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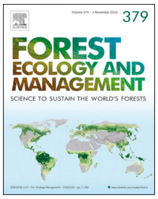

Tree diversity effects on leaf insect damage on pedunculate oak: The role of landscape context and forest stratum

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A B S T R A C T

Tree diversity has long been recognized as a major driver of insect herbivory in forest ecosystems. However, predicting the strength and direction of tree diversity effects in real-world situations has proven elusive. One likely reason is that most studies have focused on within-stand dynamics and insufficiently captured other ecological drivers of insect herbivory that can act at broader (i.e., landscape) and finer (i.e., individual trees) scales. We measured herbivory as leaf area consumed by insect herbivores in pedunculate oaks (*Quercus robur*) growing in mixed and pure forest stands in southwestern France. We assessed the effects of oak spatial isolation within the landscape, tree stand diversity, forest canopy stratification as well as the influence of leaf traits on insect herbivory. Insect herbivory increased with stand isolation regardless of tree diversity. Diversity effects were contingent upon the canopy stratum as insect herbivory in mixed stands exceeded that of pure stands only in the upper stratum. Leaf traits varied between pure and mixed stands and among canopy strata. Insect herbivory was negatively correlated with LDMC and positively with SLA. However, the observed effects of tree diversity, canopy stratum and stand isolation on insect herbivory were only partially driven by variability in oak leaf traits. Our findings illustrate that, in real-world contexts, insect herbivory can be driven by a complex interplay of multiple, scale-dependent drivers. They help step forward towards a more profound understanding of the complex forces drive insect herbivory in managed forest ecosystems.

1. Introduction

A long held view in forest ecology is that tree diversity strongly influences insect herbivory. Extensive research has demonstrated that trees are generally more prone to suffering damage when grown in monospecific stands than when associated with other tree species (Castagneyrol et al., 2014; Vehviläinen et al., 2007), although neutral (Rosado-Sánchez et al., 2018) or even opposite patterns have also been reported (Schuldt et al., 2010). The underlying phenomenon, termed associational resistance, appears to be widespread in forests (Castagneyrol et al., 2014; but see Haase et al., 2015; Schuldt et al., 2015). However, to date most empirical evidence on associational effects in forests stems from case studies of outbreaks of particular pest species and from highly controlled experiments (Castagneyrol et al., 2013; Damien et al., 2016; Schuldt et al., 2015; Vehviläinen et al., 2007). Despite their evident strengths (Grossman et al., 2018; Paquette et al., 2018; Verheyen et al., 2016), such experiments fail to properly consider the diversity and complexity of drivers that tend to affect background insect herbivory under natural, non-outbreak conditions (Guyot et al., 2016; Moreira et al., 2017; Kozlov and Zvereva 2017). This lack strongly constrains our understanding of the actual ecological relevance of associational effects on insect herbivory in real-world situations.

Patterns of insect herbivory are moulded by a variety of factors controlling plant accessibility at different scales. At the landscape scale, herbivore density tends to be highest in those habitat patches where their resource is most abundant, because the intensity of physical and chemical cues makes these patches more likely to be found and colonized (Andersson et al., 2013; Hambäck and Englund, 2005; Root, 1973). Within patches, herbivory on individual plants is influenced by the identity and diversity of their neighbours which alter the focal plant's physical and chemical apparency and its colonization (Castagneyrol et al., 2013; Finch and Collier, 2000; Moreira et al., 2016). Finally, herbivory is controlled by individual plant traits including nutritional quality and anti-herbivore defences (Castagneyrol et al., 2018b; Finch and Collier, 2000; Schoonhoven, 2005). Insects rely

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on a complex system of decision cues for selecting the plants they forage on. These can be used hierarchically or sequentially, and their role can vary depending on the animal's spatial scale of perception or nutritional status (Andersson et al., 2013; Schoonhoven, 2005). For instance, herbivores can be attracted by large patches from the distance but their final decision to feed on a given plant depends on its traits and its neighbours (Finch and Collier, 2000, 2012; Hambäck et al., 2014). While great advances have been made in our understanding of specific drivers of insect herbivory, the scale-dependent interplay between different drivers remains poorly understood.

Some such interactions have recently been hypothesized. It has for instance been suggested that resource isolation at the landscape level may cause herbivores to dedicate more time to foraging within resource patches, thus increasing damage in isolated stands as a result of reinforced small-scale effects of tree diversity on feeding decisions (Hambäck et al., 2014; Stutz et al., 2015; Underwood et al., 2014). However, observational studies demonstrating such an interaction are lacking. Within habitat patches, herbivory tends to vary along vertical gradients in forest canopy as a result of parallel changes in microclimate (in particular, higher temperatures in upper strata, Stiegel et al., 2017), leaf traits (Dudt and Shure, 1994; Stiegel et al., 2017; Thomas et al., 2010), or the diversity and activity of herbivores' enemies (Aikens et al., 2013). For instance, Stiegel et al. (2017) showed that the decrease in insect herbivory from lower to upper stratum was accounted for by the parallel increase of temperatures and decrease in nitrogen content of more sun-exposed leaves. However, the effect of stratification on herbivory has proven to vary among insect feeding guilds and forest management (Gossner et al., 2014). Tree diversity is known to alter the vertical stratification of forest canopies (Forrester, 2017; Vanhellemont et al., 2018). In turn, forest stratification will likely alter the effects of tree diversity on herbivores. For instance, both the position in the canopy (Stiegel et al., 2017) as well as the density and diversity of neighbouring trees can trigger the surface or the C:N ratio of plant leaves, two traits that are commonly related with patterns of herbivory (Castagneyrol et al., 2017; Moreira et al., 2017; Loranger et al., 2013; Pearse, 2011; Schoonhoven, 2005). However, quantifying the relative contribution of trait-dependent and trait-independent effects of plant density and diversity on insect herbivory remains challenging and the few existing studies have yielded conflicting results.

Here, we address how tree diversity effects on leaf damage caused by defoliating insect herbivores in pedunculate oak (*Quercus robur*) are shaped by ecological drivers independently and interactively acting at the individual (i.e., leaf traits), habitat (i.e., forest stratum) and landscape (i.e., forest stand isolation) scales. For this purpose, we measured leaf herbivory in oaks in the lower, intermediate and upper stratum of pure and mixed oak forest stands along a gradient of forest isolation at the landscape level across the season. We hypothesised that (i) insect herbivory would be lower in mixed oak-pine stands than in pure oak stands, and (ii) the difference would be strongest in isolated stands. We further predicted that (iii) leaf traits and insect herbivory would vary among forest strata and that (v) differences in herbivory among forest strata would be larger in pure than in mixed stands. By addressing tree diversity effects on insect herbivory at different scales, both within and between stands, our study pursues a better understanding of the hierarchical mechanisms that drive tree-herbivore interactions in real-world landscapes.

2. Materials and methods

2.1. Study site and stand selection

The study was carried out in the Landes de Gascogne region (south-western France), about 40 km southwest of Bordeaux (44°41′N, 00°51′W). This region harbours the largest plantation forest in Europe with a monoculture of maritime pine (*Pinus pinaster*) covering approximately 10 000 km^2. The remaining landscape is characterized by agricultural lands and woodlands whose most common tree species are oaks (*Quercus robur*, *Q. pyrenaica*) and birch (*Betula pendula*). These deciduous tree species are also present in variable abundance in the pine plantations.

