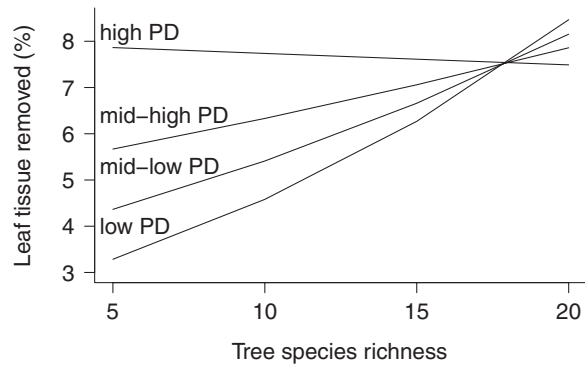


Figure 2: percentage of photosynthetic leaf tissue removed by herbivores predicted by Model 1 (see text) along the tree species richness gradient for four level of phylogenetic diversity: ‘high’ correspond to the most phylogenetically diverse plot value and low to the least phylogenetically diverse plot value. Predicted means and standard errors are not shown for the sake of readability.

after PD (PD + SR + Age) and when fitted after PD + Age (PD + Age + SR). In the tested models, average phylogenetic distance between species pairs within plots, i.e. MPD, was never significant and in contrast to PD, MPD never produced a significant interaction with SR. MPD also never produced significant effects or interactions in the feeding guild analysis (Model 2). Similar to MPD, plot elevation had no significant effects in any of the analyses and was thus never retained in a minimal model.

Tree species richness and herbivore damage at the level of individual trees

The coniferous species *P. massoniana* had significantly lower levels of herbivore damage than the other tree species and the other tree species were also significantly differently affected among each other by herbivores (Table 4, see also supplementary Appendix S4). Leaf toughness and SLA were not related to levels of average herbivore damage per tree and were thus removed from the final model. There was no interaction between species identity of the tree and tree species richness of the plot (Table 4), indicating that there were no differences in how the different tree species responded to the species diversity gradient with regard to levels of herbivore damage.

Finally, Model 3 was rerun placing the tree species richness term at the end of the model (but still before the interactions). The positive effect of tree species richness on levels of herbivore damage remained significant ($F_{1,21.1} = 6.7$, $P < 0.05$). This result means that leaves in plots with higher species richness did experience higher levels of herbivore damage than leaves in plots with lower species richness, even when we had already accounted for the different herbivory levels on the different common tree species and the different branch heights.

Feeding guild effects differed between coniferous and broadleaved tree species (Fig. 3). The broadleaved tree species themselves also varied in their responses to the different

Table 4: effects of tree species identity on levels of herbivore damage of branches in the tree crown of the dominant tree species in a plot (Model 3 in text)

	df	den df	<i>F</i>	<i>P</i>	Direction of effect
Date	1	17.7	10.9	0.004	Positive
Age	1	19.6	1.1	0.314	None
SR	1	17.0	24.6	<0.001	Positive
Age × SR	1	19.9	6.2	0.022	Stronger richness effect at younger stand stages
Con	1	70.5	87.9	<0.001	Coniferous were lower
Spec	7	87.9	15.3	<0.001	Species were different
SR × Con	1	81.6	0.5	0.481	None
SR × Spec	7	92.3	1.7	0.098	None
Height	1	808.7	12.0	<0.001	Negative
Random terms			VC	SE	Z ratio
Plot			0.0067	0.0067	1.0026
Plot:species			0.0240	0.0091	2.6300
Individual			0.0274	0.0085	3.2320
Residuals			0.1555	0.0091	17.152

The dependent variable was log+1-transformed level of herbivore damage per branch ($n = 1291$), calculated as mean damage per leaf. For further explanation, see Table 1. Abbreviations: den df = denominator degree of freedom, df = degree of freedom, SE = standard error, VC = variance components.

feeding guilds (Fig. 3; Table 5). The different feeding guilds caused different tree-species richness–herbivory relationships between the coniferous and broadleaved species (Fig. 4) but not among the different broadleaved species (Table 5). Leaf toughness and SLA did not interact with the feeding guilds and were thus not retained in the final model.

Fitted before the species identity term (Model 3), there was a tendency of tree species relative leaf biomass to negatively affect herbivory ($F_{1,54.4} = 3.5$, $P = 0.065$); the coniferous species was included with the other species in the model. This result indicates that tree species with lower relative leaf biomass within a plot experienced higher levels of herbivore damage.

The different feeding guilds responded differently to the relative leaf biomass of species within plots ($F_{3,175.8} = 30.6$, $P < 0.001$, Model 4). The chewers (generalists), the skeletonizers and the undefined feeders concentrated their damage on less abundant tree species (or were satiated with leaf biomass by the more abundant tree species), whereas the sap feeders (specialists) concentrated their damage on the dominant tree species within a plot (Fig. 5). The effect of tree species identity on the damage of the different feeding guilds remained highly significant when fitted after relative leaf biomass ($F_{24,231.3} = 28.9$, $P < 0.001$ versus $F_{24,244.8} = 32.7$, $P < 0.001$ in models without relative leaf biomass). As in the plot-level analyses, plot elevation had no significant effects and was not retained in the minimal models.

