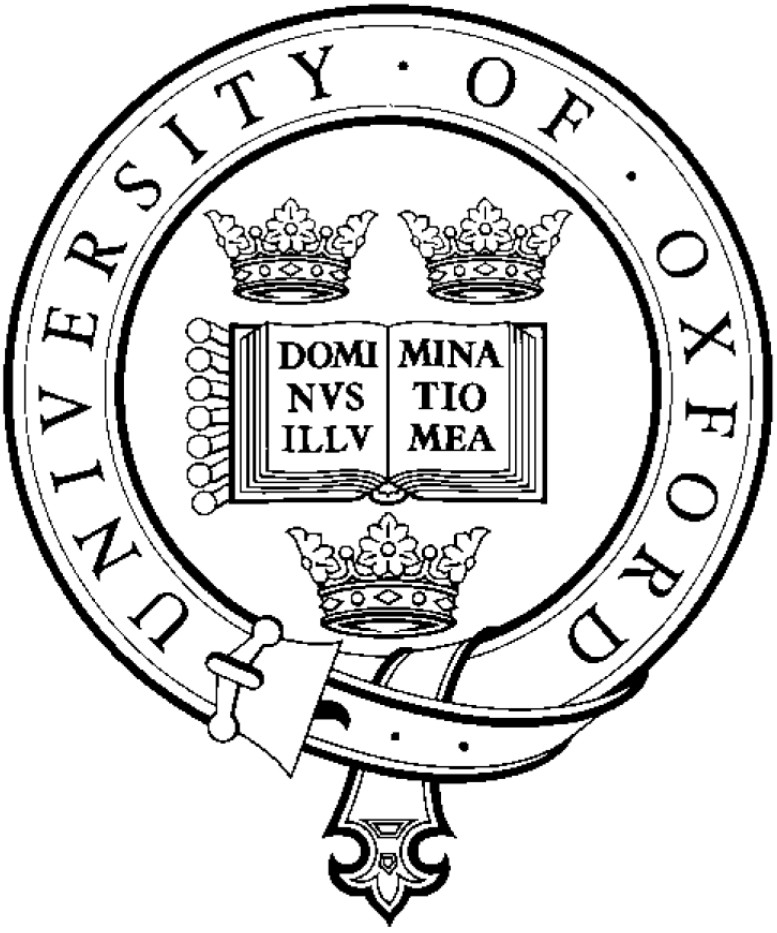
Community- and ecosystem-level effects of insect herbivory on oak (*Quercus robur*)

Kristiina Matilda Maria Visakorpi

Department of Zoology and School of Geography and the Environment

Kellogg College

University of Oxford



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Abstract

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The interactions between plants and their insect herbivores are among the most common ecological interactions. By feeding on plant tissue, insects can influence plant physiology and chemistry, with potential implications for other herbivores associated with the same host plant and for ecosystem-level processes mediated by the plant.

In this thesis, I studied the community and ecosystem-level effects of insect herbivores associated with the pedunculate oak (*Quercus robur*, L.) in temperate woodlands in Oxfordshire, UK. First, I studied how early-season moth caterpillars affect photosynthesis and selected physical and chemical leaf traits. I then examined how herbivore-induced changes in the host plant affect tree performance, the structure of the sessile herbivore community associated with the host tree, canopy-level carbon sequestration, and forest-level primary productivity.

My work shows that moth caterpillars can substantially reduce the photosynthetic rate of their host plant. These leaf-level changes can reduce canopy-level carbon sequestration, and therefore affect the primary productivity of the ecosystem. In this system, insect herbivory did not affect any of the studied leaf traits, tree performance, or the structure of the herbivore community. Leaf chemical composition varied substantially between individual trees, was affected by the growth location of the tree, and covaried significantly with the associated insect community.

My results suggest that insect herbivores can have important ecosystem-level effects by inducing small-scale changes in the primary metabolism of their host plant. My work also

emphasises the importance of abiotic conditions in influencing plant chemistry, and plant chemical composition in shaping the plant-associated insect community. I stress the need to quantify the effects of insect herbivores on carbon cycling in other systems and to include these effects in models on ecosystem productivity and biosphere-atmosphere interactions.

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"… on some level most came, in time, to recognize the futility of language. Not just in Area X but against the rightness of the lived-in moment, the instant of touch, of connection for which words were such a sorrowful disappointment, so inadequate an expression of both the finite and the infinite."

Jeff Vandermeer: Acceptance

"Most of the trees are just trees, of course; but many are half awake."

J. R. R. Tolkien: The Two Towers

Declaration of author contribution

Sofia Gripenberg and Terhi Riutta contributed to the experimental design, data analysis and commented on drafts for all chapters.

Yadvinder Malhi contributed to the data analysis in Chapters 2 and 5 and commented on drafts on Chapters 1, 2, 5 and 6.

Juha-Pekka Salminen carried out polyphenol extraction for Chapters 3 and 4.

Norma Salinas carried out quantification of leaf nitrogen and carbon content for Chapter 4.

Angélica E. Martínez-Bauer helped with data collection for Chapter 4.

Conor Bolas and Neil Harris designed the isoprene measuring equipment used in Chapter 2. Conor Bolas helped with taking the measurements and analysing the raw data. Both authors commented on Chapter 2.

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Chapter 1. General introduction

Plants play a central part in the ecology of communities and ecosystems. As primary producers they provide resources for other organisms and form the basal trophic level of most food webs. By photosynthesising, plants cycle carbon between the atmosphere and the biosphere and bind solar energy into a form that can be used by other organisms. The factors affecting plant abundance and physiology can therefore have consequences for the plant-associated food web and the wider ecosystem.

Plant-eating insects are one of the most species-rich groups on Earth (Roskov *et al.*, 2018): insects comprise ca. 62% of all 1.6 million described living species (Wiens *et al.*, 2015), and 50% of insects are herbivores (Frost, 1954). The interactions between plants and insect herbivores are thus among the most common ecological interactions (Strong *et al.*, 1984; Schoonhoven *et al.*, 2005).

By feeding on plants, insect herbivores can influence the physiology and chemistry of plant individuals, the abundance and distributions of plant populations, the species composition of plant communities, and the evolution of plant traits (Herms & Mattson, 1992; Maron & Crone, 2006; Uesugi & Kessler, 2013). These changes can further influence the community of species associated with the plant (e.g. herbivores, pollinators, pathogens) or ecosystem-level processes that are mediated by the plant (e.g. carbon or nutrient cycling). Nonetheless, in many cases the plant-herbivore interactions have been studied in isolation from this broader context: researchers have either assessed the effects of host plants on the ecology or evolution of insect herbivores, or the individual or population level effects that these herbivores have on their hosts (Maron & Crone, 2006), paying less attention to the consequences of herbivory on the wider community or ecosystem. Moreover, there has been

little overlap between studies focusing on different aspects of the plant-herbivore interaction. For example, the molecular-level changes in the host plant induced by herbivory are rarely linked to community or ecosystem-level processes. Similarly, when the consequences of herbivory are observed at the community- or ecosystem level, the underlying molecular or chemical changes in the host plant are not always investigated. In this thesis, I use methods from the fields of plant physiology, community ecology and ecosystem ecology to investigate herbivore-induced changes in host plant physiology, in host plant-associated herbivore community, and in the surrounding ecosystem-level processes.

1.1. Herbivores change the physiology of their host plants

Most plants are under threat of herbivore attack during most of their lives. Almost all plants are also able to defend themselves against the potential attackers (Murdoch, 1966; Pimm, 1991). The defences targeted against herbivores often include changes in plant chemistry or morphology and can be constantly present ("constitutive"), or specifically induced as a response to an attack (Karban & Baldwin, 1997; Gatehouse, 2002). They can occur locally in the damaged tissue (Wold & Marquis, 1997; Schmidt *et al.*, 2009), or be triggered systemically and therefore also expressed in the intact parts of the plant (Wu & Baldwin, 2009). For example, herbivores feeding on leaves can trigger defensive reactions in the roots (Baldwin & Zhang, 1997), or in intact neighbouring leaves or branches (Agrawal *et al.*, 2000; Staudt & Lhoutellier, 2007).

The metabolic pathways producing chemical compounds that deter herbivores are collectively called "secondary metabolism", while the pathways associated with vital processes such as photosynthesis and respiration constitute the "primary metabolism" of the plant. Plant secondary metabolism can produce hundreds of different compounds and compound groups (Jones *et al.*, 1991; Howe & Jander, 2008), for example cyanogenic glucosides, glucosinolates, alkaloids, phenolics and proteinase inhibitors (Bennett &

Wallsgrove, 1994). The secondary metabolites are often made from the available carbohydrates and photosynthetic end-products (Koricheva *et al.*, 1998; Schwachtje & Baldwin, 2008). They can act as feeding deterrents or cause negative fitness consequences in the herbivore. The negative effects of secondary metabolites on herbivores can result, for example, from their capacity to precipitate proteins or from their damaging effects on the herbivore gut (Salminen & Lempa, 2002), thereby making the plant less nutritious or more toxic as a food source.

The effects of secondary metabolites on herbivores and the effect of herbivore feeding on plant metabolism can be extremely specific and depend on the exact plant-herbivore interaction (Agrawal *et al.*, 2000; Kessler & Halitschke, 2007; Schaeffer *et al.*, 2018). For example, the very same compounds can act as repellents to generalist herbivores, but as attractants to specialist species that have evolved to tolerate or even benefit from the secondary chemicals of the host plant (Gatehouse, 2002; Roslin & Salminen, 2008). This diversity in the type of response to chemicals by plant enemies is thought to be the reason behind the huge chemical diversity found in plants: only a small part of compounds are active towards an herbivore at any given time (Jones *et al.*, 1991; Salazar *et al.*, 2018).

Part of the specificity of the interactions between insect herbivore and plants is caused by differences in herbivore feeding types (Erb *et al.*, 2012; Schaeffer *et al.*, 2018). For example, leaf-chewing herbivores often trigger the wounding-specific signalling network mediated by plant hormone jasmonic acid ("JA"), whereas sap-sucking by aphids triggers the salicylic acid ("SA") dependent defence pathway (Leitner *et al.*, 2005; Schweiger *et al.*, 2014). The SA-dependent pathway is also responsible for pathogen resistance (Delaney *et al.*, 1994). These differences can be extremely specific. For example, within herbivore species, individuals parasitized by one particular parasitoid species can trigger a different defensive reaction in the host plant than individuals parasitized by another parasitoid species (Poelman *et al.*, 2011). The host plant often recognizes the attacker through the oral secretions in the

herbivore saliva (Erb *et al.*, 2012). Upon recognition, the plant triggers the correct signalling cascade leading to local and/or systemic defence reaction. This can involve production of secondary metabolites, or volatile organic compounds ("VOCs"). VOCs can further transmit the signal to intact parts of the plant or to neighbouring plants (Arimura *et al.*, 2000), or attract predators of the herbivores (De Moraes *et al.*, 1998; Thaler, 1999).

In general, defenses are thought to be costly to produce, and should hence have an impact on plant growth rate and/or reproduction (Bazzaz *et al.*, 1987; Herms & Mattson, 1992; Agrawal *et al.*, 1999; Campos *et al.*, 2016). Indeed, there are accounts of plant growth and reproduction being reduced by herbivore-triggered induction of defenses (Agrawal *et al.*, 1999; Heil & Baldwin, 2002; Paul-Victor *et al.*, 2010). The negative effects of defense on growth and reproduction are traditionally thought to arise through trade-offs in allocating limited resources either to growing new tissue or manufacturing secondary metabolites (Herms & Mattson, 1992). The trade-off between growth and defense is particularly obvious in the context of plant breeding, where selecting plants for higher yield often results in lower pest tolerance (Chen *et al.*, 2015). At a molecular level, plant primary metabolism is often directly downregulated after herbivore attack. For example, photosynthesis-related genes are suppressed when defense related genes are upregulated (Bilgin *et al.*, 2010) and the defense-related signaling hormone JA can inhibit plant growth (Zhang & Turner, 2008; Attaran *et al.*, 2014). It is unclear whether these negative correlations between defense and primary metabolism are adaptive (coordinated reallocation of resources where they are most needed), or whether they arise from underlying genetic constrains due to e.g. pleiotropy (one gene affecting multiple traits) or genetic linkage (two genes being close to each other in a chromosome and inherited together) (Züst & Agrawal, 2017).

Nevertheless, the impacts of herbivory on the primary metabolism of the host plant are not always the same. Increased photosynthetic rate after herbivory ("compensatory photosynthesis") is well documented (Oleksyn *et al.*, 1998; Nykänen & Koricheva, 2004;

Halitschke *et al.*, 2011) as are compensatory increases in growth rate (Trumble *et al.*, 1993), decreases in antiherbivore defenses (Schimmel *et al.*, 2017) and sometimes even increased plant fitness (Belsky, 1986; McArt *et al.*, 2013). In a study by McArt et al. (2013), leaf herbivory increased the amount of secondary metabolites in seeds, reducing seed predation and resulting in overall increased plant fitness.

In many cases, the reported results on the effects of herbivory on plant metabolism seem to reflect the underlying scientific paradigm on plant-herbivore interactions. In a meta-analysis, Nykänen & Koricheva (2004) found that studies published around the time when the theory of induced defenses gained interest reported results of herbivory decreasing plant nutritional content and increasing the secondary metabolite concentration. Studies published later showed opposite effects, in accordance to later theories on induced susceptibility (a theory suggesting that herbivory can make the host plant more susceptible for other herbivores). The current view on the effects of herbivores on plants lies somewhere in between, focusing on understanding the conditions that affect the outcome of the interaction (Züst & Agrawal, 2017).

Despite the potential of insect herbivores to substantially affect the primary and secondary metabolism of their host plant as described above, the effects of insect herbivores on these processes have rarely been quantified in natural settings. For example, most experiments studying the effects of herbivory on photosynthesis have been conducted in laboratory conditions, using few model species or simulated damage (Kessler & Halitschke, 2007; Portillo-Estrada *et al.*, 2015). Similarly, most research on herbivore-induced VOC production has not quantified long-term changes in VOC emission profiles in the field (but see (Maja *et al.*, 2014).

1.2. Community level effects of herbivory

By changing the traits of its host plant (through the routes discussed in 1.1), one herbivore species can change the quality of the plant as a food source for other herbivores, and thus affect their abundance. These "trait-mediated indirect interactions" seem to be common between herbivores sharing the same host plant (Ohgushi, 2005; Kaplan & Denno, 2007; Eubanks & Finke, 2014), and they can be strong even if the herbivores are spatially or temporally separated from each other (Kaplan & Denno, 2007). Common examples include the effects of early-season herbivores on later arriving species (Agrawal, 1998; Tscharntke, 1999; Lill & Marquis, 2003; Van Zandt & Agrawal, 2004; Poelman *et al.*, 2008; Hernandez-Cumplido *et al.*, 2016) and root feeders affecting leaf-feeding herbivores (Arce *et al.*, 2017; Hoysted *et al.*, 2017). These interactions between herbivores sharing the same host plant can be competitive, e.g. when colonization by gall wasps decreases the growth rates of butterfly larvae (Prior & Hellmann, 2010), or mutualistic, e.g. when changes in leaf structure caused by herbivory by one caterpillar species increases abundances of other caterpillar species (Hunter, 1987).

Many of the plant-mediated indirect effects between herbivores are thought to result from the above-mentioned specificity in the defence-related signalling pathways and from the antagonistic nature between JA and SA-dependent signalling cascades (Kessler & Halitschke, 2007; Schweiger *et al.*, 2014). If a leaf-chewing herbivore has already triggered a JA-signalling pathway, the SA-dependent pathway might be suppressed, and thus the plant might be more susceptible for sap-sucking herbivores or plant pathogens (Felton & Korth, 2000; Schweiger *et al.*, 2014). Similarly, the opposite can happen: defensive reaction triggered by earlier herbivory can make the host plant more resistant to other plant enemies (Felton & Korth, 2000). These effects ("induced resistance" and "induced susceptibility") seem to be common, though not universal (Paul *et al.*, 2000; Felton & Korth, 2000).

Another possible pathway for indirect effects between herbivores are changes in the interactions with other trophic levels (e.g., Faeth, 1985). Changes in the densities of one herbivore species can cause subsequent changes in numbers of predators or parasites using that species as a food source or host. If these predators are generalist, they can reduce the population sizes of several herbivore species ("apparent competition) (Bonsall & Hassell, 1997; Morris *et al.*, 2004; Frost *et al.*, 2016). Generalist predators could also switch into feeding on the most abundant species, thereby releasing less common prey species from predation (a form of "apparent mutualism") (Bonsall & Hassell, 1997; Tack *et al.*, 2011). One pathway for changes in predation rate are the herbivore-induced VOCs, which can attract predators of the herbivores, and change the predation pressure experienced by the herbivore community associated with that host plant (Thaler, 1999; Kessler & Baldwin, 2001).

Species identified as "keystone herbivores" can have disproportionally large effects on their surrounding community through inducing changes in their host plant. Instead of affecting single species pair, keystone herbivores can trigger changes affecting several species or the whole community structure (Hunter, 1992; Van Zandt & Agrawal, 2004; Poelman *et al.*, 2010; Poelman & Kessler, 2016; Gonzalez-Megias & Gomez, 2003). For example, feeding by caterpillars of the butterfly *Pieris rapae* on Brassicae resulted in a different build-up of assemblages of other herbivore species compared to that on control plants (Poelman *et al.*, 2010). Similarly, feeding by weevil (*Rhyssomatus lineaticollis*) on milkweed (*Asclepias syriaca*) changed the subsequent colonization and insect community richness (Van Zandt & Agrawal, 2004). Many of these keystone species are also referred to as "ecosystem engineers", since their effect on other species is mediated by changes in the physical structure of the host plant (Tscharntke, 1999; Lill & Marquis, 2003).

To date, most studies investigating plant-mediated indirect effects between herbivores have been conducted on insect communities comprising a relatively small number of species (e.g. 7 species in Van Zandt & Agrawal, 2007, 4 species in Hernandez-Cumplido *et al*., 2016,

4 species in Poelman *et al.*, 2008; but see Tscharntke, 1999, Gonzales-Megia & Gomez, 2003), or in artificial laboratory conditions (e.g. Agrawal, 1998; Agrawal *et al.*, 1999). Nevertheless, most plants are naturally associated with much more diverse insect communities (Southwood, 1961; Tscharntke, 1999; Gonzalez-Megias & Gomez, 2003). Moreover, even though induced resistance has been suggested as a common pathway for keystone herbivores to affect the rest of the plant-associated community, the defence-associated plant traits that could drive any indirect effects observed are often not measured (e.g. Gonzales-Megia and Gomez 2003). It is therefore unclear whether community-wide cascading effects of individual herbivore species are common in diverse natural communities, and if they are, what mechanisms are driving any patterns observed.

1.3. Ecosystem level effects of herbivory

Photosynthesis and plant respiration are the largest fluxes in the global carbon cycle (Beer *et al.*, 2010). Thus, by affecting photosynthetic rate, herbivory has the potential to influence ecosystem-level carbon cycling. Herbivores influence photosynthesis by removing plant tissue ("direct effect" of herbivory) and by changing the photosynthetic rate in the remaining tissue ("indirect effect" of herbivory, Nabity *et al.*, 2009). Herbivores are estimated to remove on average 5% of plant biomass in temperate and tropical forests globally (Cebrian, 1999, 2004). Other estimates include for example 7% in tropical forests (Coley & Barone, 1996), 4.3-11 % in temperate forests (Edwards & Wratten, 1983) and 2% on the arctic tundra (Born & Böcher, 2001). Most studies on the effects of herbivores on ecosystem carbon cycling have concentrated on the direct effect of leaf area loss (e.g. Metcalfe *et al.*, 2014), while the indirect effects of herbivory, caused by changes in the metabolism of the host plant, have received much less attention.

Where they have been investigated, the indirect effects of herbivory have often been found to be much larger than the direct effects of leaf area loss, and the effects have been

shown to extend to larger areas outside the initial site of attack (Zangerl *et al.*, 2002; Nabity *et al.*, 2009; Meza-Canales *et al.*, 2017). By using chlorophyll imaging, Zangerl *et al.* (2002) showed that the area in which photosynthesis was altered following herbivory was six times of the area of the lost leaf tissue, and Meza-Canales *et al.* (2017) demonstrated that photosynthesis was also reduced also in undamaged, systemically affected leaves. Although these observations suggest that herbivory could drastically alter the photosynthetic rate of the whole plant, there has been no previous attempts to study the role of indirect effects on ecosystem carbon assimilation. In general, the role of biotic interactions on carbon cycling has only recently been recognized as an important research topic (Schmitz *et al.*, 2014; Monroe *et al.*, 2018).

By increasing emission of VOCs, insect herbivores can potentially change atmospheric chemistry (Karl *et al.*, 2008; Mentel *et al.*, 2013; Kravitz *et al.*, 2016). The VOCs emitted in response to biotic stresses such as herbivory (for example phenolics, green leaf volatiles and sesquiterpenins) can enhance the formation of atmospheric aerosols, which will impact climate by scattering or absorbing solar radiation or acting as condensation nuclei for ice and clouds (Mentel *et al.*, 2013). Furthermore, VOCs can react with other compounds in the atmosphere. For example, isoprene – which is a common and abundant hydrocarbon (Guenther *et al.*, 1995; Wang & Shallcross, 2000) emitted as a response to heat stress (Sharkey & Loreto, 1993; Sharkey & Singsaas, 1995; Singsaas & Sharkey, 1998) and mechanical leaf damage (Loreto & Sharkey, 1993; Portillo-Estrada *et al.*, 2017) – can react with hydroxyl radicals. Through these reactions, isoprene can be a significant source of low atmospheric ozone and other secondary pollutants (Fehsenfeld *et al.*, 1992; Fuentes *et al.*, 2000). Nevertheless, whether the herbivore-triggered changes in emissions of isoprene and other VOCs are strong enough to influence atmospheric chemistry at a significant level is currently not known.

By changing plant chemistry and physical traits, insect herbivores can also substantially affect ecosystem nutrient cycling (Hättenschwiler & Vitousek, 2000; Belovsky & Slade, 2000; Chapman *et al.*, 2003; Chomel *et al.*, 2016). For example, increased concentrations of secondary metabolites can deter soil fauna and reduce rates of litter decomposition (Hättenschwiler & Vitousek, 2000; Chomel *et al.*, 2016). On the other hand, herbivory can increase nitrogen and phosphorus content of the litter and accelerate decomposition (Chapman *et al.*, 2003). Furthermore, frass and nutrient leaching from the canopy can enhance soil nutrient content and increase growth rates on understorey vegetation or the host plant itself (Hunter, 2001; Yang & Gratton, 2014).

Most of the previous studies on the effects of insect herbivores on ecosystem-level processes have concentrated on outbreak densities of insects with cyclical population sizes (Kurz *et al.*, 2008; Clark *et al.*, 2010; Schäfer *et al.*, 2010; Lund *et al.*, 2017) have measured the effects of herbivory only through leaf area loss (Metcalfe *et al.*, 2014; Malhi *et al.*, 2014), or have concentrated on the effects of insects soil and decomposition dynamics though deposition of frass or leaf fragments (Belovsky & Slade, 2000; Chapman *et al.*, 2003; Yang & Gratton, 2014). How insect herbivores at average population densities might affect the ecosystem-level productivity though inducing changes in host plant metabolism is currently not known.

1.4. Study system and sites

The work presented in this thesis focuses on the pedunculate oak (*Quercus robur,* L.) and its associated insect herbivores. In the woodlands of UK, the pedunculate oak is common and long-lived tree species. Oaks are associated with one of the most diverse insect communities in temperate forests (over 300 species in UK, Southwood, 1961). They also experience intense herbivory early in the spring by lepidopterous caterpillars, notably winter moth (*Operophtera brumata* L.). These caterpillars emerge in synchrony with the budburst, and are

the first herbivores to attack the newly flushed leaves (Hunter, 1992). The intensity of caterpillar feeding varies between years, from defoliation of entire trees (Varley *et al.*, 1974) to more moderate levels of herbivory, depending, among other things, on the timing of the caterpillar emergence and budburst. Many of species colonizing the trees later in the season are leafminers and gall-formers that usually reach their peak abundance after the defoliating caterpillars have disappeared. These species are attacked by a diverse set of parasitoid wasps, whose abundance and species composition in turn depends on the composition of the mining and galling community.

As a likely evolutionary response to herbivory, oaks produce a diverse set of polyphenolic compounds (Feeny, 1970; Forkner *et al.*, 2004). Feeny (1970) suggested that the intense early-season herbivory by moth caterpillars is a strategy to avoid the higher concentration of proanthocyanidins (i.e. condensed tannins) in older oak leaves. Nevertheless, the view has been contested by observing that another group of phenolic compounds, hydrolysable tannins, are more abundant in oak leaves and more effective as defenses against insect herbivores than condensed tannins (Salminen *et al.*, 2004; Roslin & Salminen, 2008). Hydrolysable tannins show the opposite seasonal pattern, i.e. concentrations typically decrease during the season (Salminen *et al.*, 2004). Whether the temporal patterns in tannin concentrations and herbivory are driven by herbivores avoiding tannins or tannins being actively induced in response to herbivory has not been fully resolved.

The oak system in the UK is also well known for the long-term population dynamic studies of winter moth (Varley *et al.*, 1974) and for the cascading plant-mediated effects of early-season herbivores on late-season insect species (Hunter, 1992). The term “keystone herbivore” was first used in this system to describe the abundant early-season caterpillars affecting the survivorship of a late-season leafminer species and the abundance of late-season leaf-rolling caterpillars (West, 1985; Hunter, 1992). If the early-season caterpillars in this system influence the chemical composition of their host tree, they could act as true keystone

herbivores and have strong host-plant mediated effects not just on individual species, but to the larger community of late-season insects (Poelman & Kessler, 2016).

The experiments described in this thesis were carried out at three sites in Oxfordshire, UK (Figure 1.1): the forest of Wytham Woods, the John Krebs field station and Neptune Woods. Wytham Woods is an ancient semi-natural 390 ha woodland consisting of the most common tree species of temperate woodlands: oak (*Quercus robur*), sycamore (*Acer psuedoplatanus*), ash (*Fraxinus excelsior*) and beech (*Fagus sylvatica*) (Savill, 2011). The John Krebs field station is a research station belonging to the University of Oxford and located near the village of Wytham. At the time of the field work for this thesis, there were ca. 20 mature planted oak trees in the backyard of the station. The third field site, Neptune Woods, is a 4.5 ha oak plantation near the village of Long Wittenham and consists of ca 10000 young oak trees.

1.5. Aim and overview of research themes

The overall aim of the work presented in this thesis was to study 1) whether insect herbivory changes primary and secondary metabolism of its host plant and, if these changes occur, whether they 2) affect the host plant associated herbivore community and 3) ecosystem-level carbon cycling. To study these questions, I used early-season herbivorous caterpillars and oak trees and experimental manipulations of insect herbivory at the level of shoots and entire trees.

In Chapter 2, I investigate the direct and indirect effects of herbivory on oak photosynthesis and VOC emissions. I measure the patterns of leaf area loss in the oak canopy ("direct effects" of herbivory) and measure changes in photosynthetic rate and isoprene emission rate in the remaining leaf tissue and in the surrounding intact leaves ("indirect effects" of herbivory). To gain further insights on the possible mechanism driving any changes observed, I compare photosynthesis and VOC emissions of leaves damaged by

herbivores to leaves damaged mechanically. Finally, I estimate the implications of the effects of herbivory on the carbon assimilation of an oak canopy.

In Chapter 3, I measure whether early-season herbivory changes important chemical and structural leaf traits concurrently with the observed changes in photosynthesis. I measure leaf structure and size, leaf nutrient content, and the concentrations of 27 phenolic compounds. Using the same experimental set-up as in chapter 2, I investigate changes between intact leaves, leaves damaged by herbivores and leaves damaged mechanically. Lastly, I examine how photosynthetic rate correlates with the other leaf traits.

In Chapter 4, I test whether caterpillar herbivory early in the season affects tree performance and the structure of a late-season sessile insect community. I use a large-scale field experiment in which herbivore densities are manipulated on 72 young oak trees, and measure the effects of early-season herbivory on oak chemistry, growth and reproduction. I then investigate whether early-season herbivory causes cascading effects on the late-season leaf mining and galling community, and whether the structure of the late-season insect community on individual trees correlates with the chemical composition of the leaves.

In Chapter 5, I scale up the effects of herbivory on photosynthesis to investigate the effects of herbivory on ecosystem-level carbon cycling. I use the measures on photosynthetic rate reported in Chapter 2 to estimate oak canopy photosynthesis over the whole growing season with and without herbivores. I then use existing measurements and relationships to estimate oak primary productivity and the amount of carbon lost at the scale of a forest over an entire growing season due to the direct and indirect effects of herbivory.

In Chapter 6, which is a general discussion, I discuss the main findings of this thesis. I place my conclusions in the context of recent research on herbivore-plant interactions and on the effects of herbivores on ecosystem-level processes.

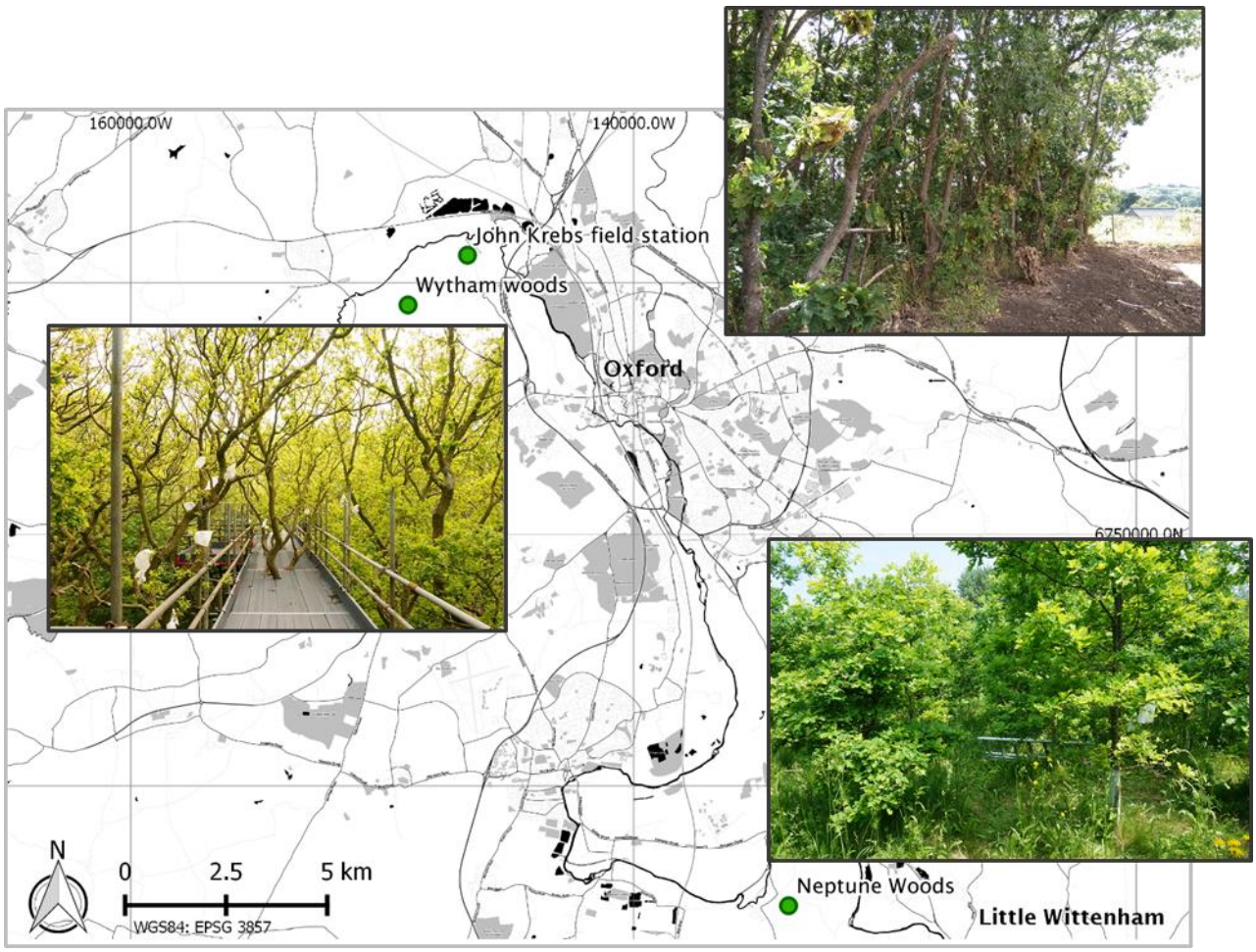
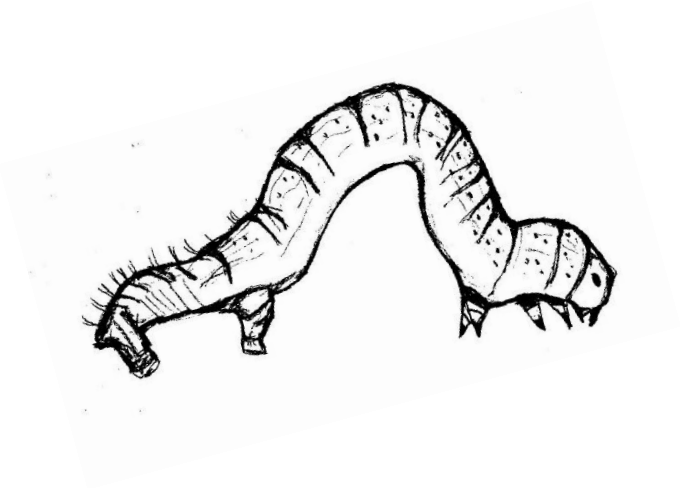


Figure 1.1. The three field sites (Wytham Woods, John Krebs field station and Neptune Woods) are all located in the vicinity of Oxford, UK. In Wytham Woods, the field work was conducted on an elevated canopy platform that allowed access to the uppermost branches.

Chapter 2: Small-scale indirect plant responses to insect herbivory could have major impacts on canopy photosynthesis and isoprene emission

Kristiina Visakorpi, Sofia Gripenberg, Yadvinder Malhi, Conor Bolas, Imma Oliveras, Neil Harris, Sami Rifai and Terhi Riutta

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Abstract

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 ± 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.

