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Insect community structure covaries with host plant chemistry but is not affected by prior herbivory

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3   prior herbivory

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21 **<u>Abstract</u>**

22 By feeding on plant tissue, insect herbivores can change several characteristics of their hosts.

23 These changes have the potential to alter the quality of the plant for other herbivore species,

24 potentially altering the structure of the community of species attacking the plant at a later

25 point in time. We tested whether herbivory early in the season changes host plant

26 performance, polyphenol chemistry, and the community structure of sessile herbivores later

27 in the season. We experimentally manipulated densities of early-season moth caterpillars on a

28 set of young oak trees and measured tree growth, reproduction, leaf chemistry, and the

29 abundance and community composition of leafmining and galling species later in the season.

30 The experimental manipulations of early-season herbivores did not affect late-season leaf

31 chemistry or tree performance. Early-season herbivores had a weak negative effect on the

32 abundance of gallers and a positive, tree-dependent effect on the overall diversity of late-

33 season sessile herbivores. The chemical composition of leaves covaried with the species

34 composition of the late-season leafmining and galling community. Both the chemical

35 composition of the host tree and the late-season insect community structure were strongly

36 affected by the growth location of the tree. Our results suggest that plant-mediated indirect

37 effects between herbivores might play a limited role in this system, whereas the underlying

38 variation in plant chemistry is an important factor structuring the associated insect

39 community. Our results emphasise that factors other than prior herbivory can be important

40 determinants of plant chemistry and the community composition of herbivores.

41

42 **Keywords:** herbivore, indirect effect, leafminer, galler, polyphenol, hydrolysable tannin,

43 flavonol, constitutive defence, induced defence, seed production, *Quercus robur*, *Acrobasis*

44 *consociella*

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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

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70   Oxfordshire, southern UK. The oaks in this region experience intense early-season herbivory

71   by leaf-chewing lepidopteran caterpillars for the few first weeks following budburst (Feeny

72   1970). Later in the season, oaks sustain a diverse community of sessile leafminers and

73   gallers. As the early-season caterpillars have been suggested to act as keystone herbivores in

74   this system (Hunter 1992), their feeding should trigger community-wide cascading effects

75   (Poelman and Kessler 2016), potentially influencing the late-season sessile herbivores.

76   Among the potential changes in oak foliage triggered by early-season herbivores are

77   changes in polyphenol content. Oaks produce a diverse set of polyphenols, including

78   hydrolysable tannins, proanthocyanidins and flavonols (Salminen et al. 2004). In particular,

79   hydrolysable tannins may act as an important defence against herbivory on oak due to their

80   high concentration in young leaves (Salminen et al. 2004, Roslin and Salminen 2008)

81   coupled with high oxidative activity in caterpillar guts (Barbehenn et al. 2009). Flavonols are

82   often induced after herbivore attack (Onkokesung et al. 2014, Ohse et al. 2017), making them

83   another interesting group in the context of plant-mediated indirect interactions between

84   herbivores. Even though there are accounts of proanthocyanidin levels increasing after

85   defoliation (Faeth 1986, Roth et al. 1998), other studies have suggested that they may not

86   always function as anti-herbivore defences (Ayres et al. 1997, Nykänen and Koricheva 2004,

87   Roslin and Salminen 2008). Many studies have reported changes in total concentrations of

88   oak phenolics after herbivory (Feeny 1970, Faeth 1986). However, the realisation that

89   defensive reactions in plants can involve specific compounds against certain types of

90   herbivores (Roslin and Salminen 2008) calls for more detail when assessing the chemical

91   profile of plants.

92   We tested experimentally how changes in the abundance of early-season herbivores

93   affect oak performance, leaf chemistry, and the late-season community of leafminers and

94   gallers. We combine large-scale experimental manipulations of herbivore densities in the

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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*

5

120   *suppression* treatment. Thus, all trees in the increased herbivory treatment experienced at

121   least a twofold increase in caterpillar abundance. To create a representative subset of leaves

122   for later surveys of leaf area loss and late-season sessile insects, we marked 16 branches on

123   each tree, evenly distributed within the tree, between 6th June and 13th June 2016.

124

125   **Early-season herbivory, tree performance and late-season herbivore surveys.** Most

126   caterpillars encountered on herbivory suppression trees and transferred to increased herbivory

127   trees were identified as *Acrobasis consociella* (Pyralidae, Hübner, 1813). These caterpillars

128   spin webs that attach several leaves together to form a small shelter. The caterpillars remain

129   in their shelters until pupation (Shaw et al. 2011, Figure 2ab). We measured the intensity of

130   early-season herbivory on each tree by counting the number of established *A. consociella*

131   shelters, and by surveying leaf area loss on leaves which were not part of these shelters, on 6th

132   - 21st June 2016. To count the shelters, we searched all branches of each tree systematically.

133   These counts gave us a direct measure of the success of our manipulations. To assess whether

134   the manipulations resulted in altered herbivory rates overall (including on leaves that were

135   not part of the shelters), we visually estimated the percentage of leaf area lost per leaf for all

136   the leaves of the 16 marked shoots on a subset (n = 39) of the experimental trees.

