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Abstract

By feeding on plant tissue, insect herbivores can change several characteristics of their hosts. These changes have the potential to alter the quality of the plant for other herbivore species, potentially altering the structure of the community of species attacking the plant at a later point in time. We tested whether herbivory early in the season changes host plant performance, polyphenol chemistry, and the community structure of sessile herbivores later in the season. We experimentally manipulated densities of early-season moth caterpillars on a set of young oak trees and measured tree growth, reproduction, leaf chemistry, and the abundance and community composition of leafmining and galling species later in the season. The experimental manipulations of early-season herbivores did not affect late-season leaf chemistry or tree performance. Early-season herbivores had a weak negative effect on the abundance of gallers and a positive, tree-dependent effect on the overall diversity of late-season sessile herbivores. The chemical composition of leaves covaried with the species composition of the late-season leafmining and galling community. Both the chemical composition of the host tree and the late-season insect community structure were strongly affected by the growth location of the tree. Our results suggest that plant-mediated indirect effects between herbivores might play a limited role in this system, whereas the underlying variation in plant chemistry is an important factor structuring the associated insect community. Our results emphasise that factors other than prior herbivory can be important determinants of plant chemistry and the community composition of herbivores.

Keywords: herbivore, indirect effect, leafminer, galler, polyphenol, hydrolysable tannin, flavonol, constitutive defence, induced defence, seed production, *Quercus robur*, *Acrobasis consociella*

Introduction

By feeding on plant tissue, insect herbivores change the characteristics of their host plant. These changes are often triggered as a defensive reaction against the attacker, and may involve changes in phenology, physical traits, or the chemical composition of the plant (Karban and Baldwin 1997, Nykänen and Koricheva 2004). Herbivory-induced changes in the plant can cause trade-offs between growth, reproduction and defence (Herms and Mattson 1992).

The changes in plant physiology caused by one herbivore species can influence the quality and quantity of the plant as a food source for other species. Such plant-mediated indirect interactions can occur between herbivore species that are temporally separated (Van Zandt and Agrawal 2004, Poelman et al. 2008, Hernandez-Cumplido et al. 2016), or attack different parts of the same host plant (McArt et al. 2013, Arce et al. 2017), and have been shown to occur in both herbaceous and woody species (Gonzalez-Megias and Gomez 2003, Ohgushi 2005, Uesugi et al. 2016). The indirect plant-mediated interactions can have unexpected consequences for plant fitness, if they result in increased or decreased resistance towards more harmful herbivores (Kessler and Baldwin 2004, McArt et al. 2013, Machado et al. 2018), or disruption of important mutualistic interactions (Kessler et al. 2011). Although a potentially important mechanism structuring herbivore communities, plant-mediated indirect effects between herbivores are not ubiquitous (see e.g., Faeth 1986, Wold and Marquis 1997, Li et al. 2016 for examples of the lack of these types of interactions). It is currently unclear under what circumstances and in what systems these interactions occur.

We investigated the effects of early-season leaf-chewing caterpillars on the performance and leaf chemistry of their host plant, and on the community structure of sessile insect herbivores colonising the same host plant later in the season. As a study system, we used the pedunculate oak (*Quercus robur* L.) and its associated insect community in

Oxfordshire, southern UK. The oaks in this region experience intense early-season herbivory by leaf-chewing lepidopteran caterpillars for the few first weeks following budburst (Feeny 1970). Later in the season, oaks sustain a diverse community of sessile leafminers and gallers. As the early-season caterpillars have been suggested to act as keystone herbivores in this system (Hunter 1992), their feeding should trigger community-wide cascading effects (Poelman and Kessler 2016), potentially influencing the late-season sessile herbivores.

Among the potential changes in oak foliage triggered by early-season herbivores are changes in polyphenol content. Oaks produce a diverse set of polyphenols, including hydrolysable tannins, proanthocyanidins and flavonols (Salminen et al. 2004). In particular, hydrolysable tannins may act as an important defence against herbivory on oak due to their high concentration in young leaves (Salminen et al. 2004, Roslin and Salminen 2008) coupled with high oxidative activity in caterpillar guts (Barbehenn et al. 2009). Flavonols are often induced after herbivore attack (Onkokesung et al. 2014, Ohse et al. 2017), making them another interesting group in the context of plant-mediated indirect interactions between herbivores. Even though there are accounts of proanthocyanidin levels increasing after defoliation (Faeth 1986, Roth et al. 1998), other studies have suggested that they may not always function as anti-herbivore defences (Ayres et al. 1997, Nykänen and Koricheva 2004, Roslin and Salminen 2008). Many studies have reported changes in total concentrations of oak phenolics after herbivory (Feeny 1970, Faeth 1986). However, the realisation that defensive reactions in plants can involve specific compounds against certain types of herbivores (Roslin and Salminen 2008) calls for more detail when assessing the chemical profile of plants.

We tested experimentally how changes in the abundance of early-season herbivores affect oak performance, leaf chemistry, and the late-season community of leafminers and gallers. We combine large-scale experimental manipulations of herbivore densities in the field with detailed measurements of polyphenol compounds and surveys of a species-rich community of leafminers and gallers. We predicted that early-season caterpillar herbivory 1) has a negative effect on tree performance by reducing growth and acorn production, 2) alters the quality of the host plant as a food source for other herbivores by changing the concentration and/or composition of polyphenols and that 3) herbivory-induced changes in the host plant affect the abundance, diversity and community composition of late-season leafminers and gallers.

Materials and methods

A detailed description of the methods is provided in Appendix S1. Below, we present a summary of the methods.