2.1 Introduction

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

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

Insect-induced changes in chemistry and metabolism can further alter the photosynthetic capacity of the remaining leaf tissue (*an indirect effect* of herbivory) (Zangerl *et al.*, 2002; Nykänen & Koricheva, 2004; Nabity *et al.*, 2009). Leaf damage often triggers upregulation of defence-related genes and down-regulation of genes related to photosynthesis (Bilgin *et al.*, 2010). Nevertheless, previous studies have found both increased ("compensatory photosynthesis") and decreased photosynthetic rate as a response to herbivory (Zangerl *et al.*, 2002; Nykänen & Koricheva, 2004; Nabity *et al.*, 2009). Similarly, VOC emission can either increase (as defensive reaction through plant-predator communication or plant-plant signalling) or decrease after leaf damage (Loreto & Sharkey, 1993; Dicke & Baldwin, 2010; Rowen & Kaplan, 2016). The exact plant response to herbivory depends on the characteristics of the specific species interaction, for example on the diet breath (e.g. specialist vs. generalist) or feeding guild (e.g. chewing vs sap-sucking) of the

herbivore (Nykänen & Koricheva, 2004; Kessler & Halitschke, 2007; Rowen & Kaplan, 2016).

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

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

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

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

2.2 Materials and methods

2.2.1 Experimental setup

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

Between 11th and 15th May 2015 and 9th and 11th May 2016, when most leaves were still newly flushed, we identified 15 shoots (of ~ 8 leaves) with only intact leaves from each study tree and enclosed each shoot in a small mesh fabric bag (Appendix 2.2, Figure S1). We randomly assigned each bag into one of the three treatments: 1) *herbivore addition*, 2) *mechanical damage*, or 3) *control*, so that each tree had five bags of each treatment. For each of the *herbivore addition* bags we added a locally collected winter moth caterpillar, and let it feed on the leaves for 3-5 days until at least two of the leaves showed signs of feeding damage. Because the effect of damage often depends on its type and amount (Wu & Baldwin, 2009; Portillo-Estrada *et al.*, 2015), each *herbivory addition* shoot was paired with a *mechanical damage* shoot immediately after the caterpillars had been removed from the mesh bags. The damage on the herbivory shoots was then replicated by tearing or punching holes with a cork borer in the leaves in the mechanical damage treatment (Appendix 2.2, Figure S2). *Control* shoots were left intact. The timing of the manipulations coincided with the peak herbivory in the area (Charmantier *et al.*, 2008). The mesh bags were left around the shoots to prevent additional herbivory until 25th June 2015 or 28th June 2016, when the amount of insect herbivory had levelled off.

One month after the application of the treatments, we randomly chose three shoots from each tree (one *herbivory addition* shoot, one *mechanical damage* shoot, and one *control* shoot) for gas exchange measurements. The few control shoots (n = 6) that showed signs of damage were excluded from further measurements. From each *herbivory addition* and *mechanical damage* shoot we measured two leaves: one damaged and one intact. From each *control* shoot we measured one intact leaf. This setup allowed us to measure five leaf-level treatments: damaged leaf in herbivory treatment, undamaged leaf in herbivory treatment, damaged leaf in mechanical treatment, undamaged leaf in mechanical treatment, and intact control leaf. We constructed photosynthetic light response curves (over the period of 28th July - 25th August 2015) for 49 leaves from ten trees and photosynthesis-CO_2 (A/C_i) -curves

(over the periods of 26th August - 10th September 2015 and 11th July - 11th August 2016) for 79 leaves from ten different trees (six of the trees were measured on both years) belonging to all the five leaf-level treatments. The timing of the gas exchange measurements corresponded to the peak photosynthetic activity of oak in the study area (Morecroft *et al.*, 2003).

On each leaf, we measured an intact part of an area of 2.5 cm^2 of the leaf with an infra-red gas analyser (CIRAS-2, PP-Systems, Hitchin, UK). For the light response curves, we took five point measurements on 15 different light levels between 2000 and 0 µmol $m^{-2}s^{-1}$ of photosynthetically active radiation (PAR). For the A/C_i curves, we measured the photosynthetic rate under ten different CO_2 concentrations between 1300 and 30 ppm. All the raw photosynthesis measurements were processed using the protocol provided by PP-Systems (ppsystems.com) for the CIRAS-2 to apply corrections for the measured variables. The resultant variable used in the analyses was photosynthetic rate per unit leaf area, expressed as µmol CO_2 m^{-2} s^{-1}. See Appendix 2.1 for details on the gas exchange measurements.

To study how herbivory and leaf damage affect the production of isoprene by the oak, we measured isoprene emission rate of 32 leaves from seven trees, using the same leaves (and thus the same five leaf-level treatments) as for the A/C_i curves with a portable gas chromatograph (iDirac, Appendix 2.3), 21st July - 9th August 2016. iDirac is a novel gas chromatograph, designed for *in-situ* use. Here we report its use for the first time in a field study. We attached the iDirac directly into the CIRAS-2 system to allow for simultaneous measurements of isoprene production and photosynthetic rate.

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

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

2.2.2 Extracting response parameters

To calculate the light-saturated photosynthesis, we fitted a Michaelis-Menten equation to the light response data for each leaf separately to estimate the parameters for the maximum light-saturated photosynthetic rate (A_{sat}) and the light intensity at which the gross photosynthetic rate is half of its maximum, K (Marino *et al.*, 2010). To obtain a measure of the mean dark respiration (R_d) for each leaf, we calculated the average photosynthetic rate on the light response curves when the light level was zero. To analyse the photosynthetic response to experimental treatments under different CO_2 concentrations, we constructed A/C_i response curves, where the photosynthetic rate (A) is modelled against the intercellular CO_2 mole fraction (C_i) (Farquhar *et al.*, 1980; Sharkey *et al.*, 2007), allowing us to estimate three important photosynthetic parameters: maximum carboxylation rate, describing the activity of Rubisco (V_{cmax}), rate of photosynthetic electron transport (J_{max}) and triose phosphate use efficiency (TPU).

After fitting, all the parameters were normalized to 25 ºC (Harley *et al.*, 1992; Sharkey *et al.*, 2007) to reduce variation caused by different ambient temperatures. For most leaves (n = 65) the Farquhar *et al.* (1980) model could be fitted to the data. For some leaves (n = 14) the model failed to estimate at least one of the parameters. These leaves were omitted from the further analyses of the treatment effects on A/C_i parameters. To study possible changes in leaf conductance, we extracted the mean stomatal conductance (g_s) recorded by the gas analyser during the A/C_i curve measurements. From those leaves of which only light response was measured (24 leaves), we used mean stomatal conductance of the light response curve. Single outlier values of stomatal conductance, K and isoprene emission were removed from further analyses. See Figure 2.2 for final sample sizes per parameter

To estimate isoprene emissions, the height of each isoprene peak in the gas chromatogram was measured and converted into mixing ratios (ppb) by using calibration measurements with known isoprene concentrations. The mixing ratios were scaled with the known air volume, area of leaf measured and flow rate to yield emission rates as nmol m^{-2} s^{-1}. Because isoprene emission is strongly influenced by temperature, we corrected the measured emission values for temperature (Guenther *et al.*, 1993, 1995), yielding the standard emission factor of isoprene (as µg m^{-2} h^{-1}), I_S (in 303 K and 1000 µmol m^{-2} s^{-1} of photosynthetically active radiation). See Appendix 2.1 for details on the model fitting and the temperature corrections.

To describe the photosynthetic rate of the study leaves in natural conditions, we extracted values from the light-response and A/C_i curves for photosynthetic rates at ambient CO_2 concentration (400 ppm) and in light intensity that corresponds to typical full light conditions (1000 µmol m^{-2} s^{-1} of photosynthetically active radiation). This parameter (A_{1000}), was used to assess the correlation between photosynthesis and isoprene emission rate, and to scale up the effects of herbivory from leaf scale to the canopy level.

2.2.3. Statistical analyses

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

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

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

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

normal distribution of the residuals. All analyses were conducted using R version 3.4.1 (R Core Team, 2018) and the packages lme4 (Bates *et al.*, 2015), multcomp (Hothorn *et al.*, 2008), nlme (Pinheiro *et al.*, 2017), gamm4 (Wood & Scheipl, 2017) and lsmeans (Lenth, 2016).

2.2.4. Quantifying the effects of herbivory on leaf and canopy scales

To estimate the effects of herbivory on photosynthesis and isoprene emission at the canopy scale, we combined three types of data: 1) the proportion of leaf area loss per leaf under natural conditions (direct effect), 2) the effect of insect herbivory on the photosynthetic rate (A_{sat}) or isoprene emission rate (I_S) per unit leaf area (indirect effect), and 3) information on natural patterns of herbivory in the oak canopy. Control leaves, which were intact leaves on intact shoots were set as a reference point to describe photosynthesis and isoprene emission in the absence of herbivory. To estimate the leaf-scale effect of herbivory on the light-saturated photosynthesis or isoprene emission rate, we first multiplied the per leaf unit area rate of a leaf damaged by herbivores with the proportion of remaining leaf area in the corresponding leaf type, yielding a "per leaf" - rate. We then compared this to a "per leaf" -rate of an intact control leaf:

$$light\ saturated\ leaf\ scale\ effect_t = \frac{A_t * (1 - D_t)}{A_{t=1}} - 1$$

(Eq. 1.)

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

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

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

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

(Eq. 2.)

where t denotes the three different leaf types and l is the proportion of leaf type t in the canopy. For photosynthesis, this model estimates the maximum potential photosynthesis in full light (as μmol m^{-2} s^{-1} of leaf_area), without considering light transmission through the canopy.

Secondly, because photosynthesis is strongly affected by the amount of available light, we estimated the effect of herbivory on canopy photosynthesis when the diffusion of light through the canopy is taken into account. To estimate this, we used the Big Leaf approach of The Joint UK Land Environment Simulator ("JULES", Clark *et al.*, 2011) to estimate canopy assimilation, combined with an estimate for canopy respiration (Mercado *et al.*, 2007). The reduction of direct light through the canopy was calculated by Beer's law (Monsi & Saeki, 1953). As a result, our model estimates instantaneous big-leaf approximated net CO_2 assimilation rate. Assimilation is reduced proportional to the transmission of light through the canopy, while leaf respiration increases as light decreases:

$$NPC = \int_{0}^{LAI} A_{sat} * \left(\frac{PAR}{K + PAR}\right) * (e^{-k*LAI}) - (0.5 - 0.05 * \ln(PAR * e^{-k*LAI})) * R_d$$

(Eq 3.)

where NPC is canopy net photosynthesis (as µmol m^{-2} s^{-1} of ground area), A_{sat} is the light-saturated photosynthetic rate, k is a light extinction coefficient, LAI is a canopy leaf area index, PAR is the light intensity at the top of the canopy and R_d is the dark respiration rate estimated from the Michaelis-Menten equation (Appendix 2.1, Eq. S1). The light extinction coefficient (k) was set to 0.5 as a previously used estimate for broadleaf forests (Clark *et al.*, 2011), leaf area index (LAI) was set to 7.8 as previously measured for this field site (Fenn *et al.*, 2015) and PAR was set to 1000 µmol m^{-2} s^{-1} as a standard daytime light intensity at the top of the canopy. We estimated canopy net photosynthesis for each leaf type (i.e. canopy consisting of only that leaf type), multiplied the estimates with the proportion of the respective leaf type observed in the canopy, and then summed these values over the three leaf types. This estimate was then compared to an estimate of a canopy with intact leaves only. Finally, we included the direct effect by subtracting the proportion of leaf area loss at canopy level:

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

(Eq. 4.)

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

2.3. Results

2.3.1. Herbivory under natural and experimental settings

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

The mesh bags successfully prevented herbivores from colonizing the experimental shoots (94 of 100 control shoots remained intact). The amount of leaf damage did not differ between the two damage treatments (10.88% ± 1.84% in mechanical and 14.13% ± 1.91% in herbivore addition, t = −0.90, df = 2, 1086, p = 0.37), but was higher in leaves with experimental herbivory compared to naturally occurring herbivory (8.45% ± 0.39%, t = 3.04, p=0.002 for herbivore addition and t = 1.72, p = 0.09 for mechanically damaged). Most leaf damage occurred at sides and tips, and only a small portion as holes (Appendix 2.2, Table S1).

2.3.2. Treatment-effects on photosynthesis and isoprene emission

Leaf treatment significantly influenced the light-saturated photosynthetic rate A_{sat} (χ^2 = 17.31, p = 0.002, df = 4, 8; Appendix 2.2, Table S2; Figure 2.1a. and 2.2a), the mean carboxylation rate V_{cmax} (χ^2 = 9.51, p = 0.05, df = 4, 11, Table S2; Figure 2.1b and 2.2d, Appendix 2.2, Figure S3), the mean electron transport rate J_{max} (χ^2 = 11.23, p = 0.02, df = 4,10, Table S2; Figure 2.1c and 2e, Figure S3), the mean stomatal conductance g_s (χ^2 = 10.48, p=0.03, df = 4,10, Table S2, Figure 2.2g) and the isoprene emission rate I_S (Lratio = 23.15, p < 0.001, df = 4,9, Table S2, Figure 2.2h). Both damaged and undamaged leaves in the herbivore addition shoots experienced a significant reduction in their A_{sat} and J_{max} compared to control leaves (z = −4.26, p < 0.001 damaged leaves and z = −4.26, p < 0.001 undamaged leaves for A_{sat}, z =

−38.92, z = −2.84, p = 0.03 damaged leaves and z = −3.24, p = 0.01 undamaged leaves for J_{max}). V_{cmax} was different between leaves damaged mechanically and intact leaves in the herbivory treatment, but the difference was only marginally significant (Tukey's test, z = 2.55, p = 0.08). Stomatal conductance (g_s) was different between control and the undamaged leaf in the herbivory treatment (z = −2.73, p = 0.049). The light intensity at which the gross photosynthetic rate is half of its maximum (K, Figure 2.2b), dark respiration (R_d, Figure 2.2c), and triose phosphate use efficiency (TPU, Figure 2.1d and 2.2f, Figure S3), on the other hand, were not influenced by leaf treatment. Mean leaf temperature significantly increased V_{cmax} (χ^2 = 4.21, p = 0.04, df = 1, 11), J_{max} (χ^2 = 9.98, p = 0.002, df = 1, 10), TPU (χ^2 = 9.93, p = 0.002, df = 1, 6), Rd (χ^2 = 8.11, p = 0.004, df = 1, 5) and g_s (χ^2 = 5.34, p = 0.02, df = 1, 10). V_{cmax}, J_{max}, TPU and g_s were significantly different between the two sites (χ^2 = 5.07, p = 0.02, df = 1, 11 for V_{cmax}; χ^2 = 5.58, p = 0.02, df = 1, 10 for J_{max}; χ^2 = 5.34, p = 0.02, df = 1, 6 for TPU and χ^2 = 5.95, p = 0.01, df = 1, 10 for g_s), and V_{cmax} differed between the two years (χ^2 = 8.82, p = 0.03, df = 1, 11).

Leaves damaged mechanically had significantly higher isoprene emission rate compared to control leaves and undamaged leaves in the herbivory treatment (t = −6.57, p < 0.007 and t = −7.16, p < 0.004, respectively). The isoprene emission rate per unit leaf area decreased with increasing percentage of leaf damage (Lratio = 8.32, p = 0.004, df = 1, 9). Isoprene emission rate correlated positively and significantly with photosynthesis (Appendix 2.2, Figure S4).

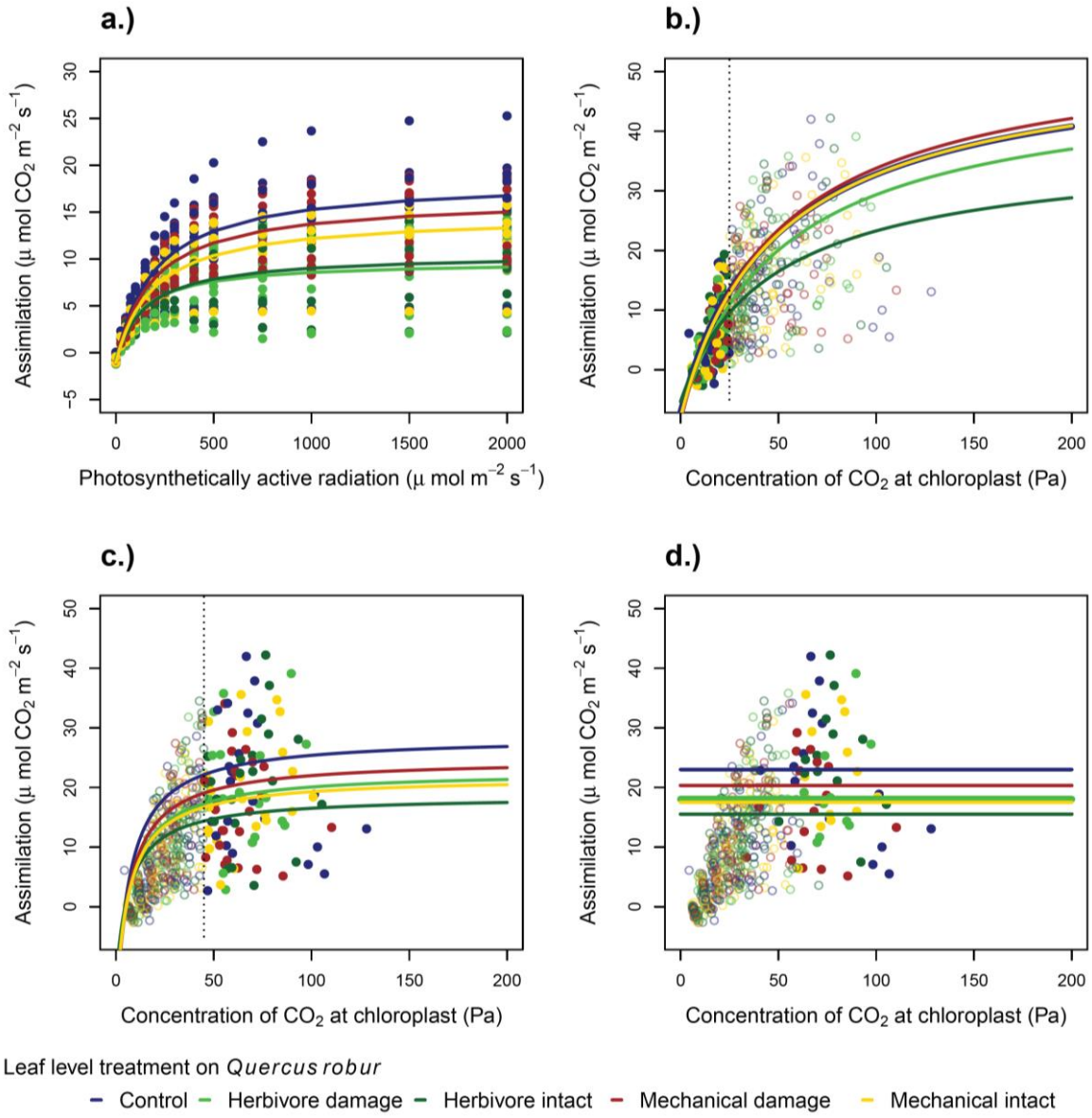


Figure 2.1. The average model predicted response curves: a) photosynthetic response to light, b) the maximum carboxylation rate (V_{cmax}), c) the maximum electron transport rate (J_{max}) and d) the maximum triose phosphate use efficiency (TPU). The original measurements are shown as points, and average model fitted parameters per treatment are shown as lines. For panels b-d, the solid points represent measurements used to estimate the corresponding parameter (*i.e.* when $[CO_2] < 25$ Pa for V_{cmax}, $[CO_2] > 45$ Pa for J_{max}, and assimilation at its maximum for TPU, see Appendix 2.1), and the circles show the remaining measurements. The dashed vertical lines at C_i concentrations of 25 (b) and 45 Pa (c) represent the points at which the limiting factor of the photosynthetic rate was assumed to change (from Rubisco limited into RuBP limited photosynthesis). The data represent measures from both field sites, and in panels b-d during both measuring years. Note that the effect of site and year has been taken into account in the statistical analyses. See Appendix 2.2, Figure S3 for the complete A/C_i response curves per treatment.

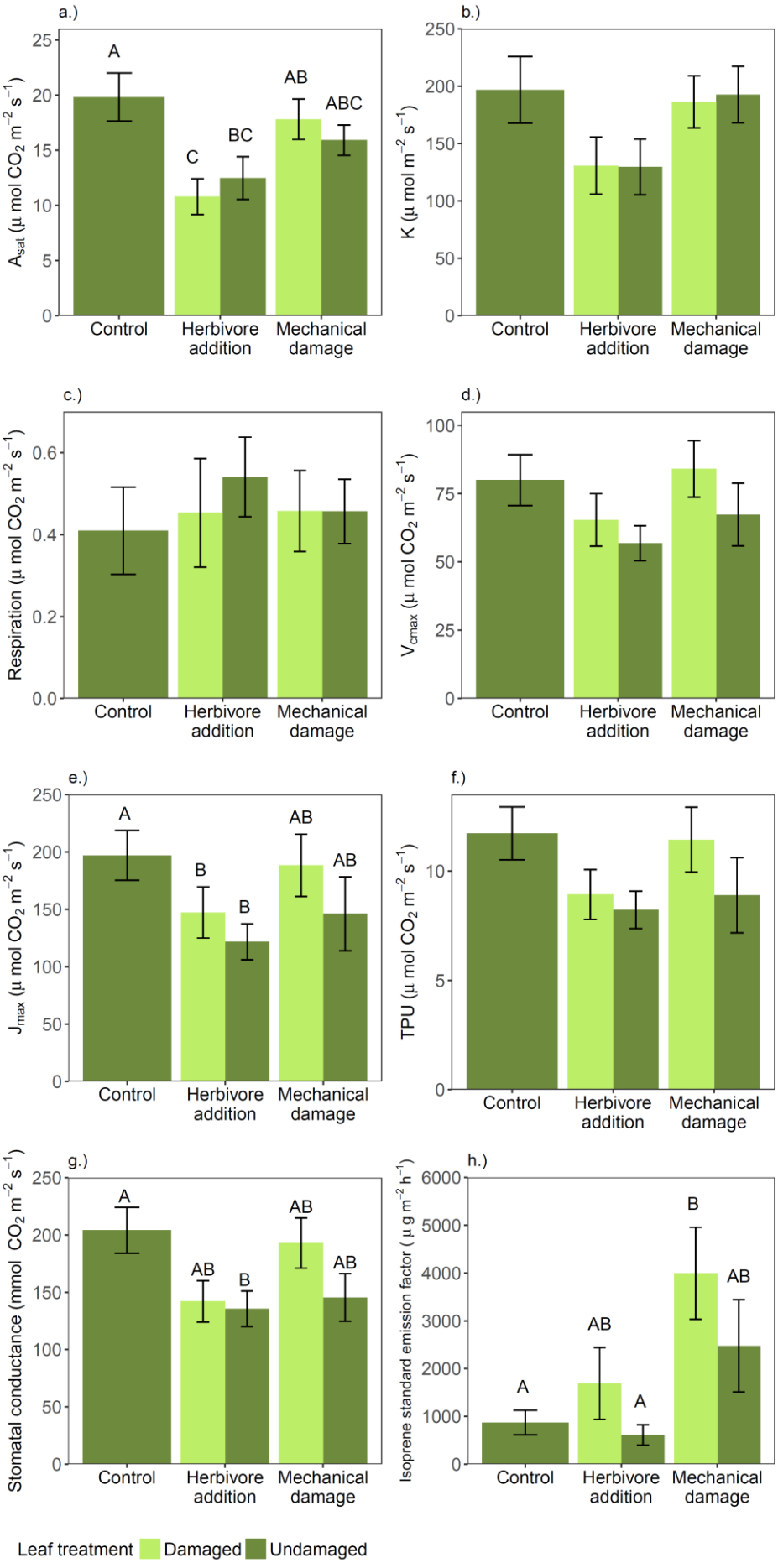

a.)
b.)
c.)
d.)
e.)
f.)
g.)
h.)
Control
Herbivore addition
Mechanical damage
Leaf treatment
Damaged
Undamaged

Figure 2.2. The average parameter values per leaf treatment on leaves of *Quercus robur* subject to herbivory by *Operophtera brumata*, mechanical damage or left as intact (control). Panel a) shows the average maximum model-fitted light-saturated photosynthetic rate (A_{sat}), b) the average light intensity at which the model-fitted photosynthetic rate is half of its maximum (K), c) the average dark respiration rate (R_d), d) the temperature-corrected average maximum carboxylation rate (V_{cmax}), e) the temperature-corrected average maximum electron transport rate (J_{max}), f) the temperature-corrected average triose phosphate use efficiency (TPU), g) the average stomatal conductance (g_s) and h) the average standard isoprene emission rate (I_S). n = 10 per leaf treatment for the figures in the panels a-c, except n=9 for the mechanically damaged leaf and n = 9 for herbivore undamaged leaf for panel b. For figures in the panels d-f, n = 15 for control, n = 13 for the herbivory treatments and n = 12 for the mechanical treatments. For panel g, n = 19 for control, n = 18 for damaged leaf in herbivore treatment and intact leaf in mechanical treatment, and n = 17 for intact leaf in the herbivore treatment and damaged leaf in the mechanical treatment. For panel h, n = 7 for control and damaged leaf in the mechanical treatment, n = 6 for undamaged leaf in the mechanical treatment and intact leaf in the herbivory treatment, and n = 4 for the damaged leaf in the herbivory treatment. Error bars are ±1 SEM. Means not sharing a letter are statistically significantly different from one another, e.g. AB and C in panel a (Tukey's test, $p < 0.05$). Note that the y-axis for respiration (panel c) is expressed as positive values (instead of the negative assimilation rates) to make the graph more intuitive. The data represent measures from both field sites, and in panels d-g during both measuring years. Note that the effect of site and year has been taken into account in the statistical analyses.

2.3.3. The effects of herbivory on leaf and canopy scales

Leaf area loss (the *direct effect* of herbivory) per leaf was 8.5% ± 0.4%. The *indirect effect* of herbivory, i.e. the herbivory-induced change in photosynthesis in the remaining leaf tissue, accounted for a 45.5% ± 10.1% reduction in the leaf-scale light-saturated photosynthesis (A_{sat}, Table 2.1). Hence, the indirect effect of herbivory was several magnitudes larger than the direct effect of leaf area loss. Within the shoots that had herbivory damage, the reduction in photosynthesis was almost identical between damaged leaves and their undamaged neighbors. When the *direct* and *indirect effects* and the proportion of damaged and undamaged leaves in the canopy were combined, 45.6% ± 7.6% of the light-saturated photosynthesis and 47.9% ± 9.5% of the net photosynthesis under diffused light was lost to herbivores at the canopy-scale

(Table 2.1). The first estimate represents a canopy consisting only of sun leaves at full light, (see Appendix 2.2, Table S3 for estimates on canopy-scale effects of herbivory on photosynthesis at lower light intensity), whereas the second estimate represent a canopy where light is reduced with increasing leaf area index due to shading. Despite the different assumptions of these estimates, the proportional change in photosynthesis due to herbivory is effectively the same.

In contrast to the photosynthesis, isoprene emission rates increased in the damaged leaves by 85.4 ± 115.6% compared to the intact control leaves, though the small number of samples and the associated large error makes drawing conclusions difficult. The shoot-level effect, where shoot-level herbivory affects undamaged leaves within the same shoot, was small (29.8 ± 32.1%) for isoprene. At the canopy-scale, the total effect of herbivory corresponded to a 52.5 ± 82.6% increase in isoprene emissions, but with large variation (Table 2.1).

Table 2.1. Total effect of the herbivory by *Operophtera brumata* on *Quercus robur* from the leaf to the canopy scale. The average percentage of leaf area loss per leaf (D_t, direct effect), the average proportion of different leaf types (t = 1, 2, 3) in the canopy, the effect of insect herbivory on the light-saturated photosynthetic rate (A_{sat}) and on the isoprene emission rate per unit leaf area (indirect effect) of the different leaf types, the estimates of the combined (direct + indirect) effects of these at leaf and canopy scales, and the canopy-scale estimates when change in the light intensity through the canopy is taken into account. The effects are expressed relative to the control treatment values (intact leaves in intact shoots). Errors are ±1 SEM derived through error propagation. See Appendix 2.2, Table S3 for values for photosynthetic rate in 1000 µmol $m^{-2}\,s^{-1}$ of photosynthetically active radiation (A_{1000}).

	Intact leaf, intact shoot (t=1)	**Intact leaf, damaged shoot (t=2)**	**Damaged leaf, damaged shoot (t=3)**	**Canopy scale total effect**
Direct effect				
Leaf area loss (%) (D_t)	0	0	−8.5 ± 0.4	
% of leaves in canopy (l_t)	1.7	27.3 ± 1.9	71.0 ± 1.9	
Canopy scale effect % (D_c)				−6.0 ± 3.8
Light saturated photosynthesis (A_{sat})				
Rate (µmol $CO_2\,m^{-2}\,s^{-1}$ of leaf area)	19.8 ± 2.2	12.5 ± 1.9	10.8 ± 1.6	
Rate (% of intact)	100	63.1 ± 11.9	54.5 ± 10.1	
Indirect effect per unit leaf area %	0	−36.9 ± 11.9	−45.5 ± 10.1	
Leaf scale effect % (direct + indirect)[Eq 1.]	0	−36.9 ± 11.9	−50.1 ± 9.5	
Canopy scale effect % (direct + indirect)[Eq 2.]				−45.6 ± 7.60

Isoprene				
Rate (µg m^{-2} h^{-1} of leaf)	871.7 ± 257.6	612.1 ± 213.5	1766.0 ± 967.0	
Rate (% of intact)	100	70.2 ± 32.1	202.6 ± 126.0	
Indirect effect per unit leaf area %	0	−29.8 ± 32.1	102.6 ± 126.0	
Leaf scale effect % (direct + indirect)[Eq 1.]	0	−29.8 ± 32.1	85.4 ± 115.6	
Canopy scale effect % (direct + indirect)[Eq 2.]				52.5 ± 82.6
Light diffused photosynthesis				
Canopy net rate per leaf type (µmol CO_2 m^{-2} s^{-1} of ground area, NPC_t)[Eq3]	29.96 ± 3.19	17.87 ± 2.59	16.92 ± 2.28	
Canopy net rate combined, weighted with the leaf type proportions (µmol CO_2 m^{-2} s^{-1} of ground area)				17.4 ± 1.83
Canopy net rate (% of intact)				58.1 ± 8.70
Canopy scale effect % (direct + indirect)[Eq 4.]				−47.9 ± 9.50

2.4. Discussion

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

2.4.1. Why does the photosynthetic rate change following leaf damage?

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

One possibility is that physical injury is inhibiting photosynthesis. Severed vein network can disrupt the transport of water and nutrients with long-lasting effects (Sack & Holbrook, 2006), simultaneously reducing stomatal conductance. Ruptures in the leaf can cause diffusion of CO_2 before it is used in the carbon-fixing reactions, lowering the efficiency

of carbon assimilation (Oleksyn *et al.*, 1998; Nabity *et al.*, 2006, 2009, 2013). Furthermore, repairing the damaged tissue uses valuable resources. Trade-offs in resource use might also occur between growth (and hence photosynthesis) and defence (Herms & Mattson, 1992). Defensive reactions against herbivores require synthesis of complex chemical compounds, which act as repellents or additional signalling molecules, using the same resources or molecular pathways than photosynthesis (Herms & Mattson, 1992; Taiz & Zeiger, 2010; Zhou *et al.*, 2015). Build-up of defensive compounds in the plant tissue might also cause the problem of auto-toxicity, lowering photosynthetic efficiency (Baldwin & Callahan, 1993; Nabity *et al.*, 2009). Damage early in the season could also "prime" the plant (Conrath *et al.*, 2002), making it more resistant to future herbivory by activating long-lasting defences. The cost of maintaining a primed state could alter primary metabolism over long-term (van Hulten *et al.*, 2006; Frost *et al.*, 2008).

2.4.2. Why does the photosynthetic rate differ between leaves damaged mechanically or by herbivores?

In this study, the mechanically damaged leaves experienced a significantly smaller reduction in their photosynthetic rate than leaves damaged by caterpillars. In previous studies, mechanical damage alone has failed to produce a response in the plant, whereas application of herbivore oral secretions, even without any physical damage, have done so (Korth & Dixon, 1997; Alborn, 1997). The herbivore-induced defensive responses depend on the species identity, specifically on the chemical make-up of the insect saliva (Alborn, 1997; Erb *et al.*, 2012). These herbivory-specific effects are usually mediated through hormonal pathways including jasmonic and salicylic acids, the activation of which also switches off photosynthesising reactions (Wasternack & Hause, 2013). These results suggest that the herbivory-inflicted photosynthetic reduction in our study is a response to the presence of

herbivores specifically, instead of leaf damage alone, and possibly actively triggered by the defence machinery of the plant (Kerchev *et al.*, 2012; Zhou *et al.*, 2015).

2.4.3. How does leaf damage affect intact neighbouring leaves?

In this study, intact and damaged leaves on the same shoots showed an almost identical degree of reduction in photosynthesis. Damage-triggered defence reactions can travel to intact plant parts through shared vasculature (Jones *et al.*, 1993), as electric signals (Sukhov, 2016), or to neighbour plants through volatile organic compounds (Arimura *et al.*, 2000). This systemic signalling can subsequently affect photosynthesis of intact plant parts (Agrawal, 2000; Barron-Gafford *et al.*, 2012; Meza-Canales *et al.*, 2017). Especially jasmonic acid can travel to systemic tissues (Baldwin & Zhang, 1997; Stratmann, 2003), and accumulate in them (Leitner *et al.*, 2005). Because in our study the systemic changes were detected within individual shoots, the signal has probably travelled through within-shoot vascular connections, which might have also restricted it from reaching the intact control shoots, or dampened the effect (Orians, 2005). The reduction in photosynthesis in neighbouring leaves might prepare the leaf for the forthcoming herbivory, either by increasing the level of defences at the expense of assimilation, or by actively shutting down the production of further carbohydrates, to provide less nutrition for herbivores (Zhou *et al.*, 2015). Herbivore-specific signalling might also explain why the mechanical treatment responded less than the herbivore addition. Our study thus shows that naturally occurring herbivory can have a considerable effect also on systemic intact leaves. These kinds of shoot-level effects have not been previously taken into account in ecosystem-scale studies.

2.4.4. Why did the isoprene emission rate increase after leaf damage?

We observed a significant positive relationship between photosynthesis and isoprene emission, concurrent with previous studies (Rasulov *et al.*, 2009; Copolovici *et al.*, 2017).

Nevertheless, the treatment-specific effects on isoprene were opposite to the effects on photosynthesis. The isoprene emission rates per unit leaf area were significantly higher in the mechanically damaged leaves than in non-damaged leaves on the intact control shoots, suggesting that the observed change might not be a response to herbivory specifically. Because the effect was not visible in the surrounding intact leaves, the damage-triggered change in isoprene emission seems to be a leaf-level response. Contrary to our results, previous studies have found *a reduction* in isoprene emission immediately after leaf damage (Loreto & Sharkey, 1993; Portillo-Estrada *et al.*, 2015; Copolovici *et al.*, 2017), but see (Ferrieri *et al.*, 2005). VOC emission profile emitted immediately after damage can substantially differ from longer-term emissions (Maja *et al.*, 2014). Nevertheless, most herbivore-induced VOCs are studied immediately after the damage occurs.