137   To measure the effects of herbivory on tree growth, we measured change in tree

138   diameter at breast height (dbh). The trees were measured before the first growing season (2nd

139   May 2016; prior to experimental manipulations), at the end of the first growing season (24th

140   August 2016), and at the end of the growing season the following year (28th August 2017).

141   To assess the effect of herbivory on tree reproduction, we counted all acorns on each tree at

142   the end of the first season (13th to 14th September 2016) and scored them either as healthy or

143   galled depending on whether they had been deformed by the seed galler *Andricus*

144 *quercuscalicis* (Burgsdorf, 1783, Figure 2c). *Andricus quercuscalicis* is a common acorn

145 galler in the area and an important seed parasite on oaks (Crawley 1985).

146   To study the effects of the experimental treatments on the abundance and diversity of

147 late-season herbivores, we surveyed the trees for leafminers and gallers between 15th August

148 and 21st September 2016. From each of the 16 marked branches on each tree, we surveyed up

149 to 50 leaves in the order they were encountered. All sessile insects were identified to genus or

150 species level based on gall or mine morphology.

151

152 **Leaf chemistry.** Based on current understanding of the role of polyphenols in plant defence

153 (see Introduction), we decided to focus our analyses on individual compounds of

154 hydrolysable tannins and flavonols. On 24th August 2016, we collected leaves from two trees

155 within each experimental cluster for phytochemical analyses (n = 36 trees). From each of

156 these trees, we collected three leaves with herbivory damage and three intact leaves

157 haphazardly from different parts of the tree. Samples were analysed in the Natural Chemistry

158 Research Group laboratory at the University of Turku (Finland) using ultra-performance

159 liquid chromatography high-resolution mass spectrometry (UPLC-HR-MS). For each sample,

160 we obtained information on concentrations of 27 polyphenol compounds (Appendix S2,

161 Table S1, Figure 2def).

162

163 **Statistical analyses.** Data on the effects of herbivory treatments on tree performance, leaf

164 chemistry and sessile insect abundance and diversity were analysed using Bayesian mixed

165 effect models with Gaussian, negative binomial (for count data, log link) or betabinomial (for

166 proportion data, logit link) error distribution and weakly informative priors. The effect of

167 treatment on leaf area loss was analysed using betaregression model (logit link). Random

168 effects in mixed effect models were cluster nested within block (tree-level data) or tree nested

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169   within cluster nested within block (leaf-level chemical data). Detailed model descriptions are

170   provided in Appendix S1. In brief, we first tested whether 1) the experimental manipulations

171   affected the density of *A. consociella* shelters by modelling the number of shelters observed

172   on each tree after the manipulations as a function of treatment (*increase*, *suppression* or

173   *control*), tree size (dbh) and their interaction. To test 2) whether leaf area loss on leaves

174   outside the shelters covaried with the abundance of *A. consociella*, we modelled the

175   proportion of leaf area loss as a function of tree size and the number of *A. consociella*

176   shelters. Tree size was included as a covariate to account for the possibility that the same

177   number of shelters might have a different effect on trees of different size. To test 3) the effect

178   of *A. consociella* herbivory on tree performance, we modelled tree growth (during the first

179   season, during the second season, or cumulative growth over two seasons), overall number of

180   acorns (healthy and infested), and the ratio of infested to healthy acorns as functions of tree

181   size, number of *A. consociella* shelters, leaf area loss at the cluster level, and the interactions

182   between the number of caterpillar shelters and all other explanatory variables. In the model

183   assessing growth rate during the second season, growth rate during the first season was

184   included as an additional explanatory variable. To test 4) the effects of *A. consociella*

185   herbivory on late-season insect abundance and diversity, we modelled the abundance or

186   diversity (Shannon's diversity index) of leafminers or gallers as a function of tree size, survey

187   date, number of *A. consociella* shelters, leaf area loss per cluster and the two-way interactions

188   between the number of *A. consociella* shelters and the other variables. To test for 5)

189   differences in leaf chemistry between trees in the different herbivory treatments and leaf

190   damage classes (*eaten* versus *intact*), we modelled the total concentration of all polyphenols,

191   the diversity of all polyphenols (Shannon's diversity index), and the concentration and

192   diversity of our two focal polyphenol subgroups (hydrolysable tannins and flavonols) as

193   functions of tree size and leaf type (*eaten* and *intact* leaves in three herbivory treatments).

194 For the Bayesian mixed effect models, the effects of individual predictor variables on the

195 response variables were assessed by examining posterior distributions and marginal effects

196 and by conducting equivalence tests (Dienes 2014, Kruschke 2018). For equivalence tests, we

197 estimated the 95% -highest density interval ("HDI") for the model and compared the

198 predictors' HDIs with the region of practical equivalence ("ROPE") to assess whether the

199 null hypothesis ("H_0", predictor not affecting response) could be accepted or rejected

200 (Kruschke 2018).

201   To assess 6) the effect of *A. consociella* abundance on the community structure of

202 late-season herbivores and 7) whether the community structure of late-season herbivores

203 covaried with the tree-level composition of polyphenols, we built generalised linear models

204 for multivariate data (Wang et al. 2012) with a negative binomial error distribution and log

205 link. The response was the tree-level abundances of individual species of leafminers and

206 gallers. Explanatory variables in model 6) were experimental block and cluster, tree size,

207 survey date and the number of *A. consociella* shelters and interactions between number of

208 shelters and date and number of shelters and tree size. In model 7) explanatory variables were

209 the experimental block and the average concentration of the different polyphenol compounds

210 per tree. The significance of explanatory variables in the multivariate models was assessed by

211 performing an analysis of deviance on the full model and assessing the significance of the

212 individual terms with likelihood ratio tests and resampled p-values. The model assumptions

213 were tested by visually examining plots of residuals against fitted values for the