Table 1
Summary of stand characteristics.

Stand	Coordinates	Stand type
Berganton	44° 45′40.85″N, 0° 49′ 37.58″W	Pure
France	44° 44′ 44.10″N, 0° 50′ 50.82″W	Pure
St Alban	44° 43′ 18.78″N, 0° 45′ 3.25″W	Pure
Barlan	44° 44′ 57.00″N, 0° 49′ 53.12″W	Pure
Castéra	44° 44′ 0.1″N, 0° 52′ 42.29″W	Pure
Croix d'Hins	44° 43′ 21.69″N, 0° 49′ 32.31″W	Pure
Hermitage	44° 44′ 50.69″N, 0° 46′ 10.78″W	Mixed
Renardière	44° 43′ 42.33″N, 0° 50′ 8.78″W	Mixed
H5	44° 43′ 8.11″N, 0° 49′ 59.83″W	Mixed
H6	44° 43′ 30.63″N, 0° 51′ 10.72″W	Mixed
H8	44° 43′ 10.51″N, 0° 50′ 36.85″W	Mixed
H20	44° 42′ 56.23″N, 0° 51′ 48.50″W	Mixed

In early 2009, we selected 12 forest stands for study: six pure stands in which *Quercus robur* was the main species, and six mixed stands consisting of pine-oak mixtures where oaks were abundant (Table 1). For each stand, we mapped the main habitat types in circular buffers of 500 m radius (*ca.* 78.5 ha). We distinguished pine plantations, deciduous forests and open habitats. Open habitats included roads, forest tracks, firebreaks, clearcuts, field and field margins, and young pine plantations (Barbaro et al., 2005). The buffer of 500 m radius provided the largest gradient of habitat variability, avoided spatial overlapping between nearby buffers, and was previously found to be suitable to study plant-herbivore-predator interactions in different landscape contexts (Barbaro et al., 2005; Chaplin-Kramer et al., 2011). Habitat mapping was based on satellite images read and analysed with QGIS version 2.18.13 (QGIS Development Team, 2017).

2.2. Leaf sampling and measurements

In each stand, we randomly selected individual oak trees and assigned them to one of three forest strata. All leaves collected below 1 m were assigned to the lower stratum, which may have included hanging low branches of adult trees as well as leaves of younger individuals. The canopies were further divided in an intermediate stratum (lower and mid-height branches) and an upper stratum (upper branches). We chose to define these strata in relative instead of absolute terms because tree height varied among the different stands.

In 2009, we sampled twice a total of 300 oak leaves per stratum in each stand, in early (July) and late (September) season. The two sampling dates were considered as temporal replicates. Although leaf herbivory is a cumulative process such that early season damage remains visible in late season, oaks produce three and up to four generations of leaves in the study area. Thus, the first and second campaigns represent independent assessments of leaf insect herbivory.

Leaves were collected on a sample of six individuals per stratum and per season, that were haphazardly selected each time to ensure statistical independence of the temporal replicates. The number of sampled leaves was held constant between pure and mixed stands and between temporal replicates, whereas the sampling methodology was adapted to the forest stratum because of technical constraints. Leaves were collected using a pruning shear for the lower stratum, and with a 10 m pole pruner for the intermediate stratum of all stands and the upper stratum of mixed stands. Rifle shooting was used to cut branches down in the upper stratum of pure stands. For the lower stratum, we assembled a 300 leaf sample by haphazardly collecting 35 leaves on one hanging branches of six different individuals, plus an additional pool of leaves from younger individuals. For intermediate and upper strata, we assembled the 300 leaf sample by collecting 30–50 leaves on six to ten

Table 2
Summary of LMM testing the effects of season, forest type, stratum and isolation on herbivory and leaf traits. P-values are indicated within brackets and significant effects are shown in bold. Marginal (R^2_m) and conditional (R^2_c) R^2 are reported for the simplified model.

Predictors	df	Total herbivory χ^2-value	Leaf surface χ^2-value	SLA χ^2-value	LDMC χ^2-value
Season	1	**121.06** (< 0.001)	0.66 (0.416)	**4.01** (0.045)	**122.03** (< 0.001)
Forest type	1	3.2 (0.072)	**4.74** (0.029)	**11.08** (< 0.001)	**13.55** (< 0.001)
Stratum	2	**129.79** (< 0.001)	**1090.22** (< 0.001)	**1240.75** (< 0.001)	**102.15** (< 0.001)
Isolation	1	**8.27** (0.004)	**3.95** (0.047)	1.00 (0.318)	0.05 (0.819)
Forest type × Stratum	2	**71.71** (< 0.001)	**26.69** (< 0.001)	**68.70** (< 0.001)	**15.45** (< 0.001)
Forest type × Isolation	1	0.86 (0.352)	0.02 (0.885)	1.59 (0.207)	2.37 (0.123)
Stratum × Isolation	2	1.08 (0.583)	2.89 (0.216)	1.26 (0.533)	2.58 (0.275)
Forest type × Stratum × Isolation	2	0.67 (0.716)	2.21 (0.331)	0.59 (0.744)	0.32 (0.854)
R^2m (R^2c)		0.36 (0.45)	0.65 (0.66)	0.68 (0.72)	0.36 (0.45)

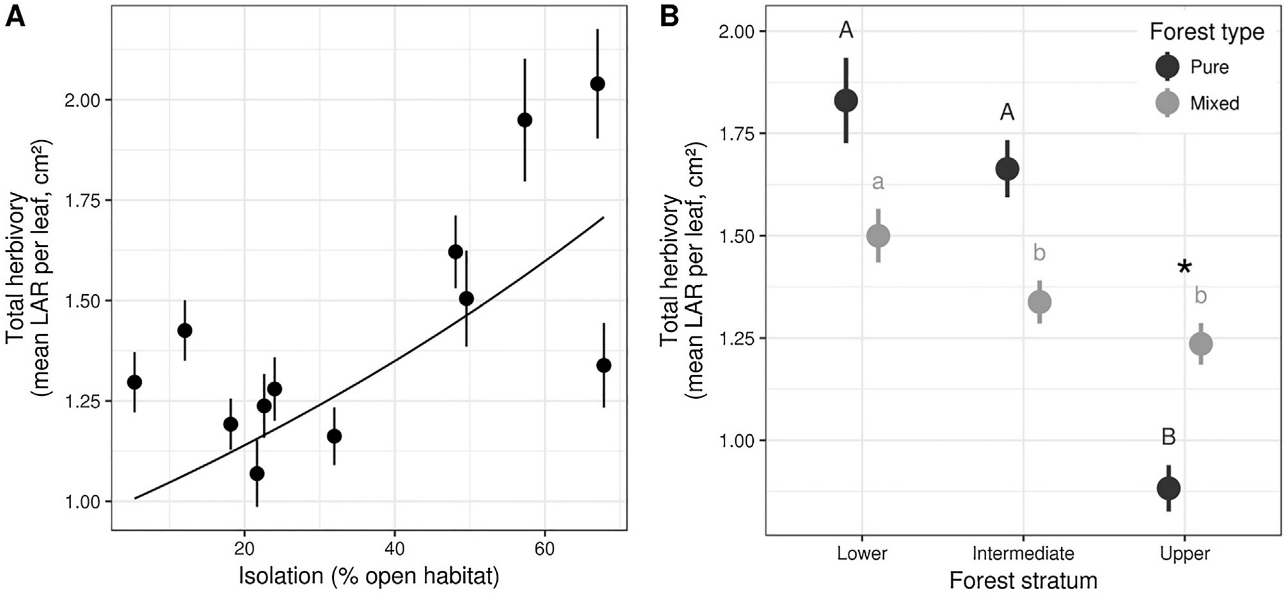

Fig. 1. Effects of stand isolation, forest type and stratum on leaf herbivory. (A) Effects of stand isolation. Each point represents leaf herbivory averaged (± SE) per stratum and season. (B) Interactive effects of forest type and stratum on leaf herbivory. Each point represents leaf herbivory averaged per season across all forest stands (± SE, n = 6 per forest type). Letters above bars indicate statistical differences between forest types.

branches of 6 different trees (1 or 2 branches per tree). In each sample, we took every tenth leaf until reaching the scheduled sample size.