Oak could be actively increasing its isoprene emission over a longer period after the damage. Physical injury to the leaf venation network could lead to increased water loss lasting for several days (Aldea *et al.*, 2005). Drought, and a release from it, have been shown to increase isoprene emissions (Sharkey & Loreto, 1993; Tattini *et al.*, 2015). If mechanical damage caused water stress at the time of the injury, this might have led to an increased isoprene emission later, once the damage had been repaired. Long-term monitoring of damaged-induced isoprene emission is needed to fully understand its response to herbivory.

2.4.5. Canopy scale effect of insect herbivory

At our study site, the *direct effect* of insect herbivory was small: insect herbivores removed 6.0% (± 3.8%) of the oak leaf area, consistent with global estimates of average herbivory rates (Cyr & Pace, 1993). The *indirect effect* of herbivory on the remaining leaf tissue of the damaged leaf, and on the neighbouring intact leaves, on the other hand, was several magnitudes larger, reducing the light-saturated photosynthesis by 46% (± 10%) and 37% (± 12%) on average, respectively. This supports the previous results on the importance of

indirect effects over direct ones (Zangerl *et al.*, 2002; Barron-Gafford *et al.*, 2012). Nevertheless, in many ecosystem-scale studies the effects of herbivory are quantified only as the amount of leaf area loss (Metcalfe *et al.*, 2014).

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

Similarly, herbivory had a large effect on isoprene emission, causing an 85% (± 116%) increase in the leaf-scale isoprene emission rate and an 53% (± 83%) increase on the canopy-scale. The large error margin makes it difficult to draw firm conclusions on the role of herbivory on canopy-level isoprene emissions. However, if our estimates are correct, this increase would be enough to counteract the predicted reduction in isoprene emissions due to climate change, increasing atmospheric CO_2 concentrations and land-use changes combined (Squire *et al.*, 2014). Despite their potential importance, biotic interactions are usually lacking from the global isoprene emission models (Müller *et al.*, 2008; Arneth *et al.*, 2008; Squire *et al.*, 2014). Previous studies have recorded higher forest-scale isoprene emissions than expected by models (Geron *et al.*, 1997; Gu *et al.*, 2017), and changes in species composition have been shown to affect forest-scale isoprene emissions (Wang *et al.*, 2017b). Our study suggests that enhanced emissions resulting from leaf damage might be leading to

underestimates of the actual forest-scale isoprene emissions, which could have significant knock-on effects on calculations of ozone and particle formation.

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

With the predicted climate change, species distributions, abundances and hence the frequencies of specific species interactions are projected to shift, and in many cases, have already shifted (Jepsen *et al.*, 2008; Kurz *et al.*, 2008). Nevertheless, insect herbivory is rarely addressed in biosphere and climate models (Kurz *et al.*, 2008). Our results clearly demonstrate that for predicting the responses of forest ecosystems to climate change, including the effects of herbivory on the carbon cycle and atmospheric chemistry is crucial. Ignoring the role of insect herbivory might thus overestimate the role of forests as carbon sinks (Kurz *et al.*, 2008; Schäfer *et al.*, 2010), or underestimate their role as isoprene emitters. We have demonstrated the importance of indirect herbivory effects for a single plant-herbivore system; there is a clear need to replicate such studies in other systems.

2.4.6. Conclusions

Moth caterpillars reduce the per unit leaf area photosynthetic rate of their host plant, both in the remaining leaf tissue of the damaged leaf, and in the intact neighbour leaves. The reduction by natural herbivory is greater than that by mechanical damage alone. This indicates the host plant can differentiate between these two types of damage, pass on the signal to undamaged parts, and respond accordingly. Isoprene emission rate is increased by mechanical leaf damage and does not seem to be an herbivory-specific reaction. These responses expressed on a scale of individual leaves and shoots have large-scale consequences on the

carbon dynamics on the scale of the forest. On a scale of a canopy, the indirect effects of herbivory emerge several times more important than the direct effect of leaf area removed. Including these effects in estimates of the interactions between biosphere and the atmosphere is crucial for better prediction of the effects of changing climate on forest ecosystems.

2.5. Acknowledgements

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Chapter 3: The effect of winter moth (*Operophtera brumata*) herbivory on physical and chemical leaf traits of pedunculate oak (*Quercus robur*)

Kristiina Visakorpi, Terhi Riutta, Yadvinder Malhi, Juha-Pekka Salminen, Norma Salinas and Sofia Gripenberg

Manuscript in preparation



Abstract

Insect herbivores have the potential to change many physical and chemical traits of their host plant. Changes in primary metabolism include changes in photosynthesis and respiration, whereas changes in secondary metabolism can result in the production of defensive chemicals. Although research on herbivory-induced defences has been conducted for decades, detailed experiments studying changes in multiple leaf traits and secondary metabolites simultaneously are still rare. In this study, we use experimental methods to test whether feeding damage by winter moth caterpillars (*Operophtera brumata*) and mechanical wounding change important physical and chemical leaf traits, including leaf mass per area, leaf area carbon and nitrogen content and the concentration of 27 polyphenol compounds on oak (*Quercus robur*) leaves. To investigate how potential changes in leaf chemistry or physical structure affect leaf functioning, we relate the studied leaf traits to rates of photosynthesis and respiration. We also provide a summary of the literature on the relationship between herbivory and the 27 polyphenol compounds measured in this study. Overall, we did not find any clear differences in physical or chemical leaf traits between the treatments. Rather, the trait variation was more driven by the leaf collection date and variation between individual trees. Only leaf nitrogen content and couple of the studied polyphenol compounds correlated with photosynthesis and leaf respiration. Our results suggest that in our study system, abiotic conditions related to the growth location, variation between individuals, and seasonal trends in plant physiology might be more important than herbivory in determining the trait composition and polyphenol chemistry of leaves.

3.1 Introduction

Insect herbivores are one of the most abundant groups of organisms on Earth, and folivory is one of the most common feeding strategies used by these insects (Forister *et al.*, 2015; Roskov *et al.*, 2018). An estimated 7-11% of leaf area is consumed annually by insect herbivores in temperate and tropical forests (Coley & Barone, 1996), but often the effect of herbivory on the host plant is larger than this loss of leaf area (Zangerl *et al.*, 2002). By feeding on leaf tissue, insect herbivores can cause changes to a range of chemical and morphological leaf traits (Karban & Baldwin, 1997; Nykänen & Koricheva, 2004). These changes are often induced as defences against the herbivores (Karban & Baldwin, 1997) and may result in further alterations in plant function, for example in photosynthetic rate (Bilgin *et al.*, 2010; Nabity *et al.*, 2013) or growth rate (Coley, 1988; Zhang & Turner, 2008). The herbivory-induced changes are often thought to arise through resource allocation trade-offs between growth and defence (Herms & Mattson, 1992; Züst & Agrawal, 2017).

Plant responses to herbivory can vary widely depending on the exact nature of the interaction. Typically, herbivory results in decreased leaf nutrient content and increased concentrations of chemical compounds known to act as plant defences (Schultz & Baldwin, 1982; Nykänen & Koricheva, 2004), reduced plant size (Zhang & Turner, 2008) and changes in several morphological traits relating to leaf toughness, waxiness and density (Lowell *et al.*, 1991; Hanley *et al.*, 2007). Of the physical leaf traits, high leaf mass per area (LMA) provides a reliable indication of plant tolerance to herbivory (Hanley *et al.*, 2007). Herbivory often changes several traits concurrently. For example, the concentration of defensive compounds can increase while nitrogen content decreases simultaneously, making the plant both more toxic and less nutritious for herbivores (Herms & Mattson, 1992; Kaitaniemi *et al.*, 1998), increased carbon deposition into defensive compounds can increase leaf mass per area (Havko *et al.*, 2016), and higher investment in defence can slow growth and leaf maturation resulting in smaller leaf size (Tuomi *et al.*, 1988; Kaitaniemi *et al.*, 1998).

Although an active field of research, many studies on effects of herbivory on the defensive chemistry of plants conducted to date have either examined a small number of compounds or summed metrics of total concentrations of groups of chemical compounds, for example "condensed tannins" (Nykänen & Koricheva, 2004; Salminen & Karonen, 2011). Nevertheless, the hundreds of different defensive compounds produced by most plants do not respond in isolation from each other, and can have contrasting effects on plant and herbivore performance (Haukioja *et al.*, 2002; Salminen & Karonen, 2011). Different compounds have been found to correlate positively, negatively, or not at all with herbivory and/or herbivore fitness, which can make summed metrics on "total concentration" less useful (Pearse, 2011; Moctezuma *et al.*, 2014). Moreover, many previous studies have been based on observational correlations between herbivory and concentrations of specific compounds, making it difficult to separate trends caused by induction of defences from herbivore host selection (e.g. (Moctezuma *et al.*, 2014; Silva *et al.*, 2015).

The changes in leaf traits triggered by herbivory can affect leaf functioning. For example, the activation of defence-related genes can downregulate genes related to photosynthesis (Bilgin *et al.*, 2010), or affect the molecular pathways needed for leaf gas exchange (Nabity *et al.*, 2013). Nitrogen is important for the proteins of the photosynthetic machinery, and thus changes in leaf nitrogen content often influence photosynthetic capacity (Lambers *et al.*, 1998). Higher LMA can reduce photosynthetic rate by increasing the lengths of diffusion paths or shading lower chloroplasts (Parkhurst, 1994; Green & Kruger, 2001). The herbivore-induced changes in leaf gas exchange, both in photosynthesis and respiration rate, can scale up and potentially affect ecosystem carbon sequestration (Visakorpi *et al.*, 2018) and changes in leaf thickness and defensive chemistry can affect nutrient cycling through altered decomposition rates (Hättenschwiler & Vitousek, 2000; Chapman *et al.*, 2003; Cornwell *et al.*, 2008; Chomel *et al.*, 2016). Herbivory-induced changes in leaf traits could thus have large-scale consequences for ecosystem-level processes.

In this study, we investigated how two distinct types of leaf damage affect selected physical and chemical leaf traits, and how the studied traits correlate with previously documented damage-induced changes in leaf photosynthetic rate (Visakorpi et al. 2018). We used a manipulative field experiment to create two types of damage: feeding damage by caterpillars and mechanical wounding. As a study system, we used the pedunculate oak (*Quercus robur* L.) and one of its most significant insect herbivores, caterpillars of the winter moth (*Operophtera brumata* L.). We measured leaf chemistry (as individual compounds of polyphenols), leaf nitrogen and carbon content, and leaf structure (leaf area and leaf mass per area). We used photosynthesis and leaf respiration measurements previously reported by Visakorpi *et al.* (2018) to assess the link between the studied chemical and structural traits and leaf functioning. In our previous study, we showed that photosynthetic rate (light-saturated photosynthesis and electron transport rate) was significantly lower in leaves damaged by herbivores and in intact leaves growing next to these damages leaves than in intact control leaves surrounded only by other intact leaves. In this study, we use the same experimental set-up to explore whether the observed changes in photosynthesis can be explained by concurrent changes leaf traits hypothesised to be influenced by herbivory. In addition, to evaluate the current evidence of the role of the studied polyphenols in plant defence against herbivores, we performed a literature review on the relationship between herbivory and the individual polyphenol compounds measured in this study. Specifically, we ask

1.) Does experimentally applied herbivory and/or mechanical leaf damage change chemical and structural leaf traits? If so, are these changes dependent on the type of damage (insect herbivory or mechanical damage) and can they also be seen in intact leaves on the same shoots?
2.) Are previously observed damage-induced changes in photosynthesis linked to parallel changes in structural and chemical leaf traits?

3.) What are the known relationships between herbivory and the polyphenol compounds? Does the published literature provide evidence for individual compounds acting as anti-herbivore defences?

Based on previous literature on polyphenols, induced defences and trade-offs between plant growth and defence, we expect 1) herbivory and/or mechanical leaf damage to result in increased investment in polyphenols (reflected in either increased concentration, diversity, or altered composition of polyphenols) and 2) photosynthetic rate to correlate negatively with investment in polyphenols. Based on previous literature on leaf nutrients and physical traits, we expect 3) leaf mass to increase after herbivory, 4) leaves subject to herbivory treatment to grow smaller and 5) leaf nitrogen content to correlate with photosynthetic rate.

3.2 Materials and methods

3.2.1. Study system and experimental design

The field experiment was carried out on ten oak trees in Oxfordshire, UK. Five of the studied oaks were mature trees (mean diameter at breast height, "dbh" 67.2 cm) located in the ForestGEO forest dynamics plot in Wytham Woods (51.7743º, -1.3379º), where their foliage could be accessed from an elevated canopy walkway. The remaining five trees were relatively young (mean dbh 13.6 cm) planted oaks by the John Krebs field station in Wytham (51.7837º, -1.3170º). On both sites, the oaks are naturally infested by caterpillars of the winter moth, which is a common generalist early-spring herbivore. The caterpillars emerge in synchrony with budburst and feed on the newly flushed leaves until early June (Hunter, 1992). Relatively few herbivore species feed on the mature oak leaves later in the season (Feeny, 1970).

The experiment was conducted during the springs and summers 2015-2016. Between 11th and 15th May 2015 and 9th and 11th May 2016, when all leaves were still young, we identified 15 shoots with only intact leaves from each of the ten study trees and enclosed each

shoot in a small mesh fabric (mesh size < 1mm) bag. We randomly assigned each bag into one of three treatments: 1) *herbivore addition*, 2) *mechanical damage*, or 3) *control*, so that each tree had five bags of each treatment. For each of the *herbivore addition* bags we added a locally collected winter moth caterpillar, and let it feed on the leaves for 3-5 days. To ensure that the artificially applied damage would mimic the herbivory treatment, the shoots assigned to the *mechanical damage* treatment were paired with the herbivore addition shoots. The type and amount of damage on the herbivory addition shoots was then replicated by tearing or punching holes in the leaves in the mechanical damage treatment. *Control* shoots were left intact. We left the mesh bags around the shoots to prevent additional herbivory until 25th June 2015 or 28th June 2016, when amount of insect herbivory had levelled off. Altogether, there were 15 experimental shoots in each of the ten trees, replicated over two seasons (n = 300 shoots altogether). For further details on the experimental set-up, see Visakorpi *et al.* (2018).

On shoots in the *herbivory addition* and *mechanical damage* treatments, we measured leaf traits for both damaged and intact leaves. Since no leaves were damaged on *control* shoots, we only measured intact leaves from these shoots. This setup allowed us to measure five leaf-level treatments: *damaged leaf in herbivory treatment*, *undamaged leaf in herbivory treatment*, *damaged leaf in mechanical treatment*, *undamaged leaf in mechanical treatment*, and *intact control leaf*.

3.2.2. Background herbivory

Because the chemical traits of the studied leaves could be affected by natural level of herbivory in other parts of the tree, we used data from surveys of background herbivory on the study trees reported in Visakorpi *et al.* (2018). These surveys were conducted by collecting 15 additional shoots from each tree four times during the season 2015 (16-28th May, 25th June, 14th July - 10th August and 18th August). The collected leaves were pressed and scanned, and

the area lost to herbivory was estimated as the percentage of missing using the ImageJ software (NIH, MD, USA).

3.2.3. Collecting leaf traits

The data collection of multiple leaf traits spanned two growing seasons, with the timing and number of replicates varying depending on the trait. The data collection stream is summarised in Figure 3.1.

Polyphenols are one of the main groups of secondary metabolites produced by oaks, thought to act both as induced and constitutive defences (Feeny, 1970; Salminen *et al.*, 2004; Roslin & Salminen, 2008). They include hydrolysable tannins, condensed tannins (also called proanthocyanidins) and flavonols (Salminen *et al.*, 2004). Previous studies have suggested that especially hydrolysable tannins could act as defences against insect herbivores due to their high concentration in young oak leaves (Salminen *et al.*, 2004) and high pro-oxidant activity in caterpillar guts (Barbehenn *et al.*, 2009; Barbehenn & Constabel, 2011). Flavonols are often induced after herbivore attack (Onkokesung *et al.*, 2014; Ohse *et al.*, 2017). Even though there are accounts of proanthocyanidin levels increasing after defoliation (Schultz & Baldwin, 1982; Faeth, 1986; Roth *et al.*, 1998), there is little evidence to suggest that they are important defences against insect herbivores (Ayres *et al.*, 1997; Osier *et al.*, 2000; Nykänen & Koricheva, 2004; Roslin & Salminen, 2008; Barbehenn & Constabel, 2011; Ohse *et al.*, 2017). Thus, we decided to concentrate on measuring hydrolysable tannins and flavonols.

To determine the chemical content of leaves representing the different experimental treatments, we measured concentrations of individual polyphenol compounds, leaf nitrogen (N) and leaf carbon (C) content. We used leaves collected from the experimental shoots during the field season of 2016 (Figure 3.1). Leaves from shoots that were used for photosynthetic measurements (three out of the 15 shoots per tree) were collected within few days after the final photosynthesis measurement on that tree had taken place (11th July to 11th

August 2016). This was done to ensure that the timing of the leaf collection would match the timing of the measured photosynthesis without disturbing the further photosynthetic measurements of that tree. The remaining leaves were collected at the end of the measuring period on 12th August 2016. Leaves were transported in a cool bag to a freezer and freeze-dried within few weeks of collection. The leaves used for photosynthetic measurements were analysed only for their polyphenol chemistry. From the remaining leaves collected at the end of the measuring period, we chose one intact and one damaged leaf from each shoot belonging to the *herbivory addition* and *mechanical damage* -treatments, and one intact leaf from each *control* shoot for polyphenol analyses. In total, we measured polyphenol content for 119 leaves from 74 shoots (Figure 3.1). Of the remaining leaves, 54 leaves from 36 shoots from 10 trees were analysed for carbon and nitrogen content. Leaves from the remaining 39 shoots were stored for potential future analyses (Figure 3.1).

Analyses of the polyphenol concentrations were carried out in the laboratory of the Natural Chemistry Research Group at the University of Turku, Finland. Shortly, 20 mg of the dry plant powder was extracted 2 x 3 h with 1.4 mL acetone/water (80:20, v:v). Acetone was evaporated from the combined extract by the Eppendorf concentrator and the water phase was freeze-dried. The lyophilized water phase was dissolved in 1 mL ultra-pure water and filtered via 0.2 µm PTFE filter. The UPLC-Orbitrap-MS analyses of individual polyphenols were acquired from the 5-time dilution of this filtered water phase. The UPLC system consisted of a binary solvent manager, sample manager, column (Acquity UPLC BEH Phenyl, 30 mm × 2.1 mm i.d., 1.7 µm, Waters Corporation), and photodiode array detector (Acquity UPLC®, Waters Corporation, Milford, MA, USA) coupled to a hybrid quadrupole-Orbitrap mass spectrometer (Q Exactive™, Thermo Fisher Scientific GmbH, Bremen, Germany; Volf *et al.* 2017). The compounds were quantified from the full scan trace by monitoring the peak areas of the most prominent ions (typically $[M-H]^-$ or $[M-2H]^{2-}$) of each compound and using external calibration curves to convert peak areas into mg/ml concentrations. For each sample,

we obtained detailed estimates of the concentration of 27 polyphenol compounds (Appendix 3.1, Table S1).

Analyses of the leaf carbon and nitrogen content were carried out in the Pontificia Universidad Católica del Perú. Dry leaf material was ground to fine powder, and C and N content was determined by combustion analysis.

To calculate leaf mass per area (LMA) and leaf area (LA), we used experimental shoots collected at the end of the field season 2015 (103 shoots, 616 leaves, Figure 3.1), on 30th October – 2nd November 2015. Leaves were scanned and dried in the oven in 70°C for 72h. Remaining leaf area, original leaf area and leaf area loss were estimated from the leaf scans using ImageJ software (NIH, MD, USA). Dried samples were weighted (Sartorius BL120S, Göttingen, Germany) to determine leaf dry weight, which was then divided by leaf area to determine LMA. A small number of control shoots that had contained damaged leaves (n = 14) were discarded from further analyses.

For photosynthetic traits, we used seven different photosynthetic parameters obtained by Visakorpi *et al.* (2018) from the same experimental setup: light-saturated photosynthetic rate (A_{sat}), light intensity where photosynthetic rate is half of its maximum (K), dark respiration rate (R_d), maximum carboxylation rate (V_{cmax}), maximum electron transport rate (J_{max}), maximum triose phosphate use efficiency (TPU), and photosynthetic rate in 1000 µmol m^{-2} s^{-1} of photosynthetically active radiation (A_{1000}), representing a standard photosynthetic rate in full daylight (Table 3.1). Photosynthetic parameters were measured for five leaves (one from each leaf-level treatment) per tree during 2015 and 2016 (Figure 3.1). For summary of all traits measured including sample sizes, see Table 3.1.

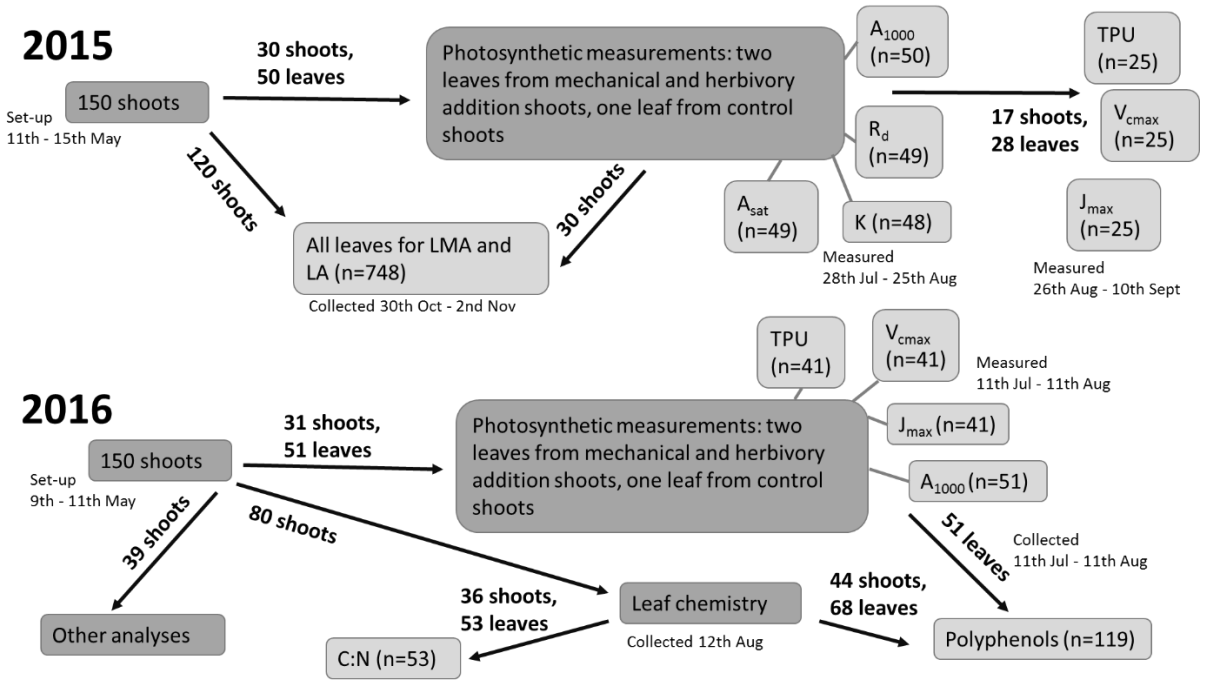


Figure 3.1. The allocation of experimental leaves into different leaf trait measurements on ten trees during the field seasons 2015 and 2016. For abbreviations of the different leaf traits, see "Collecting leaf traits" above and Table 3.1. The photosynthetic parameters (A_{sat}, K, R_d, V_{cmax}, J_{max}, TPU) were derived from photosynthesis-light and photosynthesis-CO_2 (i.e. A/C_i) response curves and subsequent model fitting (see Visakorpi *et al.* 2018). Note that model fitting to calculate some of these parameters failed, hence the final sample size is less than the number of leaves allocated for that trait (41 leaves instead of 51 leaves 2016 for V_{cmax}, J_{max}, TPU). One outlier of K was removed from further analyses.

3.2.4. Statistical analyses

To analyse differences in the background levels of herbivory between the study trees, we used linear models. To analyse the effect of the experimental treatments on the different leaf traits, we used linear mixed effect models and linear multivariate models. To test the relationships between the different leaf traits and photosynthetic rate or leaf respiration rate, we used mixed effect models, linear models and calculated correlation coefficients. To test differences in leaf traits composition between the treatments, we performed Principal Component Analyses (PCA), and Redundancy Analyses (RDA).

To select the best mixed effect model per response variable, we first chose the optimal variance structure and then assessed the significance of fixed effects (Zuur *et al.*, 2009). The

optimal variance structure was explored by building models in which the variance depended on the different explanatory variables and comparing model AICs. The model with the lowest AIC was then compared against the model with constant variance using likelihood ratio tests. Variance structure that significantly improved model fit was chosen.

To assess the significance of fixed effects in the mixed effects models, the full model for each response variable was simplified by dropping one explanatory variable or interaction at a time. The change in the model fit was assessed using likelihood ratio tests. Fixed factors that did not improve model fit were dropped from the final model (Crawley, 2007). Significance of the fixed terms in linear models was assessed by computing analyses of variance for the model fit.

Model assumptions of all linear and linear mixed effect models were assessed by visually examining plots of residuals against fitted values for the homoscedasticity of residuals, and a Quantile-Quantile plot for the normal distribution of the residuals. Below, we will describe the individual analyses and model structures in detail.

3.2.4.1 Background herbivory

To test whether the level of background herbivory differed between the study trees, we built a linear model with percentage of leaf damage as a function of tree identity. To investigate whether background level of leaf area loss correlated with tree chemistry, we built linear models with percentage of leaf damage as a function of concentration of hydrolysable tannins, concentration of flavonols, diversity of hydrolysable tannins and diversity of flavonols. In both models, percentage data was arcsine square root-transformed. As leaf area loss was estimated during a different year than tree chemistry, we used tree-level averages (n = 10) for the variables in the second model.

3.2.4.2 The effect of treatments on leaf traits

To investigate the effect of the experimental treatments on leaf polyphenol content, we built linear mixed effect models in which the response variable was either the summed concentration of all polyphenolic compounds detected in a leaf, the diversity of all compounds, or the concentration or diversity of each of the two polyphenol subgroups: hydrolysable tannins and flavonols. Polyphenol diversity was calculated as Shannon's diversity index. Since almost all compounds were found in all leaves, the diversity index reflects whether the plant is investing evenly in all the different compounds or investing disproportionately in only a few. Fixed effects were treatment, the collection site (John Krebs field station or Wytham Woods), collection date (as Julian day), and all possible two-way interactions. Variances were allowed to differ between the different study trees for the polyphenol diversity, flavonol concentration, flavonol diversity and hydrolysable tannin diversity. To account for the hierarchical study design, shoot ID nested within a tree ID was set as a random effect.

To investigate the effect of the experimental treatments on the 27 individual polyphenol compounds, we built a linear multivariate model (Warton *et al.*, 2012, 2015; Wang *et al.*, 2012), in which the matrix of all compounds was modelled as a function of the site, tree, collection date and the treatment. We then extracted the univariate test statistics for each compound separately.

To investigate the effect of treatment on leaf carbon and nitrogen content, we built a linear mixed effect model of either per-leaf nitrogen or carbon content or the log-transformed relationship between the two (C:N) as a function of treatment, the collection site and leaf mass. Interactions were omitted to avoid spurious interactions due to small sample size (n = 36, Zuur *et al.* 2009). Since the models for nitrogen content and C:N ratio showed skewed distributions of model residuals, variance was set to vary to the power of the covariate leaf mass (see description for the model selection above). Random effects were shoot nested within a tree.

To investigate the effect of the experimental treatment on LMA, we built a linear mixed effect model of LMA (as gm^{-2}) as a function of treatment, collection site, percentage of leaf damage and all two-way interactions. After carrying out model selection and examining model residuals against fitted values, we included leaf dry mass in the model as an explanatory variable to reduce heteroscedasticity of the model residuals. Shoot nested within tree was set as a random effect.

To investigate whether herbivory affected leaf size (as could be expected if trade-offs between growth and defence were present), we tested the effect of the treatment on the estimated leaf area in the absence of any damage (i.e. estimated as if the damage had not occurred, see above "Collecting leaf traits"). We built a linear mixed effect model of the estimated leaf area as a function of the experimental treatment, collection site, the percentage of leaf damage, and all possible two-way interactions. Variance structure was allowed to vary between the tree individuals to account for unequal variances between trees. Shoot nested within tree was set as a random effect.

3.2.4.3. Relationships between leaf traits and photosynthesis

To study the relationship between polyphenol chemistry and photosynthetic rate, we built a linear mixed effect model with A_{1000} as the response variable and concentration of hydrolysable tannins, diversity of hydrolysable tannins, concentration of flavonols, diversity of flavonols and the collection site as explanatory variables. Since photosynthetic rate was measured on the same leaves that were later collected for chemical analyses, we were able to build the models on leaf-level data. Total concentration of polyphenols and diversity of all polyphenols were not included in the model due to collinearity with the explanatory variables (i.e. concentrations and diversities of the two polyphenol subgroups). No interactions were included, because the possible two-way interaction combinations (interactions between the

two polyphenol groups, or between polyphenol concentration and diversity) were not biologically relevant. Random effect was shoot nested within a tree.

For each individual polyphenol compound, we investigated the correlation between its concentration and photosynthetic rate (A_{1000}), and daytime dark respiration rate ($R_{d,}$ estimated from the photosynthesis-light response curves, Visakorpi *et al.* 2018). Since leaf respiration was measured from different leaves and during a different season than leaf polyphenol content, we calculated average values for each treatment for each tree and used these tree/treatment specific averages (n = 10 per leaf-level treatment) in the analyses. We calculated Pearson's correlation coefficient for each pairwise compound-rate comparison. To visualize significant correlations, we built linear models of photosynthetic or respiration rate as a function of the concentration of the specific compound, and plotted the model predictions (Appendix 3.1, Figures S2 and S3).

To estimate the relationship with photosynthesis and LMA and photosynthesis and LA we built a linear model with A_{1000} as response variable and LMA, leaf area (as tree/treatment specific averages) and site as explanatory variables. To investigate how N and C content was related to photosynthesis, we built a separate linear model with A_{1000} as response variable and nitrogen content, carbon content and the collection site as explanatory variables as tree/treatment -specific averages. Due to collinearity, C:N ratio was not included in the model. No interactions were included in either of the models to avoid spurious interactions due to small (n = 50) sample size (Zuur *et al.*, 2009).

3.2.4.4. Leaf trait composition

To study how the individual leaf traits correlate with each other and whether they are influenced by the experimental treatments, we performed a PCA. To investigate changes in all measured leaf traits separately from changes in the polyphenol composition only, we carried out two PCAs. The first PCA included the concentration and diversity of the polyphenol

groups (all compounds, hydrolysable tannins, flavonols) and the photosynthetic (A_{1000}, A_{sat}, V_{cmax}, J_{max}, TPU, K, R_d), chemical (C, N, C:N) and physical (LMA, LA) traits. The second PCA included the concentrations of all individual polyphenols but no other traits. The first PCA was constructed using tree/treatment specific averages, whereas the second PCA was constructed using leaf-level data.

The first PCA (all traits) was carried out as an iterative PCA (Dray & Josse, 2015). This was done in order to include carbon and nitrogen content, the sample size of which was smaller than that of the other traits. In an iterative PCA missing values are replaced by random values, PCA is applied on the new data set, and the missing values are then updated using the fitted values with number of predefined dimensions. This procedure is repeated until convergence (Dray & Josse, 2015). This method has been shown to perform well on different datasets with missing values (Dray & Josse, 2015). We considered values missing at random (Rubin, 1976). Data were log-transformed, scaled and centred to improve the ordination results (Venables & Ripley, 1999). For the second PCA (individual polyphenols only), the data were logged and centred to improve the visualization of the ordination results. The data were plotted on the first five PC axis, and the resulting ordination plots were examined visually to identify clustering around treatment groups, or around the experimental trees.

To examine the statistical significance of the experimental treatment on the leaf trait composition we carried out RDAs, in which the treatment or the tree identity were set as "constraints" (Oksanen, 2015). To examine the statistical significance of the treatments while accounting for variation between the trees, we carried out a partial redundancy analysis (pRDA) where the experimental treatment was set as a constraint, and the tree identity as a condition. Partial RDA removes the variation caused by the conditioned variable, i.e. in this case the variation between the individual trees. We carried out two types of RDAs similar to the two PCAs: one for the concentration and diversity of the summed polyphenol groups (all compounds, hydrolysable tannins, flavonols), the photosynthetic (A_{1000}, A_{sat}, V_{cmax}, J_{max}, TPU,

K, R_d), chemical (C, N, C:N) and physical (LMA, LA) traits, and another for individual polyphenol compounds only. The statistical significance of the constrained variables was determined by examining the amount of constrained vs unconstrained variation revealed by the models (constrained variation referring to variation explained by the explanatory variable treatment or tree ID). The significance was tested with permutation tests (n = 999 permutations). The variation explained by the full model was then partitioned to investigate each leaf trait separately.

All analyses were conducted using R version 3.5.0 (R Core Team, 2018) and the packages vegan (PCA, RDA, variance partitioning; Oksanen *et al.*, 2017), lme4 (mixed effect models; Bates *et al.*, 2015), nlme (variance structure; Pinheiro *et al.*, 2017), missMDA (imputed PCA; Josse & Husson, 2016) and mvabund (for multivariate linear models; Wang *et al.*, 2017a).

3.2.5. Review on defensive chemistry

We carried out a literature search to summarise the current knowledge on the interactions between herbivory and the 27 polyphenol compounds detected in our study. We searched in Google Scholar and Web of Science on 25th and 26th June 2018 with search terms "*compound name*" AND herbivor*. We considered studies that had investigated the concentration of the specific compound 1) as response to experimentally inflicted herbivory, mechanical wounding or application of plant-signalling compounds known to activate anti-herbivore defences, 2) in relation to natural herbivory patterns or 3) in relation to herbivore performance. Only studies that had investigated changes in the specific compounds individually and tested the statistical significance of the studied relationships were included.

3.3. Results

In total, we recorded the concentration of 27 polyphenolic compounds in the sampled leaves. The most abundant compounds were vescalagin, cocciferin-D2 and catechin (Appendix 3.1, Table S1). The average total polyphenol content of the leaves was 39 mg g^{-1}.

3.3.1. Background herbivory

The amount of background leaf area loss was significantly different between individual study trees ($F = 9.34$, $p > 0.001$, $df = 9, 1454$). The background level of herbivory was not influenced by polyphenol chemistry ($F = 0.84$, $p = 0.40$ for concentration of hydrolysable tannins, $F = -1.37$, $p = 0.30$ for concentration of flavonols, $F = 0.01$, $p = 0.94$ for diversity of hydrolysable tannins and $F = 1.91$, $p = 0.23$ for diversity of flavonols).

