

# *Small-scale indirect plant responses to insect herbivory could have major impacts on canopy photosynthesis and isoprene emission*

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Small-scale indirect plant responses to insect herbivory could have major impacts on canopy photosynthesis and isoprene emission

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Total word count of the main text: 6498 (Introduction 732 words, Materials and Methods 2754 words, Results 982 words, Discussion 1930 words, Acknowledgements 100 words), two figures (in colour), one table, and a summary of 183 words.

Supporting Information:

Methods S1: Details on the experimental set up and on extracting the gas exchange parameters (2049 words)

Figure S1: Example of a mesh bag

Figure S2: Experimental leaves in herbivory addition and mechanical damage -treatments

Table S1: Leaf area loss at the study area and in the experiment

Figure S3: The average  $A/C_i$  response curves per leaf treatment

Figure S4: Correlation between the isoprene emission rate and photosynthetic parameters

Table S2: Coefficient estimates for mixed effects models

Table S3: Effects of herbivory on  $A_{1000}$  on leaf and canopy scales

Methods S2: iDirac overview and operation (313 words)

## Summary

- Insect herbivores cause substantial changes in the leaves they attack, but their effects on the ecophysiology of neighbouring, non-damaged leaves have never been quantified in natural canopies. We studied how winter moth (*Operophtera brumata*), a common herbivore in temperate forests, affects the photosynthetic and isoprene emission rates of its host plant, the pedunculate oak (*Quercus robur*).
- Through a manipulative experiment, we measured leaves on shoots damaged by caterpillars or mechanically by cutting, or left completely intact. To quantify the effects at the canopy scale, we surveyed the extent and patterns of leaf area loss in the canopy.
- Herbivory reduced photosynthesis both in damaged leaves and in their intact neighbours. Isoprene emission rates significantly increased after mechanical leaf damage. When scaled up to canopy-level, herbivory reduced photosynthesis by  $48 \pm 10\%$ .
- The indirect effects of herbivory on photosynthesis on undamaged leaves (40%) were much more important than the direct effects of leaf area loss (6%). If widespread across other plant-herbivore systems, these findings suggest that insect herbivory has major and previously underappreciated influences in modifying ecosystem carbon cycling, with potential effects on atmospheric chemistry.

Keywords: canopy, carbon cycling, herbivory, isoprene, photosynthesis, *Quercus robur*

## 1 **Introduction**

2 Interactions between plants and insect herbivores are among the most common ecological  
3 interactions (Strong *et al.*, 1984; Schoonhoven *et al.*, 2005). By influencing plant distribution,  
4 abundance and evolution, insect herbivores can have major impacts on community composition,  
5 primary productivity and biosphere–atmosphere interactions (Belovsky & Slade, 2000; Karl *et al.*,  
6 2008; Metcalfe *et al.*, 2014).

7 By removing plant tissue (*a direct effect* of herbivory), insect herbivores can substantially  
8 reduce photosynthesis. The loss of tissue often changes both primary (basic metabolic processes  
9 like respiration) and secondary (e.g. production of defensive chemicals) plant metabolism (Herms &  
10 Mattson, 1992; Kerchev *et al.*, 2012). This can lead to changes in the nutrient content or toxicity of  
11 the plant. Plants can also respond to herbivory by emitting volatile organic compounds (“VOCs”,  
12 Rowen & Kaplan, 2016). These changes, often triggered as defensive reactions, can spread to  
13 systemic undamaged tissue and affect all parts of the plant (Agrawal, 2000; Staudt & Lhoutellier,  
14 2007; Wu & Baldwin, 2009).

15 Insect-induced changes in chemistry and metabolism can further alter the photosynthetic  
16 capacity of the remaining leaf tissue (*an indirect effect* of herbivory, Zangerl *et al.*, 2002; Nykänen  
17 & Koricheva, 2004; Nability *et al.*, 2009). Leaf damage often triggers upregulation of defence-related  
18 genes and down-regulation of genes related to photosynthesis (Bilgin *et al.*, 2010). Nevertheless,  
19 previous studies have found both increased (“compensatory photosynthesis”) and decreased  
20 photosynthetic rate as a response to herbivory (Zangerl *et al.*, 2002; Nykänen & Koricheva, 2004;  
21 Nability *et al.*, 2009). Similarly, VOC emission can either increase (as defensive reaction through  
22 plant-predator communication or plant-plant signalling) or decrease after leaf damage (Loreto &  
23 Sharkey, 1993; Dicke & Baldwin, 2010; Rowen & Kaplan, 2016). The exact plant response to  
24 herbivory depends on the characteristics of the specific species interaction, for example on the diet

25 breath (e.g. specialist vs. generalist) or feeding guild (e.g. chewing vs sap-sucking) of the herbivore  
26 (Nykänen & Koricheva, 2004; Kessler & Halitschke, 2007; Rowen & Kaplan, 2016).

27 Isoprene is one of the most abundant plant-emitted hydrocarbons (Guenther *et al.*, 1995;  
28 Wang & Shallcross, 2000), produced by many long-lived woody species (Dani *et al.*, 2014). It is  
29 often emitted in small quantities alongside photosynthesis (Rasulov *et al.*, 2009), but also plays a  
30 key role as a stress chemical helping the plant to cope with high temperature (Sharkey & Singsaas,  
31 1995; Rasulov *et al.*, 2010). Because isoprene influences the formation and lifetime of lower  
32 tropospheric pollutants (Fehsenfeld *et al.*, 1992; Fuentes *et al.*, 2000), changes in isoprene  
33 emissions can influence atmospheric chemistry (Mentel *et al.*, 2013; Kravitz *et al.*, 2016). For  
34 estimating the effects of insect herbivory on atmospheric chemistry, quantifying herbivory-induced  
35 changes in isoprene emissions is of key interest.

36 To date, most studies assessing the link between herbivory and photosynthesis or isoprene  
37 emission have used cultivated model plant species (mostly species in the Brassicaceae or  
38 Solanaceae), simulated herbivory (Portillo-Estrada *et al.*, 2015), or controlled greenhouse  
39 environments (Kessler & Halitschke, 2007). The effect of herbivory (including its *indirect effects*)  
40 on photosynthesis or isoprene emissions in natural systems thus remains largely unknown. In  
41 addition, these effects have often been studied at the scale of individual plants or plant parts, and  
42 remain poorly quantified at larger scales. This prevents us from drawing conclusions about the  
43 large-scale influence of insect herbivory on carbon cycling and atmospheric chemistry.

44 Using a manipulative experiment, we investigated how a common insect herbivore affects  
45 photosynthesis and isoprene emission rate of its host plant in a natural broadleaf deciduous forest.  
46 As a study system, we used the pedunculate oak (*Quercus robur* L.) and caterpillars of the winter  
47 moth (*Operophtera brumata* L.), both of which are common species throughout temperate  
48 woodlands. We measured rates of photosynthesis and isoprene emissions in intact leaves, leaves  
49 eaten by herbivores, intact leaves close to eaten leaves (to quantify the systemic effects), and leaves

50 subject to mechanical damage (to gain insights into how the potential herbivory-induced responses  
51 are triggered). Specifically, we addressed the following questions: 1.) Do photosynthetic and/or  
52 isoprene emission rates of oak leaves change following leaf damage? 2.) Is the effect different  
53 between herbivore-induced damage versus mechanical wounding? 3.) Are damage-induced  
54 responses restricted to damaged leaves, or can changes in photosynthetic and/or isoprene emission  
55 rates be observed on intact leaves close to their damaged neighbour? 4.) What are the total effects of  
56 herbivory-induced leaf area loss (*direct effect*) and changes in the remaining leaf tissue (*indirect*  
57 *effect*) at the canopy scale?

58

## 59 **Materials and methods**

### 60 **Experimental setup**

61 The study was carried out during the springs and summers 2015-2016 on ten oak trees (*Quercus*  
62 *robur* L.) in Oxfordshire, UK. Five of the oaks were mature trees (mean diameter at breast height,  
63 “dbh” 67.2 cm  $\pm$  5.4 cm SEM) located in Wytham Woods (51°46' 27.48" N, 1° 20' 16.44" W, 160  
64 m.a.s.l), and the remaining five were young (mean dbh 13.6 cm  $\pm$  1.8 cm SEM) planted oaks by the  
65 John Krebs field station in Wytham (51 47' 1.32" N, 1° 19' 1.2" W, 63 m.a.sl). Oak is a strong  
66 isoprene emitter (Lehning *et al.*, 1999). On both sites, the oaks are naturally infested by caterpillars  
67 of the winter moth, which is a common generalist early-spring herbivore. The caterpillars emerge in  
68 synchrony with the budburst, and feed on the newly flushed leaves until June (Hunter, 1992).  
69 Relatively few herbivore species feed on the mature oak leaves later in the season (Feeny, 1970)  
70 Oaks in our study area do not reach their full photosynthetic capacity until late June, (Morecroft *et*  
71 *al.*, 2003), creating a time lag between the peak herbivory and the peak photosynthesis. For  
72 herbivores to have substantial impact on photosynthesis in this system, their effect should carry  
73 over until the oak has reached its full photosynthetic capacity.