Herbivory was estimated by two measurers aware of sample origin. To reduce variability among observers, we used a grid of 0.25 cm^2 (0.5 × 0.5 cm) printed on a transparent plastic sheet and overlaid on leaves. We calculated the total leaf area removed or affected by insect herbivores divided by the number of leaves analysed. We initially disentangled damage caused by different feeding guilds (chewers, skeletonizers, leaf-rollers, leaf-miners). However, some of these guilds caused too scant damages to allow separate analyses and we therefore pooled all types of damages.

We measured three leaf traits: leaf surface, Specific Leaf Area (SLA) and Leaf Dry Matter Content (LDMC). These traits were measured on 10 leaves per stratum, stand and sampling date following Cornelissen et al. (2003) after confirming that this sample size satisfyingly captures variability among strata. We only used undamaged, mature, and fully expanded leaves. Leaf surface and water-saturated fresh mass were measured with a planimeter (WinFolia Pro 2007b, Regent Instruments, Canada Inc.) and a balance (Ohaus EP114 Explorer Pro Analytical Balance). Leaf surface, SLA and LDMC were first calculated at the level of individual leaves and then averaged per replicate, stratum, stand and season.

2.3. *Statistical analyses*

We calculated the percentage of open area in buffers of 500 m radius centered on selected stands as a proxy for stand isolation at the landscape level. We preferred to use this variable instead of deciduous forest cover because pedunculate oaks commonly grow below the canopy of pine plantations without being detectable on satellite images (Gerzabek et al., 2017). The cover of deciduous forest alone therefore underestimates oak abundance in the landscape. We used linear mixed-effect models (LMM) to analyse the effects of landscape, tree diversity and canopy stratification on leaf traits and insect herbivory. Fixed effects were season (early *vs.* late), forest type (pure *vs.* mixed stand), stratum (lower, intermediate and upper) and isolation (percentage of open areas in the landscape). Season was considered as a temporal replicate. We tested all two- and three-way interactions between forest type, stratum and isolation. We declared stand identity as a random factor to account for the non-independence of samples from the same stand. For each response variable (herbivory, leaf surface, SLA and LDMC), we first built the full model and then applied model simplification by sequentially removing non-significant terms, starting with the highest-order interaction term. We made no attempt to simplify the random factor as it was imposed by the sampling design. Significant interactions between stratum and forest type were treated by estimating contrasts among strata for each forest type separately and contrasts between pure and mixed stands for each stratum independently. Finally, we estimated

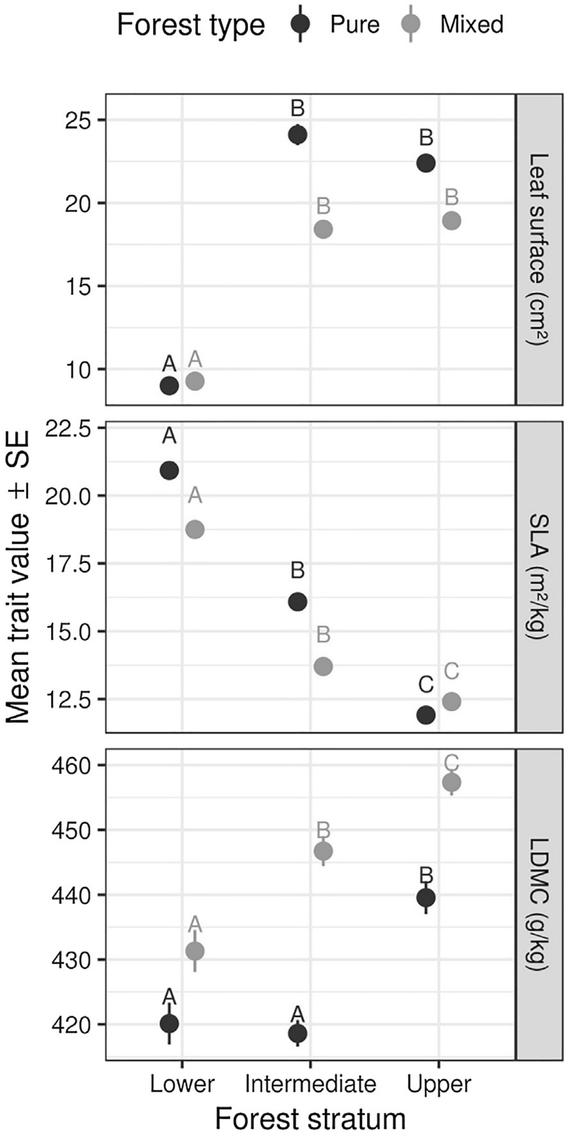

Fig. 2. Interactive effects of stratum and forest type on leaf traits. Dots and error bars represent means (± SE) across seasons and forest types. Letters above bars indicate statistical differences between strata. Contrasts between strata are shown for each forest type separately (indicated by different grey shades).

model coefficients of the simplified model and calculated R^2 for fixed effects (R_m^2) and fixed plus random effects (R_c^2). Response variables were log-transformed to improve the distribution of model residuals. We back-transformed model predictions with an exponential function to plot them on figures.

We were interested in disentangling the effects of isolation, forest type, stratum and leaf traits on herbivory. Yet, leaf traits were influenced by both forest type and stratum (see Results). In order to avoid issues arising from collinearity, we also tested the effect of leaf traits on herbivory in a separate model where we replaced the factors forest type and stratum by SLA and LDMC. We then applied the same modelling procedure as described above. SLA, LDMC and isolation were scaled and centered to allow comparing coefficient parameter estimates, although we present raw data in the figures.

Finally, we used a structural equation modelling (SEM) to confirm the indirect trait-mediated effect of tree diversity and forest stratum on herbivory. We first built a theoretical model in which herbivory was only explained by SLA and LDMC. SLA and LDMC were endogeneous variables. Tree diversity and forest stratum were exogeneous variables that only influenced SLA and LDMC. We used the piecewiseSEM package (Lefcheck, 2016) and Shipley's test of direct separation to evaluate the probability that none of the paths missing from the hypothesised network contain useful information (in particular direct paths linking forest stratum and tree diversity to herbivory). This hypothesis was considered rejected if χ^2-test of Fisher's C statistic fell below the significance level ($P < 0.05$).

All analyses were done in R version 3.3.1 (R Development Core Team, 2016) using the packages *lmer4*, *car*, *multcomp*, *MuMIn* and *piecewiseSEM* (Bartoń, 2016; Fox and Weisberg 2011; Hothorn et al., 2008; Kuznetsova et al., 2016, Lefcheck, 2016).