3.3.2. Does experimentally applied leaf damage change chemical and physical leaf traits?

3.3.2.1. Effects of the experimental treatments and leaf area loss

For coefficient estimates for models, see Appendix 3.1, Table S2. Based on the model selection of the mixed effects models, the experimental treatments did not affect nitrogen content (Lratio = 7.83, $p = 0.10$, $df = 4, 11$, Figure 3.2a) or carbon ($\chi^2 = 0.98$, $p = 0.91$, $df = 4, 9$, Figure 3.2b), C:N ratio (Lratio= 5.73, $p = 0.22$, $df = 4, 10$, Figure 3.2c), leaf mass per area ($\chi^2 = 7.93$, $p = 0.09$, $df = 4, 10$, Figure 3.2d), leaf area (Lratio = 6.03, $p = 0.20$, $df = 4, 8$, Figure 3.2e), total polyphenol concentration ($\chi^2 = 5.44$, $p = 0.25$, $df = 4, 10$, Figure 3.2f), total polyphenol diversity (Lratio = 1.10, $p = 0.89$, $df = 4, 18$, Figure 3.2g) or diversity of flavonols (Lratio = 7.21, $p = 0.13$, $df = 4, 17$, Figure 3.2i). There were also no differences in concentrations of individual polyphenol compounds between the experimental treatments, although for most compounds, the effect was only marginally non-significant (Appendix 3.1, Figure S1, Table S3).

Experimental treatment had significantly different impacts on hydrolysable tannin concentration between the different collection dates and the two sites, as indicated by retention of the interaction terms (treatment : date, Lratio = 39.9, $p < 0.001$, df = 4, 27; treatment : site, Lratio = 32.6, $p < 0.001$, df = 4, 27). The effect of treatment on the concentration of flavonols was also affected by collection date (treatment : date, Lratio = 19.0, $p < 0.001$, df = 4, 27) and site (treatment : site, Lratio = 21.5, p = 0.006, df = 27, 8). The effect of the treatment on the diversity of hydrolysable tannins was influenced by the collection date (treatment : date, Lratio = 12.4, p = 0.01, df = 22, 4).

The proportion of leaf area loss had a positive effect on LMA ($\chi^2 = 5.06$, p = 0.02, df = 1, 6) but no effect on leaf area (Lratio = 2.32, p = 0.13, df = 1, 5).

3.3.2.2. Differences in leaf traits between sites and collection day

Trees in Wytham Woods had higher nitrogen content (Lratio = 4.18, p = 0.04, df = 1, 7) and higher total polyphenol concentration ($\chi^2 = 4.06$, p = 0.04, df = 1, 6) compared to trees at the John Krebs field station (Appendix 3.1, Table S2). Concentrations of many individual polyphenol compounds differed between the two sites (Appendix 3.1, Table S3).

There was no difference between the two sites in leaf carbon content ($\chi^2 = 2.10$, p = 0.15, df = 1, 6), C:N ratio (Lratio = 0.95, p = 0.33, df = 1,7), hydrolysable tannin concentration (Lratio = 1.72, p = 0.19, df = 1, 10), total polyphenol diversity (Lratio = 0.04, p = 0.83, df = 1, 15), hydrolysable tannin diversity (Lratio = 0.003, p = 0.96, df = 1, 23), flavonol diversity (Lratio = 0.63, p = 0.43, df = 1, 14), LMA ($\chi^2 = 2.16$, p = 0.14, df = 1, 7) or leaf area (Lratio = 2.32, p = 0.13, df = 1, 5).

There was a negative association between collection date and the total concentrations of polyphenols ($\chi^2 = 5.81$, p = 0.02, df = 1, 6) and a positive association between collection date and the diversity of all polyphenols (Lratio = 11.02, $p < 0.001$, df = 1, 14, Appendix 3.1, Table S2). All individual polyphenol compounds were affected by the collection date

(Appendix 3.1, Table S3). Collection date did not affect diversity of flavonols (Lratio = 1.31, p = 0.25, df = 1, 14).

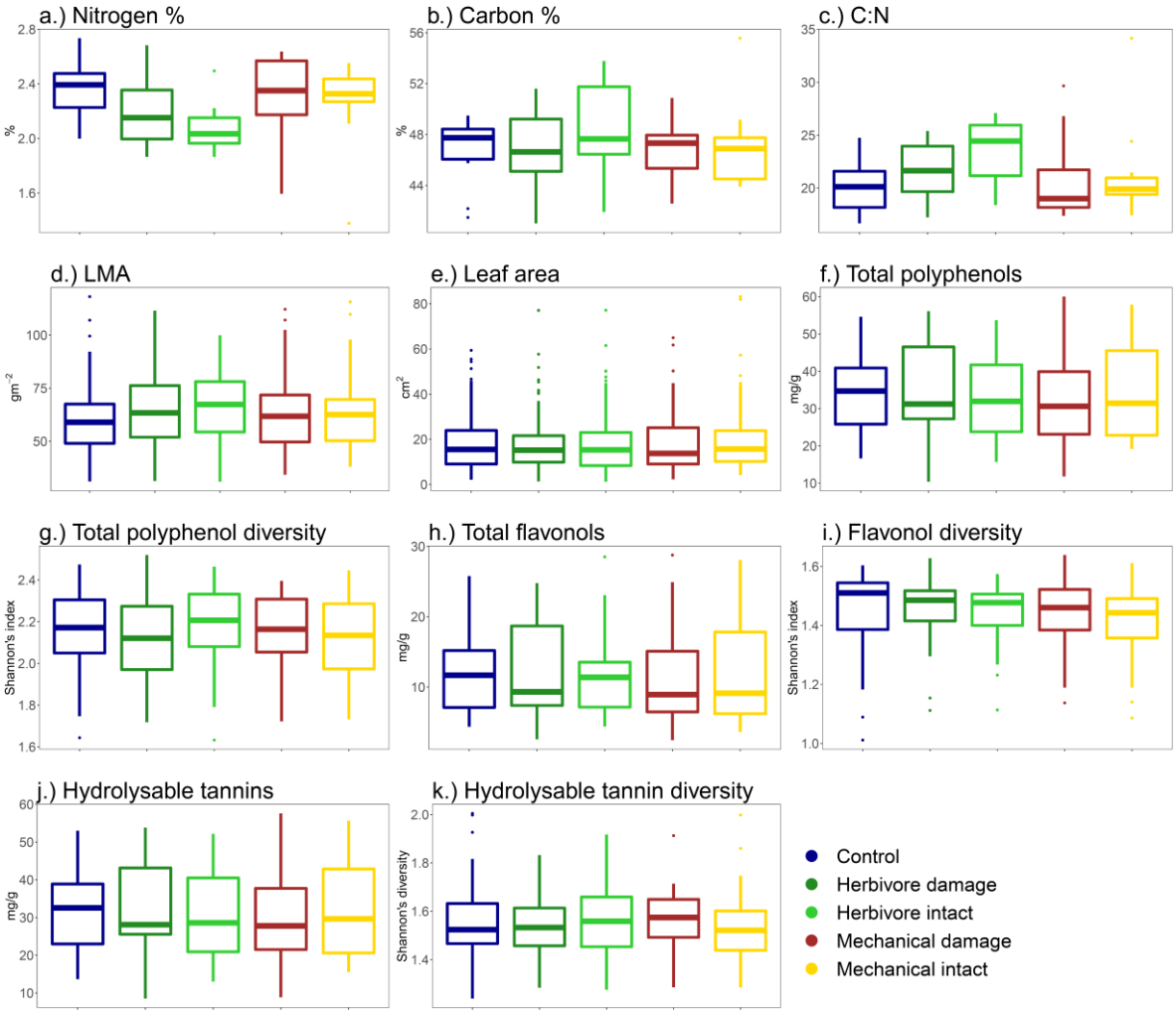


Figure 3.2. The effects of experimental treatment on the studied leaf traits. Panel a) shows nitrogen (N) concentration, b) carbon (C) content, c) C:N ratio, d) leaf mass per area (LMA), e) leaf area (LA), f) total concentration of polyphenols, g) Shannon's diversity for all polyphenols, h) total concentrations of flavonols, i) Shannon's diversity for flavonols, j) total concentrations of hydrolysable tannins and k) Shannon's diversity of hydrolysable tannins. Note that for h) and j) the interactions between treatment and collection date, and treatment and site are significant, and for k) the interaction between treatment and collection day is significant. The line at the middle of the box shows the median, the lower and upper hinges of the box show the 25th to 75th percentile. Data points outside the whiskers are determined outliers.

Table 3.1. Summary of the investigated leaf traits, their mean values across all samples and for each leaf-level treatment separately, and the total number of samples used to obtain values for each trait. The photosynthetic parameters (A_{1000} to TPU) were obtained from Visakorpi *et al.* (2018), the other traits were collected for this study. See Appendix 3.1 Table S1 for concentrations of individual polyphenol compounds. The errors are ± 1 SEM.

Leaf trait	Description	Total mean	Control	Herbivore damage	Herbivore intact	Mechanical damage	Mechanical intact	n
A_{1000}	Photosynthetic rate in 1000 PAR, µmol CO_2 m^{-2} s^{-1}	11.6 ± 0.5	10.8 ± 1.4	8.34 ± 1.2	7.10 ± 0.9	11.6 ± 1.2	9.22 ± 1.1	100
A_{sat}	Light-saturated photosynthetic rate, µmol CO_2 m^{-2} s^{-1}	15.3 ± 0.9	19.8 ± 2.2	10.8 ± 1.6	12.5 ± 1.9	17.6 ± 1.7	16.0 ± 1.5	49
K	Light intensity where photosynthetic rate is half of its maximum, µmol m^{-2} s^{-1}	168 ± 12	197 ± 29	131 ± 25	130 ± 24	189 ± 21	191 ± 28	48
R_d	Dark respiration rate, µmol CO_2 m^{-2} s^{-1}	-0.46 ± 0.1	-0.41 ± 0.1	-0.45 ± 0.1	-0.54 ± 0.1	-0.46 ± 0.1	-0.45 ± 0.1	49
V_{cmax}	Carboxylation efficiency, µmol CO_2 m^{-2} s^{-1}	70.1 ± 4.3	80 ± 9.3	65.4 ± 9.6	56.9 ± 6.4	84.1 ± 10	67.3 ± 12	65
J_{max}	Electron transport efficiency, µmol CO_2 m^{-2} s^{-1}	158.1 ± 11	197 ± 22	147 ± 22	122 ± 16	188 ± 27	146 ± 32	65
TPU	Triose phosphate use efficiency, µmol CO_2 m^{-2} s^{-1}	9.76 ± 0.6	11.7 ± 1.2	8.93 ± 1.1	8.23 ± 0.9	11.4 ± 1.5	8.89 ± 1.7	65
LMA	Leaf mass per area, gm^{-2}	63.2 ± 0.6	60.4 ± 1.2	64.4 ± 1.2	66.4 ± 1.5	62.3 ± 1.4	62.5 ± 1.6	616
LA	Estimated original leaf area, cm^2	18.1 ± 0.5	17.9 ± 1.0	17.7 ± 1.0	18.0 ± 1.3	18.0 ± 1.1	19.3 ± 1.6	616
N	Nitrogen content, %	2.24 ± 0.04	2.34 ± 0.07	2.19 ± 0.08	2.09 ± 0.08	2.30 ± 0.09	2.26 ± 0.10	53
C	Carbon content, %	47.1 ± 0.4	46.7 ± 0.9	46.8 ± 0.9	48.5 ± 1.6	46.8 ± 0.63	47.0 ± 1.0	53
Total phenols	Total concentration of all phenolic compounds, mg/g	34.0 ± 1.1	34.1 ± 2.3	35.4 ± 2.3	32.9 ± 2.4	32.6 ± 2.3	34.9 ± 2.8	119
Phenol diversity	Shannon's diversity index for all phenolic compounds	2.14 ± 0.02	2.14 ± 0.1	2.12 ± 0.04	2.16 ± 0.1	2.16 ± 0.04	2.11 ± 0.04	119
Total HT	Total concentration of hydrolysable tannins, mg/g	24.4 ± 1.2	24.2 ± 2.7	26.1 ± 2.5	22.4 ± 3.0	23.8 ± 2.41	25.1 ± 3.07	119
HT diversity	Shannon's diversity index for hydrolysable tannins	1.56 ± 0.01	1.58 ± 0.04	1.55 ± 0.03	1.59 ± 0.04	1.56 ± 0.03	1.54 ± 0.03	119
Total FL	Total concentration of flavonols, mg/g	5.16 ± 0.2	5.08 ± 0.4	5.26 ± 0.6	5.85 ± 0.5	4.46 ± 0.3	5.32 ± 0.7	119
FL diversity	Shannon's diversity index for flavonols tannins	1.43 ± 0.01	1.44 ± 0.03	1.45 ± 0.02	1.44 ± 0.03	1.44 ± 0.03	1.40 ± 0.03	119

3.3.3. Are changes in photosynthesis linked to parallel changes in leaf traits?

3.3.3.1. Relationships between the different leaf traits

Photosynthetic rate was positively related to leaf nitrogen content ($F = 8.16$, $p = 0.007$, $df = 1, 32$, Figure 3.3a, Appendix 3.1, Table S2). There was no significant relationship between photosynthesis and carbon content ($F = 0.03$, $p = 0.87$, $df = 1, 31$, Figure 3.3b), LMA ($F = 3.53$, $p = 0.07$, $df = 1, 42$, Figure 3.3c), leaf area ($F = 0.10$, $p = 0.75$, $df = 1, 42$, Figure 3.3d), flavonol concentration ($\chi^2 = 3.38$, $p = 0.07$, $df = 1, 6$, Figure 3.3e), flavonol diversity ($\chi^2 = 0.13$, $p = 0.71$, $df = 1, 6$, Figure 3.3f), hydrolysable tannin concentration ($\chi^2 = 0.06$, $p = 0.81$, $df = 1, 6$, Figure 3.3g) or hydrolysable tannin diversity ($\chi^2 = 0.48$, $p = 0.49$, $df = 1, 6$, Figure 3.3h).

Of the individual polyphenols, four hydrolysable tannins (cocciferin D2, vescavaloninic acid, tellimagrandin 1, galloly- HHDP-glucose) and one flavonol (quercetin diglycocide) correlated positively with photosynthesis (Appendix 3.1, Figure S2). Cholorogenic acid correlated negatively with photosynthesis (Figure S2). Three hydrolyzable tannins (monogalloylglucose, vescalagin, castalagin) correlated negatively with leaf respiration rate (measured at the tree level), wheres two flavonols (quercetin glucuronide, kaempferol diglycoside) correlated positively with respiration rate (Appendix 3.1, Figure S3).

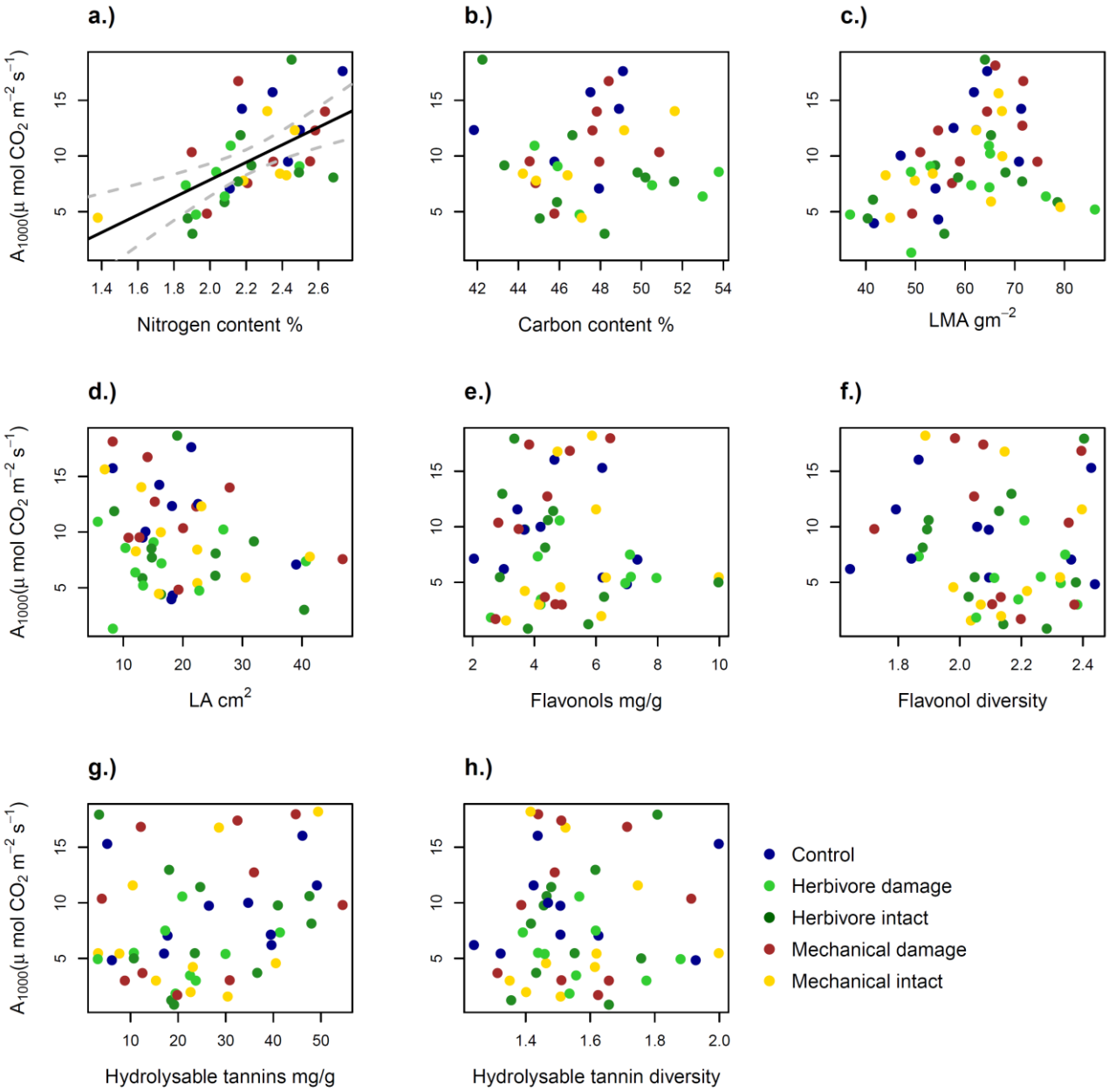


Figure 3.3. The relationship between photosynthetic rate (A_{1000}) and a) nitrogen (N) concentration b) carbon (C) content, c) leaf mass per area (LMA) d) leaf area (LA), e) total concentration of all flavonols, f) Shannon's diversity for flavonols, g) total concentrations of hydrolysable tannins and h) Shannon's diversity of hydrolysable tannins in the different experimental treatments. Note that for panels a-d the relationship represents values *per tree*, because photosynthetic rate and nutrient content have been measured from different leaves. Only the relationship in panel a) is significant.

3.3.3.2. Differences in leaf trait composition

There were no differences in the distribution of the studied leaf traits (concentration and diversity of polyphenol groups, photosynthetic parameters, C and N content and physical leaf traits) between the treatments ($F = 1.18$, $p = 0.26$, $df = 4, 45$, Figure 3.4a). Tree identity had a significant effect on leaf trait composition ($F = 5.01$, $p = 0.001$, $df = 9, 40$, Figure 3.4b). The two PCA axes were mainly determined by the leaf nutrients and photosynthetic parameters (PC1, Figure 3.4c, Appendix 3.1, Figure S4a) and the summed metrics for polyphenol compounds (PC2, Figure 3.4c, Appendix 3.1, Figure S4b).

Similarly, in the second PCA (concentrations on individual compounds only), the polyphenol profile of plants (i.e. the overall composition of polyphenol compounds as assessed using PCA) did not differ between the treatments ($F = 0.36$, $p = 0.99$, $df = 4, 114$, Figure 3.4d), but was significantly affected by the tree identity ($F = 24.62$, $p = 0.001$, $df = 9, 109$, Figure 3.4e). The first PC axis was influeced by the concentrations of the two most abundant compounds, vescavaloninic acid and cocciferin D2 (PC1, Appendix 3.1, Figure S4c). The largest portion of variation in all traits was explained by the variation between individual trees (Figure 3.5). For compounds that were found in low concentrations (Appendix 3.1, Table S1), a high proportion of the variation was unexplained (Figure 3.5).

After accounting for the variation between the study trees, the partial RDA revealed differences between the treatments in the compostion of traits other than individual polyphenols (Appendix 3.1, Figure S5a, $F = 2.28$, $p = 0.004$, $df = 4, 36$). On the first RD-axis, the leaves in the herbivory treatment (both intact and damaged) differed from rest of the leaves (control and mechnical treatment, Appendix 3.1, Figure S5a). The first axis was mainly influenced by the different photosynthetic parameters (Appendix 3.1, Figure S5b). The polyphenol composition was not different between the treatments even after accounting for differences between the trees ($F = 0.97$, $p = 0.47$ $df = 4, 105$).

The concentration of polyphenols correlated negatively with the diversity of polyphenols (Pearson's $r = -0.67$, $t = -9.81$, $df = 117$, $p < 0.001$, Figure 4c, Appendix 3.1, Figure S6a), and with the size of the leaf (Pearson's $r = -0.47$, $t = -3.66$, $df = 46$, $p < 0.001$, Figure 4c, Appendix 3.1, Figure S6).

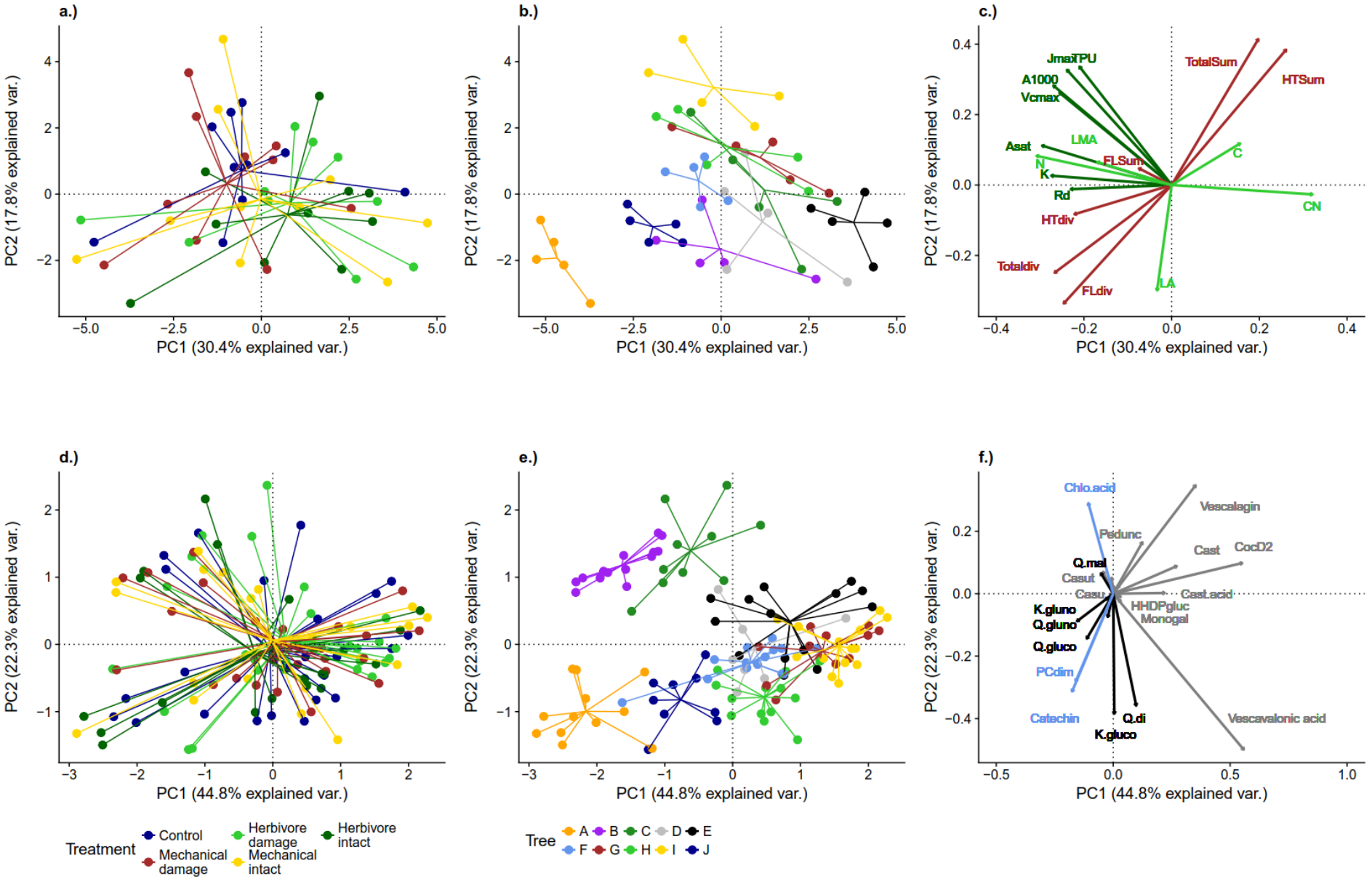

a.)
b.)
c.)
d.)
e.)
f.)
PC1 (30.4% explained var.)
PC2 (17.8% explained var.)
PC1 (44.8% explained var.)
PC2 (22.3% explained var.)
JmaxTPU
A1000
Vcmax
LMA
Asat
N
K
Rd
HTdiv
Totaldiv
FLdiv
TotalSum
HTSum
C
FLSum
CN
LA
Chlo.acid
Vescalagin
Pedunc
Cast
CocD2
Q.mal
Casut
Casu
Cast.acid
HHDPgluc
Monogal
K.gluno
Q.gluno
Q.gluco
PCdim
Catechin
Q.di
K.gluco
Vescavalonic acid
Treatment
Control
Mechanical damage
Herbivore damage
Mechanical intact
Herbivore intact
Tree
A B C D E
F G H I J

Figure 3.4. Results from PCA analyses on 1) studied leaf traits (excluding the concentrations of individual polyphenol compounds; panels a-c, see Table 3.1), and 2) polyphenol composition based on individual compounds (panels d-f, see Appendix 3.1, Table S1). Results are grouped either by experimental treatment (panels a and d) or by study tree (panels b and e). Data points in panels a-b represent averages per tree and treatment and use imputed values for missing values of nitrogen and carbon content. In panels d-e each data point is one leaf, with no missing values. Panels c) and f) show the relationships between different leaf traits. In panel c) dark green arrows represent photosynthetic parameters, brown arrows represent defensive traits, and light green arrows other traits. In panel f) black arrows show hydrolysable tannins, grey arrows show flavonols and blue arrows show other chemical compounds (see Table 3.2 and Appendix 3.1, Table S1). Note that respiration rate has been made positive to make the graph more intuitive (i.e. so that larger respiration values correspond to higher respiration rate).

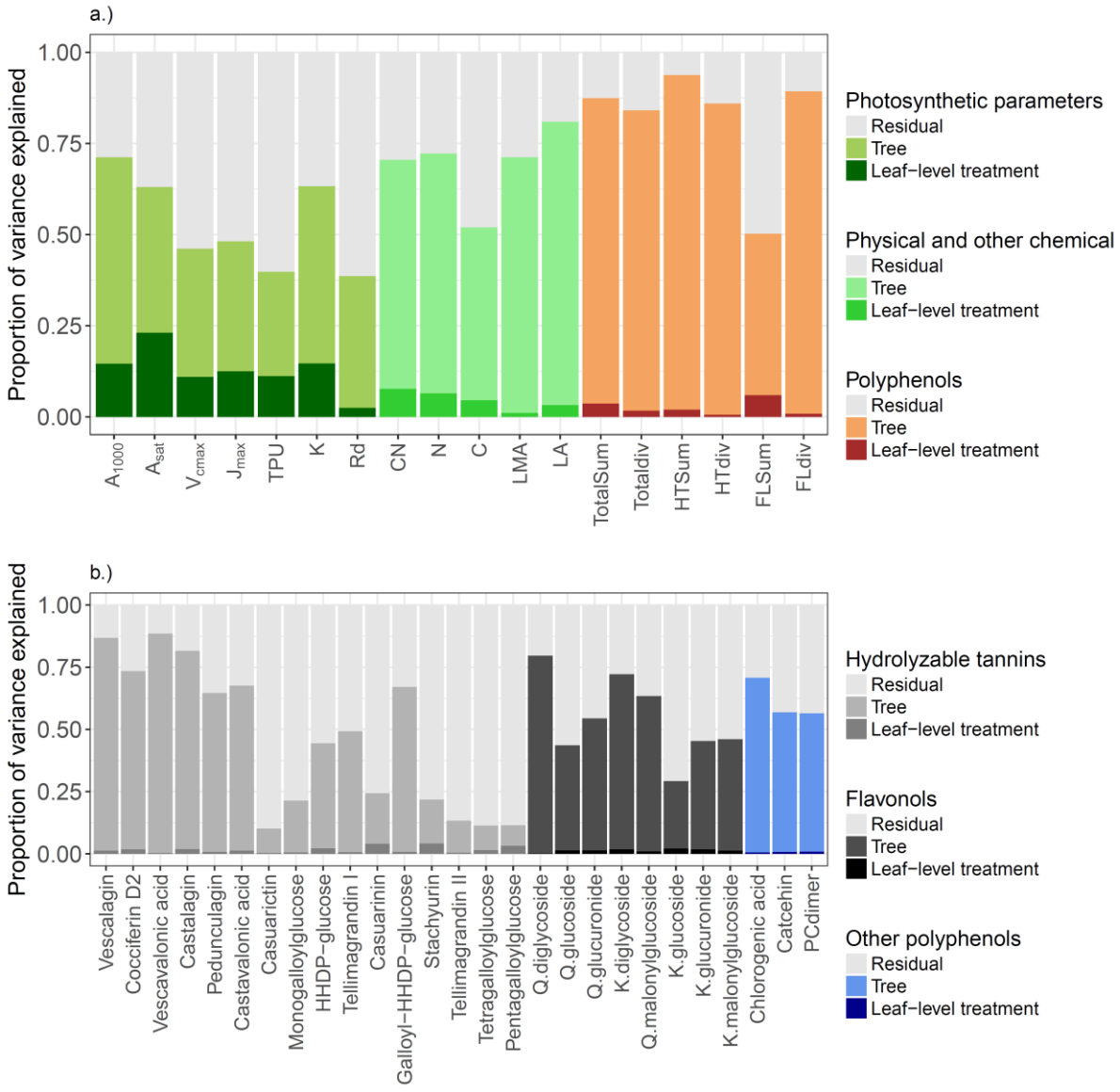


Figure 3.5. The proportion of variance in each leaf trait explained by the experimental treatment (dark colours), tree (light colours), and the unexplained variation (light grey bars) based on partitioned variance from the partial RDA analysis. Panel a.) shows photosynthetic traits (dark green), physical and non-defensive chemical traits (lighter green) and concentrations and diversities of all polyphenols and the two largest polyphenol groups (hydrolysable tannins and flavonols, orange). Panel b.) shows variation in individual polyphenols, grouped as hydrolysable tannins (light greys), flavonols (dark greys) and other compounds (blues). Within each group, the compounds are shown in order of decreasing abundance.

3.3.4. Does literature provide evidence for polyphenols acting as anti-herbivore defences?

For most compounds, we found only handful of studies reporting the relationship between the compound and herbivory. For these compounds, we report the results from all studies found in the literature search. For chlorogenic acid there were considerably more studies (n = 99). Thus, for this compound, we report only a few examples of the effects reported in the literature. For five compounds, we found no studies testing their relationship with herbivory. Thus, to our knowledge, for these compounds (casuarinin, galloyl-HHDP-glucose-1, stachyurin, quercetin malonylglucoside, kaempferol malonylglucoside) our study is the first to test the relationship between herbivory and their concentration.

Table 3.2. Summary table of all the phenolic compounds and documented relationships with herbivory from the literature search.