Experimental setup and study site. The study was carried out in Neptune Wood, Oxfordshire, UK (51°38'24"N, 1°12'02"W). Neptune Wood is a 4.5 ha plantation of ca 10000 oaks established in 2005. We selected 18 clusters of four neighbouring, similar-sized trees (n = 72 trees). The clusters were located in six experimental blocks with three clusters per block (Figure 1). Within each block, each cluster was randomly assigned to either a manipulative treatment (*suppression of herbivory* or *increased herbivory)* or left as a non-manipulated *control*. Each tree subject to the *herbivory suppression* treatment was searched through once systematically leaf-by-leaf. All caterpillars encountered were carefully removed and transferred to trees in the *increased herbivory* treatment, along with additional caterpillars collected from other oak trees at the site. All manipulations were carried out from 12th May 2016 until 30th May 2016, from the appearance of the first leaves until most caterpillars had disappeared. We transferred between 200 and 250 caterpillars to each tree in the *increased herbivory* treatment and removed 15 to 219 caterpillars from trees in the *herbivory* *suppression* treatment. Thus, all trees in the increased herbivory treatment experienced at least a twofold increase in caterpillar abundance. To create a representative subset of leaves for later surveys of leaf area loss and late-season sessile insects, we marked 16 branches on each tree, evenly distributed within the tree, between 6th June and 13th June 2016.

Early-season herbivory, tree performance and late-season herbivore surveys. Most caterpillars encountered on herbivory suppression trees and transferred to increased herbivory trees were identified as *Acrobasis consociella* (Pyralidae, Hübner, 1813). These caterpillars spin webs that attach several leaves together to form a small shelter. The caterpillars remain in their shelters until pupation (Shaw et al. 2011, Figure 2ab). We measured the intensity of early-season herbivory on each tree by counting the number of established *A. consociella* shelters, and by surveying leaf area loss on leaves which were not part of these shelters, on 6th - 21st June 2016. To count the shelters, we searched all branches of each tree systematically. These counts gave us a direct measure of the success of our manipulations. To assess whether the manipulations resulted in altered herbivory rates overall (including on leaves that were not part of the shelters), we visually estimated the percentage of leaf area lost per leaf for all the leaves of the 16 marked shoots on a subset (n = 39) of the experimental trees.

To measure the effects of herbivory on tree growth, we measured change in tree diameter at breast height (dbh). The trees were measured before the first growing season (2nd May 2016; prior to experimental manipulations), at the end of the first growing season (24th August 2016), and at the end of the growing season the following year (28th August 2017). To assess the effect of herbivory on tree reproduction, we counted all acorns on each tree at the end of the first season (13th to 14th September 2016) and scored them either as healthy or galled depending on whether they had been deformed by the seed galler *Andricus* *quercuscalicis* (Burgsdorf, 1783, Figure 2c). *Andricus quercuscalicis* is a common acorn galler in the area and an important seed parasite on oaks (Crawley 1985).

To study the effects of the experimental treatments on the abundance and diversity of late-season herbivores, we surveyed the trees for leafminers and gallers between 15th August and 21st September 2016. From each of the 16 marked branches on each tree, we surveyed up to 50 leaves in the order they were encountered. All sessile insects were identified to genus or species level based on gall or mine morphology.

Leaf chemistry. Based on current understanding of the role of polyphenols in plant defence (see Introduction), we decided to focus our analyses on individual compounds of hydrolysable tannins and flavonols. On 24th August 2016, we collected leaves from two trees within each experimental cluster for phytochemical analyses (n = 36 trees). From each of these trees, we collected three leaves with herbivory damage and three intact leaves haphazardly from different parts of the tree. Samples were analysed in the Natural Chemistry Research Group laboratory at the University of Turku (Finland) using ultra-performance liquid chromatography high-resolution mass spectrometry (UPLC-HR-MS). For each sample, we obtained information on concentrations of 27 polyphenol compounds (Appendix S2, Table S1, Figure 2def).

Statistical analyses. Data on the effects of herbivory treatments on tree performance, leaf chemistry and sessile insect abundance and diversity were analysed using Bayesian mixed effect models with Gaussian, negative binomial (for count data, log link) or betabinomial (for proportion data, logit link) error distribution and weakly informative priors. The effect of treatment on leaf area loss was analysed using betaregression model (logit link). Random effects in mixed effect models were cluster nested within block (tree-level data) or tree nested within cluster nested within block (leaf-level chemical data). Detailed model descriptions are provided in Appendix S1. In brief, we first tested whether 1) the experimental manipulations affected the density of *A. consociella* shelters by modelling the number of shelters observed on each tree after the manipulations as a function of treatment (*increase*, *suppression* or *control*), tree size (dbh) and their interaction. To test 2) whether leaf area loss on leaves outside the shelters covaried with the abundance of *A. consociella*, we modelled the proportion of leaf area loss as a function of tree size and the number of *A. consociella* shelters. Tree size was included as a covariate to account for the possibility that the same number of shelters might have a different effect on trees of different size. To test 3) the effect of *A. consociella* herbivory on tree performance, we modelled tree growth (during the first season, during the second season, or cumulative growth over two seasons), overall number of acorns (healthy and infested), and the ratio of infested to healthy acorns as functions of tree size, number of *A. consociella* shelters, leaf area loss at the cluster level, and the interactions between the number of caterpillar shelters and all other explanatory variables. In the model assessing growth rate during the second season, growth rate during the first season was included as an additional explanatory variable. To test 4) the effects of *A. consociella* herbivory on late-season insect abundance and diversity, we modelled the abundance or diversity (Shannon's diversity index) of leafminers or gallers as a function of tree size, survey date, number of *A. consociella* shelters, leaf area loss per cluster and the two-way interactions between the number of *A. consociella* shelters and the other variables. To test for 5) differences in leaf chemistry between trees in the different herbivory treatments and leaf damage classes (*eaten* versus *intact*), we modelled the total concentration of all polyphenols, the diversity of all polyphenols (Shannon's diversity index), and the concentration and diversity of our two focal polyphenol subgroups (hydrolysable tannins and flavonols) as functions of tree size and leaf type (*eaten* and *intact* leaves in three herbivory treatments).