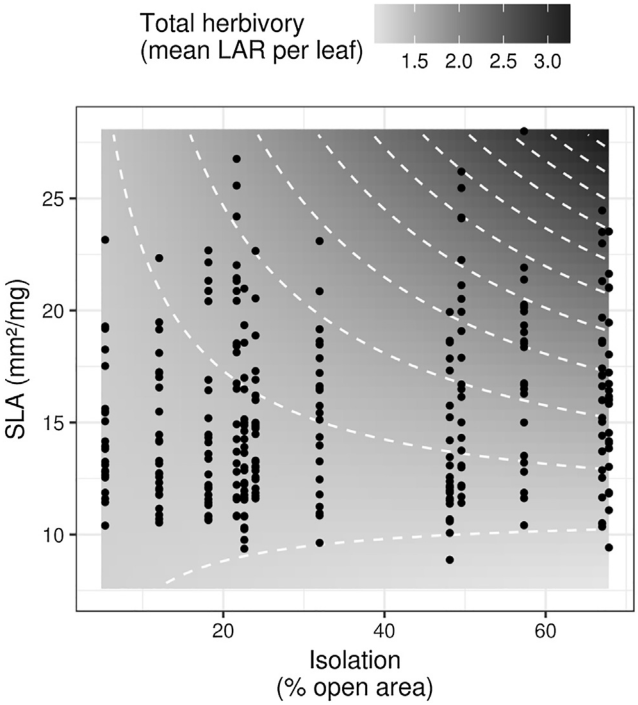

Fig. 3. Interactive effect of Specific Leaf Area (SLA) and stand isolation on leaf herbivory. Grey shades and isolines show predictions from mixed-effects models for early-season data and for an average value of LDMC. Dots show the original data.

3. Results

3.1. Effects of forest type, stratum, isolation and season on herbivory

Herbivory was on average (± SE) 1.42 ± 0.03 cm² per leaf (corresponding to *ca.* 8% leaf area), which corresponds to background insect herbivory in the study area. Herbivory was 37% higher in the late season than in the early season (Table 2). It increased with stand isolation (Fig. 1A) and decreased from the lower to the upper stratum (Fig. 1B). Herbivory did not differ between mixed and pure stands, but we observed a significant forest type × stratum interaction (Table 2) as differences between pure and mixed stands were only significant in the upper stratum (coefficient parameter estimate ± SE: 0.60 ± 0.13, Fig. 1B). In pure stands, leaf herbivory was significantly lower in the upper stratum (Fig. 1B) whereas it did not differ between the intermediate and the lower stratum. In mixed stand, leaf herbivory was higher in the lower stratum than in the intermediate and upper stratum, whereas the latter two did not differ (Fig. 1B).

3.2. Effects of forest type, stratum and isolation on leaf traits

Leaf traits varied consistently among strata and between forest types

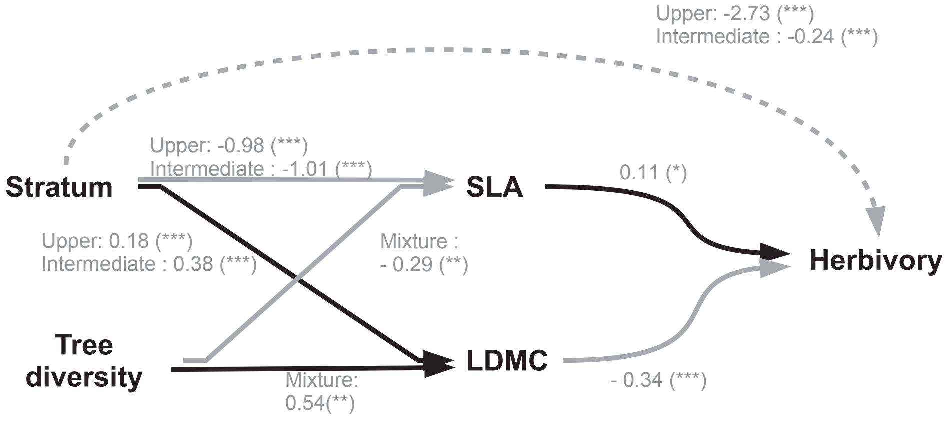

Fig. 4. Path diagrams showing the results of the piecewise SEM. Solid and dashed lines represent significant direct and indirect relationships among variables, respectively. Black and grey lines represent negative and positive correlations, respectively. Standardized coefficients and significance thresholds are shown along paths.

(Fig. 2) with a significant forest type × stratum interaction for all traits (Table 2). Leaf surface was on average (± SE) $17.5 \pm 0.3\,\text{cm}^2$. It was twice as large in the intermediate and upper stratum than in the lower stratum, both across forest types and seasons, while there were no differences between the intermediate and the upper stratum. Differences between the lower and the intermediate and upper stratum were larger in pure stands than in mixed stands (Table 2). Oaks had larger leaves in pure stands than in mixed stands, but only in the upper and intermediate strata. SLA was on average $15.40 \pm 0.15\,\text{mm}^2 \cdot \text{mg}^{-1}$. It consistently decreased from lower to upper stratum in both forest types and seasons. All contrasts between strata were significant, but differences were larger in pure than in mixed stands. SLA tended to be higher in pure stands than in mixed stands, but this difference was only significant in the lower and the intermediate stratum. LDMC was on average $436.0 \pm 1.2\,\text{mg} \cdot \text{g}^{-1}$. It consistently increased from lower to upper stratum in both seasons and forest types. However, differences among strata were contingent on forest type (Table 2). In pure stands, LDMC was greater in the upper stratum than in the intermediate and lower stratum, while the latter two did not differ. In mixed stands, all contrasts between strata were significant. LDMC was greater in mixed stands than in pure stands, but this difference was only significant in the intermediate and the upper stratum.

4. Leaf traits associated with the effects of forest type and stratum on herbivory

Replacing the factors forest type and stratum in the LMM by the leaf traits SLA and LDMC, we found that differences in herbivory between forest types and among strata could be accounted for by LDMC and SLA. Herbivory decreased with LDMC ($[82.4 \pm 24.8] \times 10^{-3}$, df = 1, $\chi^2 = 11.00$, $P = 0.001$) and increased with SLA ($[6.0 \pm 37.6] \times 10^{-3}$, df = 1, $\chi^2 = 34.90$, $P < 0.001$, Fig. 3). The effect of SLA was however contingent on stand isolation (significant SLA × isolation interaction: df = 1, $\chi^2 = 14.46$, $P < 0.001$). The positive coefficient parameter estimate (± SE) for the interaction (0.06 ± 0.02) indicated that the effect of SLA on herbivory was slightly stronger in more isolated stands and that the effect of stand isolation was in turn stronger for leaves with greater SLA. These results are consistent with our observation that SLA and herbivory both decreased from the lower to the upper stratum. Model R^2 was however lower when the factors forest type and stratum were replaced by SLA and LDMC ($R^2_m = 0.29$ and $R^2_c = 0.42$ *vs.* $R^2_m = 0.36$ and $R^2_c = 0.44$, Table 2), suggesting that the measured leaf traits account largely but not completely for differences in herbivory arising from differences between forest types and among strata.

In line with these results, the SEM analysis (Fig. 4) confirmed that there were missing paths in our *a priori* network ($C = 9.69$, df = 4, $P = 0.046$), indicating that the effects of tree diversity and forest stratum on insect herbivory were only partially mediated by their effect on the measured leaf traits. In particular, there was a significant missing path between forest stratum and herbivory, indicating an additional, trait-independent, effect of forest stratum on insect damage.