Compound	Herbivory related effects	References
Hydrolysable tannins		
Vescalagin	Increase with simulated herbivory (1). Feeding deterrent (2). Positive correlation with leafminer abundance (3). No change after psyllid feeding (4).	1. Moctezuma *et al.* 2014 2. Roslin & Salminen 2008 3. Yarnes *et al.* 2008 4. Patton *et al.* 2018
Cocciferin D2	Positive correlation with leafminers (1). No correlation with herbivory (2)	1. Yarnes *et al.* 2008 2. Moctezuma *et al.* 2014
Vescavaloninic acid	No correlation with herbivory.	Moctezuma *et al.* 2014
Castalagin	Negative correlation with leaf miners (1). No correlation with herbivory (2).	1. Yarnes *et al.* 2008 2. Moctezuma *et al.* 2014
Pedunculagin 1	Negative correlation with leaf miners (1). Increased metabolic cost for herbivores, but no effect on herbivore performance (2). Positive correlation with larval survival and growth (3). Marginally non-significant increase after herbivory (4).	1. Yarnes *et al.* 2008 2. Barbehenn *et al.* 2009 3. Haviola *et al.* 2007 4. Ruuhola *et al.* 2013
Castavaloninic acid	Negative correlation with leafminers.	Yarnes *et al.* 2008
Casuarictin	Negative correlation with herbivore weight (1). Increase after herbivory (2, 3).	1. Ruuhola *et al.* 2007 2. Ruuhola *et al.* 2013 3. Ossipov *et al.* 2014

Monogalloylglucose	No correlation with herbivory (1). Both positive and negative correlation with herbivory, depending on the level of previous herbivory (2). No correlation with winter moth caterpillar growth (4). No change after herbivory (3, 5).	1. Ruuhola *et al.* 2007 2. Lempa et al. 2004 3. Patton *et al.* 2018 4. Tikkanen & Julkunen-Tiitto 2003 5. Ruuhola *et al.* 2013
HHDP-glucose	No correlation with herbivory (1). No change after psyllid feeding (2).	1. Moctezuma *et al.* 2014 2. Patton *et al.* 2018
Tellimagrandin 1	No effect on herbivores (1). No change after psyllid feeding (2). Increase after herbivory (3, 4).	1. Haviola *et al.* 2007 2. Patton *et al.* 2018 3. Ruuhola *et al.* 2013 4. Ossipov *et al.* 2014
Casuarinin	No previous data	
Galloyl-HHDP-glucose 1	No previous data	
Stachyurin	No previous data	
Tellimagrandin II	No change after psyllid feeding (1). Increase after herbivory (2).	1. Patton *et al.* 2018 2. Ruuhola *et al.* 2013
Tetragalloylglucose	Negative effect on herbivore encapsulation (1). Lowered leaf consumption of early instars, no effect on late instars, no effect on herbivore performance (2). No change after psyllid feeding (3). Increase after herbivory (4).	1. Haviola *et al.* 2007 2. Salminen & Lempa 2002 3. Patton *et al.* 2018 4. Ruuhola *et al.* 2013
Pentagalloylglucose	Positive correlation with larval weight (1). Lowered leaf consumption of early instars, no effect on late instars, no effect on herbivore performance (2). Increase after psyllid feeding (3).	1. Haviola *et al.* 2007 2. Salminen & Lempa 2002 3. Patton *et al.* 2018
Flavonols		
Quercetin glucoside	No correlation with herbivory (1). Increase after psyllid feeding (2). No correlation with herbivore performance (4, 8). Not affected by oviposition or herbivory (5), by artificial defoliation (3), by bark beetle attack (6) or by MeJa -treatment (7).	1. Moctezuma *et al.* 2014 2. Patton *et al.* 2018 3. Keinänen *et al.* 1999 4. Tikkanen & Julkunen-Tiitto 2003 5. Beyaert *et al.* 2012 6. Schiebe *et al.* 2012 7. Ruiz-Garcia *et al.* 2013 8. Lehrman *et al.* 2012
Quercetin glucuronide	Negative correlation with beetle feeding (1). No correlation with herbivory (2). No change after psyllid feeding (3), artificial defoliation (4) or MeJa -treatment (5).	1. Johnson *et al.* 2009 2. Moctezuma *et al.* 2014 3. Patton *et al.* 2018 4. Keinänen *et al.* 1999 5. Ruiz-Garcia *et al.* 2013
Kaempferol glucoside	No correlation with herbivory (1). Increased resistance to thrips (2). No effect on herbivores (3, 5). Not induced by spruce bark beetle attack (4).	1. Moctezuma *et al.* 2014 2. Leiss et al. 2009b 3. Tikkanen & Julkunen-Tiitto 2003 4. Schiebe *et al.* 2012

		5. Lehrman *et al.* 2012
Quercetin diglycoside	No change after herbivory (1). No effect on beetle oviposition or survival (2).	1. O'Neil *et al.* 2010 2. Torp *et al.* 2013
Kaempferol glucuronide	No correlation with herbivory (1). No effect of artificial defoliation (2).	1. Johnson *et al.* 2009 2. Keinänen *et al.* 1999
Quercetin malonylglucoside	No previous data	
Kaempferol malonylglucoside	No previous data	
Kaempferol diglycoside	Increase after beetle damage (1). No change after caterpillar damage (1). No effect on herbivores (2, 3).	1. O'Neil *et al.* 2010 2. Torp *et al.* 2013 3. Lehrman *et al.* 2012
Other polyphenols		
PC dimer	Induced in phloem by simulated bark beetle attack (1). Decrease in phloem after mechanical wounding (2).	1. Mason *et al.* 2017 2. Muilenburg *et al.* 2013
Chlorogenic acid	Induced by herbivory (4, 11, 12), decrease after herbivory (2), no change after herbivory (9), no effect on herbivores (1, 7, 8, 13), negative effects on herbivores (1, 6), feeding deterrent (30), feeding stimulant (3, 10), increased resistance to herbivores (5).	1. Pascacio-Villafán *et al.* 2014 2. Kirakosyan *et al.* 2004 3. Fulcher *et al.* 1998 4. Poveda *et al.* 2012 5. Leiss *et al.* 2009 6. Felton *et al.* 1992 7. Ruuhola *et al.* 2007 8. Torp *et al.* 2013 9. Patton *et al.* 2018 10. Lempa *et al.* 2004 11. Zhang *et al.* 2018 12. Kessler & Baldwin 2004 13. Lehrman *et al.* 2012
Catechin	Induced by herbivory (3, 4, 7, 8, 9, 11, 15), decrease after herbivory or simulated herbivory (1, 10, 12, 18, 20), not affected by herbivory (16, 17), negative effects on herbivores (2, 6), positive effects on herbivores (6, 19), no effect on herbivores (14), feeding deterrent (11, 13), positive correlation with natural herbivory (1), increased resistance to herbivores (5).	1. Moctezuma *et al.* 2014 2. Ruuhola *et al*. 2007 3. Zhang *et al.* 2018 4. Bouklikha *et al.* 2018 5. Duan *et al.* 2014 6. Pascacio-Villafán *et al.* 2014 7. Rani *et al.* 2013 8. Roitto *et al.* 2009 9. Thelen *et al.* 2005 10. Kirakosyan *et al.* 2004 11. Berg 2003 12. Keinänen *et al.* 1999 13. Fulcher *et al.* 1998 14. Tikkanen & and Julkunen-Tiitto 2003 15. Ossipov *et al.* 2015 16. Beyaert *et al.* 2012 17. Schiebe *et al.* 2012

18. Ruiz-Garcia *et al.* 2013
19. Lehrman *et al.* 2012
20. Muilenburg *et al.* 2013

3.4. Discussion

In this study, we tested whether experimentally manipulated herbivory and mechanical leaf damage affect a number of chemical and structural leaf traits, and whether these traits correlate with rates of photosynthesis and respiration. We measured concentrations of 27 polyphenol compounds, leaf nitrogen and carbon content, leaf mass per area and leaf area. We also surveyed the literature on previously recorded relationships between herbivory and the individual polyphenol compounds measured in this study. To our knowledge, this is the first experimental study to test the effects of herbivory and mechanical leaf damage on several individual polyphenol compounds.

In our experiment, we detected no differences in the concentrations of individual polyphenol compounds, the overall polyphenol composition, nitrogen or carbon content, leaf mass per area or leaf area between treatments. Of the leaf physiological traits, only the photosynthetic parameters differed between the treatments, reflecting earlier results reported by Visakorpi *et al.* (2018). While we detected some effects of the experimental treatments on the overall concentration and diversity of hydrolysable tannins and the concentration of flavonols, these varied in time (depending on the timing of leaf collection for phytochemical analyses) and between the two study sites. Leaf nitrogen content and six individual polyphenol compounds correlated with photosynthesis. Different trees showed large variation in their trait composition, especially in terms of their polyphenol composition. The literature survey revealed the varying nature of the relationship between herbivory and plant polyphenol concentration: most compounds have been observed to function both as defences and

attractants or to have no relationship to herbivory at all. Below, we discuss each of these results in detail.

3.4.1. Leaf traits differed between trees, and the effect of treatment was different between the two sites

We predicted that herbivory and/or mechanical damage would result in changes in polyphenol concentration, diversity or composition, to larger leaf mass per area and/or result in leaves growing smaller. However, there were no clear differences in the studied leaf traits between experimental treatments. Experimental treatments had a significant effect on hydrolysable tannin and flavonol concentrations, but this effect was different depending in the study site and collection date (i.e. a significant site : treatment and date : treatment interactions). There were large differences in the trait composition between study trees and between the two study sites.

To investigate further how the effect of treatment and date on polyphenol concentration differed between the two sites, we analysed the effect of treatment on polyphenol chemistry for the two sites separately. Site-specific linear mixed effects models revealed that the effect of treatment differed between the collection days only at the canopy walkway for both flavonols and hydrolysable tannins. By the field station, treatment alone had a significant effect on hydrolysable tannin concentration: the intact leaves in herbivory treatment had a significantly lower hydrolysable tannin concentration that the damaged leaf in the same treatment (on average 14.5 mg g^{-1} compared to 20.4 mg g^{-1}). See Appendix 3.2 for further details on the models and results.

Previous studies have found increased concentrations of hydrolysable tannins after insect defoliation on oak (Schultz & Baldwin, 1982; Elderd *et al.*, 2013), but also no changes in plant chemistry after herbivory (Lempa *et al.*, 2004; Beyaert *et al.*, 2012), and high variation in plant chemical composition between individuals of the same species (Schultz *et*

al., 1982; Hwang & Lindroth, 1997; Osier *et al.*, 2000; Elderd *et al.*, 2013; Kazakou *et al.*, 2014; Siefert *et al.*, 2015). Some of this discrepancy could be due to variation in environmental conditions. The abiotic conditions associated with the growth location of the plant can directly affect plant chemistry (Koricheva *et al.*, 1998), and indirectly modify how effective the compounds are against herbivores. For example, the concentrations of specific compounds and/or their effectiveness as an antiherbivore defence can depend on ambient temperatures (Stamp & Osier, 1998; Top *et al.*, 2017) or soil nutrient content (Hunter & Schultz, 1995; Sampaio *et al.*, 2016). Local differences in the amount of shade and far-red radiation reflected by green plant tissue (as a proxy for competition from the surrounding vegetation) can force the plant to prioritize growth over defence and subsequently reduce the concentrations of defensive compounds (Henriksson *et al.*, 2003; Izaguirre *et al.*, 2006; de Vries *et al.*, 2017). Differences in local abiotic conditions can also affect plant chemical composition if these chemicals are used for example as protection from intense light (Fischbach *et al.*, 1999; Agati *et al.*, 2013) and temperature (Tattini *et al.*, 2015), or as defences against fungal pathogens (Bennett & Wallsgrove, 1994; Kang *et al.*, 2018) and competing plants (Li *et al.*, 2010). For example, flavonols can protect leaf tissue from excess UV-radiation, and are thus often found in higher concentrations in plant parts which receive more sunlight (Fischbach *et al.*, 1999).

There are several factors that differed between the two study sites where our experiments were carried out, which might explain the observed variation in polyphenol chemistry. The trees measured at the canopy walkway (site "Wytham Woods") were old (150-200 years) and the leaves collected from them were upper canopy sun leaves with higher nitrogen content. The trees measured by the field station (site "John Krebs field station") were young (33 years), planted, and the leaves collected from them came from the lower branches, though still exposed to the sun. Within the site by the field station, three of the trees (A-C, see Figure 3be) experienced intense sunlight for several hours during the day, whereas two of the

trees (D and E, see Figure 3be) were shaded during the warmest times of the day. These differences in growth locations most likely contributed to the differences observed in leaf traits and chemical composition. We found no relationship with the overall level of herbivory on the trees and their polyphenol concentration, so the differences between sites and trees were most likely not affected by the level of background herbivory.

We found that the effects of the treatments on the concentration and diversity of hydrolysable tannins and the concentration of flavonols also depended on the timing of the collection of leaf samples. Since assessing seasonal changes in leaf chemistry was not one of our study questions (and since we did not want to cause additional disturbance to the trees until all physiological leaf measurements had been taken), leaf samples were not collected evenly over the growing season. The unequal spread of the samples over the season limits our ability to investigate the interactive effects between herbivory and the collection date. As a "post-hoc" analysis, we built models separately for leaves collected at the end of the season (on 12th August), and for leaves collected throughout the season (before 12th August, see Appendix 3.2 for details). The effect of the experimental treatment on hydrolysable tannin concentration was significant only for leaves collected at the end of the season. The seasonal background variation in oak leaf chemistry (Feeny, 1970; Salminen *et al.*, 2004) might have altered how the treatments were affecting the measured chemical traits. If for example the concentration of hydrolysable tannins was changing over the period of leaf sample collection, the differences in their concentration between the treatments might be less visible on leaves collected at different parts of the season. It is also possible that the time lag between when herbivory took place (in May) and when the leaf samples were collected (July-August) was influencing the effect of the treatments on leaf chemistry: if herbivory induced only short-lived changes in the concentrations of some of the polyphenols, these changes might not be visible on leaves collected later in the season.

3.4.2. Correlation between leaf gas exchange and leaf traits

We found that nitrogen was positively related to photosynthesis, that five different polyphenols (cocciferin D2, vescavaloninic acid, quercetin diglycoside, tellimagrandin 1, galloyl-HHDP-glucose) correlated positively with photosynthesis, and that chlorogenic acid correlated negatively with photosynthesis. A positive relationship between nitrogen and photosynthesis is commonly observed because many of the enzymes needed for carbon-fixing (e.g. ribulose 1,5-bisphosphate) contain a large portion of the leaf nitrogen (Nykänen & Koricheva, 2004). Two compounds (quercetin glucuronide, kaempferol diglycoside) correlated positively with leaf respiration rate, and three compounds (vescalagin, castalagin, monogalloylglucose) negatively. Of the correlations between the individual polyphenol compounds and photosynthesis and polyphenols and respiration (n = 54 correlations), 11 (20%) were significant. With the false-positive rate of 5%, most of the significant correlations were truly positive.

Based on resource trade-off hypotheses (Herms & Mattson, 1992; Züst & Agrawal, 2017), we expected to find a negative correlation between photosynthetic rate and defensive chemistry. Defence-related signalling molecules can suppress photosynthesis-related pathways (Nabity *et al.*, 2013) and downregulate photosynthesis-related genes (Bilgin *et al.*, 2010). Nevertheless, in our study, most individual compounds and their summed metrics did not correlate with photosynthesis, and of the significant correlations, all but one were positive. Positive correlations between photosynthesis and leaf chemicals might not be surprising, if photosynthetic products are needed for the formation of secondary metabolites (Koricheva *et al.*, 1998; Schwachtje & Baldwin, 2008). The concentration of many secondary metabolites, including hydrolysable tannins, depend at least partly on the available carbohydrate pool, which in turn depends on photosynthetic rate (Koricheva *et al.*, 1998; Lempa *et al.*, 2004). In addition, the relationship between photosynthesis and defensive compounds can change depending on the surrounding biotic or abiotic conditions. For example, Lempa *et al.* (2004)

found that on trees with low herbivory pressure, photosynthetic rate and concentration of monogalloylglucose were positively correlated with leaf consumption, whereas on defoliated trees the trend was opposite. Thus, monogalloylglucose acted as a deterrent only on trees with higher levels of previous herbivory damage. Leaves with a high photosynthetic rates may be better protected, but also more able to compensate for the lost leaf area (Lempa *et al.*, 2004). We did not observe changes in the concentrations of the individual compounds, but it might have been possible that their function had similarly shifted from attractant to deterrent.

Some other interesting correlations between the measured leaf traits emerged from the PC analyses: the concentration of polyphenols, especially hydrolysable tannins, correlated negatively with the diversity of same compounds, and leaf area correlated negatively with summed concentration of polyphenols. The negative correlation between polyphenol concentration and diversity could be reflecting a resource trade-off in polyphenol production: it might be costly to produce many different compounds in high concentration simultaneously. Consequently, different plant individuals might be allocating resources to polyphenol production differently. The negative correlation between leaf size and polyphenol concentration might be a sign of the resource allocation trade-off between growth and defence we expected but failed to observe between photosynthesis and polyphenol concentrations.

3.4.3. Varying relationships between herbivory and polyphenols in the literature

For many of the polyphenol compounds detected in this study, the literature includes studies demonstrating positive, negative, or no effects on herbivore performance or an increase, decrease or no change in concentration following herbivory (Table 3.2). For example, in previous studies, the flavonols quercetin glucoside and quercetin glucuronide did not respond to herbivory, herbivore oviposition, artificial defoliation or application plant activator methyl jasmonate and the hydrolysable tannin pentagalloylglucose correlated positively with herbivore weight but negatively with leaf consumption rate (Table 3.2).

The outcome of a plant-herbivore interaction can be extremely specific depending on the species or genotype of the two interacting parties (Roslin & Salminen, 2008; Smilanich *et al.*, 2016). In many cases, the effects of potential defensive compounds on herbivores depend also on which other chemicals are present and in what quantities (Stamp & Osier, 1998; Barbehenn & Kochmanski, 2013; Pascacio-Villafán *et al.*, 2014). For example, catechin has been shown to have a positive effect on herbivore weight when alone, but negative when mixed with chlorogenic acid (Pascacio-Villafán *et al.*, 2014). These synergic or antagonistic effects are thought to be common (Nelson & Kursar, 1999; Barbehenn & Kochmanski, 2013), but so far there has been only few experiments testing combinations of different compounds (Stamp & Osier, 1998; Steppuhn & Baldwin, 2007; Barbehenn & Kochmanski, 2013; Pascacio-Villafán *et al.*, 2014). The effectiveness of defensive chemicals can also depend on plant nutritional content, so that some compounds act as defences only when leaf nutrient content is low (Barbehenn *et al.*, 2009). If these conditions (other leaf chemicals, leaf nutrient content) are not measured or reported, the outcome of several different studies using different species and conditions is likely to be varied. For a compound to act as a defence against herbivores, it should show negative effects on herbivore fitness or performance. Nevertheless, most of the compounds reported here do not show evidence for that.

3.4.4. Conclusions

In this study, we tested whether herbivory or mechanical leaf damage changes chemical and structural leaf traits, and whether these traits correlate with leaf photosynthesis and respiration rates. We also surveyed existing literature on the relationships between herbivory and the specific polyphenol compounds. The experimental manipulations did not induce any changes in leaf structure or nitrogen and carbon content. Leaf polyphenol chemistry was affected by the manipulations, but differently depending on the study site and time of the year. These differences were only visible on the level of the summed concentrations of hydrolysable

tannins or flavonols. Individual compounds or the chemical composition as a whole did not differ between the treatments. Most polyphenols did not correlate with photosynthesis or correlated positively, opposite of what would be expected based on hypotheses on growth-defence trade-offs. Based on the literature survey, we found little pre-existing evidence of any of the compounds acting as antiherbivore defences. We suggest that these results of the field experiment are due to variation in environmental conditions experienced by the host tree. Leaf chemistry is often affected by climate, nutrient availability and intensity of competition from surrounding plants. All these factors differed between the two study sites and might have had a stronger effect on plant chemistry than our herbivory manipulations. The specific polyphenol compounds studied here might function as constitutive rather than induced defences. Additionally, seasonal changes in the background level of plant chemicals might have further diluted the effect of the treatments on plant chemistry.

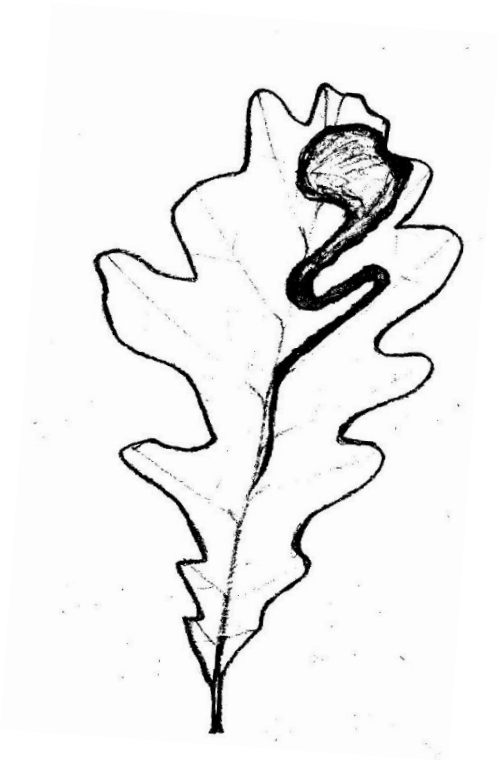
3.5. Acknowledgements

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Chapter 4: Host plant chemistry correlates with the associated insect community, but is not affected by prior herbivory

Kristiina Visakorpi, Terhi Riutta, Angelica Martinez-Bauer, Juha-Pekka Salminen and Sofia Gripenberg

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Abstract

By feeding on plant tissue, insect herbivores can change several characteristics of their hosts. These changes have the potential to alter the quality of the plant for other herbivore species, potentially altering the structure of the community of species attacking the plant at a later point in time. We tested whether herbivory early in the season changes host plant performance, polyphenol chemistry, and the community structure of sessile herbivores later in the season. We experimentally manipulated densities of early-season moth caterpillars on a set of young oak trees, and measured tree growth, reproduction, leaf chemistry, and the abundance and community composition of leafmining and galling species later in the season. The experimental manipulations of early-season herbivores did not affect late-season leaf chemistry, tree performance or the abundance or community composition of herbivores. Leaf chemistry differed between intact and eaten leaves, but only in trees with high density of early-season herbivores. The chemical composition of leaves correlated with the community structure of late-season insect herbivores. Both the chemical composition of the host tree, and the late-season insect community structure, were strongly affected by the spatial location of the tree. Our results suggest that plant-mediated indirect effects between herbivores might play a limited role in this system, whereas the underlying variation in plant chemistry could be an important factor in structuring the associated insect community.

4.1. Introduction

By feeding on plant tissue, insect herbivores change the characteristics of their host plant (Karban & Baldwin, 1997). These changes are often triggered as a defensive reaction against the attacker, and may involve changes in phenology, physical traits or in the chemical composition of the plant (Karban & Baldwin, 1997; Nykänen & Koricheva, 2004). These herbivory-induced changes can cause trade-offs between growth, reproduction and defence (Herms & Mattson, 1992a; Campos *et al.*, 2016), often negatively affecting plant fitness (Hawkes & Sullivan, 2001).

The changes in plant physiology caused by one herbivore species might influence the quality and quantity of the plant as a food source for other herbivores. Such indirect interactions between herbivores appear to be common (Ohgushi, 2005; Kaplan & Denno, 2007), and can occur between species that are temporarily separated (Van Zandt & 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). However, many of studies investigating plant-mediated effects between herbivores have been conducted in greenhouse or common garden settings (but see e.g. Faeth, 1986, Wold & Marquis, 1997), and have typically assessed the effects of a single focal herbivore species on only one or at most a handful of other species (but see e.g. Tscharntke, 1999). The extent to which individual herbivore species in natural systems affect entire communities is currently unclear. Moreover, despite the many examples of plant-mediated indirect effects between (pairs of) herbivores, the potential mechanism driving these effects (e.g. changes in host plant chemistry) are not always explored.

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

Oxfordshire, southern UK. The oaks in this region experience intense early-season herbivory by leaf-chewing lepidopteran caterpillars (Feeny, 1970; Hunter, 1992), which have been thought to act as keystone herbivores in this system (Hunter, 1992). The feeding starts from budburst and lasts for a couple of weeks. Later in the season, oaks sustain a diverse community of sessile leafminers and gallers.

Oaks produce a diverse set of polyphenols, especially ellagitannins (Salminen *et al.*, 2004), which are thought to act as constitutive and induced defences against herbivores (Rossiter *et al.*, 1988; Roslin & Salminen, 2008; McArt *et al.*, 2013). Even though many studies have reported changes in total concentrations of phenolics after herbivory (Feeny, 1970; Faeth, 1986; Forkner *et al.*, 2004), the realisation that defensive reactions in plants can involve specific compounds against certain types of herbivores (Roslin & Salminen, 2008; Smilanich *et al.*, 2016) calls for more detail when assessing the chemical profile of plants. The chemical composition of plant tissue has recently been recognized as an important factor in explaining plant-associated insect diversity both between (Richards *et al.*, 2015; Volf *et al.*, 2018; Salazar *et al.*, 2018) and within species (Poelman *et al.*, 2010; Glassmire *et al.*, 2016; Bustos-Segura *et al.*, 2017). To which extent this correlation between plant chemistry and the associated insect community is affected by earlier herbivory is currently unclear.

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

4.2. Materials and methods

4.2.1. 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 10 000 oaks established in 2005. We chose a study system of young trees to allow us to carry out herbivore manipulations at the whole tree scale. We selected 18 clusters of four neighbouring, similar-sized trees (n = 72). Within each cluster, the trees grew < 5 m apart. The clusters were located in six experimental blocks, three clusters per block (Appendix 4.1, Figure S1). 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. Despite our efforts to cause as little disturbance to the trees as possible, a small number of shoots broke off in the process of caterpillar transfer. To ensure that all the trees were exposed to the same degree of disturbance we snapped five random shoots from each *control* tree and shook the trunk and the branches. All manipulations were carried out from 12th May 2016 until 30th May 2016, i.e. from the appearance of the first leaves until most caterpillars had disappeared. To create a representative subset of leaves for later surveys of leaf area loss and late-season insects, we marked 16 branches on each tree between 6th June and 13th June 2016, half of them in lower canopy and half in upper canopy. Within each stratum, half of the shoots were on the outer tips of the branches and remaining shoots closer to the trunk. All marked shoots were spread evenly in four directions around the tree, and the exact shoot was chosen haphazardly.

4.2.2. Early-season herbivory

Most caterpillars encountered on and removed from herbivory suppression trees and transferred to increased herbivory trees were identified as *Acrobasis consociella* (Pyralidae, Hübner, 1813). These caterpillars spin web that attach several leaves together to form a small shelter. The caterpillars remain in their shelters until pupation, feeding on the leaves inside (Shaw et al. 2011, Appendix 4.1, Figure S2ab).

We measured the intensity of early-season herbivory by counting the number of *A. consociella* shelters, and by surveying leaf area loss on leaves which were not part of these shelters, on 6th - 21st June 2016. To count the caterpillar shelters, we searched all branches of each tree systematically. Since most of the caterpillars added and removed during the manipulations belonged to this shelter-spinning species, these counts gave us a direct measure of the success of our manipulations. To assess whether the manipulations resulted in altered herbivory rates overall, we visually estimated the percentage of leaf area lost for the leaves of the 16 marked branches on ca. half of the experimental trees (n = 39).

4.2.3. Tree performance

To measure the effects of herbivory on tree growth, we measured change in tree diameter at breast height (dbh) using digital callipers (Messzeuge, Germany). Two readings were taken on the opposite sides of the trunk to avoid biases due to uneven shape of the trunk. The trees were measured before the first growing season (2nd May 2016; prior to experimental manipulations), at the end of the first growing season (24th August 2016), and at the end of the growing season the following year (28th August 2017). To assess the effect of herbivory on tree reproduction, we counted all acorns on each tree at the end of the first season (13th to 14th September 2016) and scored them either as “healthy”, or “galled” depending on weather they had been deformed by the seed galler *Andricus quercuscalicis* (Burgsdorf, 1783, Appendix

4.1, Figure S2c). *Andricus quercuscalicis* is a common acorn galling species in the area, and an important seed parasite on oaks (Crawley, 1985).

4.2.4. Late-season herbivore surveys

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

Many of the surveyed gallers have both gamic and agamic generations, and many of the leafminers have several gamic generations per season. The gamic generation of galls is often on catkins or buds. For such species, we surveyed only the agamic generation which occurs on leaves from July onwards. For remaining species, we surveyed several gamic generations found on leaves. Since our experimental manipulations started immediately after leaf flush, all sessile insect species encountered on the leaves would have colonised those leaves (through oviposition by adult females) during or after the manipulations.

4.2.5. Leaf chemistry

Polyphenols are believed to be important defensive compounds on oak. They include hydrolysable tannins, condensed tannins (i.e. proanthocyanidins) and flavonols (Salminen *et al.*, 2004). Previous studies suggest that hydrolysable tannins in particular may act as an important defence against herbivory on oak due to their high concentration in young leaves (Salminen *et al.*, 2004; Roslin & Salminen, 2008) coupled with high pro-oxidative activity in caterpillar guts (Barbehenn *et al.*, 2009). Flavonols are often induced after herbivore attack (Onkokesung *et al.*, 2014; Ohse *et al.*, 2017). Even though there are accounts of proanthocyanidin levels increasing after defoliation (Faeth, 1986; Roth *et al.*, 1998), several

studies have suggested that they may not function as anti-herbivore defences (Ayres *et al.*, 1997; Nykänen & Koricheva, 2004; Roslin & Salminen, 2008). There is also evidence of both flavonols (Barbour *et al.*, 2016), and hydrolysable tannins (Yarnes *et al.*, 2008), affecting gallers and leafminers, respectively. Thus, we decided to measure individual compounds of hydrolysable tannins and flavonols instead of the total pool of proanthocyanidins.

On 24th August 2016, we collected leaves from two trees within each experimental cluster for phytochemical analyses (n = 36 trees). From each of these trees, we collected three leaves with herbivory damage and three intact leaves haphazardly from different parts of the tree. The three leaves collected per leaf type per tree were pooled and analysed as one sample. Leaves were transported in a cool bag to a freezer, freeze-dried and ground to fine powder. Samples were analysed in the Natural Chemistry Research Group laboratory at the University of Turku, Finland using ultra-performance liquid chromatography mass spectrometry (UPLC-MS). In brief, 20 mg of the dry plant powder was extracted 2 x 3 h with 1.4 mL acetone/water (80:20, v:v). Acetone was evaporated from the combined extract by the Eppendorf concentrator and the water phase was freeze-dried. The lyophilized water phase was dissolved in 1 mL ultra-pure water and filtered via 0.2 µm PTFE filter. The UPLC-Orbitrap-MS analyses of individual polyphenols were acquired from the 5-time dilution with water of this filtered water phase. The UPLC system consisted of a binary solvent manager, sample manager, column (Acquity UPLC BEH Phenyl, 30 mm × 2.1 mm i.d., 1.7 µm, Waters Corporation), and photodiode array detector (Acquity UPLC®, Waters Corporation, Milford, MA, USA) coupled to a hybrid quadrupole-Orbitrap mass spectrometer (Q Exactive™, Thermo Fisher Scientific GmbH, Bremen, Germany; Volf *et al.*, 2017). The compounds were quantified from the full scan trace by monitoring the peak areas of the most prominent ions (typically $[M-H]^-$ or $[M-2H]^{2-}$) of each compound and using external calibration curves to convert peak areas into mg/ml concentrations. For each sample, we obtained information on concentrations of 27 polyphenol compounds (Appendix 4.2, Table S1).

4.2.6. Statistical analyses

Data were analysed using generalized linear mixed effect models ("GLMM") with negative binomial (count) or betabinomial (proportion) distribution, betaregression models and linear mixed effect models ("LMM"). Random effects in mixed effect models were cluster nested within block (tree-level data) or tree nested within cluster nested within block (leaf-level chemical data). For each response, the full model was simplified by dropping one explanatory variable at a time. The change in the model fit was assessed using likelihood ratio tests and fixed factors that did not improve model fit were dropped (Crawley, 2007). Tukey's tests were used to investigate pairwise differences between treatments. Detailed model descriptions are provided in Appendix 4.3. In brief, we first tested whether 1) the experimental manipulations affected the density of *A. consociella* shelters by modelling the number of shelters per tree as a function of treatment (*increase*, *suppression* or *control*), tree size (dbh) and their interaction. To test 2) whether the abundance of *A. consociella* covaried with leaf area loss on leaves outside the shelters we modelled the proportion of leaf area loss as a function of experimental block, tree size and the number of *A. consociella* shelters. To test 3) the effect of *A. consociella* herbivory on tree performance, we modelled tree growth (during the first season, during the second season, or cumulative growth over two seasons), overall number of acorns (healthy and infested), and the ratio of healthy to infested acorns as functions of tree size, number of *A. consociella* shelters, leaf area loss at the cluster level, interactions between the number of caterpillar shelters and the above-mentioned variables. In the model assessing growth rate during the second season, growth rate during the first season was included as an additional explanatory variable. To test 4) the effects of *A. consociella* herbivory on late-season insect abundance and diversity, we modelled the abundance or diversity (Shannon's diversity index) of leafminers or gallers as a function of tree size, survey date, number of *A. consociella* shelters, leaf area loss per cluster and the two-way interactions between the number of *A. consociella* shelters and the other variables. To test for 5)

differences in leaf chemistry between trees in the different herbivory treatments (*increase, suppression, control*) and leaf damage classes (*eaten* versus *intact*), we modelled the total concentration of all polyphenols, the diversity of all polyphenols (Shannon's diversity index), and the concentration and diversity of our two focal polyphenol subgroups (hydrolysable tannins and flavonols) as functions of tree size and leaf type (two leaf types within three herbivory treatment groups yielding six treatment × damage class combinations).

To assess 6) the effect of *A. consociella* abundance on the community structure of late-season herbivores and 7) whether the community structure of late-season herbivores correlated with tree-level composition of polyphenols, we built generalised linear models ("GLM") for multivariate data (Wang *et al.*, 2012) with negative binomial distribution. The response was the tree-level abundances of individual species of leafminers and gallers. Explanatory variables in model 6) were experimental block and cluster, tree size, survey date and the number of *A. consociella* shelters and interactions between the shelters and date and shelters and tree size. In model 7) explanatory variables were the experimental block and the average concentration of the different polyphenol compounds per tree. The significance of explanatory variables in multivariate GLMs was assessed by performing an analysis of deviance on the full model and assessing the significance of the individual terms with likelihood ratio tests and resampled p-values. To examine which species (as part of the multivariate response variable) expressed significant effects, we used univariate likelihood ratio tests with unadjusted p-values. To partition the variance in late-season insect community into components explained by the different explanatory variables in models 6 and 7, we built a hierarchical Bayesian joint species distribution model (Ovaskainen *et al.*, 2017). The matrix of the late-season species abundances was set as the response and the concentrations of individual polyphenols and the number of caterpillar shelters as the environmental covariate matrix, with experimental block and cluster as random effects and assuming an overdispersed Poisson error distribution.

To test for 8) differences in chemical composition of different leaf types (*eaten* vs. *intact* from the three tree-level treatments) we performed a principal component analysis ("PCA") and extracted values for the two first PC axes. We then built LMMs in which each axis was modelled as a function of tree size and leaf type (six different treatment × damage class combinations

For all analyses, model assumptions were tested by visually examining plots of residuals against fitted values for the homoscedasticity of residuals. For LMMs and GLMMs, we also examined plots of residuals against the explanatory variables, and Quantile-Quantile plots for the normal distribution of the residuals. The latest version of R (3.5.0) and packages nlme (Pinheiro *et al.*, 2017), multcomp (Hothorn *et al.*, 2008), DHARMa (Hartig, 2017), betareg (Cribari-Neto & Zeileis, 2010), glmmADMB (Fournier *et al.*, 2012), vegan (Oksanen *et al.*, 2017), mvabund (Wang *et al.*, 2017a), HH (Heiberger, 2017) and HMSC (Ovaskainen *et al.*, 2017) were used in the analyses.

4.3. Results

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

We transferred between 200 and 250 caterpillars to each tree in the *increased herbivory* treatment and removed 15 to 219 caterpillars from trees in the *herbivory suppression* treatment. Thus, all trees in the increased herbivory treatment experienced at least a twofold increase in *A. consociella* abundance. The early-season herbivore manipulation resulted in significantly higher and lower density of *A. consociella* shelters in the corresponding treatments compared to the control ($\chi^2 = 33.78$, $p < 0.001$, df = 2, 7, Figure 4.1a). The amount of leaf area loss outside these shelters was not affected by the manipulations ($z = -1.08$, $p = 0.28$), but differed significantly between the experimental blocks ($p = 0.008$).