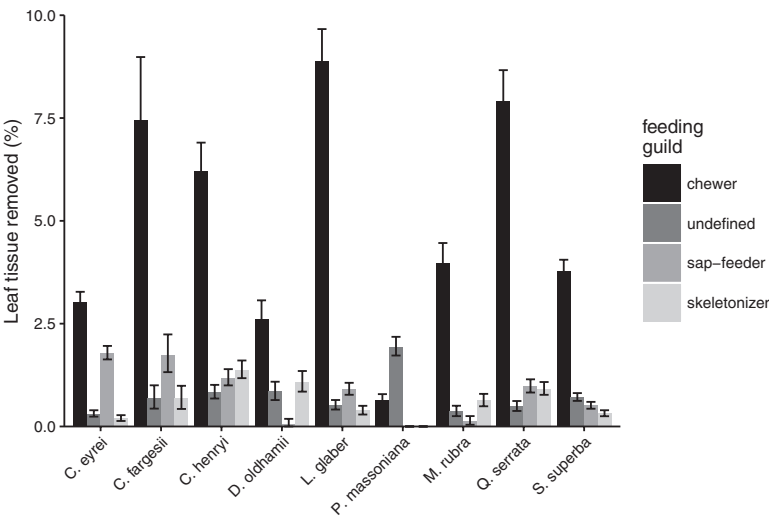


Figure 3: percentage of leaf tissue removed (mean ± SE) by the different feeding guilds predicted by Model 4 (see text) for the different tree species. Predictions were calculated by keeping the other explanatory variables at their average value. Means and standard errors are back-transformed from log+1-transformed percentages (negative predictions excluded).

Table 5: effects of tree species identity on levels of feeding guild-specific herbivore damage of branches in the tree crown of the dominant tree species in a plot (Model 4 in text)

	df	den df	F	P	Direction
Guild × Con	3	221.0	127.0	<0.001	Different feeding guild damages for coniferous compared to the other tree species
Guild × Spec	21	248.7	19.2	<0.001	Different feeding guild damages among broadleaf species
SR × Guild × Con	3	233.8	5.4	0.001	Feeding guild damages are different along the tree species richness gradient on coniferous compared to the other species
SR × Guild × Spec	28	294.2	0.97	0.509	Feeding guild damages are not different along the tree species richness gradient among the broadleaf species
Random terms			VC	SE	Z ratio
Plot			0.0028	0.0028	1.0020
Plot:species			−0.0055	0.0017	−3.1888
Individual			0.0018	0.0015	1.1653
Branch			0.0011	0.0016	0.6821
Damage:plot			0.0138	0.0047	2.9167
Damage:plot:species			0.0300	0.0047	6.4043
Damage:individual			0.0130	0.0027	4.7671
Residuals			0.0901	0.0030	29.714

Only the terms of interest are shown for the sake of simplicity. The dependent variable was log+1-transformed level of feeding guild-specific herbivore damage per branch ($n = 5164$, four guild-specific values per branch), calculated as mean of feeding guild-specific damage per leaf. For further explanations, see Table 1. Abbreviations: den df = denominator degree of freedom, df = degree of freedom, SE = standard error, VC = variance components.

Herbivory and tree species composition

Plots ordinated by tree species composition did not correlate with levels of total herbivore damage. Within feeding guilds, there was an effect of tree species composition on skeletonizer damage ($R^2 = 0.41$, $P = 0.002$) and on count of mines done by leaf miners ($R^2 = 0.25$, $P = 0.033$, supplementary Appendix S5) but not on levels of leaf miners damage. Tree species composition was also linked with a variable that was important in explaining levels of leaf herbivory, i.e. tree species richness ($R^2 = 0.46$, $P < 0.001$).

DISCUSSION

Our results support the hypothesis of a positive effect of tree diversity, i.e. species richness and phylogenetic diversity, on levels of herbivore damage, i.e. the percent damage caused by herbivores on leaves in the canopy of the studied subtropical forest plots in south-east China. Although one has to be careful on drawing conclusions from observational studies, these provide pictures of complex natural ecosystems that could not easily be generated experimentally (Leuschner et al. 2009).

Therefore, our results are valuable in helping to verify if theoretical predictions that hold for simpler experimental systems still hold in more complex natural systems. Furthermore, our study followed a comparative design, whereas most previous observational studies followed a sample survey design (Snedecor and Cochran 1989). The comparative study design had the advantage that plots were deliberately selected to

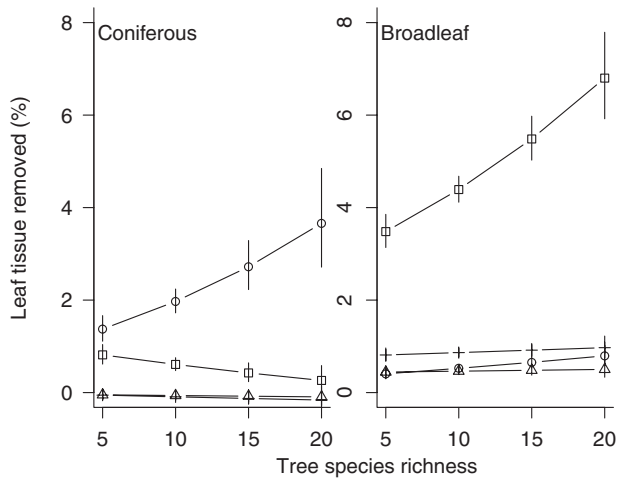


Figure 4: percentage (\pm SE) of photosynthetic leaf tissue removed by the different feeding guilds predicted by Model 4 (see text) for the coniferous tree species and the eight broadleaf tree species. Predictions were calculated by keeping the other explanatory variables at their average value. Square symbols are for chewer damage, circles for leaves damaged by undefined other feeding guilds, triangles for skeletonizer damage and crosses for sap-feeder damage. Means and standard errors are back-transformed from log+1-transformed percentages.