5. Discussion

The factorial and hierarchical sampling design of this single year-study enabled us to get a detailed insight into the complex interplay of environmental drivers that determine patterns of background insect herbivory across spatial scales (Fig. 5). We did not detect a global difference in herbivory between mixed and pure stands, yet a finer analysis revealed that such an effect of tree diversity did exist but only in certain canopy strata in pure stands. We also observed that both stand isolation and stratum influenced herbivory. Finally, both stand isolation and stratification effects were partly explained by the concomitant variation in the leaf traits SLA and LDMC. This set of interrelationships clearly illustrates the complex nature of the multiple, scale-dependent drivers of insect herbivory in real-world contexts. It calls for caution when interpreting ecological studies that address limited sets of putative drivers of insect herbivory in simplified environments.

Leaf insect herbivory was not globally reduced in mixed stands but in certain strata. We found that tree diversity effects on insect herbivory were not consistent across canopy strata. While mixed stands experienced lower herbivory than pure stands in the lower and intermediate stratum, the opposite occurred in the upper stratum. Previous studies on effects of tree diversity on insect herbivory have acknowledged potential stratum effects but, instead of quantifying them, seeked to reduce them by averaging herbivory at the level of individual trees (Castagneyrol et al., 2013; Muiruri et al., 2015; Vehviläinen et al., 2006). Our study pinpoints the pitfalls of such an approach by demonstrating that the canopy stratum can exert a significant influence on the (non-)detection of global effects of tree diversity. The pattern that we observed might be explained by the fact that insects coming from other forest stands are likely to arrive in the upper stratum. Reduced herbivory in the upper stratum of pure stands could then reflect a dilution of the recently arrived herbivores among a larger number of host trees (Bañuelos and Kollmann, 2011; Damien et al., 2016; Otway et al., 2005).

Leaf insect herbivory increased from the upper to the lower stratum. In accordance with previous studies (e.g. Reynolds and Crossley, 1997; Stiegel et al., 2017), we found that insect herbivores caused most damage in the lower stratum. This trend has several possible, non-exclusive explanations. First, vertical stratification in herbivory can be a direct consequence of stratification in leaf traits. SLA increased and LDMC decreased towards the lower stratum, suggesting that its leaves were most palatable and hence most attractive for herbivores (Le Corff and Marquis, 1999; Murakami and Wada, 1997; Stiegel et al., 2017). This assumption was fully confirmed by the distinct effects that both parameters exerted on levels of herbivory. Although we did not measure microclimate in our stands, it is likely that

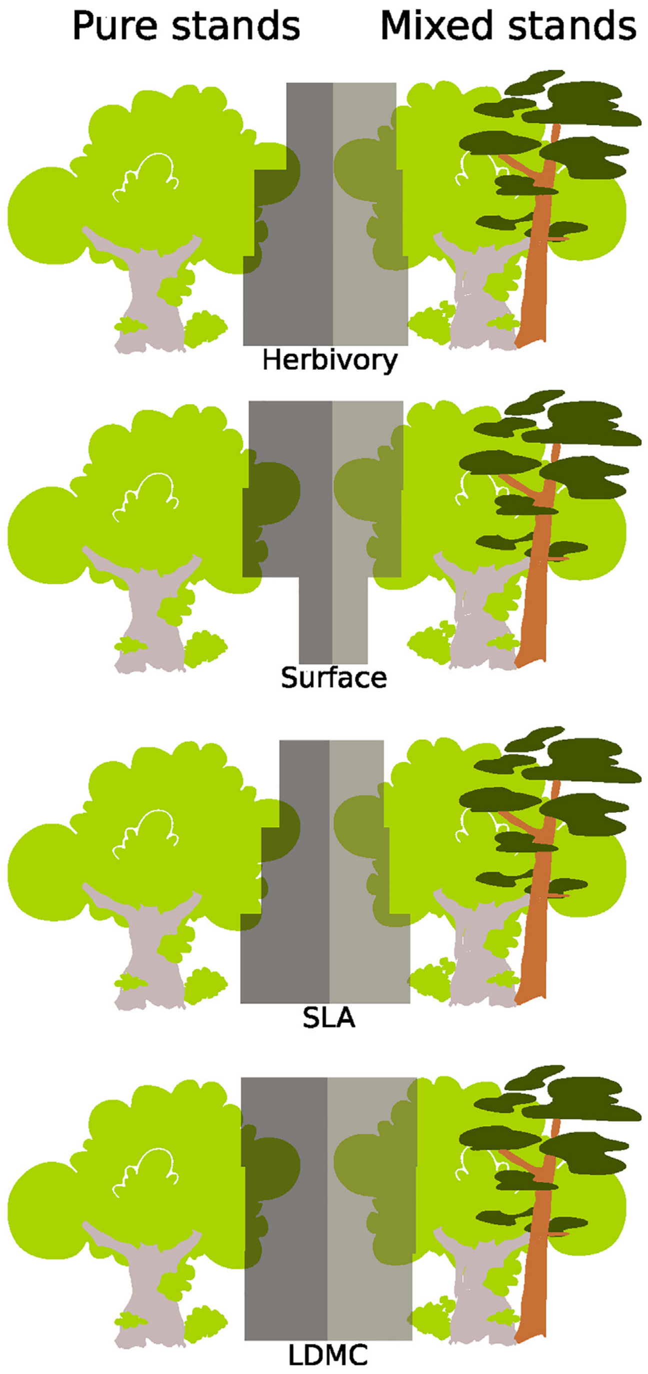

Fig. 5. Summary of herbivory and leaf trait response to forest type and stratification. Grey squares represent herbivory and traits for different forest types and strata. Differences in square size are proportional to observed changes in herbivory or traits. The figure therefore illustrates the extent of differences between forest types and among strata.

light and temperature were the primary drivers of the observed SLA and LDMC trends (Yamasaki and Kikuzawa, 2003, Stiegel et al., 2017). The elevated herbivory in the lower stratum might also be triggered to some extent by reduced levels of chemical defences in the youngest individuals (Boege and Marquis, 2005; Moreira et al., 2017). Yet this effect does not explain the observed differences between the intermediate and the upper stratum. Second, stratification of herbivory could result from stratification of herbivore communities. Several studies have reported higher abundance or richness of herbivores in lower canopy strata and linked this trend with greater leaf quality (Le Corff and Marquis, 1999; Murakami et al., 2005) or a vertical transfer of herbivores from the canopy to the understory (Murakami and Wada, 1997; White and Whitham, 2000). However, relationships between herbivore abundance or diversity and herbivore damage are not straightforward (Basset et al., 1992; Rhainds and English-Loeb, 2003; Rossetti et al., 2017); hence the relevance of this explanation is difficult to gauge in our case. Third, top-down control of herbivores by their enemies could generate lower herbivory in upper strata, if predation pressure is higher in this part of the canopy (Sobek et al., 2009; Aikens et al., 2013). Unfortunately, we possess no empirical data yet on predator abundance or activity that would enable us to test the validity of this explanation.