74           Between 11<sup>th</sup> and 15<sup>th</sup> May 2015 and 9<sup>th</sup> and 11<sup>th</sup> May 2016, when most leaves were still  
75 newly flushed, we identified 15 shoots (of ~ 8 leaves) with only intact leaves from each study tree  
76 and enclosed each shoot in a small mesh fabric bag (see Supplementary Information, Methods S1).  
77 We randomly assigned each bag into one of the three treatments: 1) *herbivore addition*, 2)  
78 *mechanical damage*, or 3) *control*, so that each tree had five bags of each treatment. For each of the  
79 *herbivore addition* bags we added a locally collected winter moth caterpillar, and let it feed on the  
80 leaves for 3-5 days until at least two of the leaves showed signs of feeding damage. Because the  
81 effect of damage often depends on its type and amount (Wu & Baldwin, 2009; Portillo-Estrada *et*  
82 *al.*, 2015), each *herbivory addition* shoot was paired with a *mechanical damage* shoot immediately  
83 after the caterpillars had been removed from the mesh bags. The damage on the herbivory shoots  
84 was then replicated by tearing or punching holes with a cork borer in the leaves in the mechanical  
85 damage treatment. *Control* shoots were left intact. The timing of the manipulations coincided with  
86 the peak herbivory in the area (Charmantier *et al.*, 2008). The mesh bags were left around the shoots  
87 to prevent additional herbivory until 25<sup>th</sup> June 2015 or 28<sup>th</sup> June 2016, when the amount of insect  
88 herbivory had levelled off.

89           One month after the application of the treatments, we randomly chose three shoots from  
90 each tree (one *herbivory addition* shoot, one *mechanical damage* shoot, and one *control* shoot) for  
91 gas exchange measurements. The few control shoots (n=6) that showed signs of damage were  
92 excluded from further measurements. From each *herbivory addition* and *mechanical damage* shoot  
93 we measured two leaves: one damaged and one intact. From each *control* shoot we measured one  
94 intact leaf. This setup allowed us to measure five leaf-level treatments: damaged leaf in herbivory  
95 treatment, undamaged leaf in herbivory treatment, damaged leaf in mechanical treatment,  
96 undamaged leaf in mechanical treatment, and intact control leaf. We constructed photosynthetic  
97 light response curves (over the period of 28th July - 25th August 2015) for 49 leaves from ten trees  
98 and photosynthesis-CO<sub>2</sub> (A/C<sub>i</sub>) -curves (over the periods of 26th August - 10th September 2015 and

99 11th July - 11th August 2016) for 79 leaves from ten different trees (six of the trees were measured  
100 on both years) belonging to all the five leaf-level treatments. The timing of the gas exchange  
101 measurements corresponded to the peak photosynthetic activity of oak in the study area (Morecroft  
102 *et al.*, 2003).

103 On each leaf, we measured an intact part of an area of 2.5 cm<sup>2</sup> of the leaf with an infra-red  
104 gas analyser (CIRAS-2, PP-Systems, Hitchin, UK). For the light response curves, we took five  
105 point measurements on 15 different light levels between 2000 and 0  $\mu\text{mol m}^{-2}\text{s}^{-1}$  of  
106 photosynthetically active radiation (PAR). For the A/C<sub>i</sub> curves, we measured the photosynthetic  
107 rate under ten different CO<sub>2</sub> concentrations between 1300 and 30 ppm. All the raw photosynthesis  
108 measurements were processed using the protocol provided by PP-Systems (ppsystems.com) for the  
109 CIRAS-2 to apply corrections for the measured variables. The resultant variable used in the  
110 analyses was photosynthetic rate per unit leaf area, expressed as  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ .

111 To study how herbivory and leaf damage affect the production of isoprene by the oak, we  
112 measured isoprene emission rate of 32 leaves from seven trees, using the same leaves (and thus the  
113 same five leaf-level treatments) as for the A/C<sub>i</sub> curves with a portable gas chromatograph (iDirac,  
114 see Supporting Information, Methods S2), 21<sup>st</sup> July - 9<sup>th</sup> August 2016. iDirac is a novel gas  
115 chromatograph, designed for *in-situ* use. Here we report its use for the first time in a field study. We  
116 attached the iDirac directly into the CIRAS-2 system to allow for simultaneous measurements of  
117 isoprene production and photosynthetic rate. See Supporting Information, Methods S1 for details  
118 on all the gas exchange measurements.

119 After measurements were taken the leaves were photographed to estimate the leaf area lost  
120 to herbivory. To estimate the natural level of insect herbivory on the study trees throughout the  
121 growing season, we collected 15 additional shoots from each tree on four time points (16-28<sup>th</sup> May,  
122 25<sup>th</sup> June, 14<sup>th</sup> July - 10<sup>th</sup> August and 18<sup>th</sup> August 2015), and pressed and scanned the leaves. The  
123 area lost to herbivory of the photographed and scanned leaves were estimated as the percentage of

124 missing area from the side of the leaf, from the tip, or as holes, using the ImageJ software (NIH,  
125 MD, USA).

126

### 127 **Extracting response parameters.**

128 To calculate the light-saturated photosynthesis, we fitted a Michaelis-Menten equation to the light  
129 response data for each leaf separately to estimate the parameters for the maximum light-saturated  
130 photosynthetic rate ( $A_{\text{sat}}$ ) and the light intensity at which the gross photosynthetic rate is half of its  
131 maximum,  $K$  (Marino *et al.*, 2010). To obtain a measure of the mean dark respiration ( $R_d$ ) for each  
132 leaf, we calculated the average photosynthetic rate on the light response curves when the light level  
133 was zero. To analyse the photosynthetic response to experimental treatments under different  $\text{CO}_2$   
134 concentrations, we constructed  $A/C_i$  response curves, where the photosynthetic rate ( $A$ ) is modelled  
135 against the intercellular  $\text{CO}_2$  mole fraction ( $C_i$ ) (Farquhar *et al.*, 1980; Sharkey *et al.*, 2007),  
136 allowing us to estimate three important photosynthetic parameters: maximum carboxylation rate,  
137 describing the activity of Rubisco ( $V_{\text{cmax}}$ ), rate of photosynthetic electron transport ( $J_{\text{max}}$ ) and triose  
138 phosphate use efficiency (TPU). See Supporting Information, Methods S2 for details on model  
139 fitting.

140 After fitting, all the parameters were normalized to 25 °C (Harley *et al.*, 1992) (Sharkey *et*  
141 *al.*, 2007) to reduce variation caused by different ambient temperatures. For most leaves ( $n = 65$ )  
142 the Farquhar *et al.* (1980) model could be fitted to the data. For some leaves ( $n = 14$ ) the model  
143 failed to estimate at least one of the parameters. These leaves were omitted from the further  
144 analyses of the treatment effects on  $A/C_i$  parameters. To study possible changes in leaf  
145 conductance, we extracted the mean stomatal conductance ( $g_s$ ) recorded by the gas analyser during  
146 the  $A/C_i$  curve measurements. From those leaves of which only light response was measured (24  
147 leaves), we used mean stomatal conductance of the light response curve. Single outlier values of

148 stomatal conductance,  $K$  and isoprene emission were removed from further analyses. See Fig. 2 for  
149 final sample sizes per parameter

150 To estimate isoprene emissions, the height of each isoprene peak in the gas chromatogram  
151 was measured and converted into mixing ratios (ppb) by using calibration measurements with  
152 known isoprene concentrations. The mixing ratios were scaled with the known air volume, area of  
153 leaf measured and flow rate to yield emission rates as  $\text{nmol m}^{-2} \text{s}^{-1}$ . Because isoprene emission is  
154 strongly influenced by temperature, we corrected the measured emission values for temperature  
155 (Guenther *et al.*, 1993, 1995), yielding the standard emission factor of isoprene (as  $\mu\text{g m}^{-2} \text{h}^{-1}$ ),  $I_s$   
156 (in 303 K and  $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$  of photosynthetically active radiation). See Supporting  
157 Information, Methods S1 for details on the temperature correction.

158 To describe the photosynthetic rate of the study leaves in natural conditions, we extracted  
159 values from the light-response and  $A/C_i$  curves for photosynthetic rates at ambient  $\text{CO}_2$   
160 concentration (400 ppm) and in light intensity that corresponds to typical full light conditions ( $1000$   
161  $\mu\text{mol m}^{-2} \text{s}^{-1}$  of photosynthetically active radiation). This parameter ( $A_{1000}$ ), was used to assess the  
162 correlation between photosynthesis and isoprene emission rate, and to scale up the effects of  
163 herbivory from leaf scale to the canopy level.

164

165 **Statistical analyses.** To test for effects of our experimental treatments on photosynthesis and  
166 isoprene emission, we built a separate linear mixed effects model for each of the key response  
167 parameters described above. Each photosynthesis-related response parameter ( $A_{\text{sat}}$ ,  $K$ ,  $R_d$ ,  $V_{\text{cmax}}$ ,  
168  $J_{\text{max}}$ ,  $\text{TPU}$ ,  $g_s$ ) was modelled as a function of leaf-level treatment (a categorical variable with five  
169 levels), site (Wytham Woods or John Krebs field station), mean leaf temperature (to account for any  
170 remaining variation by the ambient temperatures), year (2015 or 2016, for the parameters that had  
171 been measured in both years), and the percentage of leaf damage as explanatory variables. Time of  
172 the day was assumed to have a non-linear effect, and was added as general additive smoother. To

173 avoid spurious treatment effects due to small sample sizes, interactions were not included (Zuur,  
174 2009). Tree identity and shoot identity (nested within tree identity), were included as random  
175 factors (random intercepts) to account for non-independence of leaves on the same shoots and trees.  
176 Isoprene emissions ( $I_s$ ) were modelled using the same approach, except that variance structure was  
177 allowed to vary between the leaf treatments to allow for unequal variances across these groups. For  
178 each response variable, the full model was simplified by dropping one explanatory variable at a  
179 time. The change in the model fit was assessed using likelihood ratio tests. Fixed factors that did not  
180 improve model fit were dropped from the final model (Crawley, 2007). Where leaf type was  
181 significant, a post-hoc Tukey's test was applied to assess which of the five leaf treatments differed  
182 significantly from one other. Because of the adjusted variance structure in the isoprene model, the  
183 pairwise leaf treatment comparisons were carried out estimating least square means.