214 homoscedasticity of residuals. To examine which species (as part of the multivariate response

215 variable) expressed significant effects, we used univariate likelihood ratio tests with

216 unadjusted p-values. To partition the variance in late-season insect community structure into

217 components explained by the different explanatory variables in models 6 and 7, we built a

218 hierarchical Bayesian joint species distribution model (Ovaskainen et al. 2017). The matrix of

9

219   the late-season species abundances was set as the response and the concentrations of

220   individual polyphenols and the number of caterpillar shelters as the environmental covariate

221   matrix, with experimental block and cluster as random effects and assuming an overdispersed

222   Poisson error distribution with log link. To test for 8) differences in chemical composition of

223   different leaf types (*eaten* vs. *intact* from the three tree-level treatments) we performed a

224   principal component analysis ("PCA") and extracted values for the first two PC axes. We

225   then built Bayesian mixed effect models in which each axis was modelled as a function of

226   tree size and leaf type (six different treatment × damage class combinations) with tree nested

227   within a cluster nested within block as random effect.

228

229   **Results**

230   For supplementary tables and figures, see Appendix S2, for the effects of other explanatory

231   variables (tree size and collection date) see Appendix S3 and for plotted marginal effects

232   from the Bayesian mixed effect models, see Appendix S4. The primary data and the R code

233   associated with the analyses are available at Figshare

234   (https://doi.org/10.6084/m9.figshare.7938509.v1)

235

236   **The effect of treatment on the number of caterpillar shelters and leaf area loss.** The

237   early-season herbivore manipulation resulted in lower abundance of *A. consociella* shelters in

238   the *suppressed herbivory* treatment compared to the *increased herbivory* treatment (posterior

239   mean = −1.79, 95% CI = −3.23 to −0.31, 0.43% inside ROPE, H_0 rejected, Tables S3 and S4,

240   Figure 3a). Based on model predictions, this corresponds to a difference of 170 (± 121 SD)

241   caterpillar shelters per averaged-sized tree between the two treatments (compare to the

242   observed mean 103 ± 74 SD shelters in the unmanipulated *control* trees).

243   The amount of leaf area loss outside the shelters was not affected by the number of *A.*

244   *consociella* shelters on the tree (100% inside ROPE, H_0 accepted). The average leaf area loss

245   was 6.0 % ($\pm$ 0.1%) per leaf and 9.4 % ($\pm$ 0.2%) per eaten leaf (see Table S2 for patterns of

246   leaf area loss to herbivory)

247

248   **Does herbivory influence polyphenol chemistry or tree performance?** Total polyphenol

249   concentration (Figure 3b), hydrolysable tannin concentration and flavonol concentration did

250   not differ between leaf types (Tables S3 and S4). Nevertheless, the composition of

251   polyphenols as captured by the first axis of the PCA differed between intact and damaged

252   leaves in the *increased herbivory* treatment (posterior mean = −0.38, 95% CI = −0.61 to

253   −0.15; 0.4% inside ROPE, H_0 rejected; Figure S1c, Tables S3 and S4). The first PC axis was

254   defined by the variation in the concentrations of the most abundant hydrolysable tannins

255   (Figure S2). There were no clear differences in polyphenol composition between leaf types

256   along the second PC axis (H_0 unresolved, Tables S3 and S4). Experimental block had a

257   strong effect on the grouping of the data points in the resulting ordination (Figure S1d). The

258   diversity of all polyphenols, the diversity of hydrolysable tannins and the diversity of

259   flavonols did not show clear differences between leaf types (H_0 unresolved, Tables S3 and

260   S4).

261   The number of *A. consociella* shelters on a tree did not have any effect on tree growth

262   or acorn production (100% inside ROPE; H_0 accepted, Tables S3 and S4). There were no

263   clear relationships between the abundance of early-season caterpillar shelters and the

264   proportion of galled acorns; or between leaf area loss and tree growth, leaf area loss and

265   production of acorns or leaf area loss and the proportion of galled acorns (Tables S3 and S4,

266   all H_0 unresolved).

267

268 **Is the abundance, diversity and community structure of late-season herbivores affected**

269 **by prior herbivory?** We surveyed a total of 41443 oak leaves and recorded 27603 individual

270 galls and leafmines representing 20 different taxa (Table S3). The abundance of late-season

271 galls was negatively related to the number of *A. consociella* shelters (posterior mean = −0.16,

272 95% CI = −0.29 to −0.02, 1.0% inside ROPE, H_0 rejected, Tables S3 and S4, Figure 3c). The

273 number of *A. consociella* shelters also affected the overall diversity of sessile insects

274 (leafminers and gallers combined), but this effect depended on tree size (shelters × tree size

275 interaction, scaled posterior mean = −0.06, 95% CI = −0.09 to −0.02, 1.93% inside ROPE, H_0

276 rejected; Figure 3d, Tables S3 and S4): large trees with a high abundance of caterpillar

277 shelters had lower diversity of late-season herbivores than large trees with low shelter

278 abundance, whereas the opposite pattern was recorded on small trees.

279 There were no clear relationships between the number of *A. consociella* shelters and

280 the overall abundance of late-season herbivores, the abundance of leafminers (H_0 unresolved

281 for both, Table S4) and the community composition of late-season herbivores (LRT = 26.0, p

282 = 0.46, Figure 4b, Figure S1a). There were no clear relationships between the abundance and

283 diversity of late-season insects and leaf area loss on leaves that were not part of caterpillar

284 shelters (Tables S3 and S4, all H_0 unresolved).