Effects of forest type and stratum on leaf insect herbivory were partly explained by leaf traits. On the one hand, we detected an interaction between canopy stratum and tree diversity on leaf traits whereby differences in leaf traits among strata were more pronounced in pure stands. Recent studies reported that tree diversity may alter abiotic factors in individual canopies (and in particular light environment), and hence insect herbivory (Castagneyrol et al., 2018a, 2017; Muiruri and Koricheva, 2016; Rosado-Sánchez et al., 2017). Given the different growth form of oaks and pines, it is likely that the vertical stratification of abiotic factors was far more heterogeneous in mixed than in pure stands (Forrester, 2017), resulting in neater vertical gradients of leaf traits in pure stands. On the other hand, we found significant relationships between leaf traits and herbivory. In particular, leaf insect herbivory increased with increasing SLA and decreased with increasing LDMC. However, despite this direct effect of leaf traits on herbivory, we still detected a direct effect of forest stratum on leaf insect herbivory, while the effect of tree diversity on herbivory seemed to be primarily driven by an effect of tree diversity on leaf traits. To the best of our knowledge, to date no study addressing leaf trait-mediated effects of tree diversity on leaf insect herbivory has systematically compared leaves from different strata. Yet our results indicate that controlling for this effect will be critical for future studies of tree-herbivore interactions in mixed forests.

Leaf insect herbivory increased with oak isolation at the landscape scale. A long held view in ecology is that herbivory increases with the density of resources (resource concentration hypothesis, Root, 1973). Yet, we found the opposite. Whereas studies on forest fragmentation often find that herbivore abundance and richness decrease with patch size and isolation (Rossetti et al., 2017), results are less consistent for herbivory itself (Maguire et al., 2016; Rossetti et al., 2017; Simonetti et al., 2007). Our results suggest that the variation in outcomes of previous studies may to a considerable extent be caused by tree cues at the within-patch and individual tree scales that dilute among-patch trends in herbivory. For instance, virtually no studies we are aware of have to date accounted for stratification effects. Yet such effects could be far more determinant for patterns of herbivory than for herbivore richness or abundance if they affect the quality of the plant diet – which was the case in our system. On the other hand, we cannot exclude that the trend we observed was to some extent also mediated by differential top-down control of herbivores by predators, as has been reported by some authors (Gonzalez-Gomez et al., 2006; Maguire et al., 2015; Rossetti et al., 2014). Likewise, we present here the results of a single year-study that was conducted in 12 mixed and pure forest stands in southwestern France. We cannot exclude that our results may reflect particular abiotic conditions and the management applied to forest stands in this region.

6. Conclusions

Multiple independent approaches have been used so far to address the effect of tree diversity on insect herbivory. Studies on the influence of habitat diversity at the landscape level and tree diversity within forest patches mainly addressed insect movements and population dynamics at large scales. Studies focusing on a much smaller scale addressed tree-tree interactions and their effects on herbivores through changes in leaf traits. Here, we linked knowledge on tree-herbivore interactions from these different perspectives by integrating landscape-, forest type- and individual-levels effects on insect herbivores within the same study. By demonstrating that insect herbivory in mixed stands exceeded that of pure stands only in the upper stratum, we unravel the importance of considering small-scale variability of biotic and abiotic factors when addressing insect herbivory on forest trees. Our findings therefore help step forward towards a more profound understanding of the complex forces that drive insect herbivory in forests.

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Author contributions

BG conceived the study and acquired the data. BC, BG and EVC analyzed the data. BC and BG drafted the first version of the manuscript. All authors wrote the final version of the manuscript.

References

Aikens, K.R., Timms, L.L., Buddle, C.M., 2013. Vertical heterogeneity in predation pressure in a temperate forest canopy. PeerJ 1, e138. https://doi.org/10.7717/peerj.138.

Andersson, P., Löfstedt, C., Hambäck, P.A., 2013. Insect density–plant density relationships: a modified view of insect responses to resource concentrations. Oecologia 173, 1333–1344. https://doi.org/10.1007/s00442-013-2737-1.

Bañuelos, M.-J., Kollmann, J., 2011. Effects of host-plant population size and plant sex on a specialist leaf-miner. Acta Oecol. 37, 58–64. https://doi.org/10.1016/j.actao.2010.11.007.

Barbaro, L., Pontcharraud, L., Vetillard, F., Guyon, D., Jactel, H., 2005. Comparative responses of bird, carabid, and spider assemblages to stand and landscape diversity in maritime pine plantation forests. Écoscience 12, 110–121. https://doi.org/10.2980/i1195-6860-12-1-110.1.

Bartoń, K., 2016. MuMIn: Multi-Model Inference. R package version 1.15.6. < http://CRAN.R-project.org/package=MuMIn > .

Basset, Y., Aberlenc, H.-P., Delvare, G., 1992. Abundance and stratification of foliage arthropods in a lowland rain forest of Cameroon. Ecol. Entomol. 17, 310–318. https://doi.org/10.1111/j.1365-2311.1992.tb01063.x.

Boege, K., Marquis, R.J., 2005. Facing herbivory as you grow up: the ontogeny of resistance in plants. Trends Ecol. Evol. 20, 441–448. https://doi.org/10.1016/j.tree.2005.05.001.

Castagneyrol, B., Bonal, D., Damien, M., Jactel, H., Meredieu, C., Muiruri, E.W., Barbaro, L., 2017. Bottom-up and top-down effects of tree species diversity on leaf insect herbivory. Ecol. Evol. 7, 3520–3531. https://doi.org/10.1002/ece3.2950.

Castagneyrol, B., Giffard, B., Péré, C., Jactel, H., 2013. Plant apparency, an overlooked driver of associational resistance to insect herbivory. J. Ecol. 101, 418–429. https://doi.org/10.1111/1365-2745.12055.

Castagneyrol, B., Jactel, H., Moreira, X., 2018a. Anti-herbivore defences and insect herbivory: interactive effects of drought and tree neighbours. J. Ecol. https://doi.org/10.1111/1365-2745.12956. (in press).

Castagneyrol, B., Jactel, H., Vacher, C., Brockerhoff, E.G., Koricheva, J., 2014. Effects of plant phylogenetic diversity on herbivory depend on herbivore specialization. J. Appl. Ecol. 51, 134–141. https://doi.org/10.1111/1365-2664.12175.

Castagneyrol, B., Moreira, X., Jactel, H., 2018b. Drought and plant neighbourhood interactively determine herbivore consumption and performance. Sci. Rep. 8, 5930. https://doi.org/10.1038/s41598-018-24299-x.

Chaplin-Kramer, R., O'Rourke, M.E., Blitzer, E.J., Kremen, C., 2011. A meta-analysis of crop pest and natural enemy response to landscape complexity. Ecol. Lett. 14, 922–932. https://doi.org/10.1111/j.1461-0248.2011.01642.x.

Cornelissen, J.H.C., Lavorel, S., Garnier, E., Díaz, S., Buchmann, N., Gurvich, D.E., Reich, P.B., ter Steege, H., Morgan, H.D., van der Heijden, M.G.A., Pausas, J.G., Poorter, H., 2003. A handbook of protocols for standardised and easy measurement of plant functional traits worldwide. Aust. J. Bot. 51, 335–380.

Damien, M., Jactel, H., Meredieu, C., Régolini, M., van Halder, I., Castagneyrol, B., 2016. Pest damage in mixed forests: disentangling the effects of neighbor identity, host density and host apparency at different spatial scales. For. Ecol. Manage. 378, 103–110. https://doi.org/10.1016/j.foreco.2016.07.025.