reflect a balanced representation of the independent factors of influence, tree species richness and stand age. Finally, our study is one among few specifically intended to study herbivory in the canopy layer in relation to tree diversity. Because ecological conditions are different in the canopy (only a subset of the plant species present in the plot effectively reach the canopy, solar radiation is stronger, humidity is lower, wind and temperature variations are greater (Jones 1983; Oke 1987)), it is essential to measure herbivory *in situ*. It is interesting to note that our results, with an average level of herbivore damage of 7%, agree in magnitude with what has been observed in other natural forests (i.e. Neves *et al.* 2010; Schuldt *et al.* 2010; Sobek *et al.* 2009). Such damage levels have been shown to already impact plant fitness (Zvereva *et al.* 2012).

Effects of tree diversity on average levels of herbivory per plot

The positive effect of tree species richness on levels of herbivore damage found in the present study contrasts with findings of some previous studies (Jactel and Brockerhoff 2007; Massey *et al.* 2006; Sobek *et al.* 2009; Unsicker *et al.* 2006) but is in agreement with findings of other previous studies (Dinnage 2013; Loranger *et al.* 2014; Mulder *et al.* 1999; Plath *et al.* 2012; Schuldt *et al.* 2014a). The plant species richness range in all these studies varied widely and was not different between the two groups finding positive versus negative effects of plant species richness on herbivory. Therefore, the number of plant species involved is probably not the reason for the direction of the diversity effect and indeed there are hypotheses for both, positive or negative effects. Moreover, other experimental characteristics were present in both study groups (negative

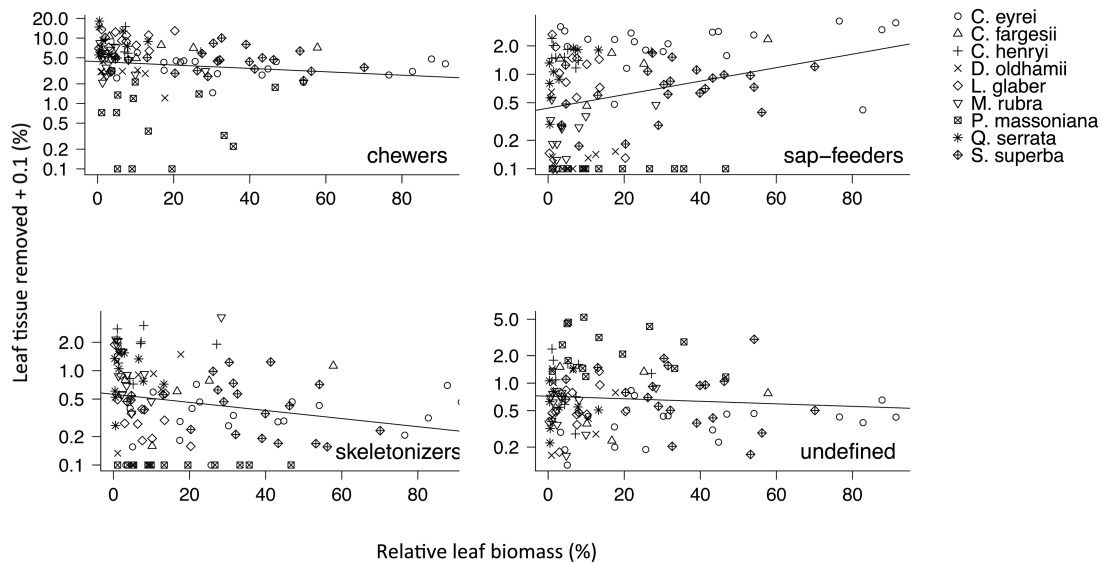


Figure 5: percentage of photosynthetic leaf tissue removed +0.1 as a function of the relative leaf biomass of each tree species within plots ($n = 116$; note log-scale of y-axis). Due to herbivory values of zero, 0.1 was added to solve the problem of logarithmic transformation. The nine tree species are marked by different symbols. The three feeding guilds and the undefined feeding guild are displayed separately.

versus positive effects of plant species richness on herbivory): natural forest (Massey *et al.* 2006 and Sobek *et al.* 2009 versus Schuldt *et al.* 2014a) or grassland (Unsicker *et al.* 2006 versus Mulder *et al.* 1999, Loranger *et al.* 2014 and Dinnage 2013). In addition, there is one study in both groups using the response of specialist herbivores to explain the increase (Jactel and Brockerhoff 2007) or decrease (Plath *et al.* 2012) of herbivory along the plant species richness gradient. Therefore, there is no evidence for a systematic difference in plant community characteristics that could separate the group of studies finding positive from the group of studies finding negative effects of plant species richness on herbivory.