285

286 **Does the abundance, diversity, and community structure of late-season herbivores**

287 **covary with polyphenol chemistry?** The community composition of late-season herbivores

288 covaried significantly with the tree-level polyphenol composition (LRT = 560.1, p = 0.02,

289 Figure 4acd, Table S6). Most compounds correlated with the abundance of at least one of the

290 late-season insect species, and most of these statistically significant pairwise correlations

291 were positive (Figure 4c). With the exception of the most abundant insect species, polyphenol

292 composition accounted for the largest proportion of the explained variation in insect

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293 abundances across trees (Figure 4d). The community composition of late-season herbivores

294 differed between experimental blocks (LRT = 333.9, p = 0.001, Figure S1be) and clusters

295 (LRT = 490.1, p = 0.001).

296

Discussion

298 In this study, we tested how early-season herbivory changes host plant performance,

299 polyphenol chemistry, and the community structure of sessile herbivores later in the season.

300 We manipulated densities of early-season shelter-building *A. consociella* caterpillars on 72

301 oak trees, creating an experimental gradient from a 2.5-fold reduction to a twofold increase in

302 caterpillar numbers, exceeding the natural variation in the number of caterpillar shelters

303 among trees at the study site. The manipulations did not affect leaf area loss on leaves that

304 were not part of the shelters, the concentration and composition of polyphenols, tree growth,

305 or reproduction. The composition of polyphenols differed between eaten and intact leaves;

306 however, only on trees in the *increased herbivory* treatment. There were few clear effects of

307 early-season caterpillars on the late-season sessile insect community, but the number of early-

308 season caterpillar shelters had a negative effect on galler abundance, and the effect of early-

309 season caterpillars on the overall diversity of late-season herbivores depended on the size of

310 the tree. The community composition of late-season insects covaried with tree-level

311 polyphenol composition, and both the community structure of late-season herbivores and the

312 tree-level polyphenol composition were strongly affected by the growth location of the host

313 tree. Below, we discuss each of these findings.

314

315 **No effect of caterpillar manipulations on leaf area loss or leaf chemistry.** Leaf area lost to

316 herbivores (on leaves outside caterpillar shelters) was not affected by the number of *A.*

317 *consociella* shelters. This suggests that *A. consociella* caterpillars feed primarily on the leaves

13

318   which are part of the shelters, and do not affect the abundance or feeding rate of other early-

319   season leaf-chewers sharing the host tree.

320   We found no differences in the concentration, diversity or composition of polyphenols

321   between trees with different amounts of *A. consociella* shelters. Nevertheless, there was

322   substantial variation in chemistry between individual trees and experimental blocks (e.g. total

323   polyphenol concentration varying 12.1 mg/g SD between trees, 9.6 mg/g SD between blocks

324   and 3.6 mg/g SD between treatments, with the mean concentration being 47 mg/g). These

325   results suggest that an increase in the number of *A. consociella* caterpillars did not induce any

326   density-dependent systemic defence reactions in their hosts, and that plant chemistry is more

327   strongly influenced by other factors than herbivory. Induced defences should be favoured

328   when herbivory pressure varies in time and when previous attack is a good indicator of future

329   herbivory (Karban 2011). This might not be the case in our study system, where the timing of

330   herbivory is predictable (most herbivory occurs within a few weeks after budburst), even

331   when its intensity varies between years. The best strategy for the plant might therefore be to

332   produce defensive compounds constitutively at the beginning of each season. Indeed, the

333   concentrations of hydrolysable tannins on oak show seasonal change, being high during the

334   months after budburst (Salminen et al. 2004). Alternatively, if low levels of herbivory are

335   sufficient for inducing defences, and if the strength of the defence reaction does not depend

336   on the intensity of herbivory, all trees in our study might have been in an induced state. The

337   herbivory-induced change in polyphenol concentration could also be too small to be

338   noticeable against the large background variation in chemistry between trees.

339   We detected differences in leaf chemistry between intact and eaten leaves on trees

340   with high abundances of *A. consociella* shelters. With high herbivore density, possibly only

341   the best-defended leaves remain uneaten. Even though previous studies have found leaf- and

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342   branch-level inductions of defences (Tuomi et al. 1988, Wold and Marquis 1997), we find it

343   unlikely that this kind of local induction would have occurred only in one of the treatments.

344

345   **Herbivory did not affect tree performance.** Consistent with the finding of no difference in

346   leaf chemistry between the treatments, we detected no effects of *A. consociella* herbivory on

347   tree growth, reproduction, and the proportion of galled acorns. The herbivory-induced

348   reduction in plant growth or reproductive output reported in previous studies (Hawkes and

349   Sullivan 2001, Hochwender et al. 2003, Nykänen and Koricheva 2004) is thought to result

350   from trade-offs between growth, reproduction and defence (Herms and Mattson 1992). Since

351   we observed no changes in leaf chemistry, it seems unlikely that any major re-allocation of

352   resources occurred following *A. consociella* herbivory. We tested if tree growth rate,

353   reproduction and investment in defences correlated, but found no significant relationship

354   between them (Appendix S3, "Patterns of tree resource allocation").