Dudt, J.F., Shure, D.J., 1994. The influence of light and nutrients on foliar phenolics and insect herbivory. Ecology 75, 86–98. https://doi.org/10.2307/1939385.

Finch, S., Collier, R.H., 2000. Host-plant selection by insects – a theory based on 'appropriate/inappropriate landings' by pest insects of cruciferous plants. Entomol. Exp. Appl. 96, 91–102. https://doi.org/10.1046/j.1570-7458.2000.00684.x.

Finch, S., Collier, R.H., 2012. The influence of host and non-host companion plants on the behaviour of pest insects in field crops. Entomol. Exp. Appl. 142, 87–96. https://doi.org/10.1111/j.1570-7458.2011.01191.x.

Forrester, D.I., 2017. Ecological and physiological processes in mixed versus monospecific stands. In: Mixed-Species Forests. Springer, Berlin, Heidelberg, pp. 73–115. https://doi.org/10.1007/978-3-662-54553-9_3.

Fox, J., Weisberg, S., 2011. An R Companion to Applied Regression, second ed. Sage, Thousand Oaks, CA. http://socserv.socsci.mcmaster.ca/jfox/Books/Companion.

Gerzabek, G., Oddou-Muratorio, S., Hampe, A., 2017. Temporal change and determinants of maternal reproductive success in an expanding oak forest stand. J. Ecol. 105, 39–48. https://doi.org/10.1111/1365-2745.12677.

Gonzalez-Gomez, P.L., Estades, C.F., Simonetti, J.A., 2006. Strengthened insectivory in a temperate fragmented forest. Oecologia 148, 137–143. https://doi.org/10.1007/s00442-005-0338-3.

Gossner, M.M., Pašalić, E., Lange, M., Lange, P., Boch, S., Hessenmöller, D., Müller, J., Socher, S.A., Fischer, M., Schulze, E.-D., Weisser, W.W., 2014. Differential responses of herbivores and herbivory to management in temperate European Beech. PLoS ONE 9, e104876. https://doi.org/10.1371/journal.pone.0104876.

Grossman, J.J., Vanhellemont, M., Barsoum, N., Bauhus, J., Bruelheide, H., Castagneyrol, B., Cavender-Bares, J., Eisenhauer, N., Ferlian, O., Gravel, D., Hector, A., Jactel, H., Kreft, H., Mereu, S., Messier, C., Muys, B., Nock, C., Paquette, A., Parker, J., Perring, M.P., Ponette, Q., Reich, P.B., Schuldt, A., Staab, M., Weih, M., Zemp, D.C., Scherer-Lorenzen, M., Verheyen, K., 2018. Synthesis and future research directions linking tree diversity to growth, survival, and damage in a global network of tree diversity experiments. Environ. Exp. Bot. https://doi.org/10.1016/j.envexpbot.2017.12.015.

Guyot, V., Castagneyrol, B., Vialatte, A., Deconchat, M., Jactel, H., 2016. Tree diversity reduces pest damage in mature forests across Europe. Biol. Lett. 12, 20151037. https://doi.org/10.1098/rsbl.2015.1037.

Haase, J., Castagneyrol, B., Cornelissen, J.H.C., Ghazoul, J., Kattge, J., Koricheva, J., Scherer-Lorenzen, M., Morath, S., Jactel, H., 2015. Contrasting effects of tree diversity on young tree growth and resistance to insect herbivores across three biodiversity experiments. Oikos 124, 1674–1685. https://doi.org/10.1111/oik.02090.

Hambäck, P.A., Englund, G., 2005. Patch area, population density and the scaling of migration rates: the resource concentration hypothesis revisited: density-area relations in sources and sinks. Ecol. Lett. 8, 1057–1065. https://doi.org/10.1111/j.1461-0248.2005.00811.x.

Hambäck, P.A., Inouye, B.D., Andersson, P., Underwood, N., 2014. Effects of plant neighborhoods on plant–herbivore interactions: resource dilution and associational effects. Ecology 95, 1370–1383. https://doi.org/10.1890/13-0793.1.

Hothorn, T., Bretz, F., Westfall, P., 2008. multcomp: simultaneous inference in general parametric models. Biom. J. 50, 346–363.

Kozlov, M.V., Zvereva, E.L., 2017. Background insect herbivory: impacts, patterns and methodology. Prog. Bot. 79, 315–355.

Kuznetsova, A., Brockhoff, P.B., Christensen, R.H.B., 2016. lmerTest: Tests in Linear Mixed Effects Models. R package version 2.0-30. < http://CRAN.R-project.org/package=lmerTest > .

Le Corff, J., Marquis, R., 1999. Differences between understorey and canopy in herbivore community composition and leaf quality for two oak species in Missouri. Ecol. Entomol. 24, 46–58. https://doi.org/10.1046/j.1365-2311.1999.00174.x.

Lefcheck, J.S., 2016. piecewiseSEM: Piecewise structural equation modelling in R for ecology, evolution, and systematics. Meth. Ecol. Evol. 7, 573–579. https://doi.org/10.1111/2041-210X.12512.

Loranger, J., Meyer, S.T., Shipley, B., Kattge, J., Loranger, H., Roscher, C., Wirth, C., Weisser, W.W., 2013. Predicting invertebrate herbivory from plant traits: polycultures show strong nonadditive effects. Ecology 94, 1499–1509.

Maguire, D.Y., Buddle, C.M., Bennett, E.M., 2016. Within and among patch variability in patterns of insect herbivory across a fragmented forest landscape. PLoS ONE 11, e0150843. https://doi.org/10.1371/journal.pone.0150843.

Maguire, D.Y., Nicole, T., Buddle, C.M., Bennett, E.M., 2015. Effect of fragmentation on predation pressure of insect herbivores in a north temperate deciduous forest ecosystem. Ecol. Entomol. 40, 182–186. https://doi.org/10.1111/een.12166.

Moreira, X., Abdala-Roberts, L., Rasmann, S., Castagneyrol, B., Mooney, K.A., 2016. Plant diversity effects on insect herbivores and their natural enemies: current thinking, recent findings, and future directions. Curr. Opin. Insect Sci. 14, 1–7. https://doi.org/10.1016/j.cois.2015.10.003.

Moreira, X., Glauser, G., Abdala-Roberts, L., 2017. Interactive effects of plant neighbourhood and ontogeny on insect herbivory and plant defensive traits. Sci. Rep. 7. https://doi.org/10.1038/s41598-017-04314-3.

Muiruri, E.W., Koricheva, J., 2016. Going undercover: increasing canopy cover around a host tree drives associational resistance to an insect pest. Oikos 126, 339–349. https://doi.org/10.1111/oik.03307.

Muiruri, E.W., Milligan, H.T., Morath, S., Koricheva, J., 2015. Moose browsing alters tree diversity effects on birch growth and insect herbivory. Funct. Ecol. 29, 724–735. https://doi.org/10.1111/1365-2435.12407.

Murakami, M., Wada, N., 1997. Difference in leaf quality between canopy trees and seedlings affects migration and survival of spring-feeding moth larvae. Can. J. For. Res. 27, 1351–1356. https://doi.org/10.1139/x97-097.

Murakami, M., Yoshida, K., Hara, H., Toda, M.J., 2005. Spatio-temporal variation in Lepidopteran larval assemblages associated with oak, *Quercus crispula*: the importance of leaf quality. Ecol. Entomol. 30, 521–531. https://doi.org/10.1111/j.0307-6946.2005.00724.x.