There are several potential explanations for positive effects of plant diversity on herbivory. Below, we show that in our case the most likely explanation is dietary mixing (Bernays *et al.* 1994). In this explanation, the increase in herbivore damage is a consequence of generalist herbivores taking advantage of a diversified diet. Positive effects of plant diversity on leaf herbivory might also occur if the tree diversity-induced increase in community leaf biomass leads to a J-shaped, i.e. greater than proportional, increase of herbivore abundance. In such a case, an increase in community leaf biomass could lead to a higher level of herbivore damage on single leaves (see supplementary Appendix S6 for an illustration of this concept). However, because we did not find a significant relation between community leaf biomass and average level of herbivore damage—in spite of a positive relation between tree species richness and community leaf biomass ($R^2 = 0.45$, $P < 0.001$)—the relation between community leaf biomass and herbivore abundance in our case most likely was a linear one.

Given the strong correlation between tree species richness and phylogenetic diversity, it was surprising that there was still a significant interaction between the two, suggesting that the positive effect of tree species richness on average levels of herbivore damage per plot was strongest at low levels of phylogenetic diversity. It is conceivable that at high phylogenetic diversity the spectrum of different food plants is too high even for generalist herbivores such that they also suffer from resource dilution. Bertheau *et al.* (2010) showed a strong decrease in herbivore fitness with increasing phylogenetic distance between the original and a new food plant. In contrast, additional similar tree species could be beneficial for these generalist herbivores due to advantages of dietary mixing. Such positive effects of dietary mixing on the fitness of generalist herbivore arthropods have been demonstrated earlier in plant species diverse grassland ecosystems (Pfisterer *et al.* 2003; Unsicker *et al.* 2010). Although forests are spatially more complex, which may affect how arthropods can switch from one host to another, our results match with the prediction of the dietary mixing hypothesis. Many of the dominant herbivores in the forests of our study region are highly mobile adult leaf chewers (including e.g. chrysomelid beetles and orthopterans; Schuldt *et al.* 2014c; J. Zhang, X. Chen, X. Xu, X. Lu and A. Schuldt, unpublished data), which are generally characterized by relatively low feeding specialization (Forister

et al. 2015; Novotny *et al.* 2010) and for which the use of multiple hosts is easily possible due to their mobility. Deviating responses of other feeding guilds to tree species richness might in part be due to lower mobility and thus lower ability for dietary mixing or higher feeding specialization (Forister *et al.* 2015; Hambäck *et al.* 2014). Our results also match with findings of Dinnage (2013) and Castagneyrol *et al.* (2014) and underline the importance of both plant species richness and phylogenetic diversity in predicting herbivore impacts. It is remarkable that, beyond these effects of tree diversity, tree species composition was not linked with levels of total herbivore damage on leaves. This emphasizes the usefulness of tree diversity as an explanatory variable in studies of variation in herbivory levels in subtropical forests. Our finding that only PD but not MPD showed significant interactions with tree species richness might reflect the average nature of the MPD measure, which does not distinguish between different distributions of phylogenetic distances among species within communities, e.g. many small and many large distances if species are phylogenetically clustered versus mostly medium distances if species are phylogenetically regularly dispersed (Allan *et al.* 2013).

Differences in levels of herbivore damage among tree species and among feeding guilds

Because a part of the herbivore species can be expected to be relatively specialized and hence to be found only on some tree species, differences in herbivore damage among tree species and among herbivore feeding guilds are expected. Furthermore, specialized herbivores could be sensible to the dilution of the resource offered by their food plant species. In our study, the different tree species investigated indeed did suffer different levels of herbivore damage and were impacted differentially by the different feeding guilds. Furthermore, the different feeding guilds were also differently affected by tree species richness or phylogenetic diversity. These results support differences in feeding guild degree of specialization. Because many chewers were possibly to a greater extent generalists (see above), the increasing chewing damage along the tree species richness gradient might be the result of better plot quality for these less specialized herbivores which would have profited from the diversity and complementarity of resources as described in the previous section and previous reports (Bernays *et al.* 1994; Dinnage 2013; Schuldt *et al.* 2014a). As discussed above, this resource-mixing hypothesis relies on herbivores actively switching among hosts to optimize their diet. Alternatively, recent models propose that plant species richness might change the whole-plot attractiveness or suitability (Hambäck *et al.* 2014), which could have increased the immigration/emigration ratio of herbivores resulting in higher damage in more diverse plots of our study. However, the fact that underrepresented tree species suffered higher levels of damage supports the dietary mixing hypothesis because damage caused by specialists was not caused at random within the plot but rather must have reflected an active host choice.

Moreover, the recently proposed models focus on relatively small patches of host plants and assume that demographic processes play a minor role at such spatial scales. In contrast, our study analyzes larger plot-level data of forest stands embedded in a matrix of surrounding, similar forest, where herbivore population dynamics might add to explaining the observed herbivory patterns. Nevertheless, a closer analysis of small-scale interactions at the plant neighborhood level, and a direct incorporation of herbivore traits, may provide valuable insight into these plant–herbivore interactions (Hambäck *et al.* 2014).

In contrast, sap-feeder damage showed a less pronounced response to tree species richness but affected the different tree species very differently. The tree species *D. oldhamii* had near zero sap-feeder damage, whereas *Castanopsis eyrei* had about 3% of its leaf area damaged by sap feeders. The sap-feeder group was also the only group showing a negative response to tree species dilution. Altogether this points to a higher degree of specialization of the sap-feeding arthropods, which is in agreement with earlier reports from tropical and sub-tropical forests (Blüthgen *et al.* 2006; Novotny *et al.* 2010; Staab *et al.* 2015). Such negative responses to tree species dilution—or alternatively positive responses to tree species concentration—also support the dietary mixing hypothesis because specialized arthropods should not take advantage of a greater variety of resources.