4.3.2. Does herbivory influence the concentration, diversity, and composition of polyphenols?

Leaf type (*eaten* vs *intact* in the three tree-level treatments) had a significant effect on total polyphenol concentration (χ^2 = 20.06, p = 0.001, df = 5, 10, Figure 4.1b), hydrolysable tannin concentration (χ^2 = 13.01, p = 0.02, df = 5, 10) and flavonol concentration (χ^2 = 16.46, p = 0.006, df = 5, 10). The significant effect of 'leaf type' was in these cases driven by differences between eaten and intact leaves in the *increased herbivory* treatment (z = 4.14, p < 0.01 all polyphenols, Figure 4.1b, z = 3.01, p = 0.02, hydrolysable tannins, z = 3.32, p < 0.01, flavonols). Similarly, polyphenol composition differed significantly between leaf types along the first PC axis (χ^2 = 16.9, p = 0.005, df = 5, Appendix 4.1, Figure S3c) due to differences between eaten and intact leaves in the *increased herbivory* treatment (z = −3.48, p = 0.005). The first PC axis explained variation in the concentrations of the most abundant hydrolysable tannins (Appendix 4.1, Figure S4). Polyphenol composition did not differ between leaf types along the second PC-axis (χ^2 = 6.48, p = 0.26, df = 5, 10). Experimental block had a strong effect on the grouping of the data points in the resulting ordination (Appendix 4.1, Figure S3d). The diversity of all polyphenols (χ^2 = 5.56, p = 0.35, df = 5, 10), the diversity of hydrolysable tannins (Lratio= 5.78, p = 0.33, df = 5, 10) and the diversity of flavonols (χ^2 = 8.88, p = 0.11, df = 5, 10) did not differ between leaf types.

4.3.3. Is tree performance affected by herbivory?

No significant relationships were detected between the number of *A. consociella* shelters and tree growth (Lratio = 0.23, p = 0.63, df = 1, 6, season 1, χ^2 = 1.82, p = 0.18, df = 1, 7, season 2, χ^2 = 1.80, p = 0.18, df = 1, 5 cumulative growth), acorn production (χ^2 = 0.41, p = 0.52, df = 1, 6) and the proportion of healthy acorns (χ^2 = 0.76, p = 0.38, df = 1, 5). Leaf area loss on leaves that were not part of *A. consociella* shelters correlated negatively with tree growth during the season following the experimental manipulations (χ^2 = 4.85, p = 0.03, df = 1, 6), and with the cumulative growth over two seasons (χ^2 = 6.01, p = 0.01, df = 1, 5, Figure 4.1c).

There were no significant relationships between leaf area loss and growth during the first season (Lratio = 1.32, p = 0.25, df = 1, 6), production of acorns (χ^2 = 0.83, p = 0.36, df = 1, 6) and the proportion of healthy acorns (χ^2 = 0.13, p = 0.71, df = 1, 5). Growth during the second season correlated strongly and negatively with growth during the first season (χ^2 = 55.11, p < 0.001, df = 1, 6, Table S2, Appendix 4.1, Figure S5).

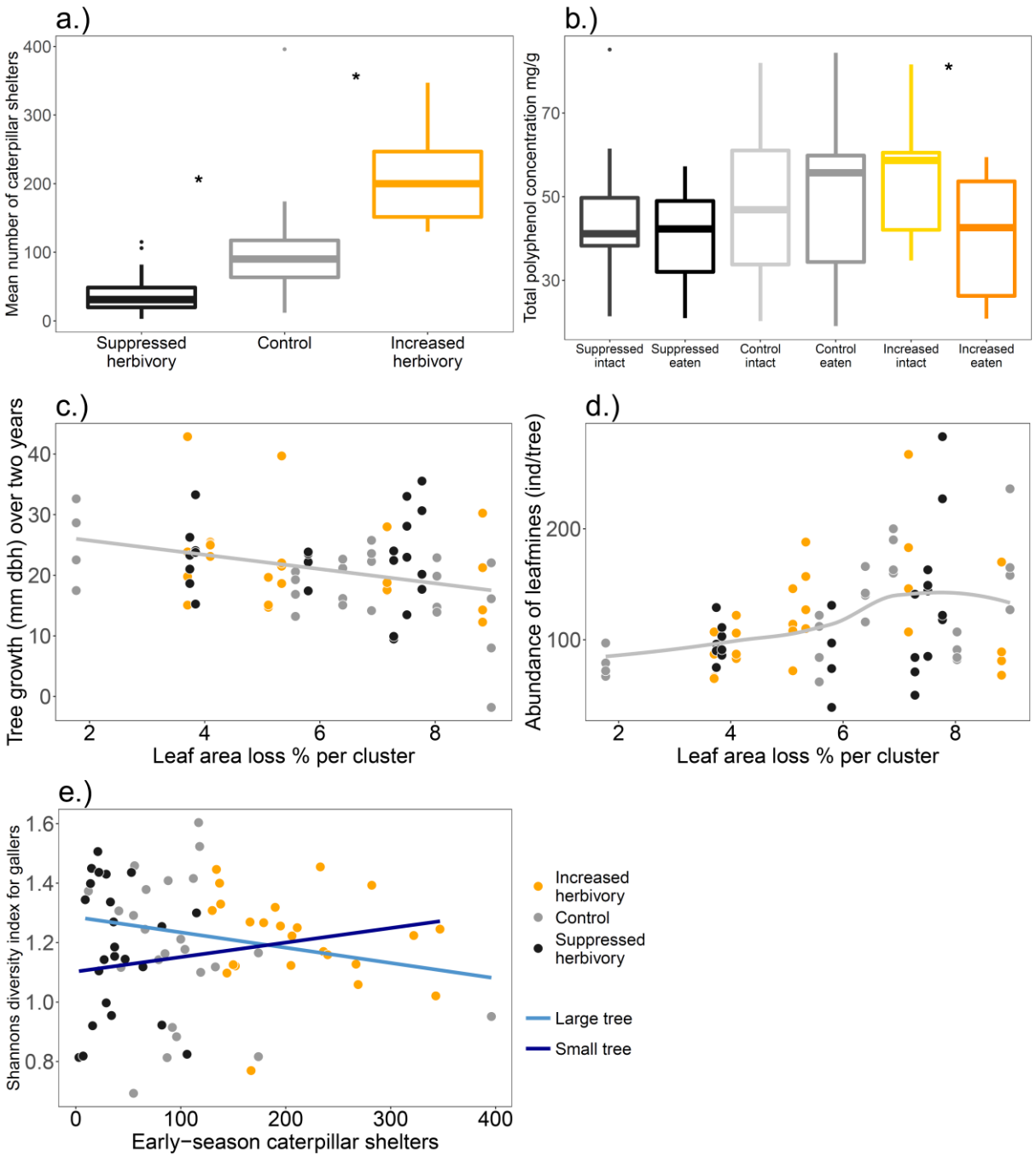


Figure 4.1. The effects of early-season herbivory on the different response variables. Panel a) the number of *A. consociella* shelters on the different treatments, b) the total concentration (mg/g) of polyphenols in the six studied leaf types, c) the correlation between leaf area loss on leaves outside *A. consociella* shelters and tree

growth (of dbh) over the two growing seasons, d) the correlation between leaf area loss and the abundance of late-season leafmines and e) the effect of *A. consociella* shelters on the diversity of late-season gallers, separated by tree size ("large" if dbh > mean dbh 43.1 mm). Statistically significant differences are denoted with asterisks (Tukey's test $p < 0.05$) in panels a and b. Lines have been drawn through model fitted points estimated from the models.

4.3.4. Is the abundance, diversity and community structure of late-season herbivores affected by prior herbivory?

We surveyed a total of 41443 oak leaves and recorded 27603 individual galls or leafmines representing 20 different taxa (Appendix 4.2, Table S3). The number of *A. consociella* caterpillars did not affect the abundance of late-season herbivores ($\chi^2 = 2.07$, $p = 0.15$, df = 1, 6 leafminers, $\chi^2 = 2.33$, $p = 0.13$, df = 1, 5 gallers) or the diversity of leafminers ($\chi^2 = 0.16$, $p = 0.69$, df = 1, 5). The diversity of gallers was affected by the number of *A. consociella* shelters, but this effect was dependent on tree size (treatment × tree size interaction $\chi^2 = 8.37$, $p = 0.004$, df = 1, 8, Figure 4.1e): large trees with a high abundance of caterpillar shelters had lower diversity of gallers, whereas the opposite pattern was recorded on small trees. The community composition of late-season herbivores was not affected by the number of *A. consociella* shelters (LRT = 26.0, $p = 0.46$, Figure 4.2b, Figure S3a).

The leaf area loss on leaves that were not part of caterpillar shelters correlated positively with the number of leafminer individuals ($\chi^2 = 4.46$, $p = 0.03$, df = 1, 5, Figure 4.1d). There was no correlation between leaf area loss and the abundance of gallers ($\chi^2 = 0.16$, $p = 0.69$, df = 1, 5), or the diversity of either group of insects ($\chi^2 = 1.12$, $p = 0.29$, df = 1, 9 gallers, $\chi^2 = 1.38$, $p = 0.24$, df = 1, 5, leafminers).

4.3.5. Does the abundance, diversity, and community structure of late-season herbivores covary with polyphenol chemistry?

The community composition of late-season herbivores covaried significantly with the tree-level polyphenol composition (LRT = 560.1, p = 0.02, Figure 4.2acd, Appendix 4.2, Table S4). Most compounds correlated with the abundance of at least one of the late-season insect species, and most of these pairwise correlations were positive (Figure 4.2c). Except for the most abundant insect species, tree-level polyphenol composition accounted for the largest proportion of the explained variation (Figure 4.2d). The community composition of late-season herbivores differed between experimental blocks (LRT = 333.9, p = 0.001, Appendix 4.1, Figure S3be) and clusters (LRT = 490.1, p = 0.001).

For coefficient estimates for all the mixed effect models, see Appendix 4.2, Table S2 and for the effects of other explanatory variables (tree size and collection date) see Appendix 4.3.

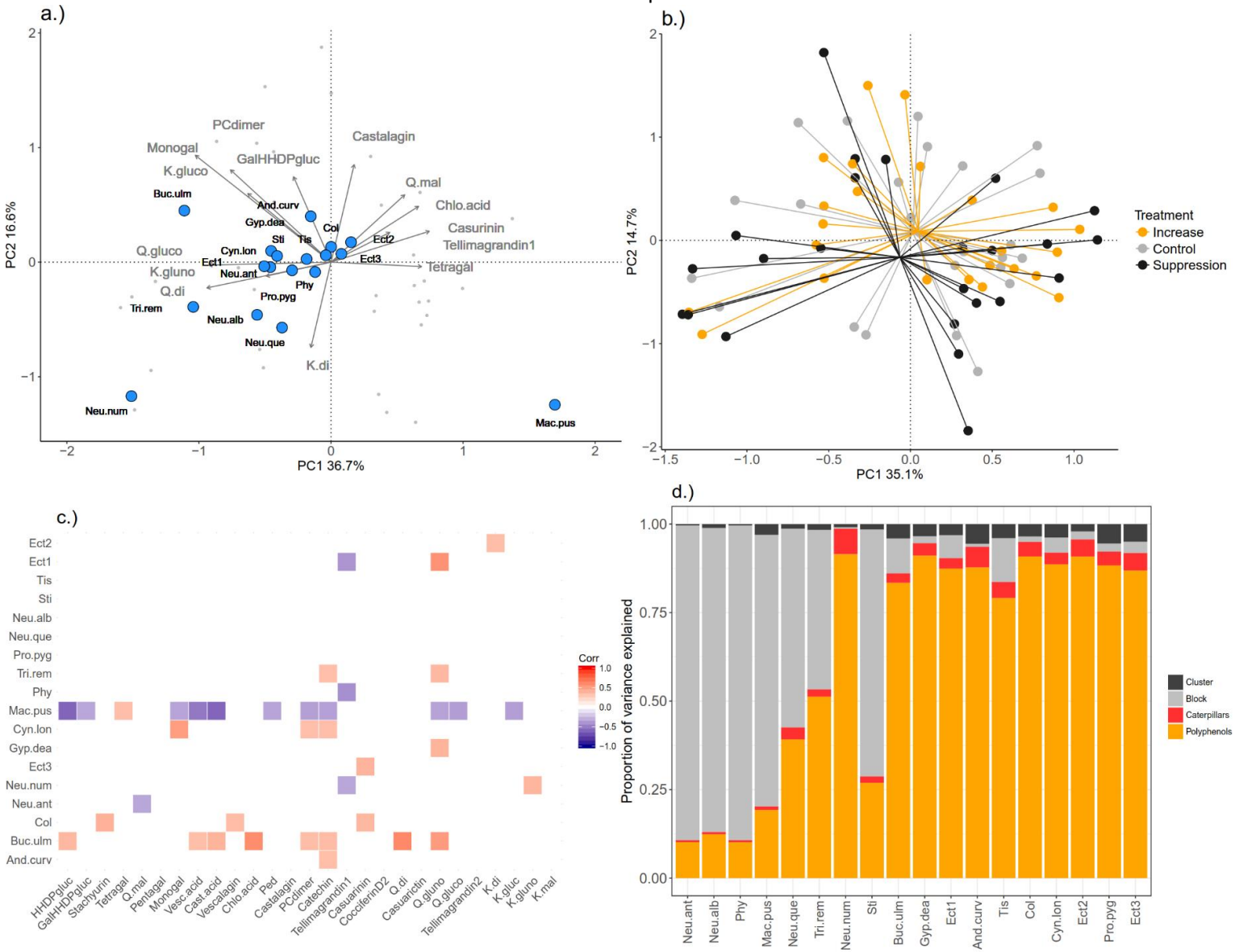

a.)
PCdimer
Monogal
GalHHDPgluc
K.gluco
Castalagin
Q.mal
Chlo.acid
Casurinin
Tellimagrandin1
Tetragal
Buc.ulm
And.curv
Gyp.dea
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Sti
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Neu.alb
Neu.que
K.di
Neu.num
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PC2 16.6%
PC1 36.7%
b.)
PC2 14.7%
PC1 35.1%
Treatment
Increase
Control
Suppression
c.)
Corr
d.)
Proportion of variance explained
Cluster
Block
Caterpillars
Polyphenols

Figure 4.2. The effect of tree-level polyphenol composition and experimental treatments on the late-season insect community. a) PCA-based ordination describing the variation in late-season insect community structure and polyphenol composition. The different insect species are shown as blue circles, the different polyphenol compounds as grey arrows and the different experimental trees (n = 36) as small grey dots. Compounds expressing collinearity have been removed. b) The late-season insect community structure using all of the experimental trees (n = 72). See Appendix 4.1, Figure S3e for grouping by experimental block and Figure S3a for ordination for half of the trees (n = 36). c) Significant ($p < 0.05$) correlation coefficients between the late-season herbivore species (as rows) and polyphenols (as columns). Positive correlations are red, and negative blue. d) Proportion of variance in the abundances of the late-season species (in order of abundance, Appendix 4.2, Table S3) explained by the different explanatory variables (polyphenols and caterpillar shelters) and random effects (block and cluster). See Appendix 4.2, Tables S1 and S3 for abbreviations.

4.4. Discussion

We manipulated densities of early-season shelter-building *A. consociella* caterpillars on 72 oak trees, creating an experimental gradient from a 2.5-fold reduction to a twofold increase in caterpillar numbers. The manipulations did not affect leaf area loss on leaves that were not part of the shelters. Neither did the manipulations have any effects on the concentration and composition of polyphenols or on tree growth or reproduction. The composition and concentration of polyphenols differed between eaten and intact leaves, but this effect was only seen on trees in the *increased herbivory* treatment. There were few clear effects of early-season caterpillars on the late-season sessile insect community: the only statistically significant effect was seen in terms of galler diversity, where diversity increased with the number of caterpillar shelters on small trees and decreased on large trees. The community composition of late-season insects correlated significantly with tree-level polyphenol composition, and both the community structure of late-season herbivores and the tree-level polyphenol composition were strongly affected by the growth location of the host tree. Below, we will discuss each of these findings in turn.

4.4.1. No effect of caterpillar manipulations on leaf area or leaf chemistry

Leaf area lost to herbivores (as measured on leaves outside caterpillar shelters) was not affected by the amount of *A. consociella* shelters. *Acrobasis consociella* caterpillars most likely only feed on the leaves which are part of the shelters, and do not affect the abundance or feeding rate of other early-season leaf-chewers associated with the same tree.

We found no differences in the concentration, diversity or composition of polyphenols between trees with different amounts of *A. consociella* shelters. Because several previous studies have shown the same compounds acting as anti-herbivore defences on oak (Rossiter *et al.*, 1988; Roslin & Salminen, 2008), the lack of such effects suggests that *A. consociella* caterpillars did not induce a systemic defence reaction in their hosts. Induced defences should be favoured when herbivory pressure varies and when previous attack is a good indicator of future herbivory (Adler & Karban, 1994). This might not be the case in our study system, in which early-season caterpillars appear every spring. Consequently, the timing of herbivory is easily predictable, and previous herbivory does not necessarily correlate with future attacks. The best strategy for the plant might therefore be to produce defensive compounds constitutively at the beginning of each season. Indeed, the concentrations of hydrolysable tannins on oak show seasonal change, being high during the months after budburst (Salminen *et al.*, 2004). The additional herbivory pressure imposed by our caterpillar manipulations might not have affected this "background" level of defences.

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

polyphenol concentration between the leaf types probably reflect herbivore feeding preferences, rather than defensive reaction by the host tree.

4.4.2. Herbivory did not affect tree performance

Consistent with the finding of no difference in polyphenol chemistry between the treatments, we detected no effects of *A. consociella* herbivory on any of the tree performance metrics: growth, reproduction, and proportion of healthy acorns. Many previous studies have reported reduced growth or reproductive output after herbivory (Hawkes & Sullivan, 2001; Nykänen & Koricheva, 2004). These are thought to result from trade-offs between growth, reproduction and defence (Herms & Mattson, 1992a; Campos *et al.*, 2016). Since we did not observe changes in polyphenol chemistry, most likely no trade-offs between growth, defence or reproduction occurred. We did observe that trees which grew more during the first season grew significantly less during the second season. Since none of the growth rates were influenced by herbivory, some other stressor might have restricted growth of some of the trees during the first summer. These trees might have then compensated the reduced growth rates by growing faster the following season. For example, drought can reduce tree growth, and induce a compensatory increase in growth rate afterwards (Orwig & Abrams, 1997).

We observed a negative association between early-season herbivory outside the caterpillar shelters and tree growth. Since this leaf area loss-gradient was observational, we cannot conclude whether tree growth was reduced by increased leaf area loss, or whether herbivores chose to feed on trees with low growth rates.

4.4.3. Effects of early-season herbivory on the late-season sessile insect community

We found no effects of early-season *A. consociella* caterpillars on the abundance or community composition of late-season sessile herbivores. Since we did not observe any change in leaf chemistry, leaf area loss or tree performance between the different treatments,

the lack of plant-mediated indirect effects is not surprising. The early-season caterpillars affected only galler diversity, and only on larger trees (Appendix 4.3, "The interaction between tree size and the diversity of late-season gallers"). Nevertheless, the effect was weak and mainly driven by a few large trees with high numbers of caterpillar shelters. Since we observed no changes in tree chemistry or performance, the mechanism behind this effect remains unclear.

Of previous studies reporting herbivory-induced defences affecting other host plant-associated species, many have been carried out in greenhouse or common garden settings with a handful of herbivore species (Van Zandt & Agrawal, 2004; Poelman *et al.*, 2009; Hernandez-Cumplido *et al.*, 2016; Arce *et al.*, 2017). Most plants, especially trees, are associated with diverse insect communities (Southwood, 1961), and evidence for plant-mediated herbivory-induced effects on community composition in these naturally diverse communities is currently sparse. Faeth (1986) and Wold & Marquis (1997) manipulated early-season herbivore densities on trees in natural settings similar to our study, and – like us – failed to find differences in the late-season insect community between differently manipulated trees.

One explanation for the lack of cascading effects between herbivores could be parallel changes in the activity at the higher trophic levels (Faeth, 1986; Kaplan & Denno, 2007). Many trees emit volatile predator-attracting compounds as a response to herbivory (Thaler, 1999; Kessler & Baldwin, 2001). Additionally, empty galls or caterpillar shelters could provide hiding places for predators (Ohgushi, 2005). The high abundance of early-season caterpillar shelters might have increased the number of predators on those trees, which might have suppressed the number or feeding activity of the caterpillars. Subsequently, the number of shelters we counted might have not reflected the feeding pressure experienced by the tree. These kinds of changes in predation pressure would not be present in controlled greenhouse

conditions or might be weaker in more artificial common garden settings, where many of the previous studies have been conducted.

We observed a positive correlation between leaf area loss outside the caterpillar shelters and late-season leafminers. This might be due to these two herbivore groups choosing similar trees to feed on, rather than due to direct effects between these two groups. Fast-growing trees might invest less in defence than slow-growing ones, and thus experience higher levels of herbivory (Coley *et al.*, 1985). We tested if plant growth rate, reproduction and investment in defences correlated, and whether these correlations were affected by early-season herbivory, but found no significant relationship between any of the three life history traits (Appendix 4.3, "Patterns of tree resource allocation").

4.4.4. The chemical composition of the host tree correlated with insect community structure

We found that the polyphenol composition correlated significantly with insect community structure. Many previous studies have observed a similar relationship between plant chemistry and the associated insect community structure, both between (Richards *et al.*, 2015; Volf *et al.*, 2018; Salazar *et al.*, 2018) and within species (Glassmire *et al.*, 2016; Bustos-Segura *et al.*, 2017). The impact of individual compounds on the different species was specific to the exact species × compound pair, and most of these pairwise correlations were positive.

Interactions between plants and their insect herbivores can be extremely specific. Even though polyphenols deter feeding (Roslin & Salminen, 2008) and reduce herbivore performance (Haviola *et al.*, 2007; Ruuhola *et al.*, 2007), some herbivores can benefit from secondary chemicals produced by their host plant (Roslin & Salminen, 2008; Smilanich *et al.*, 2016). Since most of the late-season species in our study were specialists on the genus *Quercus* (Appendix 4.2, Table S3), they might have been adapted to the different oak-specific compounds, which might explain the observed positive relationships. Both the insect community structure and the tree-level chemical composition were affected by the growth

location of the host tree. Several studies on oak have shown how leaf chemistry or insect community structure is influenced by microclimate and soil properties (Fine, 2004; Moreira *et al.*, 2017). At our study site, small-scale environmental heterogeneity might have created variation in host tree chemical composition, affecting the associated insect community. Since the observed relationship between insect community structure and chemistry was correlative, it is possible that the environment was affecting both compositions independently.

4.4.5. Conclusions

In this study, changes in early-season insect abundance had no detectable effects on tree chemistry, tree performance or the abundance or community composition of late-season herbivores. We found that host tree chemistry correlated with the associated insect community structure. Both chemical and insect community composition were strongly influenced by the growth location of the host tree. Interactions with other trophic levels and the effect of small-scale variation in environmental conditions on plant chemistry might be diluting herbivore-triggered indirect effects on community structure in natural settings. These conditions are rarely replicated in greenhouse or common garden experiments, in which many previous studies on plant-insect interactions are based. Large-scale changes in plant chemistry and community-wide cascading effects between herbivore species might be rare in natural systems. Abiotic factors, rather than earlier herbivory, might be more important in determining the quality of the host plant and thus the structure of the associated insect community in this system.

4.5. Acknowledgements.

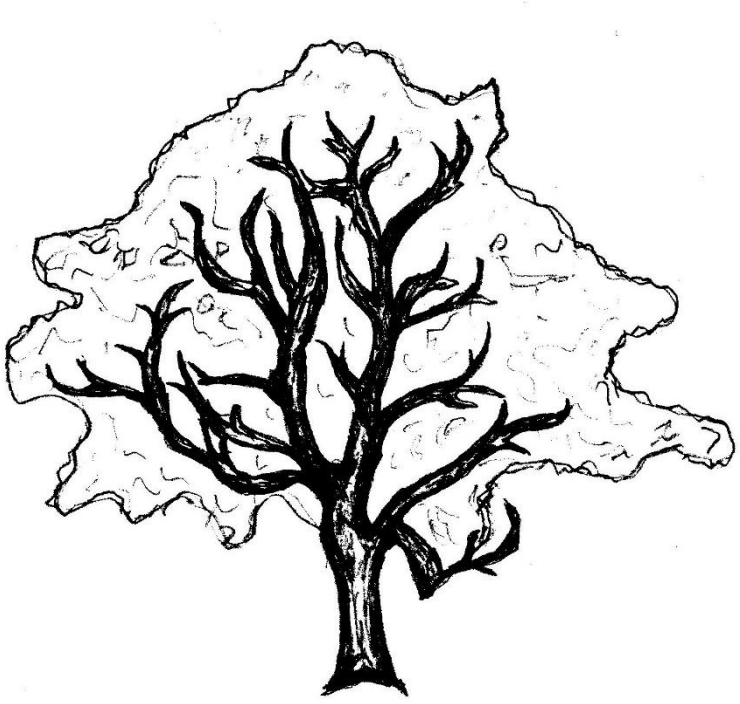
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Chapter 5: Does insect herbivory suppress ecosystem productivity? Evidence from a temperate woodland

Kristiina Visakorpi, Sofia Gripenberg, Yadvinder Malhi and Terhi Riutta

Manuscript in preparation



Abstract

Insect herbivores can affect the photosynthetic capacity of their host plants directly by reducing the area of photosynthesising leaf tissue, or indirectly by changing the rates of photosynthesis and respiration in remaining plant tissue. These changes can further influence rates of carbon sequestration and loss. Nevertheless, the effects of herbivory on ecosystem carbon cycling through indirect changes in plant gas exchange have rarely been quantified. In this study, we estimate the amount of carbon lost to insect herbivory in a temperate deciduous woodland both through the direct effect of leaf area loss and through the indirect changes in leaf gas exchange. We also assess how ecosystem productivity changes with decreasing and increasing herbivory pressure.

As a study system, we used the pedunculate oak (*Quercus robur*) and spring-feeding moth caterpillars in Wytham Woods (Oxfordshire, UK). We combine data on leaf photosynthesis and respiration in the presence or absence of caterpillar herbivory with information on leaf area loss, stem respiration, seasonal changes in leaf cover and photosynthetic rate, and meteorological data collected over a growing season. We quantify the carbon budget of an individual oak tree and a hectare of forest under normal levels of herbivory, and in the hypothetical situation of complete absence of herbivory. These estimates are compared to estimates on oak net primary productivity (NPP) derived from surveys of woody growth, and from earlier biometric measures at the study site.

We estimate net primary productivity of an oak forest to be 1.8 – 4.9 Mg C ha^{-1}. Herbivores remove 0.12 Mg C ha^{-1} over the growing season by removing leaf area and prevent 5.4 Mg C ha^{-1} from being assimilated through changes in plant primary metabolism. Our estimate on the effect of herbivory on canopy gas exchange is larger than previous estimates based only on leaf area loss. We propose that the effect of herbivory on ecosystem productivity is non-linear and depends on the proportion of leaves affected by herbivory. We

call for replicated studies in other systems and with other species to validate the relationship between insect herbivory and ecosystem productivity described here.

5.1 Introduction

Almost every terrestrial plant species is attacked by insect herbivores (Strong *et al.*, 1984; Schoonhoven *et al.*, 2005; Forister *et al.*, 2015). By consuming plants, insect herbivores reduce plant biomass and change the area of photosynthesising tissue. Herbivores can cause further changes in the remaining plant tissue by changing plant primary metabolism, resource allocation, or trigger the production of induced defences (Karban & Baldwin, 1997; Nykänen & Koricheva, 2004; Schwachtje & Baldwin, 2008). Herbivory-mediated changes in plant metabolism in the remaining tissue can substantially influence rates of photosynthesis and respiration (Nykänen & Koricheva, 2004; Nabity *et al.*, 2009; Bilgin *et al.*, 2010) with potential consequences for carbon sequestration and loss. These indirect effects of herbivores on plant gas exchange can potentially have wide consequences for ecosystem-level carbon cycling and primary productivity (Strickland *et al.*, 2013; Visakorpi *et al.*, 2018).

The effects of insect herbivores on tree growth and mortality are well documented (e.g. Kosola *et al.*, 2001; Aukema *et al.*, 2006). It is also known that insect herbivores can affect ecosystem nutrient cycling through increased deposits of frass and leaf fragments from the canopy (Belovsky & Slade, 2000; Hunter, 2001; Meehan *et al.*, 2014). Apart from these effects, relatively little is known about how insect herbivores affect ecosystem carbon cycling. By quantifying leaf area loss in a tropical forest, Metcalfe *et al.* (2014) estimated that insect herbivores remove 12-19% of foliar production. In contrast, Dungan *et al.* (2007) estimated that phloem-feeding by scale insects increased photosynthesis by 4%, compensating any loss of carbon to herbivory. Other studies have shown that under specific circumstances, the effect of herbivory on forest carbon cycling can be surprisingly large, enough to shift the ecosystem from being a carbon sink into a carbon source (Kurz *et al.*, 2008; Heliasz *et al.*, 2011; Lund *et*

al., 2017). Many of the large effects of insects on forest carbon cycling documented to date are due to increased tree mortality caused by outbreaks of insect pests and subsequent increases in decomposition rates.

Despite their potential importance, interactions between insect herbivores and their host plants have only recently been included in ecosystem-scale studies and in many cases, the effects of animals on the ecosystem-level carbon cycle remain poorly quantified (Schmitz *et al.*, 2014). Despite their potential importance, changes in canopy or ecosystem CO_2 exchange resulting from indirect effects of herbivory (i.e. plant responses to herbivory in the remaining tissue) under non-outbreak densities of insects, have generally been neglected (but see Strickland *et al.*, 2013, Visakorpi *et al.*, 2018). Overlooking the effects of insect herbivory could lead to biased estimations of ecosystem productivity, or of the role of forests as carbon sinks (Kurz *et al.*, 2008; Campioli *et al.*, 2016).

In this study, we quantify the effects of insect herbivory on tree- and forest-level gross and net primary productivity in a temperate maritime woodland in southern England, UK. In a previous study in this system, we found that rates of photosynthesis of oak (*Quercus robur*, L.) leaves subject to herbivory by winter moth (*Operophtera brumata*, L.) caterpillars as well as intact leaves growing on the same shoots as these damaged leaves were substantially lower than rates of photosynthesis on intact leaves surrounded only by other intact leaves (Visakorpi *et al.*, 2018). We estimated that herbivory can reduce canopy-level photosynthesis by as much as 50%, with most of this effect attributable to reduced photosynthetic rate in intact leaf tissue. Whether these changes in carbon assimilation affect tree and stand level productivity remains unknown. Here, we use the measurements of herbivory-inflicted leaf area loss and indirect changes in photosynthetic rate (Visakorpi *et al.*, 2018) to estimate the effects of herbivory on oak primary production over an entire growing season. Specifically, we ask 1) what is the overall effect of insect herbivory on net primary production over the whole growing season; 2) how much carbon is lost by herbivores reducing leaf area *versus* changing

plant metabolism; and 3) how does the effect of herbivory on forest productivity change with changing herbivory pressure?

5.2. Materials and methods

5.2.1. Site and study system description

All experiments and measurements took place in Wytham Woods (51º.47' N, 1º 20' W, 160 m.a.s.l), located in Oxfordshire, UK. Wytham Woods is an ancient semi-natural woodland of 390 ha, consisting of the most common tree species of temperate woodlands in Europe. The forest is dominated by sycamore (*Acer pseudoplatanus*, ca. 50% of the basal area), oak (*Quercus robur*, ca. 20% of the basal area) and ash (*Fraxinus excelsior*, ca. 20% of the basal area) (Butt *et al.*, 2009; Savill, 2011). Most parts of the woodland have experienced long-standing management practices (e.g. coppicing, selective logging) until 50 year ago (Butt *et al.*, 2009). Recruitment is low, and established vegetation accounts for most of the carbon uptake (Thomas *et al.*, 2011). The specific area within Wytham Woods in which our study took place has been a woodland at least since the 18th century, and most likely since the last ice age (Butt *et al.*, 2009).

Our focal study area within Wytham Woods hosts a 18-ha forest dynamics monitoring plot, which has been a target of extensive surveys of forest structure and ecosystem-scale fluxes and is a part of the Smithsonian Global Earth Observation network (www.sigeo.si.edu). The 18-ha plot was surveyed for tree growth, recruitment and mortality in 2008, 2010 and 2017. The carbon cycle of the plot has been measured 2007-2009 using the eddy covariance technique (Thomas *et al.*, 2011), by biometric quantification of leaf and woody biomass ("bottom-up") in a 1 ha intensive plot (Fenn *et al.*, 2015). According to these estimates, the gross primary production (GPP, assimilation of carbon by photosynthesis) of the site is 21.1-21.9 Mg C ha^{-1} yr^{-1}, net primary production (NPP, GPP – autotrophic respiration) 7.05 Mg C ha^{-1} yr^{-1} and net ecosystem productivity (NEP, net carbon balance) is 1.2 Mg C ha^{-1} yr^{-1}

(Table 5.1). These values are similar to other temperate forests (global average NPP 7.38 ± 0.55 Mg C ha^{-1} y^{-1} for deciduous temperate humid forests, (Luyssaert *et al.*, 2007), but slightly higher than for other ancient woodlands, probably due to the past management practices (Thomas *et al.*, 2011). The effect of herbivory on the carbon cycle of the plot has not been quantified before.

In Wytham Woods, lepidopterous caterpillars are the first herbivores to attack the newly flushed leaves of most of the tree species (Feeny, 1970). They emerge in synchrony with the budburst, and feed until early June (Hunter, 1992). Relatively few herbivore species feed on the mature leaves later in the season (Feeny, 1970). Of the early-spring herbivores, winter moth is one of the most common species (Feeny, 1970). The winter moth population reaches outbreak densities approximately every 10 years. The last high abundance year was in 2010 (285 caterpillars per m^2 of oak canopy, Lionel Cole; unpublished data). Since 2013, the population size has been small, for example 5 caterpillars per m^2 in 2014 (Lionel Cole, unpublished data). Winter moth is a native, generalist herbivore that feeds on most of the woody species in the area.

Pedunculate oak is among the most common tree species in Wytham Woods, accounting for 20 % of the total basal area (Butt *et al.*, 2009) and ca. 11% of the leaf area index (LAI) (Fenn 2010) in the 18 ha forest dynamics plot. Previous work at the site has revealed seasonal dynamics in oak photosynthesis, which peaks on average 63 (± 6) days after budburst (Morecroft *et al.*, 2003).

Most of the previous physiological measurements on oaks at the site have been taken from the upper canopy leaves of five mature trees accessed from a canopy platform (Stokes, 2000; Morecroft *et al.*, 2003; Visakorpi *et al.*, 2018). We use measurements from these trees throughout our study (Table 5.1) and scale up the leaf-level gas exchange to tree-level estimates using existing information from these five trees. We estimate leaf area loss, canopy photosynthesis, canopy respiration, stem respiration and root respiration for each tree

separately. By using hourly meteorological data, we scale these values for each day of the growing season (from budburst until 100% leaf fall, Julian day 109 to 326, corresponding to 19th April to 22nd November, Table 5.1). We then extrapolate these estimates to values per one hectare of the plot. We use two different estimates for plot-level values: first, we estimate oak NPP and the effect of herbivory for a hypothetical forest comprising only of mature oak trees, assigning the total basal area of the plot to oaks only. Second, we estimate herbivory and oak NPP for our specific plot, using information on the observed oak basal area. We will refer to these two approaches as 'oak plot' and 'real plot'. We estimate the oak NPP for two scenarios: 1.) for a completely intact canopy using gas exchange measurements from completely intact leaves only (no herbivory) and 2.) for a canopy under the observed level of herbivory, using gas exchange measurements from intact, damaged and systemically affected leaves scaled to their observed proportions in the oak canopy.