184 To analyse the relationship between isoprene emission and the photosynthetic parameters  
185 measured simultaneously ( $A_{1000}$ ,  $V_{cmax}$ ,  $J_{max}$  and TPU), we built linear, exponential and quadratic  
186 models in which the isoprene emission rate was modelled as a function of each selected  
187 photosynthetic parameter. We then estimated the model fit by comparing the adjusted  $r^2$ -values  
188 between the different models (linear, exponential and quadratic), and selected the model with the  
189 highest  $r^2$  value for each of the parameters.

190 To test for the differences in the amount of leaf damage between the two damage treatments  
191 (mechanical and herbivory) and naturally occurring damaged leaves, we built a linear model with  
192 proportion of damage as a function of damage type (herbivore addition, mechanical, natural). To  
193 test for patterns in natural herbivory levels, we built a linear model of proportion of damage as a  
194 function of the site and the collection date. Proportions were arcsine-square root –transformed in  
195 order not to violate model assumptions (Crawley, 2007). For all models, the model assumptions  
196 were tested by visually examining plots of residuals against fitted values for the homoscedasticity of  
197 residuals, and a Quantile-Quantile plot for the normal distribution of the residuals. All analyses

198 were conducted using R version 3.4.1 (R Core Team, 2017) and the packages lme4 (Bates *et al.*,  
199 2015), multcomp (Hothorn *et al.*, 2008), nlme (Pinheiro *et al.*, 2017), gamm4 (Wood & Scheipl,  
200 2017) and lsmeans (Lenth, 2016).

201

202 **Quantifying the effects of herbivory on leaf and canopy scales.** To estimate the effects of  
203 herbivory on photosynthesis and isoprene emission at the canopy scale, we combined three types of  
204 data: 1) the proportion of leaf area loss per leaf under natural conditions (direct effect), 2) the effect  
205 of insect herbivory on the photosynthetic rate ( $A_{\text{sat}}$ ) or isoprene emission rate ( $I_s$ ) per unit leaf area  
206 (indirect effect), and 3) information on natural patterns of herbivory in the oak canopy. Control  
207 leaves, which were intact leaves on intact shoots were set as a reference point to describe  
208 photosynthesis and isoprene emission in the absence of herbivory. To estimate the leaf-scale effect  
209 of herbivory on the light-saturated photosynthesis or isoprene emission rate, we first multiplied the  
210 per leaf unit area rate of a leaf damaged by herbivores with the proportion of remaining leaf area in  
211 the corresponding leaf type, yielding a “per leaf” - rate. We then compared this to a “per leaf” -rate  
212 of an intact control leaf:

213

$$214 \text{ light saturated leaf scale effect}_t = \frac{A_t * (1 - D_t)}{A_{t=1}} - 1$$

215

216 (Eq. 1.)

217

218 where A is the light-saturated assimilation rate ( $A_{\text{sat}}$ ) or the isoprene emission rate, D is the  
219 proportion of leaf area loss per leaf type (= direct effect, between 0 and 1) and t denotes the three  
220 different leaf types (1 = intact leaf in a completely intact shoot, 2 = intact leaf in an herbivory  
221 treatment, 3 = damaged leaf). For the intact leaves in the herbivory treatment, the leaf scale effect

222 was simply the percentage change in the photosynthetic or isoprene emission rate, indicating a  
223 “shoot-level effect” of herbivory spreading from the damaged leaves to the intact neighbours.

224 We estimated the effect of herbivory at the level of the canopy with two different methods.  
225 Firstly, to estimate the herbivory effect at the level of the canopy for the maximum light-saturated  
226 photosynthesis and isoprene emission rate, we multiplied the light saturated leaf-scale effect of each  
227 leaf type by the proportion of the respective leaf type in the canopy, and then summed these values  
228 over the three leaf types:

$$229 \text{ light saturated canopy effect} = \sum_{t=1}^3 \text{leaf scale effect}_t * l_t$$

230 (Eq. 2.)

231

232 where t denotes the three different leaf types and l is the proportion of leaf type t in the canopy. For  
233 photosynthesis, this model estimates the maximum potential photosynthesis in full light (as  $\mu\text{mol}$   
234  $\text{m}^{-2} \text{s}^{-1}$  of leaf\_area), without considering light transmission through the canopy.

235 Secondly, because photosynthesis is strongly affected by the amount of available light, we  
236 estimated the effect of herbivory on canopy photosynthesis when the diffusion of light through the  
237 canopy is taken into account. To estimate this, we used the Big Leaf approach of The Joint UK  
238 Land Environment Simulator (“JULES”, Clark *et al.*, 2011) to estimate canopy assimilation,  
239 combined with an estimate for canopy respiration (Mercado *et al.*, 2007). The reduction of direct  
240 light through the canopy was calculated by Beer's law (Monsi & Saeki, 1953). As a result, our  
241 model estimates instantaneous big-leaf approximated net CO<sub>2</sub> assimilation rate. Assimilation is  
242 reduced proportional to the transmission of light through the canopy, while leaf respiration  
243 increases as light decreases:

244

245 
$$NPC = \int_0^{LAI} A_{sat} * \left( \frac{PAR}{K + PAR} \right) * (e^{-k*LAI}) - (0.5 - 0.05 * \ln(PAR * e^{-k*LAI})) * R_d$$

246 (Eq 3.)

247

248 where NPC is canopy net photosynthesis (as  $\mu\text{mol m}^{-2} \text{s}^{-1}$  of ground area),  $A_{sat}$  is the light-saturated  
 249 photosynthetic rate,  $k$  is a light extinction coefficient, LAI is a canopy leaf area index, PAR is the  
 250 light intensity (“photosynthetically active radiation”) at the top of the canopy and  $R_d$  is the dark  
 251 respiration rate estimated from the Michaelis-Menten equation (Supporting Information Methods  
 252 S1, Eq. S2). The light extinction coefficient ( $k$ ) was set to 0.5 as a previously used estimate for  
 253 broadleaf forests (Clark *et al.*, 2011), leaf area index (LAI) was set to 7.8 as previously measured  
 254 for this field site (Fenn *et al.*, 2015) and PAR was set to  $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$  as a standard daytime  
 255 light intensity at the top of the canopy. We estimated canopy net photosynthesis for each leaf type  
 256 (i.e. canopy consisting of only that leaf type), multiplied the estimates with the proportion of the  
 257 respective leaf type observed in the canopy, and then summed these values over the three leaf types.  
 258 This estimate was then compared to an estimate of a canopy with intact leaves only. Finally, we  
 259 included the direct effect of leaf area loss by subtracting the proportion of leaf area loss at canopy  
 260 level:

261

262 canopy effect at diffused light =  $\left( \frac{\sum_{t=1}^3 NPC_t * l_t}{NPC_{t=1}} - D_c \right) - 1$

263 (Eq. 4.)

264

265 where  $t$  denotes the three different leaf types,  $l$  is the proportion of leaf type  $t$  in the canopy and  $D_c$   
 266 is the proportion of leaf area loss (=direct effect) at the canopy scale.

267

268 **Results**

269 **Herbivory under natural and experimental settings.** There was no difference between the natural  
270 levels of herbivory between the two study sites ( $t = -0.55$ ,  $df = 2$ ,  $1461$ ,  $p = 0.58$ ) and no change  
271 throughout the growing season ( $t = -1.65$ ,  $sf = 2$ ,  $1461$ ,  $p = 0.10$ ), indicating that early-season  
272 herbivory is the dominant type of insect herbivory in the study system. Almost all shoots surveyed  
273 for natural herbivory levels had at least one damaged leaf: of the 175 shoots surveyed, only three  
274 (1.7%) were completely intact.

275 The mesh bags successfully prevented herbivores from colonizing the experimental shoots  
276 (94 of 100 control shoots remained intact). The amount of leaf damage did not differ between the  
277 two damage treatments ( $10.88\% \pm 1.84\%$  in mechanical and  $14.13\% \pm 1.91\%$  in herbivore addition,  
278  $t = -0.90$ ,  $df = 2$ ,  $1086$ ,  $p = 0.37$ ), but was higher in leaves with experimental herbivory compared  
279 to naturally occurring herbivory ( $8.45\% \pm 0.39\%$ ,  $t = 3.04$ ,  $p = 0.002$  for herbivore addition and  $t =$   
280  $1.72$ ,  $p = 0.09$  for mechanically damaged). Most leaf damage occurred at sides and tips, and only a  
281 small portion as holes (Supporting Information, Table S1).