Otway, S.J., Hector, A., Lawton, J.H., 2005. Resource dilution effects on specialist insect herbivores in a grassland biodiversity experiment. J. Anim. Ecol. 74, 234–240. https://doi.org/10.1111/j.1365-2656.2005.00913.x.

Paquette, A., Hector, A., Castagneyrol, B., Vanhellemont, M., Koricheva, J., Scherer-Lorenzen, M., Verheyen, K., TreeDivNet, 2018. A million and more trees for science. Nat. Ecol. Evol. 2, 763–766. https://doi.org/10.1038/s41559-018-0544-0.

Pearse, I.S., 2011. The role of leaf defensive traits in oaks on the preference and performance of a polyphagous herbivore, *Orgyia vetusta*. Ecol. Entomol. 36, 635–642. https://doi.org/10.1111/j.1365-2311.2011.01308.x.

QGIS Development Team, 2017. QGIS Geographic Information System. Open Source Geospatial Foundation.

R Core Team, 2016. R: a language and environment for statistical computing. R fundation for statistical computing, Vienna, Austria.

Reynolds, B.C., Crossley, D.A., 1997. Spatial variation in herbivory by forest canopy arthropods along an elevation gradient. Environ. Entomol. 26, 1232–1239. https://doi.org/10.1093/ee/26.6.1232.

Rhainds, M., English-Loeb, G., 2003. Testing the resource concentration hypothesis with tarnished plant bug on strawberry: density of hosts and patch size influence the interaction between abundance of nymphs and incidence of damage. Ecol. Entomol. 28, 348–358. https://doi.org/10.1046/j.1365-2311.2003.00508.x.

Root, R.B., 1973. Organization of a plant-arthropod association in simple and diverse habitats: the fauna of collards (*Brassica Oleracea*). Ecol. Monogr. 43, 95. https://doi.org/10.2307/1942161.

Rosado-Sánchez, S., Parra-Tabla, V., Betancur-Ancona, D., Moreira, X., Abdala-Roberts, L., 2017. Tree species diversity alters plant defense investment in an experimental forest plantation in southern Mexico. Biotropica. https://doi.org/10.1111/btp.12527.

Rosado-Sánchez, S., Parra-Tabla, V., Betancur-Ancona, D., Moreira, X., Abdala-Roberts, L., 2018. Effects of tree species diversity on insect herbivory and leaf defences in Cordia dodecandra. Ecol. Entomol. https://doi.org/10.1111/een.12648.

Rossetti, M.R., Gonzalez, E., Salvo, A., Valladares, G., 2014. Not all in the same boat: trends and mechanisms in herbivory responses to forest fragmentation differ among insect guilds. Arthropod-Plant Int. 8, 593–603. https://doi.org/10.1007/s11829-014-9342-z.

Rossetti, M.R., Tscharntke, T., Aguilar, R., Batáry, P., 2017. Responses of insect herbivores and herbivory to habitat fragmentation: a hierarchical meta-analysis. Ecol. Lett. 20, 264–272. https://doi.org/10.1111/ele.12723.

Schoonhoven, L.M., 2005. Insect-Plant Biology, second ed. Oxford University Press, Oxford ; New York.

Schuldt, A., Barrufol, M., Böhnke, M., Bruelheide, H., Härdtle, W., Lang, A.C., Nadrowski, K., von Oheimb, G., Voigt, W., Zhou, H., Assmann, T., 2010. Tree diversity promotes insect herbivory in subtropical forests of south-east China. J. Ecol. 98, 917–926. https://doi.org/10.1111/j.1365-2745.2010.01659.x.

Schuldt, A., Bruelheide, H., Härdtle, W., Assmann, T., Li, Y., Ma, K., von Oheimb, G., Zhang, J., 2015. Early positive effects of tree species richness on herbivory in a large-scale forest biodiversity experiment influence tree growth. J. Ecol. 103, 563–571. https://doi.org/10.1111/1365-2745.12396.

Simonetti, J.A., Grez, A.A., Celis-Diez, J.L., Bustamante, R.O., 2007. Herbivory and seedling performance in a fragmented temperate forest of Chile. Acta Oecol.-Int. J. Ecol. 32, 312–318. https://doi.org/10.1016/j.actao.2007.06.001.

Sobek, S., Scherber, C., Steffan-Dewenter, I., Tscharntke, T., 2009. Sapling herbivory, invertebrate herbivores and predators across a natural tree diversity gradient in Germany's largest connected deciduous forest. Oecologia 160, 279–288. https://doi.org/10.1007/s00442-009-1304-2.

Stiegel, S., Entling, M.H., Mantilla-Contreras, J., 2017. Reading the leaves' palm: leaf traits and herbivory along the microclimatic gradient of forest layers. PLoS ONE 12 (1), e0169741. https://doi.org/10.1371/journal.pone.0169741.

Stutz, R.S., Banks, P.B., Dexter, N., McArthur, C., 2015. Herbivore search behaviour drives associational plant refuge. Acta Oecol. 67, 1–7. https://doi.org/10.1016/j.actao.2015.05.004.

Thomas, S.C., Sztaba, A.J., Smith, S.M., 2010. Herbivory patterns in mature sugar maple: variation with vertical canopy strata and tree ontogeny. Ecol. Entomol. 35, 1–8.

Underwood, N., Inouye, B.D., Hambäck, P.A., 2014. A conceptual framework for associational effects: when do neighbors matter and how would we know? Q. Rev. Biol. 89, 1–19.

Vanhellemont, M., Bijlsma, R.-J., De Keersmaeker, L., Vandekerkhove, K., Verheyen, K., 2018. Species and structural diversity affect growth of oak, but not pine, in uneven-aged mature forests. Basic Appl. Ecol. 27, 41–50. https://doi.org/10.1016/j.baae.2018.01.003.

Vehviläinen, H., Koricheva, J., Ruohomäki, K., 2007. Tree species diversity influences herbivore abundance and damage: meta-analysis of long-term forest experiments. Oecologia 152, 287–298. https://doi.org/10.1007/s00442-007-0673-7.

Vehviläinen, H., Koricheva, J., Ruohomäki, K., Johansson, T., Valkonen, S., 2006. Effects of tree stand species composition on insect herbivory of silver birch in boreal forests. Basic Appl. Ecol. 7, 1–11. https://doi.org/10.1016/j.baae.2005.05.003.

Verheyen, K., Vanhellemont, M., Auge, H., Baeten, L., Baraloto, C., Barsoum, N., Bilodeau-Gauthier, S., Bruelheide, H., Castagneyrol, B., Godbold, D., Haase, J., Hector, A., Jactel, H., Koricheva, J., Loreau, M., Mereu, S., Messier, C., Muys, B., Nolet, P., Paquette, A., Parker, J., Perring, M., Ponette, Q., Potvin, C., Reich, P., Smith, A., Weih, M., Scherer-Lorenzen, M., 2016. Contributions of a global network of tree diversity experiments to sustainable forest plantations. Ambio 45, 29–41. https://doi.org/10.1007/s13280-015-0685-1.

White, J.A., Whitham, T.G., 2000. Associational susceptibility of cottonwood to a box elder herbivore. Ecology 81, 1795–1803.

Yamasaki, M., Kikuzawa, K., 2003. Temporal and spatial variations in leaf herbivory within a canopy of *Fagus crenata*. Oecologia 131, 226–232. https://doi.org/10.1007/s00442-003-1337-x.