5.2.2. Leaf area loss

To estimate the level of insect herbivory on the study trees throughout the growing season, we used earlier estimates of the proportion of leaf area loss on the five oak trees and the proportion of three different leaf types per each tree: completely intact leaves which are growing on shoots with only other intact leaves ("completely intact"), intact leaves growing on shoots with damaged leaves on them ("systemically affected"), and leaves damaged by herbivores ("damaged") (Visakorpi *et al.*, 2018). To estimate the amount of carbon lost through the eaten leaf tissue, we used estimates for leaf mass per area ("LMA", gm^{-2}) and leaf carbon content (% of dry mass), which have been measured for the same three leaf types from the same five trees (see Chapter 3 in this thesis).

Table 5.1. Characteristics of the study site, and the five oak trees used for the gas exchange measurements and herbivory manipulations. Error are ± 1 SEM.

Plot-level measures	**Value**	**Year**	**Reference**
Annual rainfall	637 mm	2015	Rennie *et al.* 2017
Mean annual temperature	10.2 °C	2015	Rennie *et al.* 2017
Mean daily summer temperature	13.8 °C	2015	Rennie *et al.* 2017
Mean nightly summer temperature	10.7 °C	2015	Rennie *et al.* 2017
Total stem density (>1 cm dbh)	1128.2 ha^{-1}	2008	Butt *et al.* 2009
Total basal area (>10 cm dbh)	33.3 m^2ha^{-1}	2008	Butt *et al.* 2009
Peak leaf area index LAI	7.8 m^2m^{-2}	2008	Thomas *et al.* 2011
	6.5 m^2m^{-2}	2007	Fenn 2010
	ca. 6.5 m^2m^{-2}		Nils Rutjes 2016, unpublished data
Stem area index	1.94 m^2m^{-2}	2008	Fenn 2010
Average oak budburst	19th April	1996, 1999, 2000	Morecroft *et al.* 2003
100% oak leaf fall	22nd November	2007	Fenn 2010
Peak seasonal oak photosynthesis	4th July	1999	Morecroft *et al.* 2003
Percentage of oak of total LAI	11 ± 3 %	2007	Fenn 2010
Oak basal area	6.71 m^2ha^{-1}	2008	Butt *et al.* 2009
Biometric GPP	21.9 Mg C ha^{-1} yr^{-1}	2008	Fenn *et al.* 2015
Eddy flux GPP	21.1 Mg C ha^{-1} yr^{-1}		Thomas *et al.* 2011
Biometric NPP	7.04 Mg C ha^{-1} yr^{-1}	2008	Fenn *et al.* 2015
Eddy flux NEP	1.2 Mg C ha^{-1} yr^{-1}	2008	Thomas *et al.* 2011
Biometric R_{eco}	20.3 Mg C ha^{-1} yr^{-1}	2008	Fenn *et al.* 2015
Eddy flux R_{eco}	19.8 Mg C ha^{-1} yr^{-1}		Thomas *et al.* 2011
The five intensively studied oak trees			
Age	150 - 200 years		Morecroft *et al.* 2003
Mean dbh	67.2 cm ± 5.4 cm	2017	Y. Malhi, unpublished analysis
Mean height	21.6 m ± 0.6 m	2017	this study
Mean % of herbivory	5.92 % ± 0.38 %	2015	Visakorpi *et al.* 2018
Mean light-saturated photosynthesis (A_{sat})	16.13 ± 1.20 µmol m^{-2} s^{-1}	2015	Visakorpi *et al.* 2018
Mean stem area	471 ± 76 m^2	2017	this study
Mean crown area	57.6 ± 7.3 m^2	2017	this study
Mean leaf mass per area	64.6 ± 0.9 gm^{-2}	2015	this study
Mean C % in leaf tissue	47.8 ± 0.8 %	2016	this study

5.2.3. Estimating oak NPP

We used three methods to estimate oak NPP. First, NPP was estimated as a difference between GPP and autotrophic respiration. We upscaled leaf photosynthesis and respiration

measures and combined these with estimates on oak stem and root respiration at the site for the whole growing season of 2015 ("*NPP through canopy upscaling*"). Second, we used census data on tree growth at the site during 2010-2016 to estimate woody growth, and allometric equations for oak to estimate leaf production and belowground production ("*NPP through tree growth census*"). Third, we used earlier estimates of oak above- and belowground NPP at the site ("*NPP through biometric measurements*") taken during 2007-2008 (Fenn, 2010). We estimate NPP for two scenarios: for an oak tree experiencing the observed amount of leaf area loss (hereafter "*Normal herbivory*") and an oak tree with no herbivory at all ("*Intact canopy*"). The difference in NPP between these two scenarios describes the effect of herbivory on oak NPP in this system. In summary, we used three different upscaling methods (canopy upscaling, tree census, biometric), two different ways to characterise the plot (oak plot, real plot) and two different herbivory scenarios (normal, intact). We describe the procedures briefly here and in more detail in Appendix 5.1.

NPP through canopy upscaling: To estimate oak NPP through canopy upscaling, we used measures on photosynthesis-light response curves measured from the three different leaf types (completely intact, systemically affected and damaged, (Visakorpi *et al.*, 2018). We used the Big Leaf approach of The Joint UK Land Environment Simulator ("JULES", (Clark *et al.*, 2011) to estimate canopy assimilation, combined with an estimate for daytime canopy respiration (Mercado *et al.*, 2007), while taking into account leaf area loss and the proportions of the three different leaf types as described in Visakorpi *et al.* (2018). The rates were scaled to hourly light data, to seasonal changes in the photosynthetic rate of oak (Morecroft *et al.*, 2003) and to seasonal changes in LAI (Stokes, 2000; Fenn, 2010). To estimate night-time canopy respiration, we measured nightly respiration rates from the five oak trees and the three leaf types in Wytham Woods during two nights in July 2015. From these measures, we estimated canopy-scale respiration rate over the whole growing season taking into account the

proportion of different leaf types in the canopy, the different respiration rates at different canopy layers (Griffin *et al.*, 2001), and the seasonal changes in LAI. The estimates were further adjusted to hourly temperature taking into account different temperature responses at different canopy heights (Turnbull *et al.*, 2003), and to the daytime photosynthetic rate of the day before (Whitehead *et al.*, 2004). To estimate stem respiration, we used earlier measurements of oak stem respiration at the site (Walker, 2017). Both leaf and stem respiration rates have a similar positive relationship with the photosynthesis rate (Wertin & Teskey, 2008). Therefore, to estimate the stem respiration rate in the absence of herbivory, we assumed that stem respiration followed the same pattern as leaf respiration, mirroring the increase in photosynthesis. Stem respiration per unit stem area was scaled to hourly temperature data for the whole year and multiplied with a stem area estimate for each of the five trees. Root respiration was assumed to be 13% of stem respiration, based on earlier measurements at the site (Fenn *et al.*, 2015). To achieve an estimate of oak NPP for each of the five trees separately, the estimates of canopy photosynthesis and respiration (per m^2 of canopy) were multiplied with the average crown area and combined with tree-level estimates of stem and root respiration. These tree-specific estimates were then scaled to per hectare of forest (assumed to consist of oak trees only) and per hectare of our specific study site (where oak comprises 20% of the total plot basal area, Table 5.1). For all meteorological data, we used hourly data recorded on an open site at Wytham Woods during years 2014 and 2015 (Rennie *et al.*, 2017).

NPP through tree growth census: To estimate oak NPP through tree growth measurements, we used data of dbh measurements on all oak trees (355 stems, >10 cm dbh) within the 18 ha forest dynamics plot. Using data from the censuses in 2010 and 2016 (Y. Malhi, unpublished analysis) we estimated the yearly growth as increase in aboveground woody biomass. We multiplied the estimated change in woody biomass by the carbon content of oak wood (0.47;

Butt *et al.*, 2009) to estimate woody NPP. We then used oak-specific allometric relationships obtained from previous measurements at the site to estimate oak leaf NPP and belowground NPP (Fenn, 2010) (Appendix 5.3, Table S3). To estimate the effect of herbivory on oak NPP, we estimated NPP in the absence of herbivory assuming that the total NPP under normal herbivory situation was reduced to the same extent (ca. 56%, Appendix 5.3, Table S3) as canopy gas exchange in the canopy upscaling calculations described above. We then extrapolated the values per tree to estimates for one hectare of a plot with only oak trees, and for a hectare of the actual study site.

NPP through biometric measurements: To estimate oak NPP through biometric measurements, we combined data from aboveground oak NPP in an area of one hectare in Wytham Woods with information on oak-specific ratio of wood NPP to leaf NPP and oak-specific ratio of aboveground biomass to belowground biomass (Fenn, 2010; see Appendix 5.3, Table S3). We combined these relationships for an estimate of whole oak NPP as Mg C yr^{-1} per ha of the actual study site. By using the oak basal area in the plot, we then scaled this estimate for each of the five trees, and per hectare of a plot with only oak trees. To estimate the effect of herbivory on oak NPP, we again assumed that the total NPP was reduced to the same extent as canopy gas exchange in the canopy upscaling calculations above.

All errors were derived through standard error propagation rules and reflect uncertainty in the different scaling equations and sampling errors of the raw measurements. For detailed descriptions of the canopy upscaling, error propagation and the different oak NPP estimates, see Appendix 5.1 (“Detailed methods”).

5.2.4. Predicting the effects of herbivory on NPP under varying levels of herbivory

To predict the effect of herbivory on oak NPP under different levels of caterpillar herbivory, we first simulated changes in the proportions of the three different leaf types (intact,

systemically affected, damaged) in the oak canopy. We assumed that the ratio of damaged leaves to systemically affected leaves and the proportion of leaf area loss per damaged leaf stay at the same levels as observed in our herbivory surveys (0.43 and 8.53% respectively) until all shoots had at least one damaged leaf. After this, we let the proportion of systemically affected leaves decrease until all leaves were damaged. Finally, we assumed that the proportion of leaf area loss per leaf increases, until the tree was completely defoliated. In other words, we assumed that the caterpillar damage first spreads evenly to all shoots, then to all leaves, and then increases per leaf. We linked these changes in leaf area loss with winter moth caterpillar densities at two known time points: during our herbivory surveys 2015 (5 individuals m^{-2}; Lionel Cole, unpublished data) and a maximum reported caterpillar density which we assumed to lead to a complete defoliation (1200 individuals m^{-2}, Feeny 1970). We scaled LAI with the amount of leaf area loss assuming that the full LAI of the plot (6.5) represents LAI under the observed level of herbivory (5.92 %). We then estimated canopy photosynthesis for each different herbivory scenario and compared these estimates to an estimate of canopy photosynthesis with absence of herbivores (0% leaf area loss).

5.3. Results

The average leaf area loss per tree was 5.92 % ± 0.38 % (Table 5.1). We estimate that insect herbivores remove on average 0.70 (± 0.1) kg of carbon over the growing season per tree and 0.12 (± 0.02) Mg C ha yr^{-1} (assuming a forest of only oak trees) through leaf area loss.

The canopy photosynthesis peaked with the seasonal peak in photosynthetic rate per unit leaf area on 11th July (Figure 5.1a, Appendix 5.3, Figure S1a). Night-time canopy respiration rate (Figure 5.1b) and stem respiration rate (Appendix 5.3, Figure S5c) were highest during the two warmest nights and days (2nd July and 23nd August). Canopy NPP and whole tree NPP (Figure 5.1c) both followed the changes in photosynthetic rate. The canopy photosynthesis was lower and canopy respiration higher in the absence of herbivory than

under normal herbivory rates. Since leaf respiration rate was small compared to photosynthetic rate, the net CO_2 exchange was still higher for the intact canopy. For a description of the meteorological conditions at the site during the growing season 2015, see Appendix 5.2 ("Meteorological conditions at the site").

By using canopy upscaling, we estimate that under the observed level of herbivory, mature oak trees sequester on average between 22 ± 10 kg C yr^{-1} per tree (Table 5.2), 1.8 ± 0.8 Mg C ha^{-1} yr^{-1} in a forest comprised of oak trees only (Figure 5.2) and between 0.4 ± 0.2 Mg C ha^{-1} yr^{-1} at our specific study site. The estimates derived from the tree growth census were very similar to the canopy upscaling values while the estimates based on earlier biometric measurements were higher than those derived by either of the other two methods (Table 5.3, Appendix 5.3, Figure S7).

On average, the NPP of an oak tree under observed level of herbivory was between 45 ± 22% and 73 ± 30% lower than NPP of an intact canopy, depending on the estimation method. In terms of carbon, this corresponds to a reduction of between 35 ± 13 and 75 ± 23 kg C yr^{-1} per tree, or between 2.8 ± 1.9 and 6.2 ± 1.3 Mg C ha^{-1} yr^{-1} for the plot of only oak trees and between 0.69 ± 0.02 and 1.1 ± 0.3 Mg C ha^{-1} yr^{-1} at our study site, compared to the potential maximum NPP in the absence of herbivory.

We estimate that as the level of herbivory increases, the proportion of intact, unaffected leaves decrease until the level of ca. 5% herbivory (Appendix 5.3, Figure S6a). At this point, all shoots would be likely to have at least one damaged leaf, and consequently all leaves would be either damaged or systemically affected. The effect of herbivory increases linearly until this point. When all leaves are affected by herbivory (directly or systemically), the canopy photosynthesis is only further affected by the amount of leaf area loss and decreasing LAI (Figure 5.3, Appendix 5.3, Figure S6).

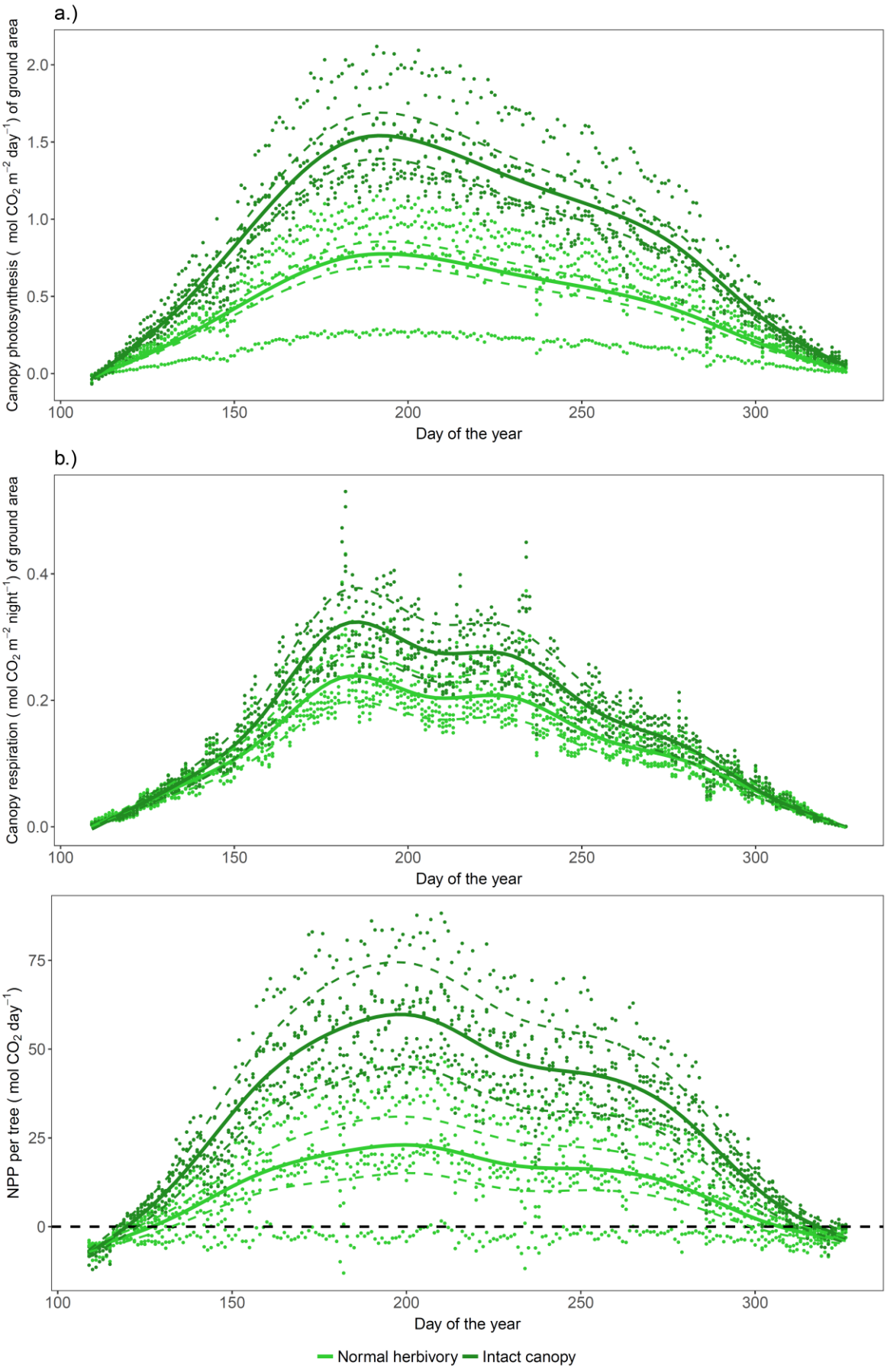

a.)
Canopy photosynthesis (mol CO_2 m^{-2} day^{-1}) of ground area
2.0
1.5
1.0
0.5
0.0
100
150
200
250
300
Day of the year
b.)
Canopy respiration (mol CO_2 m^{-2} $night^{-1}$) of ground area
0.4
0.2
0.0
100
150
200
250
300
Day of the year
NPP per tree (mol CO_2 day^{-1})
75
50
25
0
100
150
200
250
300
Day of the year
Normal herbivory
Intact canopy

Figure 5.1. Daily a) canopy photosynthesis and b) night-time canopy respiration per m^2 of ground area and c) net primary productivity (NPP) per tree based on data from five oak trees in Wytham Woods during the growing season 2015 (19th April to 22nd November). The model for canopy photosynthesis takes into account the effect of reduced light through the canopy layers and daytime respiration rate per unit leaf area (Visakorpi *et al.*, 2018), seasonality in photosynthetic rate per unit leaf area (Morecroft *et al.*, 2003), seasonality in LAI, change in light intensity during each day and daylength over the season, and photosynthetic response to light (Visakorpi *et al.*, 2018). The "intact canopy" scenario is based on measurements on intact leaves surrounded by only intact leaves, whereas the estimates under "normal herbivory" are on photosynthesis and respiration rates of three different leaf types (intact, damaged, systemically affected), weighted with their observed abundance. The canopy respiration model takes into account different respiration rates at different canopy layers, the effect of temperature on respiration scaled for hourly temperature data, the differences in temperature responses at different part of the canopy, the effect of daytime photosynthesis on night-time respiration and seasonality of LAI. In all panels, each data point is a modelled estimate for one tree for one day. Whole tree NPP is calculated by subtracting canopy, stem and root respiration from canopy photosynthesis. The dashed black line in panel c shows the level of zero NPP (negative values indicate that the tree is losing carbon). The dashed coloured lines represent the propagated measuring uncertainty.

Table 5.2. The average carbon budget per oak with and without herbivory based on canopy upscaling. The NPP per tree is a combination of these estimates. Carbon loss through reduced assimilation represents the average difference in the whole tree NPP between the normal herbivory and intact canopy scenarios. Carbon loss through missing leaf area is based on the estimate of the amount of leaf area loss to herbivory and the carbon content of oak leaves. The means are averages over the estimates of five trees and the errors are propagated measuring uncertainties.

	Normal herbivory		**Intact canopy**	
	Mean	**± error**	**Mean**	**± error**
Canopy photosynthesis (mol CO_2 yr^{-1} per tree)	5617	714	11097	1409
Canopy respiration (mol CO_2 yr^{-1} per tree)	1607	205	2060	262
Canopy NPP (mol CO_2 yr^{-1} per tree)	4010	743	9037	1433
Stem respiration (mol CO_2 yr^{-1} per tree)	1921	390	2463	559
Root respiration (mol CO_2 yr^{-1} per tree)	251	93	322	123
Autotrophic respiration (mol CO_2 yr^{-1} per tree)	3779	450	4845	630
NPP whole tree (mol CO_2 yr^{-1} per tree)	1838	844	6252	1543
NPP whole tree (C g yr^{-1} per tree)	22077	10141	75094	18535
Carbon loss through reduced assimilation (g yr^{-1} per tree)	53017	1759	0	0
Carbon loss through missing leaf area (g yr^{-1} per tree)	698	100	0	0

Table 5.3. Oak NPP for intact canopy and canopy under normal level of herbivory and the effect of herbivory on the NPP estimated with different methods and at different scales (tree, oak plot, real plot). The errors are propagated measuring errors. See also Appendix 5.3, Figure S7 for visual representation.

	NPP Normal herbivory	± error	NPP Intact canopy	± error	Herbivory effect, C	± error	Herbivory effect %	± error
Per tree, kg C yr-1								
Canopy upscaling	22.08	10.14	75.09	18.54	53.02	21.13	70.6	33.1
Biometric measurements	59.65	14.58	134.42	17.94	74.77	23.12	55.63	18.73
Tree census	28.02	3.67	63.14	12.53	35.12	13.06	55.63	23.44
Per plot of only oak, Mg ha-1 yr-1								
Canopy upscaling	1.83	0.84	6.23	1.54	4.4	1.76	70.6	33.13
Biometric measurements	4.95	1.21	11.16	1.49	6.21	1.92	55.63	18.73
Tree census	3.38	0.4	6.17	1.24	2.79	1.3	45.19	22.9
Per real plot (counting only oak trees), Mg ha-1 yr-1								
Canopy upscaling	0.36	0.17	1.38	0.31	1.02	0.35	73.67	30.31
Biometric measurements	0.87	0.21	1.97	0.26	1.1	0.34	55.63	18.73
Tree census	0.55	0.01	1.23	0.02	0.69	0.02	55.63	2.2

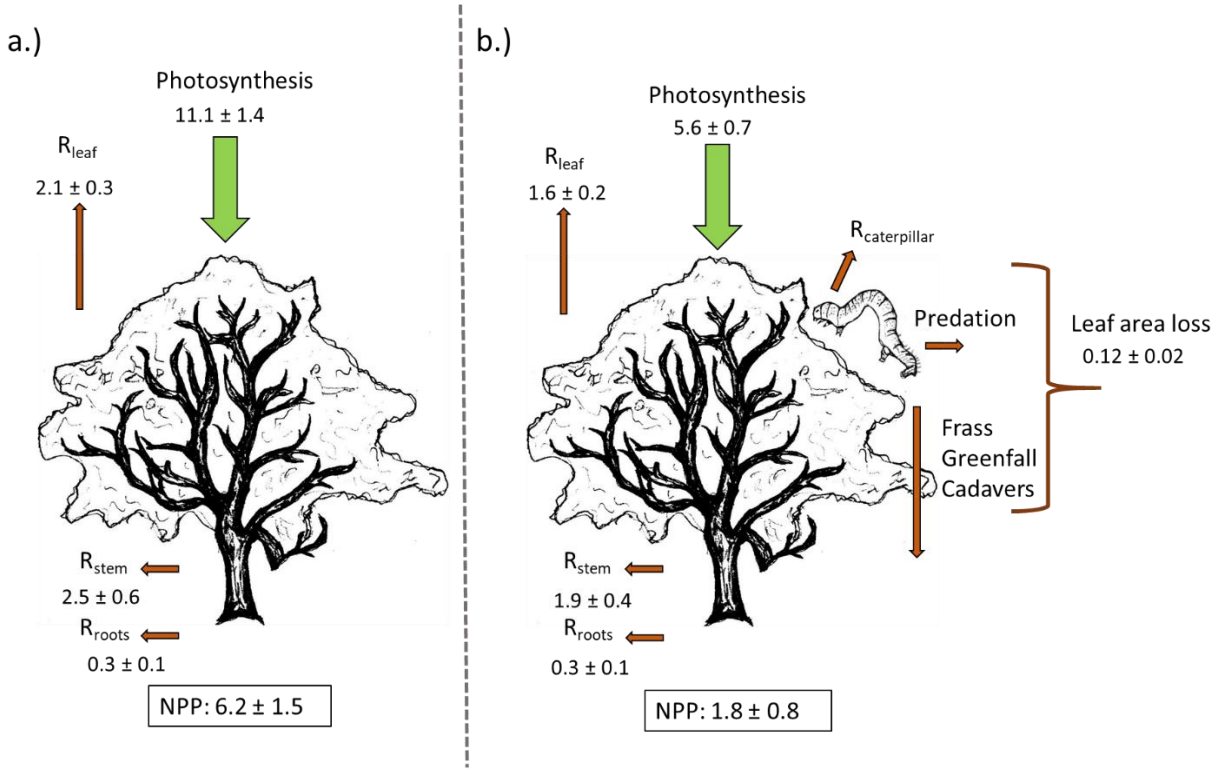


Figure 5.2. The combined carbon budget a) without and b) with herbivory for an oak forest as Mg C ha^{-1} yr^{-1} based on canopy upscaling. The carbon flux of leaf area loss (0.12 Mg C ha^{-1} yr^{-1}) will continue its cycle either by entering soil as frass or insect cadavers, being respired by herbivores, or being eaten by higher trophic levels preying on caterpillars. Errors are propagated measuring uncertainties.

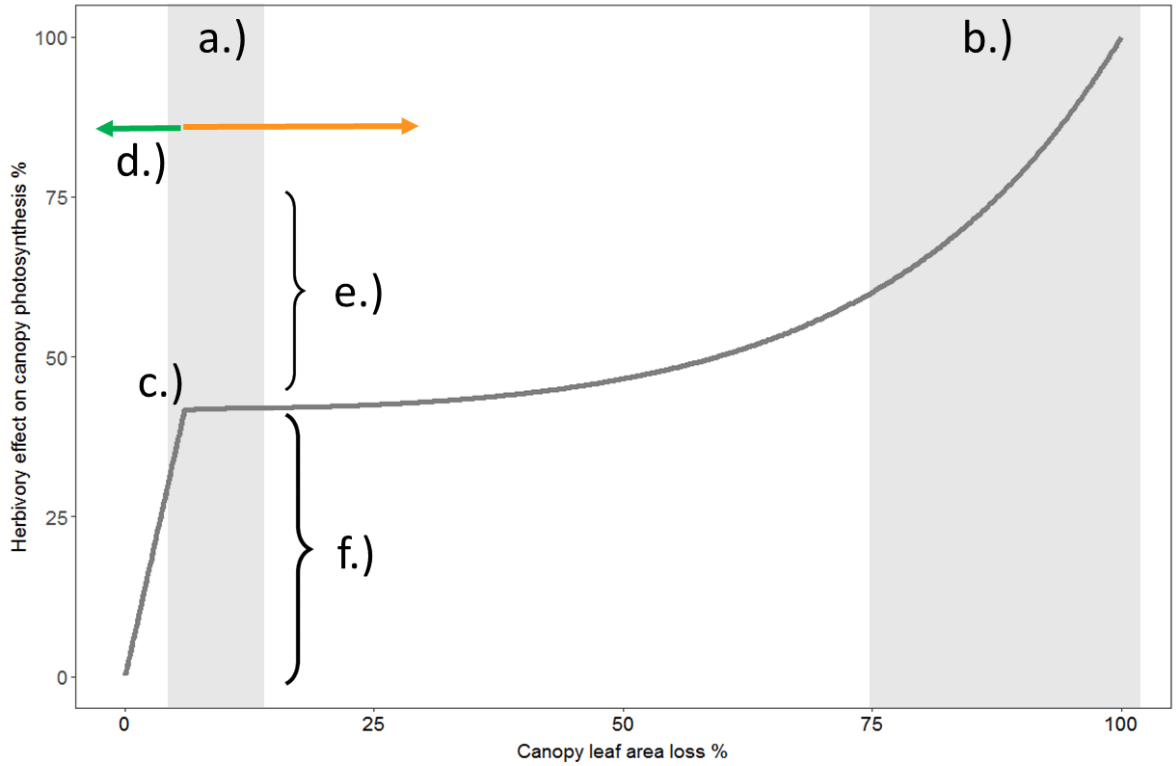


Figure 5.3. The effect of leaf area loss on canopy photosynthesis, as % reduction from the full photosynthetic capacity. The first shaded area (a) represents normal level of herbivory in temperate forests (5-15% of leaf area loss, Cebrian 1999, 2004). The second shaded area (b) shows leaf area loss during an insect herbivore outbreak (75-100% leaf area loss, e.g. Clark *et al.*, 2010; Schäfer *et al.* 2010). Above the level where all leaves are affected by herbivory (c), the changes in the effect of herbivory are driven by increasing leaf area loss (orange arrow). Below this level (d), the herbivory effect on photosynthesis is mainly driven by the spread of systemic changes in leaf gas exchange in the undamaged leaf tissue (green arrow, "indirect effects"). Most previous studies on the effects of herbivory on net primary productivity have compared outbreak densities of herbivores to a normal level of herbivory (e). Nevertheless, we predict this effect is smaller than comparing normal levels of herbivory to a completely intact canopy (f). See Appendix 5.3, Figure S6 for description of the changes in the proportion of different leaf types and leaf area loss with changing herbivore densities.

Table 5.4. Earlier estimates on the effects of insect herbivores on ecosystem carbon cycling. The baseline estimate represents productivity under normal level of herbivory (e.g. before an insect outbreak), or in the absence of herbivores (this study and Dungan *et al.*, 2007). Herbivory level is percentage of leaf area loss, unless otherwise stated.

Reference	Baseline Mg C ha^{-1} yr^{-1}	Baseline measure	Herbivore effect Mg C ha^{-1} yr^{-1}	Herbivore effect %	Method	Study system	Herbivore species	Herbivory level
Nardini *et al.* 2004	5	NPP	-4.25	85%	Gas exchange and canopy model	Horse chestnut *(Aesculus hippocastanum)*	Leafminer (*Cameraria ohridella)*	25-60% of LAI
Dungan *et al.* 2007	17.2	Canopy photosynthesis	0.688	4%	Sap flow model	Beech (*Nothofagus solandri*)	Scale insect *(Ultracoelostoma assimile*)	0
Cook *et al.* 2008	3.73	NEP	-2.955	79%	Model and eddy covariance	Temperate deciduous forest	Tent caterpillar (*Malacosoma disstria*)	34%
Kurz *et al.* 2008	-	Net biome production	-0.36	-	Forest ecosystem model	Pine /spruce	Pine beetle (*Dendroctunus ponderosae*)	-
Clark *et al.* 2010	1.87	NEP	-0.65-4.8	34%-257%	Eddy covariance and biometric methods	Oak /pine	Gypsy moth (*Lymantria dispar*)	> 75%
Schäfer *et al.* 2010	12.4	Canopy photosynthesis	-3	24%	Sap-flow based canopy modelling	Oak /pine	Gypsy moth (*Lymantria dispar*)	> 75%
Heliasz *et al.* 2011	1.05	NEP	-0.93	89%	Eddy covariance	Mountain birch (*Betula pubescens*)	Autumnal moth (*Epirrita autumnata)*	100%
Malhi *et al.* 2014	14.2-15	NPP	-1.46	11%	Estimation of leaf area loss	Tropical forest	Several	18.80%

Metcalfe *et al.* 2013	2-5	Foliar production	-0.3-0.9	12%-19%	Estimation of leaf area loss	Tropical forest	Several	19-20%
Stickland et al. 2013	-	-	-33%	33%	^{13}C pulse chace experiment	Meadow	Grasshopper (*Melanoplus femurrubrum)*	32gm^{-2} of biomass
Lund et al. 2017	4.5	NEP	-2.61	58%	Chamber-based CO_2 exchange	Tundra	Moth (*Eurois occulta)*	100%
this study	**11.1**	**Oak NPP**	**-4.4**	**71%**	**Leaf gas exchange**	**Oak *(Quercus robur)***	**Early-season moth caterpillars**	**6%**
			-0.12		**Leaf area loss**			

5.4. Discussion

We estimate that insect herbivores remove 0.12 (± 0.02) Mg ha yr^{-1} of carbon through leaf area loss and prevent 4.4 (± 1.8) Mg ha^{-1} yr^{-1} of carbon from being assimilated in a temperate oak forest. Even with relatively low levels of herbivory (6% leaf area loss), the oak NPP was low and occasionally negative. In a hypothetical situation with no herbivory at all, we estimate that the oak forest could be a considerably higher carbon sink. While previous studies (Kurz *et al.*, 2008; Clark *et al.*, 2010; Schäfer *et al.*, 2010; Heliasz *et al.*, 2011; Strickland *et al.*, 2013; Metcalfe *et al.*, 2014; Lund *et al.*, 2017) have shown that herbivore outbreaks can result in a considerable loss of carbon from the ecosystem, our study suggests that insect herbivores can have a considerable impact on the forest carbon budget even at relatively low population densities.

We predict that the increase in the effect of herbivory on ecosystem NPP is most pronounced at low levels of herbivory. When the level of herbivory is low, even small increases in the proportion of leaves attacked will cause the indirect effects of herbivory to spread to a large proportion of the canopy. Once herbivory reaches a level where every leaf is affected (either by being subject to herbivory or by being located on shoots where other leaves are attacked), subsequent increases in leaf area loss contribute only little to the overall reduction in canopy-level photosynthesis. Consequently, we suggest that the relationship between the intensity of herbivory and its effect on ecosystem productivity is non-linear and described by two stages: first, a rapid increase in the effect of herbivory due to the spread of indirect effects in the canopy, and second, an exponential increase with increasing leaf area loss.

Our study illustrates the potential magnitude of the effect of insect herbivores on forest carbon cycling. Nevertheless, our estimates are based on upscaling of leaf-level measurements and on allometric relationships, rather than on direct canopy measurements. Consequently, we cannot be sure that the true effects of different levels of herbivory would follow our predictions.

Below, we will compare our estimate to previous estimates of forest NPP and discuss reasons for the possible discrepancies between our simulation and the real-world effects of herbivores on ecosystem productivity.