355   Indirect effects between host-plant associated species can influence plant reproduction

356   and fitness if induction of defences causes cross-resistance (Kessler and Baldwin 2004,

357   McArt et al. 2013), induced susceptibility (Poelman et al. 2010, Machado et al. 2018) or

358   disruption of beneficial species interactions (Kessler et al. 2011). It is possible that oaks in

359   this system have been selected to avoid triggering production of chemicals in response to

360   herbivory, if these come with fitness costs (Poelman and Kessler 2016). Fitness costs could

361   rise for example if certain chemicals serve as cues for host location for acorn gallers, or make

362   acorns less likely to be dispersed to new growth locations. Reliance on constitutive rather

363   than induced defences could be one way to avoid these costs.

364

365   **The effects of early-season herbivory on the late-season sessile insect community.** We

366   found that early-season *A. consociella* caterpillars had a negative effect on the abundance of

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367 late-season gallers. We also found that the effect of early-season caterpillars on late-season

368 herbivore diversity depended on the size of the host tree: an increase in the number of

369 caterpillar shelters increased herbivore diversity, but only on small trees (see Appendix S3).

370 Based on the model predictions, the addition of 170 early-season *A. consociella* shelters (the

371 model-predicted difference between *increase* and *suppression* treatments) to a tree would

372 result in 0.2 ($\pm$ 1.2 SD) more galler individuals per leaf on average-sized trees and an 0.2 ($\pm$

373 0.2 SD) increase in Shannon's diversity on small trees (compare to observed background

374 diversity of 1.6 ± 0.2 SD). Assuming that each tree has an average of ca. 2000 leaves (see

375 Appendix S1 for estimation of leaf number), 170 additional caterpillar shelters would result

376 in a decrease of 389 ($\pm$ 2392 SD) galler individuals per tree (against the estimated

377 background level of 2138 ± 718 SD gallers per *control* tree of 2000 leaves). Since the effects

378 of *A. consociella* shelters on late-season herbivore abundance and diversity are smaller or of

379 the same size as natural variation in these responses at the site, they might not be ecologically

380 very important. In addition, the effect on diversity was only seen on small trees, and errors

381 around both trends were large. Since we did not observe any change in leaf chemistry, leaf

382 area loss or tree performance between the different treatments, the drivers behind the trends

383 in the abundance of gallers and overall diversity of late-season herbivores are uncertain.

384   Several studies have reported host-plant mediated indirect effects between herbivores

385 (e.g. Van Zandt and Agrawal 2004, Poelman et al. 2010, Hernandez-Cumplido et al. 2016,

386 Arce et al. 2017), though other studies have not found any evidence for these effects (e.g.

387 Faeth 1986, Wold and Marquis 1997, Li et al. 2016). The presence and the strength of host

388 plant-mediated effects might depend on the characteristics of the species involved in the

389 studied interactions. For example, woody plants rely more on quantitative defences than

390 herbaceous species (Smilanich et al. 2016). Rather than having a lethal effect on herbivores,

391 quantitative defences reduce herbivore feeding rate (Feeny 1976), and might thus not

16

392   generate any clear effects on densities of sessile herbivores during a single season.

393   Additionally, compared to fast-growing species, slow-growing species often rely more on

394   constitutive than on induced defences (Endara and Coley 2011, Karban 2011). Therefore,

395   long-lived woody plants might depend primarily on quantitative, constitutive defences, which

396   might less likely mediate indirect interactions between herbivores than the more flexible

397   defences induced in fast-growing herbaceous species. Another factor affecting the occurrence

398   of plant-mediated indirect effects could be the longevity of the induced chemicals. If the

399   chemical compounds induced early in the season are no longer present when the late-season

400   species arrive (see e.g., Edwards et al. 1986), chemically mediated indirect interactions

401   between temporally separated herbivores might not occur. Nevertheless, since there are many

402   examples of indirect effects between early- and late-season herbivores (e.g., Van Zandt and

403   Agrawal 2004, Poelman et al. 2008, Hernandez-Cumplido et al. 2016), this mechanism might

404   only be relevant in the context of some plant-herbivore systems. Lastly, some herbivores can

405   suppress the defence and herbivore-recognition pathways of their host (Chung et al. 2013),

406   which could prevent indirect effects between plant-associated species from occurring.

407   One factor influencing the strength of plant-mediated indirect effects between

408   herbivores could be parallel changes in the activity of natural enemies of the herbivores

409   (Faeth 1986, Kaplan and Denno 2007). Many trees emit volatile predator-attracting

410   compounds as a response to herbivory (Thaler 1999, Kessler and Baldwin 2001).

411   Additionally, empty galls or caterpillar shelters could provide hiding places for predators and

412   other arthropods, thus influencing arthropod diversity and abundance (Lill and Marquis 2003,

413   Ohgushi 2005). For example, in our study the number of predators might have been highest

414   on trees with a high abundance of early-season caterpillar shelters. Increased predation or

415   behavioural changes to avoid predators might have suppressed the feeding activity of the

17

416   caterpillars (Steffan and Snyder 2010). Consequently, the number of shelters might not have

417   reflected the feeding pressure experienced by the tree.

418

419   **Tree-level chemical composition covaried with insect community structure.** We found

420   that tree-level polyphenol composition covaried with the community structure of sessile

421   insects. The chemical composition of plant tissue has recently been recognized as an

422   important factor explaining plant-associated insect diversity both between (Abrahamson et al.

423   2003, Richards et al. 2015, Salazar et al. 2018) and within species (Poelman et al. 2010,

424   Glassmire et al. 2016, Bustos-Segura et al. 2017), but exactly what drives the observed

425   phenotypic variation in plant chemistry is often not known.