For the Bayesian mixed effect models, the effects of individual predictor variables on the response variables were assessed by examining posterior distributions and marginal effects and by conducting equivalence tests (Dienes 2014, Kruschke 2018). For equivalence tests, we estimated the 95% -highest density interval (“HDI”) for the model and compared the predictors’ HDIs with the region of practical equivalence (“ROPE”) to assess whether the null hypothesis (“H_0”, predictor not affecting response) could be accepted or rejected (Kruschke 2018).

To assess 6) the effect of *A. consociella* abundance on the community structure of late-season herbivores and 7) whether the community structure of late-season herbivores covaried with the tree-level composition of polyphenols, we built generalised linear models for multivariate data (Wang et al. 2012) with a negative binomial error distribution and log link. The response was the tree-level abundances of individual species of leafminers and gallers. Explanatory variables in model 6) were experimental block and cluster, tree size, survey date and the number of *A. consociella* shelters and interactions between number of shelters and date and number of shelters and tree size. In model 7) explanatory variables were the experimental block and the average concentration of the different polyphenol compounds per tree. The significance of explanatory variables in the multivariate models was assessed by performing an analysis of deviance on the full model and assessing the significance of the individual terms with likelihood ratio tests and resampled p-values. The model assumptions were tested by visually examining plots of residuals against fitted values for the homoscedasticity of residuals. To examine which species (as part of the multivariate response variable) expressed significant effects, we used univariate likelihood ratio tests with unadjusted p-values. To partition the variance in late-season insect community structure into components explained by the different explanatory variables in models 6 and 7, we built a hierarchical Bayesian joint species distribution model (Ovaskainen et al. 2017). The matrix of the late-season species abundances was set as the response and the concentrations of individual polyphenols and the number of caterpillar shelters as the environmental covariate matrix, with experimental block and cluster as random effects and assuming an overdispersed Poisson error distribution with log link. To test for 8) differences in chemical composition of different leaf types (*eaten* vs. *intact* from the three tree-level treatments) we performed a principal component analysis ("PCA") and extracted values for the first two PC axes. We then built Bayesian mixed effect models in which each axis was modelled as a function of tree size and leaf type (six different treatment × damage class combinations) with tree nested within a cluster nested within block as random effect.

<u>**Results**</u>

For supplementary tables and figures, see Appendix S2, for the effects of other explanatory variables (tree size and collection date) see Appendix S3 and for plotted marginal effects from the Bayesian mixed effect models, see Appendix S4. The primary data and the R code associated with the analyses are available at Figshare (https://doi.org/10.6084/m9.figshare.7938509.v1)

The effect of treatment on the number of caterpillar shelters and leaf area loss. The early-season herbivore manipulation resulted in lower abundance of *A. consociella* shelters in the *suppressed herbivory* treatment compared to the *increased herbivory* treatment (posterior mean = −1.79, 95% CI = −3.23 to −0.31, 0.43% inside ROPE, H_0 rejected, Tables S3 and S4, Figure 3a). Based on model predictions, this corresponds to a difference of 170 (± 121 SD) caterpillar shelters per averaged-sized tree between the two treatments (compare to the observed mean 103 ± 74 SD shelters in the unmanipulated *control* trees).

The amount of leaf area loss outside the shelters was not affected by the number of *A. consociella* shelters on the tree (100% inside ROPE, H_0 accepted). The average leaf area loss was 6.0 % (± 0.1%) per leaf and 9.4 % (± 0.2%) per eaten leaf (see Table S2 for patterns of leaf area loss to herbivory)

Does herbivory influence polyphenol chemistry or tree performance? Total polyphenol concentration (Figure 3b), hydrolysable tannin concentration and flavonol concentration did not differ between leaf types (Tables S3 and S4). Nevertheless, the composition of polyphenols as captured by the first axis of the PCA differed between intact and damaged leaves in the *increased herbivory* treatment (posterior mean = −0.38, 95% CI = −0.61 to −0.15; 0.4% inside ROPE, H_0 rejected; Figure S1c, Tables S3 and S4). The first PC axis was defined by the variation in the concentrations of the most abundant hydrolysable tannins (Figure S2). There were no clear differences in polyphenol composition between leaf types along the second PC axis (H_0 unresolved, Tables S3 and S4). Experimental block had a strong effect on the grouping of the data points in the resulting ordination (Figure S1d). The diversity of all polyphenols, the diversity of hydrolysable tannins and the diversity of flavonols did not show clear differences between leaf types (H_0 unresolved, Tables S3 and S4).

The number of *A. consociella* shelters on a tree did not have any effect on tree growth or acorn production (100% inside ROPE; H_0 accepted, Tables S3 and S4). There were no clear relationships between the abundance of early-season caterpillar shelters and the proportion of galled acorns; or between leaf area loss and tree growth, leaf area loss and production of acorns or leaf area loss and the proportion of galled acorns (Tables S3 and S4, all H_0 unresolved).

Is the abundance, diversity and community structure of late-season herbivores affected by prior herbivory? We surveyed a total of 41443 oak leaves and recorded 27603 individual galls and leafmines representing 20 different taxa (Table S3). The abundance of late-season galls was negatively related to the number of *A. consociella* shelters (posterior mean = −0.16, 95% CI = −0.29 to −0.02, 1.0% inside ROPE, H_0 rejected, Tables S3 and S4, Figure 3c). The number of *A. consociella* shelters also affected the overall diversity of sessile insects (leafminers and gallers combined), but this effect depended on tree size (shelters × tree size interaction, scaled posterior mean = −0.06, 95% CI = −0.09 to −0.02, 1.93% inside ROPE, H_0 rejected; Figure 3d, Tables S3 and S4): large trees with a high abundance of caterpillar shelters had lower diversity of late-season herbivores than large trees with low shelter abundance, whereas the opposite pattern was recorded on small trees.