282

283 **Treatment-effects on photosynthesis and isoprene emission.** Leaf treatment significantly  
284 influenced the light-saturated photosynthetic rate  $A_{\text{sat}}$  ( $\chi^2 = 17.31$ ,  $p = 0.002$ ,  $df = 4,8$ ; Supporting  
285 Information, Table S2; Fig. 1a. and 2a), the mean carboxylation rate  $V_{\text{cmax}}$  ( $\chi^2 = 9.51$ ,  $p = 0.05$ ,  $df =$   
286  $4,11$ , Table S2; Fig. 1b and 2d), the mean electron transport rate  $J_{\text{max}}$  ( $\chi^2 = 11.23$ ,  $p = 0.02$ ,  $df =$   
287  $4,10$ , Table S2; Fig. 1c and 2e), the mean stomatal conductance  $g_s$  ( $\chi^2 = 10.48$ ,  $p = 0.03$ ,  $df = 4,10$ ,  
288 Table S2. Fig. 2g) and the isoprene emission rate  $I_s$  ( $L_{\text{ratio}} = 23.15$ ,  $p < 0.001$ ,  $df = 4,9$ , Table S2;  
289 Fig. 2h). Both damaged and undamaged leaves in the herbivore addition shoots experienced a  
290 significant reduction in their  $A_{\text{sat}}$  and  $J_{\text{max}}$  compared to control leaves ( $z = -4.26$ ,  $p < 0.001$   
291 damaged leaves and  $z = -4.26$ ,  $p < 0.001$  undamaged leaves for  $A_{\text{sat}}$ ,  $z = -38.92$ ,  $z = -2.84$ ,  $p = 0.03$   
292 damaged leaves and  $z = -3.24$ ,  $p = 0.01$  undamaged leaves for  $J_{\text{max}}$ ).  $V_{\text{cmax}}$  was different mainly

293 between leaves damaged mechanically and intact leaves in the herbivory treatment, but the  
294 difference (revealed by the Tukey's test) was only marginally significant ( $z = 2.55$ ,  $p = 0.08$ ).  
295 Stomatal conductance ( $g_s$ ) was different between control and the undamaged leaf in the herbivory  
296 treatment ( $z = -2.73$ ,  $p = 0.049$ ). The light intensity at which the gross photosynthetic rate is half of  
297 its maximum (K, Fig. 2b), dark respiration ( $R_d$ , Fig. 2c), and triose phosphate use efficiency (TPU,  
298 Fig. 1d and 2f), on the other hand, were not influenced by leaf treatment. Mean leaf temperature  
299 significantly increased  $V_{cmax}$  ( $\chi^2 = 4.21$ ,  $p = 0.04$ ,  $df = 1$ , 11),  $J_{max}$  ( $\chi^2 = 9.98$ ,  $p = 0.002$ ,  $df = 1$ , 10),  
300 TPU ( $\chi^2 = 9.93$ ,  $p = 0.002$ ,  $df = 1$ , 6),  $R_d$  ( $\chi^2 = 8.11$ ,  $p = 0.004$ ,  $df = 1$ , 5) and  $g_s$  ( $\chi^2 = 5.34$ ,  $p = 0.02$ ,  
301  $df = 1$ , 10).  $V_{cmax}$ ,  $J_{max}$ , TPU and  $g_s$  were significantly different between the two sites ( $\chi^2 = 5.07$ ,  $p =$   
302  $0.02$ ,  $df = 1$ , 11 for  $V_{cmax}$ ;  $\chi^2 = 5.58$ ,  $p = 0.02$ ,  $df = 1$ , 10 for  $J_{max}$ ;  $\chi^2 = 5.34$ ,  $p = 0.02$ ,  $df = 1$ , 6 for  
303 TPU and  $\chi^2 = 5.95$ ,  $p = 0.01$ ,  $df = 1$ , 10 for  $g_s$ ), and  $V_{cmax}$  differed between the two measuring years  
304 ( $\chi^2 = 8.82$ ,  $p = 0.03$ ,  $df = 1$ , 11).

305 Leaves damaged mechanically had significantly higher isoprene emission rate compared to  
306 control leaves and undamaged leaves in the herbivory treatment ( $t = -6.57$ ,  $p < 0.007$  and  $t = -7.16$ ,  
307  $p < 0.004$ , respectively). The isoprene emission rate per unit leaf area decreased with increasing  
308 percentage of leaf damage ( $L_{ratio} = 8.32$ ,  $p = 0.004$ ,  $df = 1$ , 9). Isoprene emission rate correlated  
309 positively and significantly with the photosynthetic parameters (Supporting Information, Fig. S4).

310

311 **The effects of herbivory on leaf and canopy scales.** Leaf area loss (the *direct effect* of herbivory)  
312 per leaf was  $8.5\% \pm 0.4\%$ . The *indirect effect* of herbivory, i.e. the herbivory-induced change in  
313 photosynthesis in the remaining leaf tissue, accounted for a  $45.5\% \pm 10.1\%$  reduction in the leaf-  
314 scale light-saturated photosynthesis ( $A_{sat}$ , Table 1). Hence, the indirect effect of herbivory was  
315 several magnitudes larger than the direct effect of leaf area loss. Within the shoots that had  
316 herbivory damage, the reduction in photosynthesis was almost identical between damaged leaves  
317 and their undamaged neighbors. When the *direct* and *indirect effects* and the proportion of damaged

318 and undamaged leaves in the canopy were combined,  $45.6\% \pm 7.6\%$  of the light-saturated  
319 photosynthetic potential and  $47.9\% \pm 9.5\%$  of the net photosynthesis under diffused light was lost  
320 to herbivores at the canopy-scale (Table 1). The first estimate represents a canopy consisting only of  
321 sun leaves at full light, (see Supporting Information, Table S3 for estimates on canopy-scale effects  
322 of herbivory on photosynthesis at lower light intensity), whereas the second estimate represent a  
323 canopy where light is reduced with increasing leaf area index due to shading. Despite the different  
324 assumptions of these estimates, the proportional change in photosynthesis due to herbivory is  
325 effectively the same.

326 In contrast to the photosynthesis results, isoprene emission rates increased in the damaged  
327 leaves by  $85.4 \pm 115.6\%$  compared to the intact control leaves, though the small number of samples  
328 and the associated large error makes drawing conclusions difficult. The shoot-level effect, where  
329 shoot-level herbivory affects undamaged leaves within the same shoot, was small ( $29.8 \pm 32.1\%$ )  
330 for isoprene. At the canopy-scale, the total effect of herbivory corresponded to a  $52.5 \pm 82.6\%$   
331 increase in isoprene emissions, but with large variation (Table 1).

332

## 333 **Discussion**

334 In this study herbivory substantially reduced photosynthesis in damaged leaves and in their intact  
335 neighbours. Isoprene emission rates significantly increased after mechanical leaf damage. At the  
336 canopy-scale, these results indicate that even a relatively moderate level of herbivory (6% of  
337 canopy leaf area), leads to a 48% reduction in the potential photosynthesis and a 53% increase in  
338 isoprene emission rate, although the effect on isoprene emission was not statistically significant at  
339 the canopy-scale. Below, we will discuss each of our findings in turn.

340

341 **Why does the photosynthetic rate change following leaf damage?** Previous studies on the  
342 indirect effects of herbivory on photosynthesis have reported increases (Oleksyn *et al.*, 1998;

343 Nykänen & Koricheva, 2004), decreases (Oleksyn *et al.*, 1998; Nabity *et al.*, 2009) and no changes  
344 (Peterson *et al.*, 2004) in the assimilation rates after leaf damage. In this study, leaf damage by  
345 herbivores lowered the maximum light-saturated photosynthetic rate ( $A_{\text{sat}}$ ), maximum carboxylation  
346 rate ( $V_{\text{cmax}}$ ) and the maximum electron transport rate ( $J_{\text{max}}$ ). As stomatal conductance ( $g_s$ ) correlates  
347 with photosynthesis (Wong *et al.*, 1979; Gago *et al.*, 2016), its responses to the treatments were  
348 similar to that of photosynthesis. These effects were visible several months after the initial damage.  
349 It is unclear whether photosynthesis had remained low during the entire period, or whether the  
350 reduction became observable only late in the season. Other studies have reported delayed effects of  
351 herbivory on plant physiology, which can be visible several weeks (Gibberd *et al.*, 1988; Meyer,  
352 1998) or even seasons (Kaitaniemi *et al.*, 1998) after the initial damage.

353         One possibility is that physical injury is inhibiting photosynthesis. Severed vein network  
354 can disrupt the transport of water and nutrients with long-lasting effects (Sack & Holbrook, 2006),  
355 simultaneously reducing stomatal conductance. Ruptures in the leaf can cause diffusion of  $\text{CO}_2$   
356 before it is used in the carbon-fixing reactions, lowering the efficiency of carbon assimilation  
357 (Oleksyn *et al.*, 1998; Nabity *et al.*, 2006, 2009, 2013). Furthermore, repairing the damaged tissue  
358 uses valuable resources. Trade-offs in resource use might also occur between growth (and hence  
359 photosynthesis) and defence (Herms & Mattson, 1992). Defensive reactions against herbivores  
360 require synthesis of complex chemical compounds, which act as repellents or additional signalling  
361 molecules, using the same resources or molecular pathways than photosynthesis (Herms &  
362 Mattson, 1992; Taiz & Zeiger, 2010; Zhou *et al.*, 2015). Build-up of defensive compounds in the  
363 plant tissue might also cause the problem of auto-toxicity, lowering photosynthetic efficiency  
364 (Baldwin & Callahan, 1993; Nabity *et al.*, 2009). Damage early in the season could also “prime” the  
365 plant (Conrath *et al.*, 2002), making it more resistant to future herbivory by activating long-lasting  
366 defences. The cost of maintaining a primed state could alter primary metabolism over long-term  
367 (van Hulst *et al.*, 2006; Frost *et al.*, 2008).

368

369 **Why does the photosynthetic rate differ between leaves damaged mechanically or by**  
370 **herbivores?** In this study, the mechanically damaged leaves experienced a significantly smaller  
371 reduction in their photosynthetic rate than leaves damaged by caterpillars. In previous studies,  
372 mechanical damage alone has failed to produce a response in the plant, whereas application of  
373 herbivore oral secretions, even without any physical damage, have done so (Korth & Dixon, 1997;  
374 Alborn, 1997). The herbivore-induced defensive responses depend on the species identity,  
375 specifically on the chemical make-up of the insect saliva (Alborn, 1997; Erb *et al.*, 2012). These  
376 herbivory-specific effects are usually mediated through hormonal pathways including jasmonic and  
377 salicylic acids, the activation of which also switches off photosynthesising reactions (Wasternack &  
378 Hause, 2013). These results suggest that the herbivory-inflicted photosynthetic reduction in our  
379 study is a response to the presence of herbivores specifically, instead of leaf damage alone, and  
380 possibly actively triggered by the defence machinery of the plant (Kerchev *et al.*, 2012; Zhou *et al.*,  
381 2015).