426   One important factor determining plant chemistry and the associated arthropod

427   community is plant genetic variation (Wimp et al. 2005, Bangert et al. 2005). Since

428   polyphenol chemistry is heritable (Johnson et al. 2009), genetic differences between our

429   study trees might have contributed to the observed patterns of leaf chemistry and the structure

430   of the late-season insect community (see Whitham et al. 2006). Nevertheless, host genetics is

431   unlikely to be the only factor of importance: both the insect community structure and tree

432   chemistry were affected by the growth location of the tree. Several studies on oak have

433   shown how leaf chemistry or insect community structure are influenced by microclimate and

434   soil properties (Fine 2004, Moreira et al. 2017). At our study site, small-scale environmental

435   heterogeneity might have created variation in host tree chemical composition, affecting the

436   associated insect community. Lastly, since the observed relationship between insect

437   community structure and chemistry was correlative, it is also possible that the environment

438   was affecting each of them independently.

439   Our results show that the impact of individual compounds on the different herbivores

440   was specific to the exact compound by species pair. Interestingly, most of these pairwise

18

441   correlations were positive. The effect of individual chemicals can be extremely system-

442   specific, to the extent that a specific plant chemical is only effective to specific herbivore

443   species (Kessler and Halitschke 2007). Even though polyphenols can deter feeding and

444   reduce herbivore performance (Haviola et al. 2007, Ruuhola et al. 2007), some herbivores

445   can benefit from the secondary chemicals of their hosts (Roslin and Salminen 2008,

446   Smilanich et al. 2016). Since most of the late-season species in our study were specialists on

447   the genus *Quercus* (Table S4), they might have been attracted to the different oak-specific

448   compounds, if these compounds serve as cues for locating a host (Poelman et al. 2010).

449

450   **Conclusions***. In this study, changes in early-season insect herbivore abundance had no

451   detectable effects on tree chemistry or performance. The abundance of early-season

452   herbivores was negatively associated with the abundance of gallers, and positively with the

453   overall diversity of late-season sessile herbivores, although the latter effect was only seen on

454   small trees, and both trends were small. We found that host tree chemistry covaried with the

455   associated insect community structure and that both the chemical and insect community

456   compositions were strongly influenced by the growth location of the host tree. The lack of

457   effects of early-season herbivores on plant chemistry and the community composition of late-

458   season insect herbivores might be due to lack of induced changes in the host plant by early-

459   season caterpillars. If the timing of the herbivory relative to budburst is predictable,

460   constitutive defences might be favoured over induced ones. Additionally, if induced defences

461   create negative fitness consequences through for example attracting specialist plant enemies,

462   or disrupting beneficial species interactions, they might have been selected against in this

463   system. In this study, the chemistry of oak leaves appears to be more influenced by variation

464   in environmental conditions than by changes in herbivory pressure. Plant chemotype and

465   microclimatic conditions associated with the growth location are likely to be more important

19

466 than previous herbivory in determining the quality of the host plant and thus the structure of

467 the associated insect community in our study system.

468

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645

646   Figure 1. The experimental setup consisted of six experimental blocks (A-F), three clusters

647   within each block (n = 18) and four trees within each cluster (n = 72). Each block had one

648   cluster representing each of the three treatments: *suppression of herbivory*, *increased*

649   *herbivory* and *control*. On each tree,16 branches were marked and used for herbivory surveys

650   (see the main text and Appendix S1).

651

652   Figure 2. a) and b) *Acrobasis consociella* ("Broad-barred Knot-horn") caterpillar shelter on

653   oak. c) Acorn deformed by the seed galler *Andricus quercuscalicis* ("Knopper gall") next to a

654   healthy acorn. The structures of three hydrolysable tannins that were found abundant in the

655   samples: d) pedunculagin, e) tellimagrandin II and f) vescalagin.

656

657   Figure 3. The number of *A. consociella* shelters on trees in the different treatments (panel a)

658   and effects of early-season herbivory on selected response variables (panels b-d): b) the total

659   concentration (mg/g) of polyphenols in the six studied leaf types, c) the relationship between

660   early-season *A. consociella* shelters and the late-season abundance of late-season gallers and

661   d) the effect of *A. consociella* shelters on the diversity of late-season leafminers and gallers,

662   separated by tree size ("large" if dbh > mean dbh 43.1 mm). In panels c and d, the solid lines

663   have been drawn through model predictions, and the dotted lines represent model estimated

664   standard deviations.

665

666   Figure 4. The effect of tree-level polyphenol composition and experimental treatments on the

667   late-season insect community. Panel a) shows results from a PCA-based ordination

668   describing the variation in late-season insect community structure and polyphenol

669   composition. The insect species are shown as blue circles, the polyphenol compounds as grey

670   arrows and the experimental trees (n = 36) as small grey dots. Compounds expressing

29

671   collinearity have been removed. Panel b) shows the late-season insect community structure in

672   the data set comprising all experimental trees (n = 72). See Appendix S2, Figure S1e for

673   grouping by experimental block and Figure S1a for ordination for a subset of the trees (n =

674   36). Panel c) shows Pearson's correlations between the late-season herbivore species (as

675   rows) and polyphenols (as columns). Significant ($p < 0.05$) correlations are shown in red

676   (positive) and blue (negative). Panel d) shows the proportion of variance in the abundances of

677   the late-season sessile herbivores (in order of abundance, Appendix S2, Table S5) explained

678   by the different explanatory variables (polyphenols and caterpillar shelters) and random