There were no clear relationships between the number of *A. consociella* shelters and the overall abundance of late-season herbivores, the abundance of leafminers (H_0 unresolved for both, Table S4) and the community composition of late-season herbivores (LRT = 26.0, p = 0.46, Figure 4b, Figure S1a). There were no clear relationships between the abundance and diversity of late-season insects and leaf area loss on leaves that were not part of caterpillar shelters (Tables S3 and S4, all H_0 unresolved).

Does the abundance, diversity, and community structure of late-season herbivores covary with polyphenol chemistry? The community composition of late-season herbivores covaried significantly with the tree-level polyphenol composition (LRT = 560.1, p = 0.02, Figure 4acd, Table S6). Most compounds correlated with the abundance of at least one of the late-season insect species, and most of these statistically significant pairwise correlations were positive (Figure 4c). With the exception of the most abundant insect species, polyphenol composition accounted for the largest proportion of the explained variation in insect abundances across trees (Figure 4d). The community composition of late-season herbivores differed between experimental blocks (LRT = 333.9, p = 0.001, Figure S1be) and clusters (LRT = 490.1, p = 0.001).

Discussion

In this study, we tested how early-season herbivory changes host plant performance, polyphenol chemistry, and the community structure of sessile herbivores later in the season. We manipulated densities of early-season shelter-building *A. consociella* caterpillars on 72 oak trees, creating an experimental gradient from a 2.5-fold reduction to a twofold increase in caterpillar numbers, exceeding the natural variation in the number of caterpillar shelters among trees at the study site. The manipulations did not affect leaf area loss on leaves that were not part of the shelters, the concentration and composition of polyphenols, tree growth, or reproduction. The composition of polyphenols differed between eaten and intact leaves; however, only on trees in the *increased herbivory* treatment. There were few clear effects of early-season caterpillars on the late-season sessile insect community, but the number of early-season caterpillar shelters had a negative effect on galler abundance, and the effect of early-season caterpillars on the overall diversity of late-season herbivores depended on the size of the tree. The community composition of late-season insects covaried with tree-level polyphenol composition, and both the community structure of late-season herbivores and the tree-level polyphenol composition were strongly affected by the growth location of the host tree. Below, we discuss each of these findings.

No effect of caterpillar manipulations on leaf area loss or leaf chemistry. Leaf area lost to herbivores (on leaves outside caterpillar shelters) was not affected by the number of *A. consociella* shelters. This suggests that *A. consociella* caterpillars feed primarily on the leaves which are part of the shelters, and do not affect the abundance or feeding rate of other early-season leaf-chewers sharing the host tree.

We found no differences in the concentration, diversity or composition of polyphenols between trees with different amounts of *A. consociella* shelters. Nevertheless, there was substantial variation in chemistry between individual trees and experimental blocks (e.g. total polyphenol concentration varying 12.1 mg/g SD between trees, 9.6 mg/g SD between blocks and 3.6 mg/g SD between treatments, with the mean concentration being 47 mg/g). These results suggest that an increase in the number of *A. consociella* caterpillars did not induce any density-dependent systemic defence reactions in their hosts, and that plant chemistry is more strongly influenced by other factors than herbivory. Induced defences should be favoured when herbivory pressure varies in time and when previous attack is a good indicator of future herbivory (Karban 2011). This might not be the case in our study system, where the timing of herbivory is predictable (most herbivory occurs within a few weeks after budburst), even when its intensity varies between years. The best strategy for the plant might therefore be to produce defensive compounds constitutively at the beginning of each season. Indeed, the concentrations of hydrolysable tannins on oak show seasonal change, being high during the months after budburst (Salminen et al. 2004). Alternatively, if low levels of herbivory are sufficient for inducing defences, and if the strength of the defence reaction does not depend on the intensity of herbivory, all trees in our study might have been in an induced state. The herbivory-induced change in polyphenol concentration could also be too small to be noticeable against the large background variation in chemistry between trees.

We detected differences in leaf chemistry between intact and eaten leaves on trees with high abundances of *A. consociella* shelters. With high herbivore density, possibly only the best-defended leaves remain uneaten. Even though previous studies have found leaf- and branch-level inductions of defences (Tuomi et al. 1988, Wold and Marquis 1997), we find it unlikely that this kind of local induction would have occurred only in one of the treatments.

Herbivory did not affect tree performance. Consistent with the finding of no difference in leaf chemistry between the treatments, we detected no effects of *A. consociella* herbivory on tree growth, reproduction, and the proportion of galled acorns. The herbivory-induced reduction in plant growth or reproductive output reported in previous studies (Hawkes and Sullivan 2001, Hochwender et al. 2003, Nykänen and Koricheva 2004) is thought to result from trade-offs between growth, reproduction and defence (Herms and Mattson 1992). Since we observed no changes in leaf chemistry, it seems unlikely that any major re-allocation of resources occurred following *A. consociella* herbivory. We tested if tree growth rate, reproduction and investment in defences correlated, but found no significant relationship between them (Appendix S3, "Patterns of tree resource allocation").

Indirect effects between host-plant associated species can influence plant reproduction and fitness if induction of defences causes cross-resistance (Kessler and Baldwin 2004, McArt et al. 2013), induced susceptibility (Poelman et al. 2010, Machado et al. 2018) or disruption of beneficial species interactions (Kessler et al. 2011). It is possible that oaks in this system have been selected to avoid triggering production of chemicals in response to herbivory, if these come with fitness costs (Poelman and Kessler 2016). Fitness costs could rise for example if certain chemicals serve as cues for host location for acorn gallers, or make acorns less likely to be dispersed to new growth locations. Reliance on constitutive rather than induced defences could be one way to avoid these costs.