382

383 **How does leaf damage affect intact neighbouring leaves?** In this study, intact and damaged  
384 leaves on the same shoots showed an almost identical degree of reduction in photosynthesis.  
385 Damage-triggered defence reactions can travel to intact plant parts through shared vasculature  
386 (Jones *et al.*, 1993), as electric signals (Sukhov, 2016), or to neighbour plants through volatile  
387 organic compounds (Arimura *et al.*, 2000). This systemic signalling can subsequently affect  
388 photosynthesis of intact plant parts (Agrawal, 2000; Barron-Gafford *et al.*, 2012; Meza-Canales *et*  
389 *al.*, 2017). Especially jasmonic acid can travel to systemic tissues (Baldwin & Zhang, 1997;  
390 Stratmann, 2003), and accumulate in them (Leitner *et al.*, 2005). Because in our study the systemic  
391 changes were detected within individual shoots, the signal has probably travelled through within-  
392 shoot vascular connections, which might have also restricted it from reaching the intact control

393 shoots, or dampened the effect (Orians, 2005). The reduction in photosynthesis in neighbouring  
394 leaves might prepare the leaf for the forthcoming herbivory, either by increasing the level of  
395 defences at the expense of assimilation, or by actively shutting down the production of further  
396 carbohydrates, to provide less nutrition for herbivores (Zhou *et al.*, 2015). Herbivore-specific  
397 signalling might also explain why the mechanical treatment responded less than the herbivore  
398 addition. Our study thus shows that naturally occurring herbivory can have a considerable effect  
399 also on systemic intact leaves. These kinds of shoot-level effects have not been previously taken  
400 into account in ecosystem-scale studies.

401

402 **Why did the isoprene emission rate increase after leaf damage?** We observed a significant  
403 positive relationship between photosynthesis and isoprene emission, concurrent with previous  
404 studies (Rasulov *et al.*, 2009; Copolovici *et al.*, 2017). Nevertheless, the treatment-specific effects  
405 on isoprene were opposite to the effects on photosynthesis. The isoprene emission rates per unit leaf  
406 area were significantly higher in the mechanically damaged leaves than in non-damaged leaves on  
407 the intact control shoots, suggesting that the observed change might not be a response to herbivory  
408 specifically. Because the effect was not visible in the surrounding intact leaves, the damage-  
409 triggered change in isoprene emission seems to be a leaf-level response. Contrary to our results,  
410 previous studies have found *a reduction* in isoprene emission immediately after leaf damage  
411 (Loreto & Sharkey, 1993; Portillo-Estrada *et al.*, 2015; Copolovici *et al.*, 2017), but see Ferrieri *et*  
412 *al.*, 2005). VOC emission profile emitted immediately after damage can substantially differ from  
413 longer-term emissions (Maja *et al.*, 2014). Nevertheless, most herbivore-induced VOCs are studied  
414 immediately after the damage occurs.

415 Oak could be actively increasing its isoprene emission over a longer period after the  
416 damage. Physical injury to the leaf venation network could lead to increased water loss lasting for  
417 several days (Aldea *et al.*, 2005). Drought, and a release from it, have been shown to increase

418 isoprene emissions (Sharkey & Loreto, 1993; Tattini *et al.*, 2015). If mechanical damage caused  
419 water stress at the time of the injury, this might have led to an increased isoprene emission later,  
420 once the damage had been repaired. Long-term monitoring of damaged-induced isoprene emission  
421 is needed to fully understand its response to herbivory.

422

423 **Canopy scale effect of insect herbivory.** At our study site, the *direct effect* of insect herbivory was  
424 small: insect herbivores removed 6.0% ( $\pm 3.8\%$ ) of the oak leaf area, consistent with global  
425 estimates of average herbivory rates (Cyr & Pace, 1993). The *indirect effect* of herbivory on the  
426 remaining leaf tissue of the damaged leaf, and on the neighbouring intact leaves, on the other hand,  
427 was several magnitudes larger, reducing the light-saturated photosynthesis by 46% ( $\pm 10\%$ ) and  
428 37% ( $\pm 12\%$ ) on average, respectively. This supports the previous results on the importance of  
429 indirect effects over direct ones (Zangerl *et al.*, 2002; Barron-Gafford *et al.*, 2012). Nevertheless, in  
430 many ecosystem-scale studies the effects of herbivory are quantified only as the amount of leaf area  
431 loss (Metcalf *et al.*, 2014).

432 By combining indirect effects with the leaf area loss ( $8.5\% \pm 0.4\%$  per leaf), we estimate  
433 that every damaged leaf has its photosynthetic rate reduced by 50% ( $\pm 10\%$ ). Surveying the natural  
434 level of herbivory in the area, only 1.7% of shoots per tree were completely intact. Therefore, most  
435 of the oak canopy (98.3%) is photosynthesising below its potential. Effectively no tree in natural  
436 settings is devoid of this herbivory-influenced suppression of photosynthesis. On a scale of the  
437 canopy, then, only 52% ( $\pm 10\%$ ) of the photosynthesis is realised. Previous studies have not  
438 considered the combined direct and indirect effects on ecosystem-level carbon cycle. We show that  
439 herbivores can reduce the canopy-scale carbon sequestration considerably, and the shoot-level  
440 effect observed in the intact neighbour leaves is a major contributor to this reduction.

441 Similarly, herbivory had a large effect on isoprene emission, causing an 85% ( $\pm 116\%$ )  
442 increase in the leaf-scale isoprene emission rate and an 53% ( $\pm 83\%$ ) increase on the canopy-scale.

443 The large error margin makes it difficult to draw firm conclusions on the role of herbivory on  
444 canopy-level isoprene emissions. However, if our estimates are correct, this increase would be  
445 enough to counteract the predicted reduction in isoprene emissions due to climate change,  
446 increasing atmospheric CO<sub>2</sub> concentrations and land-use changes combined (Squire *et al.*, 2014).  
447 Despite their potential importance, biotic interactions are usually lacking from the global isoprene  
448 emission models (Müller *et al.*, 2008; Arneth *et al.*, 2008; Squire *et al.*, 2014). Previous studies  
449 have recorded higher forest-scale isoprene emissions than expected by models (Geron *et al.*, 1997;  
450 Gu *et al.*, 2017), and changes in species composition have been shown to affect forest-scale  
451 isoprene emissions (Wang *et al.*, 2017). Our study suggests that enhanced emissions resulting from  
452 leaf damage might be leading to underestimates of the actual forest-scale isoprene emissions, which  
453 could have significant knock-on effects on calculations of ozone and particle formation.

454         Because emission of isoprene is temperature-sensitive, measurements of temperature change  
455 through the different canopy layers would be needed for a more realistic estimate on canopy-level  
456 isoprene emissions. Also, further studies on differences between sun and shade leaves and  
457 herbivory rates across the canopy, and direct canopy measurements are needed to improve the  
458 estimates on canopy photosynthesis and isoprene emissions under herbivory.

459         With the predicted climate change, species distributions, abundances and hence the  
460 frequencies of specific species interactions are projected to shift, and in many cases, have already  
461 shifted (Jepsen *et al.*, 2008; Kurz *et al.*, 2008). Nevertheless, insect herbivory is rarely addressed in  
462 biosphere and climate models (Kurz *et al.*, 2008). Our results clearly demonstrate that for predicting  
463 the responses of forest ecosystems to climate change, including the effects of herbivory on the  
464 carbon cycle and atmospheric chemistry is crucial. Ignoring the role of insect herbivory might thus  
465 overestimate the role of forests as carbon sinks (Kurz *et al.*, 2008; Schäfer *et al.*, 2010), or  
466 underestimate their role as isoprene emitters. We have demonstrated the importance of indirect

467 herbivory effects for a single plant-herbivore system; there is a clear need to replicate such studies  
468 in other systems.

469

470 **Conclusions.** Moth caterpillars reduce the per unit leaf area photosynthetic rate of their host plant,  
471 both in the remaining leaf tissue of the damaged leaf, and in the intact neighbour leaves. The  
472 reduction by natural herbivory is greater than that by mechanical damage alone. This indicates the  
473 host plant can differentiate between these two types of damage, pass on the signal to undamaged  
474 parts, and respond accordingly. Isoprene emission rate is increased by mechanical leaf damage, and  
475 does not seem to be an herbivory-specific reaction. These responses expressed on a scale of  
476 individual leaves and shoots have large-scale consequences on the carbon dynamics on the scale of  
477 the forest. On a scale of a canopy, the indirect effects of herbivory emerge several times more  
478 important than the direct effect of leaf area removed. Including these effects in estimates of the  
479 interactions between biosphere and the atmosphere is crucial for better prediction of the effects of  
480 changing climate on forest ecosystems.

481

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493 and analysing. CB, IO and SR contributed to the data analyses. CB and NH designed the isoprene  
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495

## 496 **References**

497 **Agrawal AA. 2000.** Specificity of induced resistance in wild radish: causes and consequences for two  
498 specialist and two generalist caterpillars. *Oikos* **89**: 493–500.

499 **Alborn HT. 1997.** An elicitor of plant volatiles from beet armyworm oral secretion. *Science* **276**: 945–949.

500 **Aldea M, Hamilton JG, Resti JP, Zangerl AR, Berenbaum MR, DeLucia EH. 2005.** Indirect effects of insect  
501 herbivory on leaf gas exchange in soybean. *Plant, Cell and Environment* **28**: 402–411.

502 **Arimura G, Ozawa R, Shimoda T, Nishioka T, Boland W, Takabayashi J. 2000.** Herbivory-induced volatiles  
503 elicit defence genes in lima bean leaves. *Nature* **406**: 512–515.

504 **Arneth A, Monson RK, Schurgers G, Niinemets Ü, Palmer PI. 2008.** Why are estimates of global terrestrial  
505 isoprene emissions so similar (and why is this not so for monoterpenes)? *Atmospheric Chemistry and*  
506 *Physics* **8**: 4605–4620.