679   effects (block and cluster). See Appendix S2, Tables S1 and S5 for abbreviations.
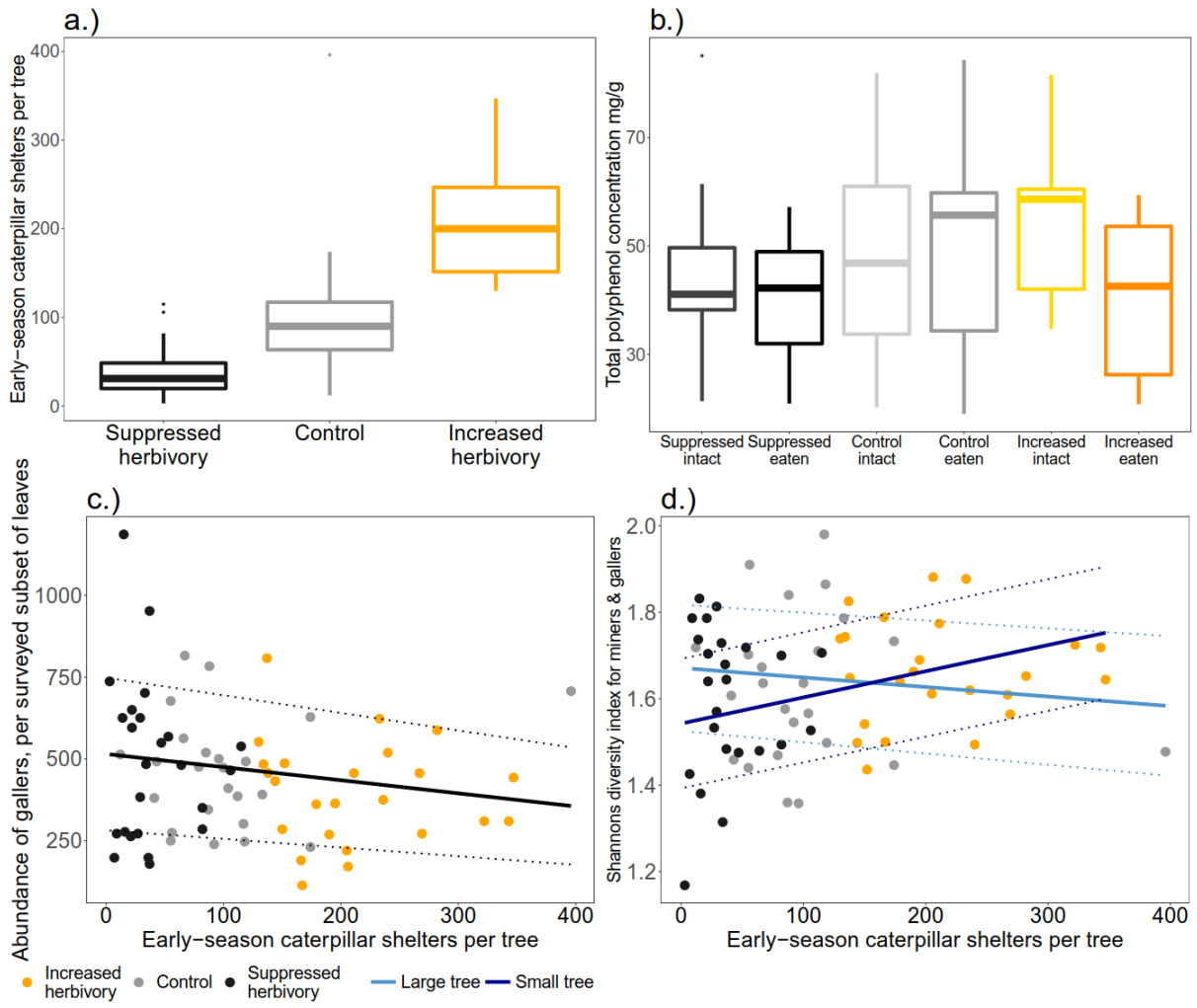
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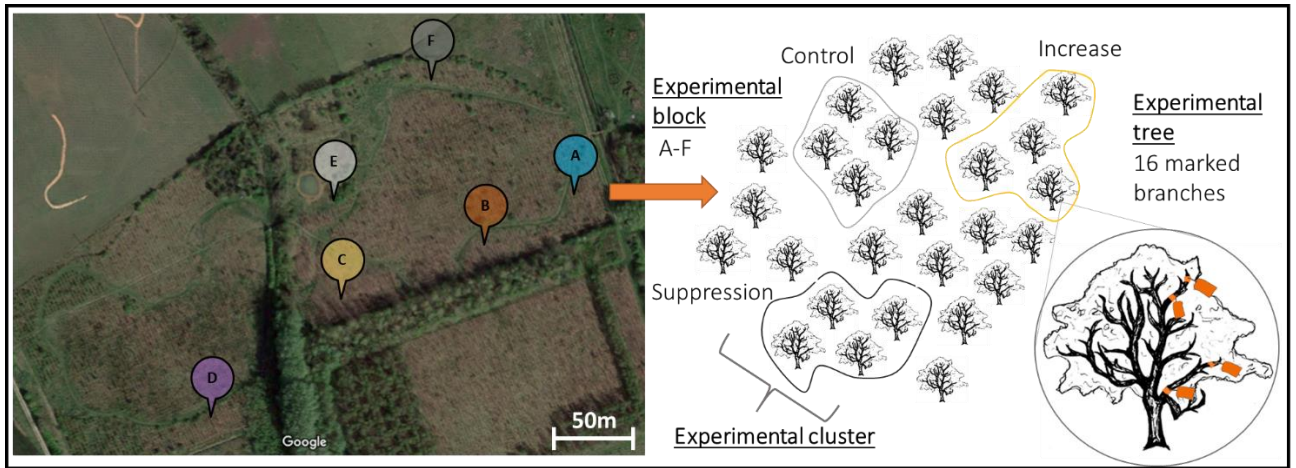
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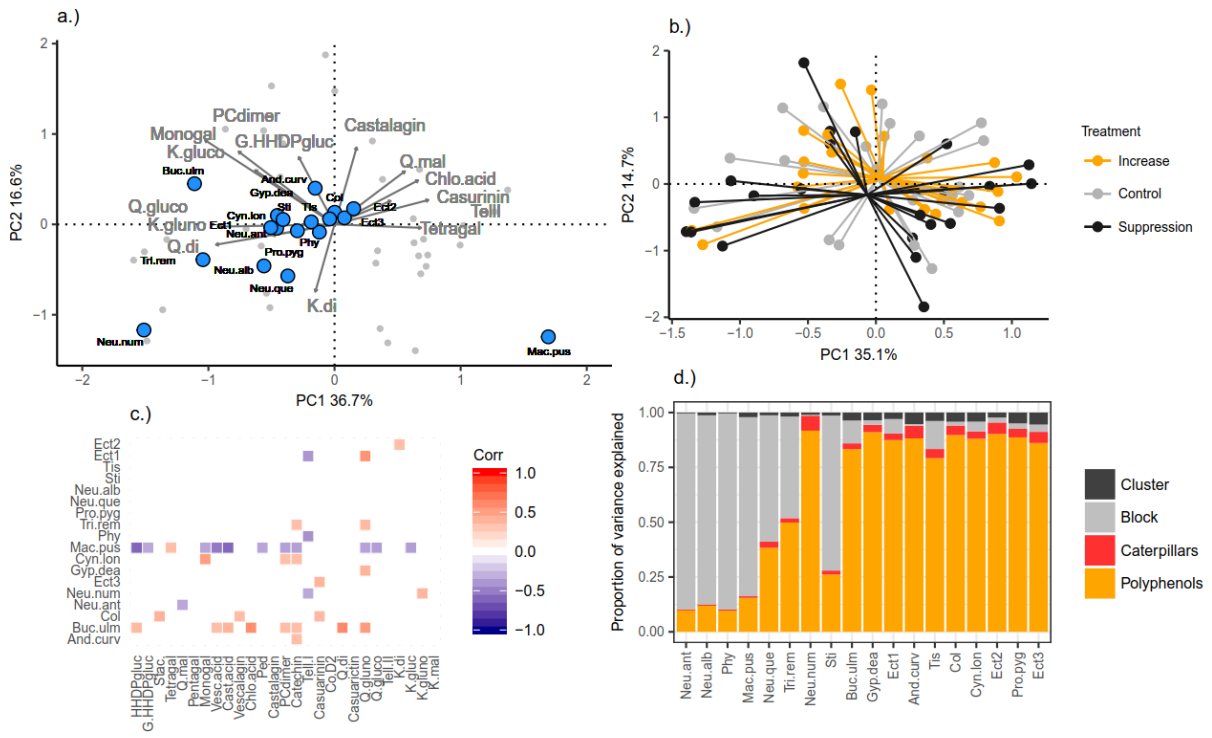682   Figure 1.