In contrast to the prediction of the resource dilution hypothesis, tree species with lower relative leaf biomass in a plot did not have lower but rather higher levels of herbivore damage than did tree species with higher relative leaf biomass in a plot. This unexpected result was caused by the chewer and skeletonizer feeding guilds (see Fig. 5), which together were responsible for the major part of the herbivore damage. The result may be due in part to a dietary mixing strategy: herbivores could maximize dietary mixing by biasing their feeding toward plant species with low relative leaf biomass. An alternative explanation would be that the tree species with the highest relative leaf biomass in a plot may actually have had lower levels of herbivore damage per leaf because of a satiation of herbivores analogous to predator satiation by mass production of seeds (see e.g. Tong *et al.* 2017 and Veller *et al.* 2015 for two recent references). Alternatively, because we did not discriminate between relative and total leaf biomass, we cannot completely exclude that tree species with the higher relative biomass have less damage because arthropod load may increase more slowly than leaf biomass resulting in herbivore dilution; a similar phenomenon was described by Otway *et al.* (2005).

Herbivory-related leaf traits can vary among (Schuldt *et al.* 2012) and additionally within tree species (Ruhnke *et al.* 2009; Suomela and Ayres 1994). Our attempts to explain the different levels of herbivore damage within the nine tree species investigated with functional traits potentially related to leaf palatability, SLA and leaf toughness, were not successful because these variables were not retained in any of

our statistical models. We tested these two leaf traits at the within species level (i.e. the species mean differences were first removed by fitting the species identity term before the leaf traits measured on individual branches). Our results indicate that the variation of these two traits within tree species did not explain variations of the levels of herbivore damage within tree species. In a separate analysis (Appendix S7, see online supplementary material), SLA and leaf toughness were fitted before the species term, therefore, testing for variation among and within species. We again found no effect of leaf toughness but a strong effect of SLA, meaning that differences in levels of herbivory between tree species could in part be explained by their different mean values of SLA. Several studies (Carmona *et al.* 2011; Pearse and Hipp 2009) suggest that the particular combination of several defense traits might be the major determinant of levels of herbivore damage among tree species. It is thus remarkable that the single leaf trait SLA in our study explains such a large variation in levels of herbivore damage between tree species. Ruhnke *et al.* (2009) showed that levels of herbivore damage vary within and among tree individuals, but not in relation to within-species variation in single leaf traits. It is conceivable that in our study a combination of multiple traits would have allowed a better prediction of the level of herbivore damage within tree species.

Implications of herbivory for plant diversity–ecosystem functioning relationships

Increased levels of herbivore damage along tree species richness gradients and differences in levels of herbivore damage among tree species might have consequences on ecosystem functioning and tree species coexistence in species-rich forests. The levels of herbivory reported in our study can already negatively impact plant fitness (Zvereva *et al.* 2012) but are unlikely to cause tree mortality as other studies reported higher levels of herbivory without increased mortality (Plath *et al.* 2012; Tong *et al.* 2003; Zvereva *et al.* 2012). Nevertheless, the herbivore damage that we report could still play a major role in shaping the tree community. This could happen because herbivory can reduce the number or the viability of seeds produced (Marquis 1988; Smith and Hough-Goldstein 2014) and can reduce plant growth (Kim *et al.* 2013; Schuldt *et al.* 2015; Zvereva *et al.* 2012), which in turn may reduce plant competitive abilities to reach additional resources. However, it is worth underlining that the relative leaf biomass of a species in a plot explained only a small part of the total variation in levels of herbivore damage and thus could not be the dominating force ruling this ecosystem. Manipulative experiments in grasslands proved that herbivory could promote plant species richness or evenness (Mulder *et al.* 1999; Stein *et al.* 2010). In forests, a manipulative experiment showed that arthropod herbivory could modify the tree species composition by altering seedling recruitment (Bagchi *et al.* 2014). These causal relationships are strong indications that the increased herbivory along the plant species richness gradient was not only

a result but might also have been a driving force in maintaining tree species richness in the studied subtropical forest in south-east China.

SUPPLEMENTARY MATERIAL

Supplementary material is available at *Journal of Plant Ecology* online.

ACKNOWLEDGEMENTS

We wish to thank the European project BACCARA funded by the European Commission's Seventh Framework Program (FP7/2007–2013) under Grant Agreement No. 226299 for financial support. Our thanks are also directed to the administration of the Gutianshan National Nature Reserve and to the members of the BEF-China consortium for logistic and intellectual support. We are indebted to the National Science Foundation of China (NSFC 30710103907 and 30930005) and the German Research Foundation (DFG FOR 891) that granted and permitted the establishment of the experiment. *Conflict of interest statement.* None declared.