507 **Baldwin IT, Callahan P. 1993.** Autotoxicity and chemical defense: nicotine accumulation and carbon gain in  
508 solanaceous plants. *Oecologia* **94**: 534–541.

509 **Baldwin IT, Zhang Z-P. 1997.** Transport of [2- <sup>14</sup>C] jasmonic acid from leaves to roots mimics wound-  
510 induced changes in endogenous jasmonic acid pools in *Nicotiana sylvestris*. *Planta* **203**: 436–441.

511 **Barron-Gafford GA, Rascher U, Bronstein JL, Davidowitz G, Chaszar B, Huxman TE. 2012.** Herbivory of wild  
512 *Manduca sexta* causes fast down-regulation of photosynthetic efficiency in *Datura wrightii*: an early  
513 signaling cascade visualized by chlorophyll fluorescence. *Photosynthesis Research* **113**: 249–260.

514 **Bates D, Mächler M, Bolker B, Walker S. 2015.** Fitting Linear Mixed-Effects Models Using **lme4**. *Journal of*  
515 *Statistical Software* **67**.

516 **Belovsky GE, Slade JB. 2000.** Insect herbivory accelerates nutrient cycling and increases plant production.  
517 *Proceedings of the National Academy of Sciences* **97**: 14412–14417.

518 **Bilgin DD, Zavala JA, Zhu J, Clough SJ, Ort DR, DeLucia EH. 2010.** Biotic stress globally downregulates  
519 photosynthesis genes: Biotic stress downregulates photosynthesis. *Plant, Cell & Environment* **33**: 1597–  
520 1613.

521 **Charmantier A, McCleery RH, Cole LR, Perrins C, Kruuk LEB, Sheldon BC. 2008.** Adaptive phenotypic  
522 plasticity in response to climate change in a wild bird population. *Science* **320**: 800–803.

523 **Clark DB, Mercado LM, Sitch S, Jones CD, Gedney N, Best MJ, Pryor M, Rooney GG, Essery RLH, Blyth E, et**  
524 **al. 2011.** The Joint UK Land Environment Simulator (JULES), model description – Part 2: Carbon fluxes and  
525 vegetation dynamics. *Geoscientific Model Development* **4**: 701–722.

526 **Conrath U, Pieterse CMJ, Mauch-Mani B. 2002.** Priming in plant–pathogen interactions. *Trends in Plant*  
527 *Science* **7**: 210–216.

528 **Copolovici L, Pag A, Kännaste A, Bodescu A, Tomescu D, Copolovici D, Soran M-L, Niinemets Ü. 2017.**  
529 Disproportionate photosynthetic decline and inverse relationship between constitutive and induced volatile  
530 emissions upon feeding of *Quercus robur* leaves by large larvae of gypsy moth (*Lymantria dispar* ).  
531 *Environmental and Experimental Botany* **138**: 184–192.

532 **Crawley MJ. 2007.** *The R book*. Chichester, England; Hoboken, N.J.: Wiley.

533 **Cyr H, Pace ML. 1993.** Magnitude and patterns of herbivory in aquatic and terrestrial ecosystems. *Nature*  
534 **361:** 148–150.

535 **Dani KGS, Jamie IM, Prentice IC, Atwell BJ. 2014.** Evolution of isoprene emission capacity in plants. *Trends*  
536 *in Plant Science* **19:** 439–446.

537 **Dicke M, Baldwin IT. 2010.** The evolutionary context for herbivore-induced plant volatiles: beyond the ‘cry  
538 for help’. *Trends in Plant Science* **15:** 167–175.

539 **Erb M, Meldau S, Howe GA. 2012.** Role of phytohormones in insect-specific plant reactions. *Trends in Plant*  
540 *Science* **17:** 250–259.

541 **Farquhar GD, von Caemmerer S, Berry JA. 1980.** A biochemical model of photosynthetic CO<sub>2</sub> assimilation  
542 in leaves of C<sub>3</sub> species. *Planta* **149:** 78–90.

543 **Feeny P. 1970.** Seasonal changes in oak leaf tannins and nutrients as a cause of spring feeding by Winter  
544 moth caterpillars. *Ecology* **51:** 565–581.

545 **Fehsenfeld F, Calvert J, Fall R, Goldan P, Guenther AB, Hewitt CN, Lamb B, Liu S, Trainer M, Westberg H,**  
546 **et al. 1992.** Emissions of volatile organic compounds from vegetation and the implications for atmospheric  
547 chemistry. *Global Biogeochemical Cycles* **6:** 389–430.

548 **Fenn K, Malhi Y, Morecroft M, Lloyd C, Thomas M. 2015.** The carbon cycle of a maritime ancient  
549 temperate broadleaved woodland at seasonal and annual scales. *Ecosystems* **18:** 1–15.

550 **Ferrieri RA, Gray DW, Babst BA, Schueller MJ, Schlyer DJ, Thorpe MR, Orians CM, Lerdau M. 2005.** Use of  
551 carbon-11 in *Populus* shows that exogenous jasmonic acid increases biosynthesis of isoprene from recently  
552 fixed carbon. *Plant, Cell and Environment* **28:** 591–602.

553 **Frost CJ, Mescher MC, Carlson JE, De Moraes CM. 2008.** Plant Defense Priming against Herbivores: Getting  
554 Ready for a Different Battle. *Plant Physiology* **146:** 818–824.

555 **Fuentes JD, Gu L, Lerdau M, Atkinson R, Baldocchi D, Bottenheim JW, Ciccioli P, Lamb B, Geron C,**  
556 **Guenther A, et al. 2000.** Biogenic hydrocarbons in the atmospheric boundary layer: a review. *Bulletin of the*  
557 *American Meteorological Society* **81**: 1537–1575.

558 **Gago J, Daloso D de M, Figueroa CM, Flexas J, Fernie AR, Nikoloski Z. 2016.** Relationships of leaf net  
559 photosynthesis, stomatal conductance, and mesophyll conductance to primary metabolism: a multispecies  
560 meta-analysis approach. *Plant Physiology* **171**: 265–279.

561 **Geron CD, Nie D, Arnsts RR, Sharkey TD, Singaas EL, Vanderveer PJ, Guenther A, Sickles JE, Kleindienst TE.**  
562 **1997.** Biogenic isoprene emission: Model evaluation in a southeastern United States bottomland deciduous  
563 forest. *Journal of Geophysical Research: Atmospheres* **102**: 18889–18901.

564 **Gibberd R, Edwards PJ, Wratten SD. 1988.** Wound-induced changes in the acceptability of tree-foliage to  
565 Lepidoptera: within-leaf effects. *Oikos* **51**: 43.

566 **Gu D, Guenther AB, Shilling JE, Yu H, Huang M, Zhao C, Yang Q, Martin ST, Artaxo P, Kim S, et al. 2017.**  
567 Airborne observations reveal elevational gradient in tropical forest isoprene emissions. *Nature*  
568 *Communications* **8**: 15541.

569 **Guenther A, Hewitt CN, Erickson D, Fall R, Geron C, Graedel T, Harley P, Klinger L, Lerdau M, Mckay WA,**  
570 **et al. 1995.** A global model of natural volatile organic compound emissions. *Journal of Geophysical*  
571 *Research* **100**: 8873.

572 **Guenther AB, Zimmerman PR, Harley PC, Monson RK, Fall R. 1993.** Isoprene and monoterpene emission  
573 rate variability: Model evaluations and sensitivity analyses. *Journal of Geophysical Research* **98**: 12609.

574 **Harley PC, Thomas RB, Reynolds JF, Strain BR. 1992.** Modelling photosynthesis of cotton grown in elevated  
575 CO<sub>2</sub>. *Plant, Cell and Environment* **15**: 271–282.

576 **Herms DA, Mattson WJ. 1992.** The dilemma of plants: to grow or defend. *The Quarterly Review of Biology*  
577 **67:** 283–335.

578 **Hothorn T, Bretz F, Westfall P. 2008.** Simultaneous inference in general parametric models. *Biometrical*  
579 *Journal* **50:** 346–363.

580 **van Hulten M, Pelsler M, van Loon LC, Pieterse CMJ, Ton J. 2006.** Costs and benefits of priming for defense  
581 in Arabidopsis. *Proceedings of the National Academy of Sciences* **103:** 5602–5607.

582 **Hunter MD. 1992.** Interactions within herbivore communities mediated by the host plant: the keystone  
583 herbivore concept. In: Effects of resource distribution on animal–plant interactions. Elsevier, 287–325.

584 **Jepsen JU, Hagen SB, Ims RA, Yoccoz NG. 2008.** Climate change and outbreaks of the geometrids  
585 *Operophtera brumata* and *Epirrita autumnata* in subarctic birch forest: evidence of a recent outbreak range  
586 expansion. *Journal of Animal Ecology* **77:** 257–264.

587 **Jones CG, Hopper RF, Coleman JS, Krischik VA. 1993.** Control of systemically induced herbivore resistance  
588 by plant vascular architecture. *Oecologia* **93:** 452–456.

589 **Kaitaniemi P, Ruohomäki K, Ossipov V, Haukioja E, Pihlaja K. 1998.** Delayed induced changes in the  
590 biochemical composition of host plant leaves during an insect outbreak. *Oecologia* **116:** 182–190.

591 **Karl T, Guenther A, Turnipseed A, Patton EG, Jardine K. 2008.** Chemical sensing of plant stress at the  
592 ecosystem scale. *Biogeosciences* **5:** 1287–1294.

593 **Kerchev PI, Fenton B, Foyer CH, Hancock RD. 2012.** Plant responses to insect herbivory: interactions  
594 between photosynthesis, reactive oxygen species and hormonal signalling pathways: Redox-mediated  
595 crosstalk in insect herbivory. *Plant, Cell & Environment* **35:** 441–453.