The effects of early-season herbivory on the late-season sessile insect community. We found that early-season *A. consociella* caterpillars had a negative effect on the abundance of late-season gallers. We also found that the effect of early-season caterpillars on late-season herbivore diversity depended on the size of the host tree: an increase in the number of caterpillar shelters increased herbivore diversity, but only on small trees (see Appendix S3). Based on the model predictions, the addition of 170 early-season *A. consociella* shelters (the model-predicted difference between *increase* and *suppression* treatments) to a tree would result in 0.2 (± 1.2 SD) more galler individuals per leaf on average-sized trees and an 0.2 (± 0.2 SD) increase in Shannon's diversity on small trees (compare to observed background diversity of 1.6 ± 0.2 SD). Assuming that each tree has an average of ca. 2000 leaves (see Appendix S1 for estimation of leaf number), 170 additional caterpillar shelters would result in a decrease of 389 (± 2392 SD) galler individuals per tree (against the estimated background level of 2138 ± 718 SD gallers per *control* tree of 2000 leaves). Since the effects of *A. consociella* shelters on late-season herbivore abundance and diversity are smaller or of the same size as natural variation in these responses at the site, they might not be ecologically very important. In addition, the effect on diversity was only seen on small trees, and errors around both trends were large. Since we did not observe any change in leaf chemistry, leaf area loss or tree performance between the different treatments, the drivers behind the trends in the abundance of gallers and overall diversity of late-season herbivores are uncertain.

Several studies have reported host-plant mediated indirect effects between herbivores (e.g. Van Zandt and Agrawal 2004, Poelman et al. 2010, Hernandez-Cumplido et al. 2016, Arce et al. 2017), though other studies have not found any evidence for these effects (e.g. Faeth 1986, Wold and Marquis 1997, Li et al. 2016). The presence and the strength of host plant-mediated effects might depend on the characteristics of the species involved in the studied interactions. For example, woody plants rely more on quantitative defences than herbaceous species (Smilanich et al. 2016). Rather than having a lethal effect on herbivores, quantitative defences reduce herbivore feeding rate (Feeny 1976), and might thus not generate any clear effects on densities of sessile herbivores during a single season. Additionally, compared to fast-growing species, slow-growing species often rely more on constitutive than on induced defences (Endara and Coley 2011, Karban 2011). Therefore, long-lived woody plants might depend primarily on quantitative, constitutive defences, which might less likely mediate indirect interactions between herbivores than the more flexible defences induced in fast-growing herbaceous species. Another factor affecting the occurrence of plant-mediated indirect effects could be the longevity of the induced chemicals. If the chemical compounds induced early in the season are no longer present when the late-season species arrive (see e.g., Edwards et al. 1986), chemically mediated indirect interactions between temporally separated herbivores might not occur. Nevertheless, since there are many examples of indirect effects between early- and late-season herbivores (e.g., Van Zandt and Agrawal 2004, Poelman et al. 2008, Hernandez-Cumplido et al. 2016), this mechanism might only be relevant in the context of some plant-herbivore systems. Lastly, some herbivores can suppress the defence and herbivore-recognition pathways of their host (Chung et al. 2013), which could prevent indirect effects between plant-associated species from occurring.

One factor influencing the strength of plant-mediated indirect effects between herbivores could be parallel changes in the activity of natural enemies of the herbivores (Faeth 1986, Kaplan and Denno 2007). Many trees emit volatile predator-attracting compounds as a response to herbivory (Thaler 1999, Kessler and Baldwin 2001). Additionally, empty galls or caterpillar shelters could provide hiding places for predators and other arthropods, thus influencing arthropod diversity and abundance (Lill and Marquis 2003, Ohgushi 2005). For example, in our study the number of predators might have been highest on trees with a high abundance of early-season caterpillar shelters. Increased predation or behavioural changes to avoid predators might have suppressed the feeding activity of the caterpillars (Steffan and Snyder 2010). Consequently, the number of shelters might not have reflected the feeding pressure experienced by the tree.

Tree-level chemical composition covaried with insect community structure. We found that tree-level polyphenol composition covaried with the community structure of sessile insects. The chemical composition of plant tissue has recently been recognized as an important factor explaining plant-associated insect diversity both between (Abrahamson et al. 2003, Richards et al. 2015, Salazar et al. 2018) and within species (Poelman et al. 2010, Glassmire et al. 2016, Bustos-Segura et al. 2017), but exactly what drives the observed phenotypic variation in plant chemistry is often not known.

One important factor determining plant chemistry and the associated arthropod community is plant genetic variation (Wimp et al. 2005, Bangert et al. 2005). Since polyphenol chemistry is heritable (Johnson et al. 2009), genetic differences between our study trees might have contributed to the observed patterns of leaf chemistry and the structure of the late-season insect community (see Whitham et al. 2006). Nevertheless, host genetics is unlikely to be the only factor of importance: both the insect community structure and tree chemistry were affected by the growth location of the tree. Several studies on oak have shown how leaf chemistry or insect community structure are influenced by microclimate and soil properties (Fine 2004, Moreira et al. 2017). At our study site, small-scale environmental heterogeneity might have created variation in host tree chemical composition, affecting the associated insect community. Lastly, since the observed relationship between insect community structure and chemistry was correlative, it is also possible that the environment was affecting each of them independently.

Our results show that the impact of individual compounds on the different herbivores was specific to the exact compound by species pair. Interestingly, most of these pairwise correlations were positive. The effect of individual chemicals can be extremely system-specific, to the extent that a specific plant chemical is only effective to specific herbivore species (Kessler and Halitschke 2007). Even though polyphenols can deter feeding and reduce herbivore performance (Haviola et al. 2007, Ruuhola et al. 2007), some herbivores can benefit from the secondary chemicals of their hosts (Roslin and Salminen 2008, Smilanich et al. 2016). Since most of the late-season species in our study were specialists on the genus *Quercus* (Table S4), they might have been attracted to the different oak-specific compounds, if these compounds serve as cues for locating a host (Poelman et al. 2010).