REFERENCES

- Allan E, Jenkins T, Fergus AJF, *et al.* (2013) Experimental plant communities develop phylogenetically overdispersed distributions during assembly. *Ecology* **94**:465–77.
- Andrew NR, Roberts IR, Hill SJ (2012) Insect herbivory along environmental gradients. *Open Journal of Ecology* **2**:202–13.
- Bagchi R, Gallery RE, Gripenberg S, *et al.* (2014) Pathogens and insect herbivores drive rainforest plant diversity and composition. *Nature* **506**:85–8.
- Barrufol M, Schmid B, Bruelheide H, *et al.* (2013) Biodiversity promotes tree growth during succession in subtropical forest. *PLOS ONE* **8**:e81246.
- Basset Y, Novotny V, Miller SE, Kitching RL (eds) (2003) *Arthropods of Tropical Forests: Spatio-Temporal Dynamics and Resource Use in the Canopy*. Cambridge, UK: Cambridge University Press.
- Bernays EA, Bright KL, Gonzalez N, *et al.* (1994) Dietary mixing in a generalist herbivore: tests of two hypotheses. *Ecology* **75**:1997–2006.
- Bertheau C, Brockerhoff EG, Roux-Morabito G, *et al.* (2010) Novel insect-tree associations resulting from accidental and intentional biological 'invasions': a meta-analysis of effects on insect fitness. *Ecol Lett* **13**:506–15.
- Blüthgen N, Mezger D, Linsenmair KE (2006) Ant-hemipteran trophobioses in a Bornean rainforest – diversity, specificity and monopolisation. *Insect Soc* **53**:194–203.
- Brezzi M (2015) Influence of tree species richness on arthropod community patterns and foliar herbivory plus allometric equations to predict tree biomass. *Ph.D. Thesis*. University of Zürich.
- Bruelheide H, Böhnke M, Both S, *et al.* (2011) Community assembly during secondary forest succession in a Chinese subtropical forest. *Ecol Monogr* **81**:25–41.
- Bruelheide H, Nadrowski K, Assmann T, *et al.* (2014) Designing forest biodiversity experiments: general considerations illustrated by a new large experiment in subtropical China. *Methods Ecol Evol* **5**:74–89.
- Cardinale BJ, Matulich KL, Hooper DU, *et al.* (2011) The functional role of producer diversity in ecosystems. *Am J Bot* **98**:572–92.
- Carmona D, Lajeunesse MJ, Johnson MTJ (2011) Plant traits that predict resistance to herbivores. *Funct Ecol* **25**:358–67.
- Castagneyrol B, Giffard B, Péré C, *et al.* (2013) Plant apparency, an overlooked driver of associational resistance to insect herbivory. *J Ecol* **101**:418–29.
- Castagneyrol B, Jactel H, Vacher C, *et al.* (2014) Effects of plant phylogenetic diversity on herbivory depend on herbivore specialization. *J Appl Ecol* **51**:134–41.
- Chave J, Réjou-Méchain M, Búrquez A, *et al.* (2014) Improved allometric models to estimate the aboveground biomass of tropical trees. *Glob Chang Biol* **20**:3177–90.
- Chi XL, Guo Q, Fang JY, *et al.* (2017) Seasonal characteristics and determinants of tree growth in a Chinese subtropical forest. *J Plant Ecol* **10**:4–12.
- Coley PD, Barone JA (1996) Herbivory and plant defenses in tropical forests. *Annu Rev Ecol Syst* **27**:305–35.
- Dinnage R (2013) Phylogenetic diversity of plants alters the effect of species richness on invertebrate herbivory. *PeerJ* **1**:e93.
- Eichhorn MP, Fagan KC, Compton SG, *et al.* (2007) Explaining leaf herbivory rates on tree seedlings in a Malaysian rain forest. *Biotropica* **39**:416–21.
- Floren A, Wetzel W, Staab M (2014) The contribution of canopy species to overall ant diversity (Hymenoptera:Formicidae) in temperate and tropical ecosystems. *Myrmecol News* **19**:65–74.
- Forister ML, Novotny V, Panorska AK, *et al.* (2015) The global distribution of diet breadth in insect herbivores. *Proc Natl Acad Sci USA* **112**:442–7.
- Garibaldi LA, Kitzberger T, Chaneton EJ (2011) Environmental and genetic control of insect abundance and herbivory along a forest elevational gradient. *Oecologia* **167**:117–29.
- Geißler C, Kühn P, Böhnke M, *et al.* (2012) Splash erosion potential under tree canopies in subtropical SE China. *Catena* **91**:85–93.
- Gilmour AR, Gogel BJ, Cullis BR, *et al.* (2009) *ASReml User Guide Release 3.0*. VSN International Ltd, Hemel Hempstead, HP1 1ES, UK. www.vsnl.co.uk.
- Hahn CZ, Niklaus PA, Bruelheide H, *et al.* (2017) Opposing intraspecific vs. interspecific diversity effects on herbivory and growth in subtropical experimental tree assemblages. *J Plant Ecol* **10**:242–51.
- Hambäck PA, Inouye BD, Andersson P, *et al.* (2014) Effects of plant neighborhoods on plant-herbivore interactions: resource dilution and associational effects. *Ecology* **95**:1370–83.
- Jactel H, Brockerhoff EG (2007) Tree diversity reduces herbivory by forest insects. *Ecol Lett* **10**:835–48.
- Jones HG (1983) *Plants and Microclimate: A Quantitative Approach to Environmental Plant Physiology*. Cambridge, UK: Cambridge University Press.
- Kenward MG, Roger JH (1997) The precision of fixed effects estimates from restricted maximum likelihood. *Biometrics* **53**:983–97.
- Kim TN, Underwood N, Inouye BD (2013) Insect herbivores change the outcome of plant competition through both inter- and intraspecific processes. *Ecology* **94**:1753–63.
- Kitajima K, Poorter L (2010) Tissue-level leaf toughness, but not lamina thickness, predicts sapling leaf lifespan and shade tolerance of tropical tree species. *New Phytol* **186**:708–21.