596 **Kessler A, Halitschke R. 2007.** Specificity and complexity: the impact of herbivore-induced plant responses  
597 on arthropod community structure. *Current Opinion in Plant Biology* **10:** 409–414.

598 **Korth KL, Dixon RA. 1997.** Evidence for chewing insect-specific molecular events distinct from a general  
599 wound response in leaves. *Plant Physiology* **115**: 1299–1305.

600 **Kravitz B, Guenther AB, Gu L, Karl T, Kaser L, Pallardy SG, Peñuelas J, Potosnak MJ, Seco R. 2016.** A new  
601 paradigm of quantifying ecosystem stress through chemical signatures. *Ecosphere* **7**: e01559.

602 **Kurz WA, Dymond CC, Stinson G, Rampley GJ, Neilson ET, Carroll AL, Ebata T, Safranyik L. 2008.** Mountain  
603 pine beetle and forest carbon feedback to climate change. *Nature* **452**: 987–990.

604 **Lehning A, Zimmer I, Steinbrecher R, Brüggemann N, Schnitzler J-P. 1999.** Isoprene synthase activity and  
605 its relation to isoprene emission in *Quercus robur* L. leaves. *Plant, Cell & Environment* **22**: 495–504.

606 **Leitner M, Boland W, Mithöfer A. 2005.** Direct and indirect defences induced by piercing-sucking and  
607 chewing herbivores in *Medicago truncatula*. *New Phytologist* **167**: 597–606.

608 **Lenth RV. 2016.** Least-Squares Means: The R Package lsmeans. *Journal of Statistical Software* **69**: 1–33.

609 **Loreto F, Sharkey TD. 1993.** Isoprene emission by plants is affected by transmissible wound signals. *Plant,*  
610 *Cell and Environment* **16**: 563–570.

611 **Maja MM, Kasurinen A, Yli-Pirila P, Joutsensaari J, Klemola T, Holopainen T, Holopainen JK. 2014.**  
612 Contrasting responses of silver birch VOC emissions to short- and long-term herbivory. *Tree Physiology* **34**:  
613 241–252.

614 **Marino G, Aqil M, Shipley B. 2010.** The leaf economics spectrum and the prediction of photosynthetic light-  
615 response curves: Leaf economics spectrum and light-response. *Functional Ecology* **24**: 263–272.

616 **Mentel TF, Kleist E, Andres S, Dal Maso M, Hohaus T, Kiendler-Scharr A, Rudich Y, Springer M, Tillmann R,**  
617 **Uerlings R, et al. 2013.** Secondary aerosol formation from stress-induced biogenic emissions and possible  
618 climate feedbacks. *Atmospheric Chemistry and Physics* **13**: 8755–8770.

619 **Mercado LM, Huntingford C, Gash JHC, Cox PM, Jogireddy V. 2007.** Improving the representation of  
620 radiation interception and photosynthesis for climate model applications. *Tellus B: Chemical and Physical*  
621 *Meteorology* **59**: 553–565.

622 **Metcalfe DB, Asner GP, Martin RE, Silva Espejo JE, Huasco WH, Farfán Amézquita FF, Carranza-Jimenez L,**  
623 **Galiano Cabrera DF, Baca LD, Sinca F, et al. 2014.** Herbivory makes major contributions to ecosystem  
624 carbon and nutrient cycling in tropical forests. *Ecology Letters* **17**: 324–332.

625 **Meyer GA. 1998.** Pattern of defoliation and its effect on photosynthesis and growth of Goldenrod.  
626 *Functional Ecology* **12**: 270–279.

627 **Meza-Canales ID, Meldau S, Zavala JA, Baldwin IT. 2017.** Herbivore perception decreases photosynthetic  
628 carbon assimilation and reduces stomatal conductance by engaging 12-oxo-phytodienoic acid, mitogen-  
629 activated protein kinase 4 and cytokinin perception: Photosynthesis regulation by herbivore perception.  
630 *Plant, Cell & Environment* **40**: 1039–1056.

631 **Monsi M, Saeki T. 1953.** Ueber den Lichtfaktor in den Pflanzengesellschaften und seine Bedeutung fuer die  
632 Stoffproduktion. *Journal of Japanese Botany*: 22–52.

633 **Morecroft MD, Stokes VJ, Morison JIL. 2003.** Seasonal changes in the photosynthetic capacity of canopy  
634 oak (*Quercus robur*) leaves: the impact of slow development on annual carbon uptake. *International*  
635 *Journal of Biometeorology* **47**: 221–226.

636 **Müller J-F, Stavrakou T, Wallens S, De Smedt I, Van Roozendaal M, Potosnak MJ, Rinne J, Munger B,**  
637 **Goldstein A, Guenther AB. 2008.** Global isoprene emissions estimated using MEGAN, ECMWF analyses and  
638 a detailed canopy environment model. *Atmospheric Chemistry and Physics* **8**: 1329–1341.

639 **Nabity PD, Heng-Moss TM, Higley LG. 2006.** Effects of insect herbivory on physiological and biochemical  
640 (oxidative enzyme) responses of the halophyte *Atriplex subspicata* (Chenopodiaceae). *Environmental*  
641 *Entomology* **35**: 1677–1689.

642 **Nabity PD, Zavala JA, DeLucia EH. 2009.** Indirect suppression of photosynthesis on individual leaves by  
643 arthropod herbivory. *Annals of Botany* **103**: 655–663.

644 **Nabity PD, Zavala JA, DeLucia EH. 2013.** Herbivore induction of jasmonic acid and chemical defences  
645 reduce photosynthesis in *Nicotiana attenuata*. *Journal of Experimental Botany* **64**: 685–694.

646 **Nykänen H, Koricheva J. 2004.** Damage-induced changes in woody plants and their effects on insect  
647 herbivore performance: a meta-analysis. *Oikos* **104**: 247–268.

648 **Oleksyn J, Karolewski P, Giertych MJ, Zytkowskiak R, Reich PB, Tjoelker MG. 1998.** Primary and secondary  
649 host plants differ in leaf-level photosynthetic response to herbivory: evidence from *Alnus* and *Betula* grazed  
650 by the alder beetle, *Agelastica alni*. *New Phytologist* **140**: 239–249.

651 **Orians C. 2005.** Herbivores, Vascular Pathways, and Systemic Induction: Facts and Artifacts. *Journal of*  
652 *Chemical Ecology* **31**: 2231–2242.

653 **Peterson RKD, Shannon CL, Lenssen AW. 2004.** Photosynthetic responses of legume species to leaf-mass  
654 consumption injury. *Environmental Entomology* **33**: 450–456.

655 **Pinheiro J, Bates D, DebRoy S, Sarkar D, R Core Team. 2017.** *nlme: Linear and Nonlinear Mixed Effects*  
656 *Models*.

657 **Portillo-Estrada M, Kazantsev T, Talts E, Tosens T, Niinemets Ü. 2015.** Emission timetable and quantitative  
658 patterns of wound-induced volatiles across different leaf damage treatments in aspen (*Populus tremula*).  
659 *Journal of Chemical Ecology* **41**: 1105–1117.

660 **R Core Team. 2017.** *R: A language and environment for statistical computing. R Foundation for Statistical*  
661 *Computing, Vienna, Austria*.

662 **Rasulov B, Huve K, Bichele I, Laisk A, Niinemets Ü. 2010.** Temperature response of isoprene emission in  
663 vivo reflects a combined effect of substrate limitations and isoprene synthase activity: a kinetic analysis.  
664 *Plant Physiology* **154**: 1558–1570.

665 **Rasulov B, Huve K, Valbe M, Laisk A, Niinemets Ü. 2009.** Evidence that light, carbon dioxide, and oxygen  
666 dependencies of leaf isoprene emission are driven by energy status in hybrid aspen. *Plant Physiology* **151**:  
667 448–460.

668 **Rowen E, Kaplan I. 2016.** Eco-evolutionary factors drive induced plant volatiles: a meta-analysis. *New*  
669 *Phytologist* **210**: 284–294.

670 **Sack L, Holbrook NM. 2006.** Leaf hydraulics. *Annual Review of Plant Biology* **57**: 361–381.

671 **Schäfer KVR, Clark KL, Skowronski N, Hamerlynck EP. 2010.** Impact of insect defoliation on forest carbon  
672 balance as assessed with a canopy assimilation model. *Global Change Biology* **16**: 546–560.

673 **Schoonhoven LM, Loon JJA van, Dicke M. 2005.** *Insect-plant biology*. Oxford ; New York: Oxford University  
674 Press.

675 **Sharkey TD, Bernacchi CJ, Farquhar GD, Singaas EL. 2007.** Fitting photosynthetic carbon dioxide response  
676 curves for C<sub>3</sub> leaves. *Plant, Cell & Environment* **30**: 1035–1040.

677 **Sharkey TD, Loreto F. 1993.** Water stress, temperature, and light effects on the capacity for isoprene  
678 emission and photosynthesis of kudzu leaves. *Oecologia* **95**: 328–333.

679 **Sharkey TD, Singaas EL. 1995.** Why plants emit isoprene. *Nature* **374**: 769–769.

680 **Squire OJ, Archibald AT, Abraham NL, Beerling DJ, Hewitt CN, Lathière J, Pike RC, Telford PJ, Pyle JA.**  
681 **2014.** Influence of future climate and cropland expansion on isoprene emissions and tropospheric ozone.  
682 *Atmospheric Chemistry and Physics* **14**: 1011–1024.

683 **Staudt M, Lhoutellier L. 2007.** Volatile organic compound emission from holm oak infested by gypsy moth  
684 larvae: evidence for distinct responses in damaged and undamaged leaves. *Tree Physiology* **27**: 1433–1440.

685 **Stratmann J. 2003.** Long distance run in the wound response – jasmonic acid is pulling ahead. *Trends in*  
686 *Plant Science* **8**: 247–250.