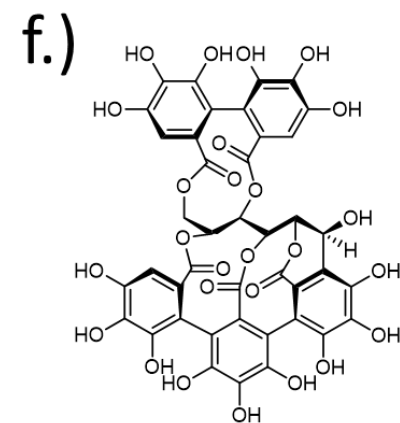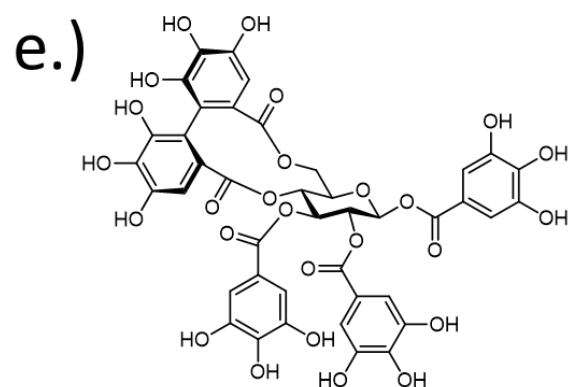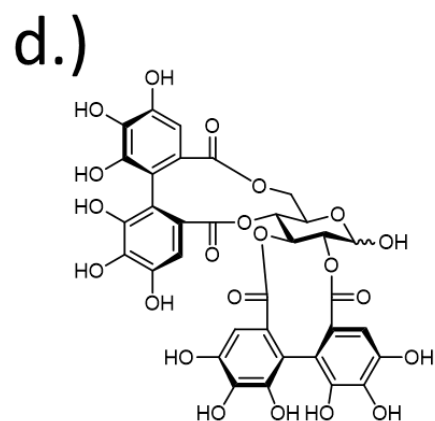
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684 Figure 2.

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687   Figure 3.

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690   Figure 4.