- Leuschner C, Jungkunst HF, Fleck S (2009) Functional role of forest diversity: pros and cons of synthetic stands and across-site comparisons in established forests. *Basic Appl Ecol* **10**:1–9.
- Loranger H, Weisser WW, Ebeling A, *et al.* (2014) Invertebrate herbivory increases along an experimental gradient of grassland plant diversity. *Oecologia* **174**:183–93.
- Lowman MD (2009) Canopy research in the twenty-first century: a review of arboreal ecology. *Trop Ecol* **50**:125–36.
- Marquis RJ (1988) Intra-crown variation in leaf herbivory and seed production in striped maple, *Acer pensylvanicum* L (Aceraceae). *Oecologia* **77**:51–5.
- Massey FP, Massey K, Press MC, *et al.* (2006) Neighbourhood composition determines growth, architecture and herbivory in tropical rain forest tree seedlings. *J Ecol* **94**:646–55.
- Michalski S, Durka W (2013) *Phylogenetic Tree of the Tree Species Found in the CSPs*. <http://china.befdata.biow.uni-leipzig.de/datasets/240>.
- Mulder CPH, Koricheva J, Huss-Danell K, *et al.* (1999) Insects affect relationships between plant species richness and ecosystem processes. *Ecol Lett* **2**:237–46.
- Neves FS, Araújo LS, Espírito-Santo MM, *et al.* (2010) Canopy herbivory and insect herbivore diversity in a dry forest-savanna transition in Brazil. *Biotropica* **42**:112–8.
- Novotny V, Miller SE, Baje L, *et al.* (2010) Guild-specific patterns of species richness and host specialization in plant-herbivore food webs from a tropical forest. *J Anim Ecol* **79**:1193–203.
- Oke TR (1987) *Boundary Layer Climates*, 2nd edn. London :Methuen Publishing Ltd, 110–54.
- Oksanen J, Blanchet FG, Kindt R, *et al.* (2012) *vegan: Community Ecology Package*. R package version 2.2–1. <http://cran.r-project.org/package=vegan>.
- Otway SJ, Hector A, Lawton JH (2005) Resource dilution effects on specialist insect herbivores in a grassland biodiversity experiment. *J Anim Ecol* **74**:234–40.
- Pearse IS, Hipp AL (2009) Phylogenetic and trait similarity to a native species predict herbivory on non-native oaks. *Proc Natl Acad Sci USA* **106**:18097–102.
- Peng SY, Schmid B, Haase J, *et al.* (2017) Leaf area increases with species richness in young experimental stands of subtropical trees. *J Plant Ecol* **10**:128–35.
- Pérez-Harguindeguy N, Díaz S, Vendramini F, *et al.* (2003) Leaf traits and herbivore selection in the field and in cafeteria experiments. *Austral Ecol* **28**:642–50.
- Petchey OL, Gaston KJ (2006) Functional diversity: back to basics and looking forward. *Ecol Lett* **9**:741–58.
- Pfisterer AB, Diemer M, Schmid B (2003) Dietary shift and lowered biomass gain of a generalist herbivore in species-poor experimental plant communities. *Oecologia* **135**:234–41.
- Plath M, Dorn S, Riedel J, *et al.* (2012) Associational resistance and associational susceptibility: specialist herbivores show contrasting responses to tree stand diversification. *Oecologia* **169**:477–87.
- R Development Core Team (2012) *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing. <http://www.r-project.org/>.
- Riihimäki J, Kaitaniemi P, Koricheva J, *et al.* (2005) Testing the enemies hypothesis in forest stands: the important role of tree species composition. *Oecologia* **142**:90–7.
- Root RB (1973) Organization of a plant-arthropod association in simple and diverse habitats: the fauna of collards (*Brassica oleracea*). *Ecol Monogr* **43**:95–124.
- Ruhnke H, Schädler M, Klotz S, *et al.* (2009) Variability in leaf traits, insect herbivory and herbivore performance within and among individuals of four broad-leaved tree species. *Basic Appl Ecol* **10**:726–36.
- Russel EP (1989) Enemies hypothesis: a review of the effect of vegetational diversity on predatory insects and parasitoids. *Environ Entomol* **18**:590–9.
- Scherber C, Eisenhauer N, Weisser WW, *et al.* (2010) Bottom-up effects of plant diversity on multitrophic interactions in a biodiversity experiment. *Nature* **468**:553–6.
- Schmid B, Baruffol M, Wang Z, *et al.* (2017) A guide to analyzing biodiversity experiments. *J Plant Ecol* **10**:91–110.
- Schmid B, Hector A, Huston MA, *et al.* (2002) The design and analysis of biodiversity experiments. In Loreau M, Naeem S, Inchausti P (eds). *Biodiversity and Ecosystem Functioning: Synthesis and Perspectives*. Oxford: Oxford University Press, 61–75.
- Schuldt A, Assmann T, Bruehlheide H, *et al.* (2014b) Functional and phylogenetic diversity of woody plants drive herbivory in a highly diverse forest. *New Phytol* **202**:864–73.
- Schuldt A, Baruffol M, Böhnke M, *et al.* (2010) Tree diversity promotes insect herbivory in subtropical forests of south-east China. *J Ecol* **98**:917–26.
- Schuldt A, Baruffol M, Bruehlheide H, *et al.* (2014c) Woody plant phylogenetic diversity mediates bottom-up control of arthropod biomass in species-rich forests. *Oecologia* **176**:171–82.
- Schuldt A, Both S, Bruehlheide H, *et al.* (2011) Predator diversity and abundance provide little support for the enemies hypothesis in forests of high tree diversity. *PLOS ONE* **6**:e22905.
- Schuldt A, Bruehlheide H, Durka W, *et al.* (2012) Plant traits affecting herbivory on tree recruits in highly diverse subtropical forests. *Ecol Lett* **15**:732–9.
- Schuldt A, Bruehlheide H, Durka W, *et al.* (2014c) Tree diversity promotes functional dissimilarity and maintains functional richness despite species loss in predator assemblages. *Oecologia* **174**:533–43.
- Schuldt A, Bruehlheide H, Härdtle W, *et al.* (2015) Early positive effects of tree species richness on herbivory in a large-scale forest biodiversity experiment influence tree growth. *J Ecol* **103**:563–71.
- Siemann E, Tilman D, Haarstad J, *et al.* (1998) Experimental tests of the dependence of arthropod diversity on plant diversity. *Am Nat* **152**:738–50.
- Smith JR, Hough-Goldstein J (2014) Impact of herbivory on mile-a-minute weed (*Persicaria perfoliata*) seed production and viability. *Biol Control* **76**:60–4.
- Snedecor GW, Cochran WG (1989) *Statistical Methods*. Ames, IA: Iowa State University Press.
- Snowdon P, Raison J, Keith H, *et al.* (2002) *Protocol for Sampling Tree and Stand Biomass*. Canberra, Australia. <http://www.greenhouse.gov.au/ncas>.
- Sobek S, Scherber C, Steffan-Dewenter I, *et al.* (2009) Sapling herbivory, invertebrate herbivores and predators across a natural tree diversity gradient in Germany's largest connected deciduous forest. *Oecologia* **160**:279–88.