Conclusions. In this study, changes in early-season insect herbivore abundance had no detectable effects on tree chemistry or performance. The abundance of early-season herbivores was negatively associated with the abundance of gallers, and positively with the overall diversity of late-season sessile herbivores, although the latter effect was only seen on small trees, and both trends were small. We found that host tree chemistry covaried with the associated insect community structure and that both the chemical and insect community compositions were strongly influenced by the growth location of the host tree. The lack of effects of early-season herbivores on plant chemistry and the community composition of late-season insect herbivores might be due to lack of induced changes in the host plant by early-season caterpillars. If the timing of the herbivory relative to budburst is predictable, constitutive defences might be favoured over induced ones. Additionally, if induced defences create negative fitness consequences through for example attracting specialist plant enemies, or disrupting beneficial species interactions, they might have been selected against in this system. In this study, the chemistry of oak leaves appears to be more influenced by variation in environmental conditions than by changes in herbivory pressure. Plant chemotype and microclimatic conditions associated with the growth location are likely to be more important than previous herbivory in determining the quality of the host plant and thus the structure of the associated insect community in our study system.

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Figure 1. The experimental setup consisted of six experimental blocks (A-F), three clusters within each block (n = 18) and four trees within each cluster (n = 72). Each block had one cluster representing each of the three treatments: *suppression of herbivory*, *increased herbivory* and *control*. On each tree,16 branches were marked and used for herbivory surveys (see the main text and Appendix S1).

Figure 2. a) and b) *Acrobasis consociella* (”Broad-barred Knot-horn”) caterpillar shelter on oak. c) Acorn deformed by the seed galler *Andricus quercuscalicis* (“Knopper gall”) next to a healthy acorn. The structures of three hydrolysable tannins that were found abundant in the samples: d) pedunculagin, e) tellimagrandin II and f) vescalagin.

Figure 3. The number of *A. consociella* shelters on trees in the different treatments (panel a) and effects of early-season herbivory on selected response variables (panels b-d): b) the total concentration (mg/g) of polyphenols in the six studied leaf types, c) the relationship between early-season *A. consociella* shelters and the late-season abundance of late-season gallers and d) the effect of *A. consociella* shelters on the diversity of late-season leafminers and gallers, separated by tree size (“large” if dbh > mean dbh 43.1 mm). In panels c and d, the solid lines have been drawn through model predictions, and the dotted lines represent model estimated standard deviations.

Figure 4. The effect of tree-level polyphenol composition and experimental treatments on the late-season insect community. Panel a) shows results from a PCA-based ordination describing the variation in late-season insect community structure and polyphenol composition. The insect species are shown as blue circles, the polyphenol compounds as grey arrows and the experimental trees (n = 36) as small grey dots. Compounds expressing

collinearity have been removed. Panel b) shows the late-season insect community structure in the data set comprising all experimental trees (n = 72). See Appendix S2, Figure S1e for grouping by experimental block and Figure S1a for ordination for a subset of the trees (n = 36). Panel c) shows Pearson’s correlations between the late-season herbivore species (as rows) and polyphenols (as columns). Significant ($p < 0.05$) correlations are shown in red (positive) and blue (negative). Panel d) shows the proportion of variance in the abundances of the late-season sessile herbivores (in order of abundance, Appendix S2, Table S5) explained by the different explanatory variables (polyphenols and caterpillar shelters) and random effects (block and cluster). See Appendix S2, Tables S1 and S5 for abbreviations.

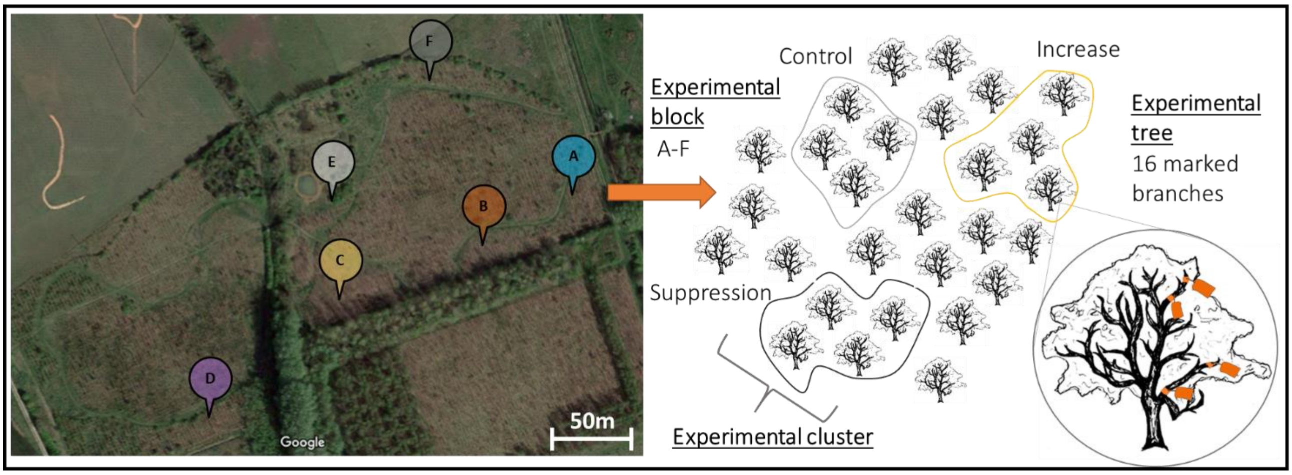
F
E
A
B
C
D
Google
50m
Control
Increase
Experimental block
A-F
Experimental tree
16 marked branches
Suppression
Experimental cluster

Figure 1.