687 **Strong DR, Lawton JH, Southwood R. 1984.** *Insects on Plants*. Southampton, United Kingdom: Blackwells  
688 Scientific Publications.

689 **Sukhov V. 2016.** Electrical signals as mechanism of photosynthesis regulation in plants. *Photosynthesis*  
690 *Research* **130**: 373–387.

691 **Taiz L, Zeiger E. 2010.** *Plant physiology*. MA, U.S.A: Sinauer Associates Inc.

692 **Tattini M, Loreto F, Fini A, Guidi L, Brunetti C, Velikova V, Gori A, Ferrini F. 2015.** Isoprenoids and  
693 phenylpropanoids are part of the antioxidant defense orchestrated daily by drought-stressed *Platanus* ×  
694 *acerifolia* plants during Mediterranean summers. *New Phytologist* **207**: 613–626.

695 **Wang K-Y, Shallcross D. 2000.** Modelling terrestrial biogenic isoprene fluxes and their potential impact on  
696 global chemical species using a coupled LSM–CTM model. *Atmospheric Environment* **34**: 2909–2925.

697 **Wang B, Shugart HH, Lerdau MT. 2017.** An individual-based model of forest volatile organic compound  
698 emissions—UVAFME-VOC v1.0. *Ecological Modelling* **350**: 69–78.

699 **Wasternack C, Hause B. 2013.** Jasmonates: biosynthesis, perception, signal transduction and action in plant  
700 stress response, growth and development. An update to the 2007 review in *Annals of Botany*. *Annals of*  
701 *Botany* **111**: 1021–1058.

702 **Wong SC, Cowan IR, Farquhar GD. 1979.** Stomatal conductance correlates with photosynthetic capacity.  
703 *Nature* **282**: 424–426.

704 **Wood S, Scheipl F. 2017.** *gamm4: Generalized Additive Mixed Models using ‘mgcv’ and ‘lme4’*.

705 **Wu J, Baldwin IT. 2009.** Herbivory-induced signalling in plants: perception and action. *Plant, Cell &*  
706 *Environment* **32**: 1161–1174.

707 **Zangerl AR, Hamilton JG, Miller TJ, Crofts AR, Oxborough K, Berenbaum MR, DeLucia EH. 2002.** Impact of  
708 folivory on photosynthesis is greater than the sum of its holes. *Proceedings of the National Academy of*  
709 *Sciences* **99**: 1088–1091.

710 **Zhou S, Lou Y-R, Tzin V, Jander G. 2015.** Alteration of plant primary metabolism in response to insect  
711 herbivory. *Plant Physiology* **169**: 1488–1498.

712 **Zuur AF (Ed.). 2009.** *Mixed effects models and extensions in ecology with R*. New York, NY: Springer.

713

714 Supporting Information:

715 Methods S1: Details on the experimental set up and on extracting the gas exchange parameters

716 Figure S1: Example of a mesh bag

717 Figure S2: Experimental leaves in herbivory addition and mechanical damage -treatments

718 Table S1: Leaf area loss at the study area and in the experiment

719 Figure S3: The average A/Ci response curves per leaf treatment

720 Figure S4: Correlation between the isoprene emission rate and photosynthetic parameters

721 Table S2: Coefficient estimates for mixed effects models

722 Table S3: Effects of herbivory on A<sub>1000</sub> on leaf and canopy scales

723 Methods S2: iDirac overview and operation

724

725

726

727 Figure 1. The average model predicted response curves. Panel a) shows photosynthetic response to  
728 light, b) the maximum carboxylation rate ( $V_{\text{cmax}}$ ), c) the maximum electron transport rate ( $J_{\text{max}}$ ) and  
729 d) the maximum triose phosphate use efficiency (TPU). The original measurements are shown as  
730 points, and average model fitted parameters per treatment are shown as lines. For panels b-d, the  
731 solid points represent measurements used to estimate the corresponding parameter (*i.e.* when  $[\text{CO}_2]$   
732  $< 25$  Pa for  $V_{\text{cmax}}$ ,  $[\text{CO}_2] > 45$  Pa for  $J_{\text{max}}$ , and assimilation at its maximum for TPU, see Supporting  
733 Information, Methods S1 for details), and the circles show the remaining measurements. The data  
734 represent measures from both field sites, and in panels b-d during both measuring years. Note that  
735 the effect of site and year has been taken into account in the statistical analyses.

736

737 Figure 2. The average parameter values per leaf treatment. Panel a) shows the average maximum  
738 model-fitted light-saturated photosynthetic rate ( $A_{\text{sat}}$ ), b) the average light intensity at which the  
739 model-fitted photosynthetic rate is half of its maximum ( $K$ ), c) the average dark respiration rate  
740 ( $R_d$ ), d) the temperature-corrected average maximum carboxylation rate ( $V_{\text{cmax}}$ ), e) the temperature-  
741 corrected average maximum electron transport rate ( $J_{\text{max}}$ ), f) the temperature-corrected average  
742 triose phosphate use efficiency (TPU), g) the average stomatal conductance ( $g_s$ ) and h) the average  
743 standard isoprene emission rate ( $I_s$ ).  $n=10$  per leaf treatment for the figures in the panels a-c, except  
744  $n=9$  for the mechanically damaged leaf and  $n=9$  for herbivore undamaged leaf for panel b. For  
745 figures in the panels d-f,  $n=15$  for control,  $n=13$  for the herbivory treatments and  $n=12$  for the  
746 mechanical treatments. For panel g,  $n=19$  for control,  $n=18$  for damaged leaf in herbivore treatment  
747 and intact leaf in mechanical treatment, and  $n=17$  for intact leaf in the herbivore treatment and  
748 damaged leaf in the mechanical treatment. For panel h,  $n=7$  for control and damaged leaf in the  
749 mechanical treatment,  $n=6$  for undamaged leaf in the mechanical treatment and intact leaf in the  
750 herbivory treatment, and  $n=4$  for the damaged leaf in the herbivory treatment. Error bars are  $\pm 1$   
751 SEM. Means not sharing a letter are statistically significantly different from one another, e.g. AB

752 and C in panel a (Tukey's test,  $p < 0.05$ ). Note that the y-axis for respiration (panel c) is expressed  
753 as positive values (instead of the negative assimilation rates) to make the graph more intuitive. The  
754 data represent measures from both field sites, and in panels d-g during both measuring years. Note  
755 that the effect of site and year has been taken into account in the statistical analyses.

756

757 Table 1. Total effect of the herbivory from the leaf to the canopy scale. The average percentage of  
758 leaf area loss per leaf ( $D_t$ , direct effect), the average proportion of different leaf types ( $t=1,2,3$ ) in  
759 the canopy, the effect of insect herbivory on the light-saturated photosynthetic rate ( $A_{sat}$ ) and on the  
760 isoprene emission rate per unit leaf area (indirect effect) of the different leaf types, the estimates of  
761 the combined (direct + indirect) effects of these at leaf and canopy scales, and the canopy-scale  
762 estimates when change in the light intensity through the canopy is taken into account. The effects  
763 are expressed relative to the control treatment values (intact leaves in intact shoots). Errors are  $\pm 1$   
764 SEM derived through error propagation. See Supporting Information, Table S3 for values for  
765 photosynthetic rate in  $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$  of photosynthetically active radiation ( $A_{1000}$ ).

766

	Intact leaf, intact shoot (t=1)	Intact leaf, damaged shoot (t=2)	Damaged leaf, damaged shoot (t=3)	Canopy scale total effect
<b>Direct effect</b>				
Leaf area loss (%) ( $D_l$ )	0	0	$-8.5 \pm 0.4$	
% of leaves in canopy ( $l_l$ )	1.7	$27.3 \pm 1.9$	$71.0 \pm 1.9$	
Canopy scale effect % ( $D_c$ )				$-6.0 \pm 3.8$
<b>Light saturated photosynthesis (<math>A_{sat}</math>)</b>				
Rate ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ of leaf area)	$19.8 \pm 2.2$	$12.5 \pm 1.9$	$10.8 \pm 1.6$	
Rate (% of intact)	100	$63.1 \pm 11.9$	$54.5 \pm 10.1$	
Indirect effect per unit leaf area %	0	$-36.9 \pm 11.9$	$-45.5 \pm 10.1$	
Leaf scale effect % (direct + indirect) <sup>Eq 1.</sup>	0	$-36.9 \pm 11.9$	$-50.1 \pm 9.5$	
Canopy scale effect % (direct + indirect) <sup>Eq 2.</sup>				$-45.6 \pm 7.60$
<b>Isoprene</b>				
Rate ( $\mu\text{g m}^{-2} \text{ h}^{-1}$ of leaf)	$871.7 \pm 257.6$	$612.1 \pm 213.5$	$1766.0 \pm 967.0$	
Rate (% of intact)	100	$70.2 \pm 32.1$	$202.6 \pm 126.0$	
Indirect effect per unit leaf area %	0	$-29.8 \pm 32.1$	$102.6 \pm 126.0$	
Leaf scale effect % (direct + indirect) <sup>Eq 1.</sup>	0	$-29.8 \pm 32.1$	$85.4 \pm 115.6$	
Canopy scale effect % (direct + indirect) <sup>Eq 2.</sup>				$52.5 \pm 82.6$
<b>Light diffused photosynthesis</b>				
Canopy net rate per leaf type ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ of ground area, $\text{NPC}_l$ ) <sup>Eq 3</sup>	$29.96 \pm 3.19$	$17.87 \pm 2.59$	$16.92 \pm 2.28$	
Canopy net rate combined, weighted with the leaf type proportions ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ of ground area)				$17.4 \pm 1.83$
Canopy net rate (% of intact)				$58.1 \pm 8.70$
Canopy scale effect % (direct + indirect) <sup>Eq 4.</sup>				$-47.9 \pm 9.50$