- Srivastava DS, Lawton JH (1998) Why more productive sites have more species: an experimental test of theory using tree-hole communities. *Am Nat* **152**:510–29.
- Staab M, Blüthgen N, Klein AM (2015) Tree diversity alters the structure of a tri-trophic network in a biodiversity experiment. *Oikos* **124**:827–34.
- Staab M, Schuldt A, Assmann T, et al. (2014) Tree diversity promotes predator but not omnivore ants in a subtropical Chinese forest. *Ecol Entomol* **39**:637–47.
- Stein C, Unsicker SB, Kahmen A, et al. (2010) Impact of invertebrate herbivory in grasslands depends on plant species diversity. *Ecology* **91**:1639–50.
- Stork NE, Grimbacher PS (2006) Beetle assemblages from an Australian tropical rainforest show that the canopy and the ground strata contribute equally to biodiversity. *Proc Biol Sci* **273**:1969–75.
- Suomela J, Ayres MP (1994) Within-tree and among-tree variation in leaf characteristics of mountain birch and its implications for herbivory. *Oikos* **70**:212–22.
- Tong YE, Lee SY, Morton B (2003) Effects of artificial defoliation on growth, reproduction and leaf chemistry of the mangrove *Kandelia candel*. *J Trop Ecol* **19**:397–406.
- Tong X, Zhang Y-X, Wang R, et al. (2017) Habitat fragmentation alters predator satiation of acorns. *J Plant Ecol* **10**:67–73.
- Unsicker SB, Baer N, Kahmen A, et al. (2006) Invertebrate herbivory along a gradient of plant species diversity in extensively managed grasslands. *Oecologia* **150**:233–46.
- Unsicker SB, Franzke A, Specht J, et al. (2010) Plant species richness in montane grasslands affects the fitness of a generalist grasshopper species. *Ecology* **91**:1083–91.
- Vehviläinen H, Koricheva J, Ruohomäki K (2007) Tree species diversity influences herbivore abundance and damage: meta-analysis of long-term forest experiments. *Oecologia* **152**:287–98.
- Vehviläinen H, Koricheva J, Ruohomäki K, et al. (2006) Effects of tree stand species composition on insect herbivory of silver birch in boreal forests. *Basic Appl Ecol* **7**:1–11.
- Veller C, Nowak MA, Davis CC (2015) Extended flowering intervals of bamboos evolved by discrete multiplication. *Ecol Lett* **18**:653–9.
- Webb CO, Ackerly DD, McPeck MA, et al. (2002) Phylogenies and community ecology. *Annu Rev Ecol Syst* **33**:475–505.
- Whiles MR, Charlton RE (2006) The ecological significance of tall-grass prairie arthropods. *Annu Rev Entomol* **51**:387–412.
- Wright DH (1983) Species-energy theory: an extension of species-area theory. *Oikos* **41**:496–506.
- Yu M, Hu Z, Yu J, et al. (2001) Forest vegetation types in Gutianshan Natural Reserve in Zhejiang. *J Zhejiang Univ (Agric & Life Sci)* **27**:375–80.
- Zhang Y, Adams J (2011) Top-down control of herbivores varies with ecosystem types. *J Ecol* **99**:370–2.
- Zvereva EL, Zverev V, Kozlov MV (2012) Little strokes fell great oaks: minor but chronic herbivory substantially reduces birch growth. *Oikos* **121**:2036–43.