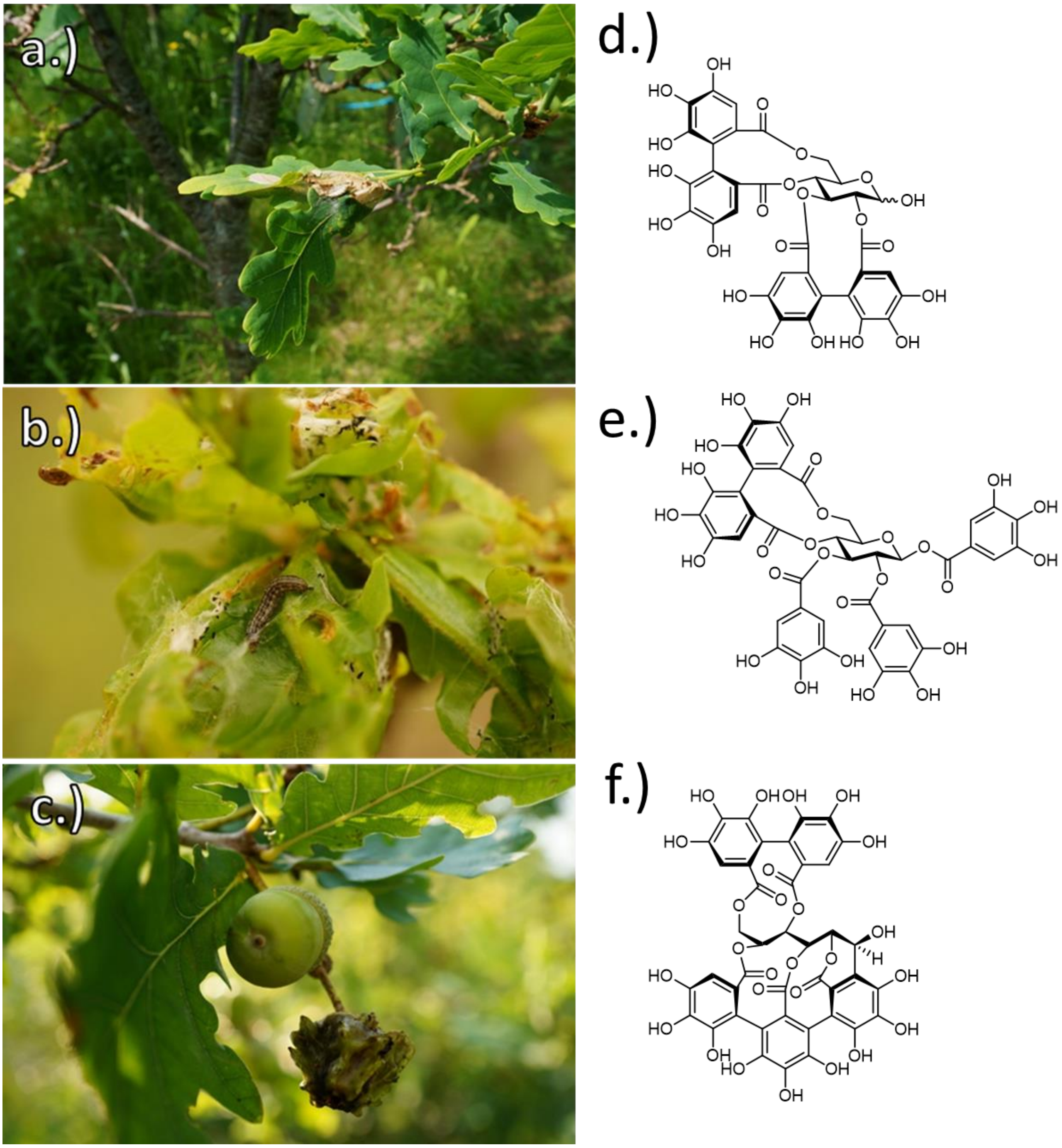

Figure 2.

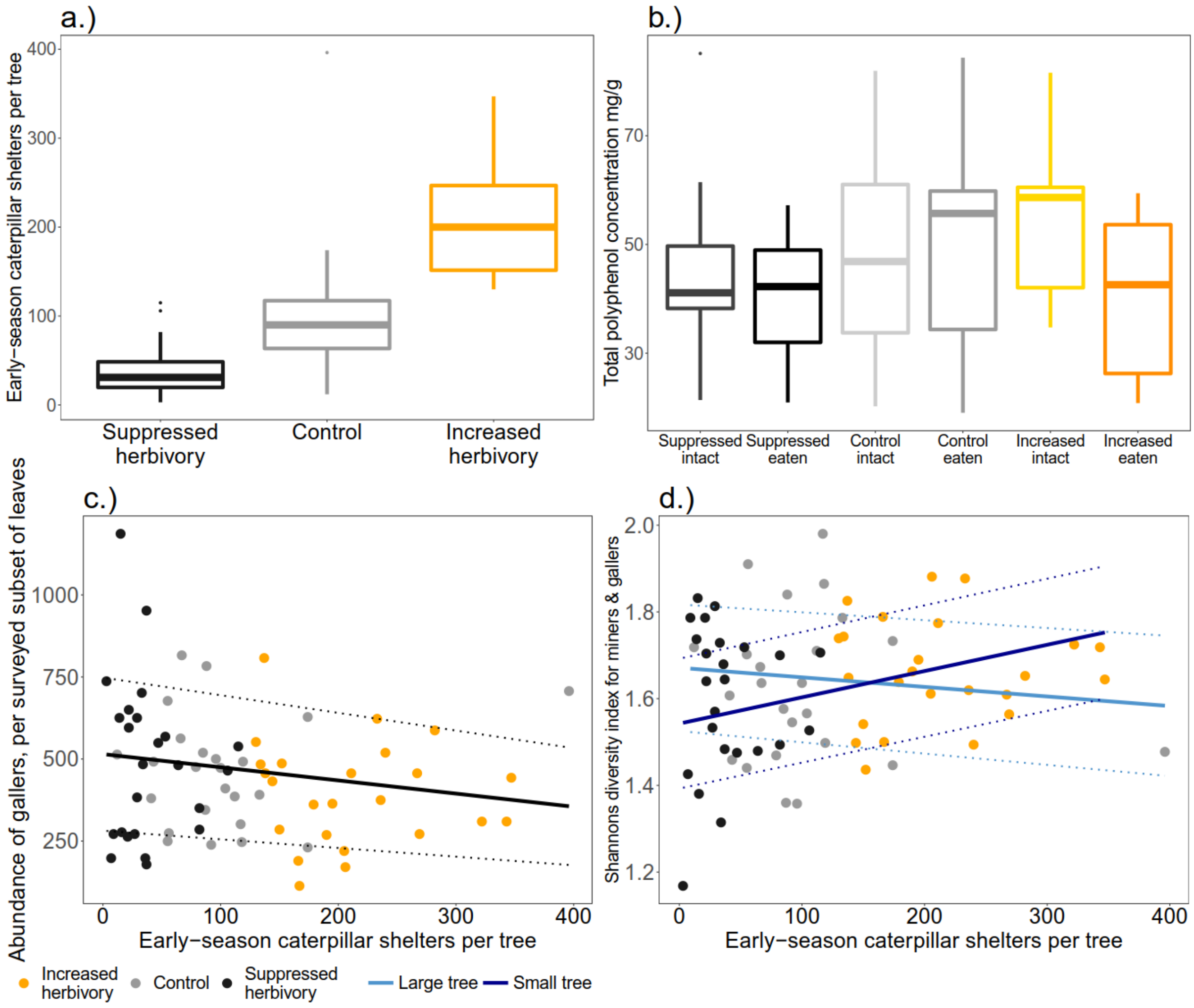
a.)
Early−season caterpillar shelters per tree
0
100
200
300
400
Suppressed herbivory
Control
Increased herbivory
b.)
Total polyphenol concentration mg/g
30
50
70
Suppressed intact
Suppressed eaten
Control intact
Control eaten
Increased intact
Increased eaten
c.)
Abundance of gallers, per surveyed subset of leaves
250
500
750
1000
0
100
200
300
400
Early−season caterpillar shelters per tree
d.)
Shannons diversity index for miners & gallers
1.2
1.4
1.6
1.8
2.0
0
100
200
300
400
Early−season caterpillar shelters per tree
● Increased herbivory
● Control
● Suppressed herbivory
Large tree
Small tree

Figure 3.

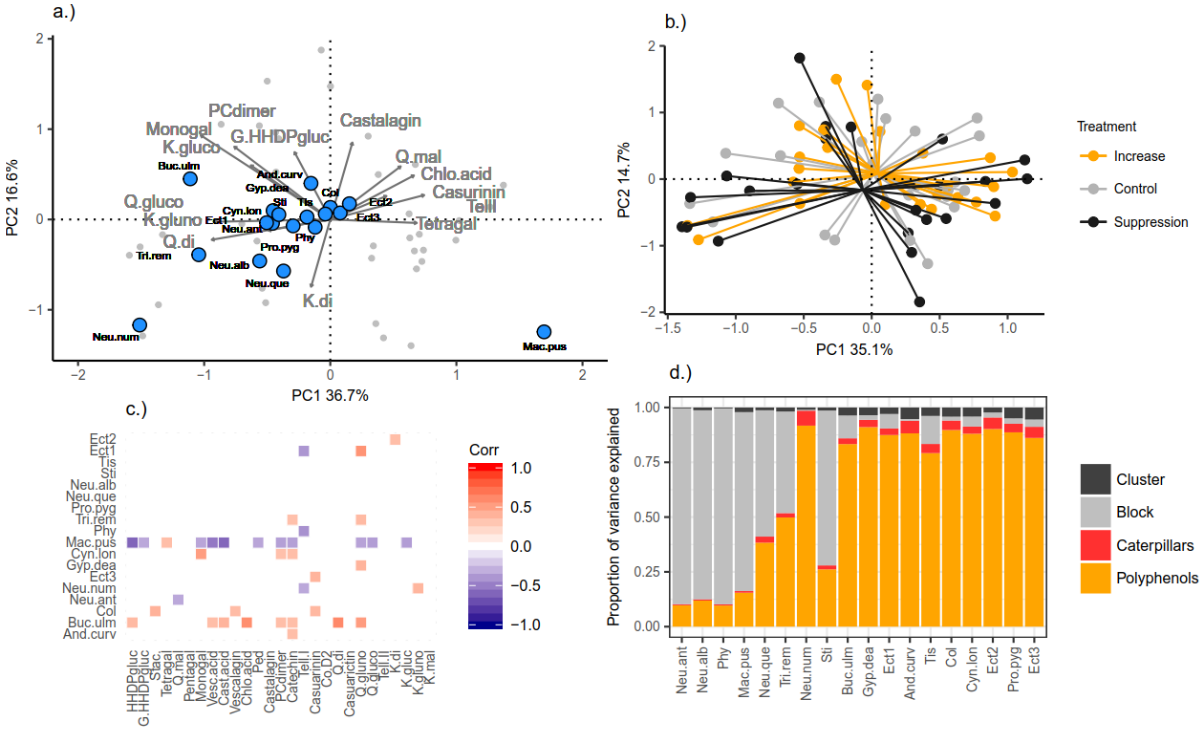
a.)
PC1 36.7%
PC2 16.6%
PCdimer
Monogal
K.gluco
G.HHDPgluc
Castalagin
Q.mal
Chlo.acid
Casurinin
Telli
Tetragal
Q.gluco
K.gluno
Q.di
K.di
Buc.ulm
And.curv
Gyp.dea
Col
Sti
Tis
Ect2
Ect3
Ect1
Cyn.lon
Neu.ant
Phy
Pro.pyg
Tri.rem
Neu.alb
Neu.que
Neu.num
Mac.pus
b.)
PC1 35.1%
PC2 14.7%
Treatment
Increase
Control
Suppression
c.)
Corr
1.0
0.5
0.0
−0.5
−1.0
d.)
Proportion of variance explained
Cluster
Block
Caterpillars
Polyphenols

Figure 4.