5.4.1. Previous estimates of oak NPP and of the effects of insect herbivores on carbon cycling

Based on canopy upscaling, we estimated the NPP in an oak plot to be 1.8 (± 0.8) Mg C ha^{-1} yr^{-1} with herbivory and 6.2 (± 1.5) Mg C ha^{-1} yr^{-1} without herbivory. This estimate is lower than an earlier estimate (Fenn, 2010) based on biometric measurements of oak NPP (4.95 Mg C ha^{-1} yr^{-1} when scaled for an oak plot). The NPP estimated with data from the tree growth census was very similar to that derived through canopy upscaling. If we scale our estimate derived from canopy upscaling to a hectare of canopy area with LAI 6.5 (instead of hectare of plot by basal area), we estimate the oak canopy photosynthesis to be 11.7 Mg C ha^{-1} yr^{-1} and canopy NPP (canopy photosynthesis – canopy respiration) 8.3 Mg C ha^{-1}. These estimates are very similar to for example, Whitehead *et al.* (2004), who estimated the net CO_2 exchange of a *Q. rubra* canopy to be 9.90 Mg C ha^{-1} yr^{-1} and Schäfer *et al.* (2010), who estimated canopy photosynthesis of 12.4 Mg C ha^{-1} yr^{-1} for an oak/pine mixture.

The observed leaf area loss was 5.92 %, which is close to the reported global average for forests (5%; Cebrian, 1999, 2004). We estimated that 5.4 (± 1.6) Mg of carbon per hectare of oak forest over the growing season does not get assimilated because of insect herbivory. Of this, only a small proportion, 0.12 (± 0.02) Mg C ha^{-1} is due to leaf area loss, while the rest is caused by the indirect effects of herbivores on photosynthetic rate. When changes in respiration rates caused by herbivory are considered, the difference in NPP between intact trees and trees with canopies experiencing herbivory is 4.4 (± 1.8) Mg C ha^{-1} yr^{-1}. This is our estimate for the total effect of herbivory for the oak forest NPP in this system, based on canopy upscaling.

In a tropical rainforest, with leaf area loss rates of ca. 20%, Metcalfe *et al.* (2014) estimated that 0.3 - 0.9 Mg C ha^{-1} yr^{-1} is lost through herbivores feeding on leaf tissue. Our estimate of the carbon loss through direct leaf area loss compares well with this estimate (Table 5.4). Based on observed changes in sap flow and canopy modelling, Schäfer *et al.* (2010) estimated that gypsy moth outbreak reduced canopy assimilation of an oak/pine -forest by 24%. Focusing on the very same gypsy moth outbreak, Clark *et al.* (2010) estimated on average 55% reduction in net ecosystem CO_2 exchange using eddy covariance and biometric measurements (Table 5.4). In our system, the change in canopy gas exchange was 56%, and effect of herbivory on whole tree NPP was 45-71%, depending on the estimation method. The larger effect of herbivory on NPP obtained through canopy upscaling (71%) arises partly because stem and root respiration were assumed to respond to herbivory similarly than leaf respiration, and because the difference between the two scenarios (intact and with herbivory) was smaller for leaf respiration than for photosynthesis. How woody respiration responds to leaf herbivory is one of the biggest unknowns in our calculations. Had we not assumed stem respiration to increase with increased photosynthesis on intact trees, our estimate for the effect of herbivory on oak NPP would have been even larger.

Many of the previous studies on the effects of herbivory on forest NPP have compared herbivore outbreak years to non-outbreak years. This comparison might not be completely analogous to the situation in our system: even in non-outbreak years, the forest canopies are still likely to experience some herbivory (Cebrian, 1999, 2004). Our estimate of no herbivory therefore represents an unnatural, hypothetical situation, which is unlikely to occur outside controlled laboratory conditions. Since the indirect suppression of photosynthesis in the intact plant tissue was the biggest driver of the effect of herbivory on NPP, comparing a natural level of

herbivory (when all leaves are affected) to a completely intact canopy might reveal a larger herbivory effect than comparing normal levels of herbivory to outbreak densities (Figure 5.3).

5.4.2. The effect of increasing herbivory pressure on forest productivity

Our estimate of the effect of herbivores on oak NPP was measured during a year with a low winter moth population density. Previous reports of winter moth outbreaks in Wytham Woods include records of complete defoliation of trees (Feeny, 1970). Intuitively, we might expect increasing herbivory pressure to have a linearly increasing effect on ecosystem productivity. Nevertheless, the herbivore-induced changes in leaf gas exchange per unit leaf area appear not to depend on the amount of damage per leaf (Visakorpi *et al.*, 2018), and the vast majority of leaves show some signs of feeding damage even under low herbivore densities. Thus, increased herbivore density from the observed level would likely not increase the magnitude of the indirect effect, nor change the proportion of leaves affected by indirect effects of herbivory. Consequently, we predict that increases in herbivore densities above those observed in our study are unlikely to result in large changes in the magnitude of the effect of herbivory on ecosystem NPP, except in outbreak years with very high proportion of leaf area loss (Figure 5.3). We predict that as densities of insect herbivores increase, the importance of the direct leaf area loss on canopy photosynthesis will also increase. After the majority of leaf area (ca. 75% or more) is lost to herbivores, the effect of herbivory starts to increase exponentially, until all leaves have been eaten. If the systemic plant responses observed in our system are widespread, many forests are likely to be at a stage where a large part of the photosynthesising plant tissue is indirectly affected by leaf damage. Consequently, detecting herbivore-induced changes in ecosystem productivity might be difficult.

There are several factors that might change how herbivory affects ecosystem productivity which have not been considered in our predictions. First, the reduced leaf area caused by herbivory would increase light penetration to lower canopy layers (Anten & Ackerly, 2001). Since even small changes in the available light can strongly influence photosynthesis (Brodersen *et al.*, 2007), the effect of increased light penetration might compensate for some of the loss of leaf tissue (e.g. 5-30% of the lost leaf area according to Anten & Ackerly, 2001). Second, increased herbivory is likely to result in increased deposits of frass, insect tissue, leaf fragments ('greenfall') and nutrient leaching from damaged leaves ("throughfall). The additional nutrient inputs could increase productivity and compensate for the loss of carbon to herbivory (Lund *et al.*, 2017). Increased herbivory on leaves might increase carbon allocation to other parts of the tree, for example to roots (Dyer *et al.*, 1991), which might increase soil and root respiration (Holland *et al.*, 1996). Third, previous studies have shown how the total litterfall might remain unchanged even after severe defoliation due to compensating second leaf flushes (Grace, 1986; Clark *et al.*, 2010). If the second leaf flush ("lammas shoots" on oak) produces more leaves as a response to early-season herbivory, or if these new leaves photosynthesise with a higher rate, they might compensate for the effects of herbivory on the canopy gas exchange. Quantifying the compensatory plant responses to herbivory and the photosynthetic rate of leaves on lammas shoots would be an important topic for future studies.

5.4.3. The effect of decreasing herbivory pressure on forest productivity

We predict that if herbivory decreases from the level observed in our study, the indirect effects of herbivory on photosynthesis would become more important. When only a small part of the canopy is affected by herbivores, even small increases in herbivory cause a large change in the canopy photosynthesis through changes in the proportion of intact leaves. Situations of lower

levels of herbivory than those observed in our study might be rare in natural conditions and the rapid changes in productivity with small changes in herbivory pressure might in practice not exist outside laboratory conditions or simulations.

Predicting the effects of reduced levels of early-season herbivory on forest carbon cycling is not straightforward. Reduced densities of herbivores early in the season could result in altered investments in plant defences (but see Chapters 3 and 4) and change the level of herbivory experienced by the host plan at later point (Agrawal, 2000; Poelman *et al.*, 2008). Changes in the frequency of insect herbivory could also change susceptibility to plant pathogens (Felton & Korth, 2000; De Vos *et al.*, 2006), which can decrease leaf photosynthesis (Copolovici *et al.*, 2014). How the interplay between different plant enemies affects carbon sequestration and cycling is currently unknown.

In the presence of herbivory, the herbivory-induced reduction in photosynthetic rate of individual leaves might be compensated for by increased photosynthesis in completely intact leaves. In the absence of herbivores, all leaves might be photosynthesising with similar rate, and the average rate of canopy gas exchange might change only little. Insect feeding can increase transport of photosynthetic end-products away from certain leaves, which would otherwise limit the rate of photosynthesis (Retuerto *et al.*, 2004; Schwachtje & Baldwin, 2008). These types of sink-source dynamics might change the distribution of photosynthetic efficiency within the canopy.

Lastly, the seasonal changes in photosynthesis experienced by oak in our study system (Morecroft *et al.*, 2003) might depend on early-season herbivore densities. The slow start and the late peak of the oak photosynthesis could be a strategy to avoid losing photosynthesised sugars to early-season caterpillars and could be driven by phenotypic plasticity in the responses to the intensity of early-season herbivores. Thus, if the spring-feeding caterpillars are absent or occur at

low density, it is possible that oak photosynthesis could develop faster, changing the amount of carbon sequestrated over the season.

5.4.4. Conclusions

We estimate that insect herbivores can, even at low densities, have a large effect on ecosystem NPP in a temperate deciduous forest, mainly through indirect changes in the rates of canopy gas exchange. We propose that most forest ecosystems are naturally in a state where photosynthesis is reduced by the effects of herbivory. The relationship between the intensity of herbivory and its effects on the host plant is non-linear. At low herbivore densities, the effect of herbivory on NPP is mostly driven by indirect effects, while the contribution of direct effects increases with increasing herbivore abundances. We predict that comparisons of productivity between completely intact plants and plants with even a small amount of herbivory-inflicted damage are likely to yield large estimates on the effects of herbivory on plant productivity. On the other hand, comparisons between plants experiencing different levels of herbivory are likely to result in low estimates on the effect of herbivory. Further studies including different study systems and species and accounting for effects such as compensatory plant responses are crucially needed to validate whether the proposed relationship between intensity of herbivory and its effect on plant productivity holds. The distributions and abundances of insect herbivores are likely to change with the changing climate (Ayres & Lombardero, 2000; Bale *et al.*, 2002; Jepsen *et al.*, 2008; Kurz *et al.*, 2008; Charmantier *et al.*, 2008). Quantifying the effect of insect herbivores carbon cycling is therefore particularly important.

Chapter 6: General discussion

In this thesis, I set out to investigate how insect herbivory changes primary and secondary metabolism of its host plant, and how herbivory-induced changes in plant function or chemistry influence community- and ecosystem-level processes associated with the plant. In my first data chapter (Chapter 2), I showed that insect herbivores can have a strong effect on photosynthesis of their host plant. The indirect effects of herbivory on photosynthetic capacity overshadowed the direct effect of leaf area loss. Leaf damage also influenced the emission of an important volatile organic compound, isoprene. I also showed that the changes in leaf gas exchange observed at the level of individual leaves have the potential to substantially influence photosynthesis at the level of the whole tree canopy, with implications for ecosystem carbon sequestration and atmospheric chemistry. I suggested that one possible pathway leading to reduced photosynthesis after insect herbivory could be a trade-off between plant growth and defence.

In my second data chapter (Chapter 3), I studied the effects of insect herbivory and mechanical leaf damage on physical and chemical leaf traits, focusing on the concentration and composition of polyphenol compounds thought to act as defensive compounds. Contrary to predictions based on results in Chapter 2, insect herbivory had little impact on measured physical and chemical leaf traits. Instead, the variation between individual trees explained most of the variation in the chemical composition of the leaves. There was a significant effect of herbivory on some of the studied chemical traits, but this effect depended on study site and date of leaf sampling. The observed changes in chemical traits did not reflect the changes in photosynthetic rate observed in Chapter 2.

In my third data chapter (Chapter 4), I studied if changes in the densities of early-season herbivores at the scale of entire trees affect leaf chemistry, tree performance and the community structure of sessile insect herbivores on the same host tree. Contrary to my predictions, the altered densities of the focal herbivore did not influence any of the measured variables. Nevertheless, leaf chemistry (independently of herbivore pressure) significantly covaried with the structure of the insect community. Again, the spatial location of the host tree was more important than herbivory in determining host plant chemical composition and the structure of the associated sessile insect community.

In my last data chapter (Chapter 5), I returned to the data on herbivory-induced changes in photosynthesis reported in my first data chapter. I quantified the herbivore-induced change in photosynthesis at the whole forest ecosystem scale over the growing season of 2015 in Wytham Woods and calculated net primary productivity by subtracting autotrophic respiration of leaves, stems and roots (estimates available from previous studies) from photosynthesis. Through this approach, I predicted the impact of insect herbivores on gross and net primary productivity at different levels of herbivory pressure. My estimates suggest that insect herbivores can affect ecosystem productivity through limiting rates of carbon sequestration from the canopy. I propose that the relationship between the intensity of herbivory and its effect on productivity is non-linear and depends on whether direct effects (leaf area loss) or indirect effects (physiological changes in the remaining plant tissue) are dominating.

Below, I discuss the main findings from my thesis work (section 6.1). First, since the chemical analyses in Chapters 3 and 4 provide little evidence for growth-defence trade-offs driving the observed herbivory-induced changes in photosynthesis, I begin by examining a number of alternative explanations (Section 6.1.1). Second, I examine possible reasons for the lack of induced defensive reactions in either of the two experiments where I expected to find

them and discuss some of the recent trends in plant defence literature (Section 6.1.2). Third, I briefly discuss factors causing variation in plant chemistry (Section 6.1.3). Fourth, I discuss why indirect interactions between herbivores in this study system seemed weak, and why I found no evidence for keystone herbivores (Section 6.1.5). Fifth, I expand the discussion on the role of insect herbivores in controlling ecosystem productivity and combine some of the knowledge on community- and ecosystem-level dynamics in this study system (Section 6.1.5). Finally, I discuss the implications of these findings in the face of the current climate change (Section 6.2) and propose important areas for future research (Section 6.3).

6.1. Main findings

6.1.1 The photosynthetic rate of oak leaves was reduced following herbivory.

In my first data chapter, I showed that insect herbivory reduces the photosynthetic rate of oak leaves. As an explanation, I proposed that the lower photosynthesis may be due to underlying trade-offs between growth and defence. Nevertheless, since I observed no changes in the concentration and composition of defensive chemicals in Chapters 3 and 4, this explanation might not be correct. Below, I propose three alternative explanations for the observed relationship between herbivory and photosynthesis.

Leaf damage by herbivory might cause changes in the onset of leaf senescence, which could alter photosynthesis. Jasmonic acid (JA), which is induced after herbivore attack, can accelerate senescence (Lim *et al.*, 2007). Water loss from cut edges can lead to dehydration, which can also trigger senescence signalling (Lim *et al.*, 2007; Nabity *et al.*, 2009). Since photosynthesis often declines with senescence, accelerated senescence following herbivory could decrease photosynthetic rate. Since production of JA can also be induced in leaves in the vicinity of attacked leaves, this mechanism could explain the lower photosynthetic rate in both damaged

and systemically affected leaves. Furthermore, photosynthetic rate per unit leaf area changes seasonally at our study site (Morecroft *et al.*, 2003; see Appendix 5.2, Figure S2a), which might be linked to leaf age or developmental stage. If herbivory induces changes in the onset of leaf senescence, it might have caused herbivory-affected leaves to be in a different seasonal phase than leaves on intact shoots. These leaves might have then expressed photosynthetic rates that were not in synchrony with the actual season.

Another possible explanation for reduced photosynthesis following herbivory is the activation of constitutively stored defence compounds upon herbivore attack. When activated, these compounds can be harmful for the plant itself and damage the photosynthetic machinery (Zangerl *et al.*, 2002; Nabity *et al.*, 2009). This "autotoxicity" has been reported for example with nicotine in tobacco and polyphenols like furanocoumarins in parsnips (Zangerl *et al.*, 2002; Nabity *et al.*, 2009). If constitutive defence compounds were stored in every leaf, but only activated following herbivore attack, this might have resulted in the pattern of reduced photosynthetic rate in affected leaves, but no difference in the concentration of the defensive compounds themselves.

Lastly, reduced photosynthesis after herbivory could be a defensive strategy in itself, without any simultaneous changes in other defensive traits. Reduced photosynthesis might make the leaf less palatable for herbivores by reducing concentration of sugars. Carbon could be actively transported away from leaves that are under attack and stored for example in roots (Schwachtje & Baldwin, 2008). The induction of this type of a "sink metabolism" can reduce photosynthesis (Wingler & Roitsch, 2008).

6.1.2. No evidence for induced defences.

In Chapters 3 and 4, I measured the concentrations of 27 different compounds of hydrolysable tannins and flavonols thought to act as defensive compounds and hypothesised to increase in concentration following herbivory. Nevertheless, neither shoot-level (Chapter 3) nor whole-tree (Chapter 4) herbivory manipulations showed any clear differences in the concentrations of any of these compounds. In Chapter 4, I discussed the potential explanations for the lack of induced defences in this system. Below, I discuss what might have limited the amount of herbivory-inflicted leaf damage on oak in the absence of induced defences and provide an overview of recent trends in the plant defence literature.

Localized induced changes in plant chemistry are thought to restrict insect feeding on attacked leaves, resulting in leaf damage being spread across multiple leaves (Edwards & Wratten, 1983). Without any local induced changes in leaf quality, we might expect herbivores to continue feeding on the specific leaf they attack until the whole leaf has been consumed. In our system, leaf damage was evenly spread on the oak leaves (the average area loss per leaf was only 8%). If induced defences were not present in our system, something else might have forced the caterpillars to stop feeding on attacked leaves and move to neighbouring leaves or shoots. For example, caterpillars might have been forced to move quickly from one leaf to another to avoid predators like birds or parasitoid wasps. By sending predator-attracting VOCs, plants can defend themselves against herbivores indirectly (De Moraes *et al.*, 1998; Thaler, 1999) and might not need to induce changes in leaf chemistry. A recent meta-analysis suggests that top-down forces by predators and parasitoids are often stronger than bottom-up forces on herbivore fitness (Vidal & Murphy, 2018). If this is true in our system as well, predator-attracting defence mechanisms might be more successful than induced changes in leaf chemistry. Since we observed increased

emission of isoprene from the damaged leaves, it is possible that herbivory triggered a general production of VOCs.

Overall, based on my results in Chapters 3 and 4, herbivory does not seem to induce long-term changes in polyphenol concentration in this study system. The polyphenols measured in this thesis might act as constitutive defences, likely being present regardless of changes in herbivory pressure. Whether constitutive defences are more common than induced defences across different plant-herbivore systems is difficult to assess. Much of the current literature on plant defences emphasises induced defences (Wittstock & Gershenzon, 2002; Gatehouse, 2002), which might result in the impression that induction of defences is more common in nature than what they actually are. Under natural conditions, where plants are attacked by a wide variety of herbivores, plants might be constantly in an induced stage (Heil & Baldwin, 2002). Moreover, the defensive compounds involved in constitutive and induced defence mechanisms are often the same (Gatehouse, 2002). If this is the case, there might in practice be no differences between constitutive and induced defences under natural conditions. Even experimental methods like those described in Chapter 3 and 4 might fail to separate these two types of defences, because the underlying variation in the levels of constitutive defences might make it impossible to record more subtle changes caused by induction (discussed in Chapter 4).

For a plant to induce defences, it needs to actively recognize the attacker and decide on a correct response, whereas constitutive defences could occur without any detailed sensing of the environment or decision-making. There has recently been more emphasis on plants as active players in herbivore-plant interactions (Kessler & Halitschke, 2007; Karban, 2015; Poelman & Kessler, 2016). There is evidence that plants can sense their environment to an astonishing level of detail (Braam, 2004; Heil, 2009; Ghosh *et al.*, 2016; Yang *et al.*, 2018), "communicate" with surrounding plants (Baldwin & Schultz, 1983; Arimura *et al.*, 2000), "call for help" to attract

predators of the attacking herbivores (De Moraes *et al.*, 1998; Thaler, 1999; Kessler & Baldwin, 2001), and rapidly send signals between intact and damaged plant parts (Wu & Baldwin, 2009; Choi *et al.*, 2017; Tunc-Ozdemir *et al.*, 2018), much like neural signals in animals (Fromm & Lautner, 2007; Toyota *et al.*, 2018). This emphasis of plants as actively sensing and modifying their environment might be part of the resurfaced focus on plant sensing and behaviour (Brenner *et al.*, 2006; but see Firn, 2004 and Alpi *et al.*, 2007 for critique) with even suggestions of plants being in some sense sentient (Calvo *et al.*, 2017) and having cognitive abilities (Gross, 2016; Gagliano *et al.*, 2018). Using these mechanisms, plants could influence both the rates of herbivory on themselves, but also the rates of predation that the herbivores are experiencing. On the other hand, there are several accounts of herbivores rapidly evolving to tolerate defensive chemicals (Després *et al.*, 2007), suppressing the defensive reactions induced by the host plant (Schimmel *et al.*, 2017) or re-using defensive chemicals from their host plants to protect themselves from predators (Nishida, 2002). Plants, as sedentary and slow-evolving organisms (e.g. long-lived tree species) might have adapated to tolerate and cope with the herbivores attacking them. As in many ecological questions, the way plants cope with enemy attack seems to be context-dependent. Plants can repel herbivores and attract predators through active defence mechanisms, but depending for example on the right environmental conditions, on the herbivore community attacking the plant and on the type and amount of herbivory the plant has experienced before (Moreira & Abdala-Roberts, 2019). Herbivores can adapt to plant defences and shape the ecology and evolution of their host plants but depending for example on the specific plant and on the herbivore species, feeding type and developmental stage.

6.1.3. Plant chemistry varied between individuals and was affected by the growth location.

In Chapters 3 and 4, the growth location of the trees had a strong effect on polyphenol chemistry and in Chapter 3, the variation in chemical composition was large between the individual trees. I suggested that this effect might be due to different environmental factors associated with growth locations affecting plant chemistry. The effect of abiotic factors on plant chemistry should not come as a surprise. The effects of growth location (which could be driven by e.g. soil type, latitude, elevation, surrounding vegetation, climate and biotic interactions) on plant chemistry are obvious for anyone working with agricultural systems, where plant chemical composition might be a crucial determinant of quality or yield (e.g. the effect of environmental conditions on the quality of grapes; Fraga *et al.*, 2014). The mechanisms resulting in different chemical compositions depending on environmental factors most likely take place in all plant species producing secondary metabolites.

In addition to environmental variation, genetic differences between individuals can influence the chemical composition of the host plant and the composition of the associated insect community (Whitham *et al.*, 2006; Busby *et al.*, 2015; Barbour *et al.*, 2016). At our study site in Neptune Wood (Chapter 4), it is possible that trees growing close together might have been genetically more similar. Similarly, in Chapter 3, the differences in physical and chemical leaf traits between the trees might have been a result of genetic differences between the individuals.

6.1.4. Plant mediated-indirect interactions between herbivores were weak with no evidence for keystone herbivores.

In Chapter 4, I studied whether changes in the intensity of early-season herbivory by moth caterpillars change the community composition of late-season leafminers and gallers. Early-season caterpillars have previously been suggested to act as important keystone herbivores in this

system, i.e. affecting the surrounding community or ecosystem disproportionately compared to their abundance (Hunter, 1992; Poelman & Kessler, 2016). Nevertheless, I observed no indirect effects between these two herbivore groups. In Chapter 4, I discussed the role of natural enemies in preventing plant-mediated indirect effects between herbivores. Below, I will discuss the earlier studies identifying the early-season caterpillars as important keystone species, how host-plant mediated effects between herbivores might be restricted to so called "ecosystem engineers" and the importance of intra-specific variation in the community and ecosystem –level effects of organisms.

Working in the very same study system, Hunter (1992) suggested that early-season caterpillars on oak would function as keystone herbivores. This suggestion was based on studies in which the survival of *Phyllonorycter* leafminers was lower on damaged leaves (West, 1985) and late-season leaf-chewing caterpillars were more abundant on trees with higher abundance of early-season caterpillars (Hunter, 1992). The reason for the lower survival of leafminers on damaged leaves was unclear, and could have been attributed to higher predation rates on these leaves (Faeth, 1985). The effects of early-season herbivores on late-season caterpillars appeared not to be caused by changes in leaf chemistry but occurred because the later arriving caterpillars were able to build leaf shelters faster on leaves that had earlier feeding damage on them.

Most studies reporting chemically-mediated interactions between herbivores feeding on the same host plant have been conducted in laboratory or common garden settings (Van Zandt & Agrawal, 2004; Poelman *et al.*, 2008; Hernandez-Cumplido *et al.*, 2016; Arce *et al.*, 2017). Most studies reporting community-wide plant-mediated effects between herbivores in natural conditions have – like the study by Hunter (1992) – involved species that modify the host plant physically (Tscharntke, 1999; Uesugi *et al.*, 2016; Ikemoto *et al.*, 2017; Zhong *et al.*, 2017). These two observations suggest that keystone herbivores might mainly be found among species

which cause physical changes in their surroundings (i.e. species that are 'ecosystem engineers'). The lack of chemically-mediated indirect interactions might not be surprising, if the induction of defences is a rare phenomenon in natural conditions (discussed above, Section 6.1.2).

In addition to keystone species, there has lately been a call to identify "keystone individuals" (Modlmeier *et al.*, 2014; Start, 2018). This concept emphasizes the variation between individuals in terms of how they affect the communities and ecosystems they are part of (as opposed to interspecific variation in community and ecosystem-level effects, i.e. keystone species). Keystone individuals are individuals with extreme phenotypes whose presence can influence trophic and community dynamics. In terms of plant-insect interactions, a keystone individual could for example be a tree with unusual chemical composition, shaping the insect community structure in a different way compared to other tree individuals. Since we found large differences in leaf chemistry between individuals (Chapter 3), and a significant correlation between leaf chemical composition and insect community structure (Chapter 4) in this study system, it might have been more likely to observe keystone plant individuals than keystone herbivore species.

6.1.5. Insect herbivores might control ecosystem productivity.

In my final data chapter, I quantified the effect of insect herbivory on ecosystem primary productivity in Wytham Woods and predicted how the effect of herbivory on forest productivity might change with changing herbivory intensity. I showed that even at low population sizes, insect herbivores might have a large impact on forest productivity. Small-scale changes in species interactions (e.g. the level of herbivory) could have large-scale consequences for ecosystem-level processes.

Traditionally, much of the literature on plant-based ecosystem-level processes has focused on the role of abiotic effects, like climate. Nevertheless, a growing body of literature is now recognizing the effects of animals on ecosystem-scale processes like carbon or nutrient cycling (Schmitz *et al.*, 2010; Estes *et al.*, 2011; Schmitz *et al.*, 2014). In addition to recognizing that herbivores can affect primary productivity through altering plant biomass, several recent studies have examined the effects of predation on herbivores on ecosystem-level carbon sequestration (Strickland *et al.*, 2013; Leroux & Schmitz, 2015; Wilmers & Schmitz, 2016; Schmid *et al.*, 2017). Since many herbivore populations are controlled by their predators (Schmitz *et al.*, 2000), predators have the potential to affect ecosystem-level cycles (Hawlena *et al.*, 2012; Strickland *et al.*, 2013). As in traditional trophic cascades (Schmitz *et al.*, 2000), the effects of predators on lower trophic levels can be a result of lower prey densities, or changes in prey behaviour (Hawlena *et al.*, 2012; Strickland *et al.*, 2013). Acknowledging the role of herbivores on ecosystem-level processes, and the role of predators controlling herbivores gives the opportunity for studies linking food web dynamics with carbon cycle dynamics. As species feed on each other, the amount of carbon sequestrated by primary producers travels further in the food web. This flow of carbon through species interaction web could be monitored and quantified. Detailed knowledge of species interactions could thus help us to understand the controls and limits of carbon cycling.

As an example (Figure 6.1), in the oak-caterpillar study system in Wytham Woods, I estimated that 0.12 Mg of carbon (ha^{-1} yr^{-1}) will be eaten by the early-season caterpillars. Based on earlier studies on the amounts of greenfall (falling leaf fragments due to herbivore feeding), roughly 75% of the lost leaf area will be ingested by the caterpillars (Russell *et al.*, 2004). Based on studies of insect energetics, approximately 15% of the carbon ingested by lepidopteran caterpillars will be respired, 60% will be turned into frass and transported into the soil, and the

rest will turn into insect tissue (Wiegert & Petersen, 1983). This insect tissue will subsequently either fall down as cadavers or be eaten by predators. The population dynamics of winter moth have been recorded for decades (Varley *et al.*, 1974 and references therein; Lionel Cole, unpublished data), and especially the mortality of larvae and pupa has been documented in detail, often to the level of exact predator species (East, 1974). Using this information on interactions between winter moth and other species in Wytham Woods, it would be possible estimate the pathways of carbon from plants to herbivores and subsequently to each predator species separately. This would allow us to recognize the importance of different species and trophic levels for carbon cycling and to predict how changes in species abundances might affect ecosystem function.

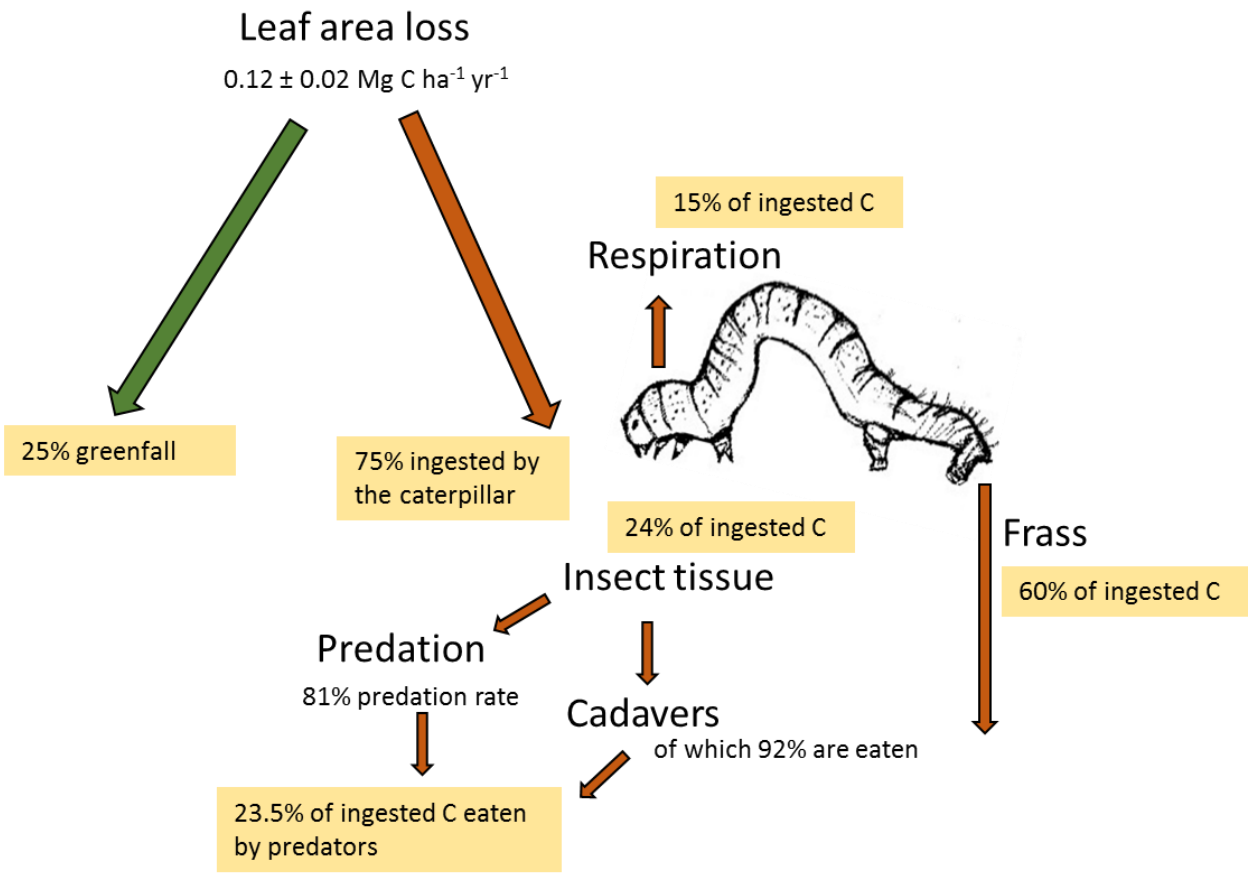


Figure 6.1. Estimating the fate of the carbon lost through eaten leaf in the oak-caterpillar study system in Wytham Woods. 23.5% of the carbon ingested by the caterpillar will be eaten by different predators.

6.2. The importance of studying plant-herbivore interactions in a changing world

Across the globe, many ecosystems are currently subject to major changes caused by rapidly increasing CO_2 concentrations, changing climate and altered ecological communities (IPCC, 2014; Steffen *et al.*, 2018). Environmental change might disrupt current patterns of plant-herbivore and herbivore-predator interactions by causing changes in species abundances (Hallmann *et al.*, 2017), distributional ranges (Jepsen *et al.*, 2008) or phenology (Charmantier *et al.*, 2008). Below, I discuss recent evidence for dramatic changes in insect abundances, potential ecosystem-scale effects of climate-mediated changes in species interactions, and possible eco-evolutionary feedbacks related to climate change.

As an example of the anthropogenic disturbance of important species interactions, there are recent reports on declines in insect abundances in forests in Germany (Hallmann *et al.*, 2017), in the Arctic tundra of Greenland (Loboda *et al.*, 2018), and among nocturnal insect food webs in Europe (Sanders *et al.*, 2016; van Langevelde *et al.*, 2018). The cascading effects of these reductions in insect numbers have already been observed as declines in insect-feeding bird populations (Inger *et al.*, 2015). The reasons for the declines in insect population sizes are not yet fully known, but it has been suggested that at least in mainland Europe, intensive agriculture and pesticides might be the main cause. The importance of understanding plant-insect interactions can be seen here: the use of pesticides (artificial plant defences) has resulted in lower insect abundance (the effect of plant chemistry on herbivore community) and affected bird populations (cascading plant-mediated effects on the third trophic level). To understand how changes in species abundances might affect ecosystem-level processes, studies investigating the effects of different population densities of species on ecosystem functioning (e.g. the simulations of the effects of different herbivore densities on ecosystem productivity in Chapter 5) are important.

Similarly, more detailed knowledge on the effects of species interactions on ecosystem-level processes like carbon cycling (demonstrated above in Section 6.4.2, Figure 6.1) would be needed.

As one of the most prominent human-caused environmental change, climate change will alter the environmental conditions affecting the interactions between plants and their insect herbivores. For example, droughts are expected to become more frequent (Allen *et al.*, 2010; Dai, 2013), which might make plants more susceptible to insect herbivory (Jactel *et al.*, 2012; Castagneyrol *et al.*, 2018a,b). Additionally, warmer climate might allow larger population sizes or distributional ranges of some insect species (Bale *et al.*, 2002; Jepsen *et al.*, 2008). These factors combined could lead to increased frequencies of insect outbreaks (Raffa *et al.*, 2008; Jönsson *et al.*, 2009; Gaylord *et al.*, 2013), resulting in reduced carbon sequestration and increased emission of VOCs into the atmosphere from forests. Lower carbon sequestration could accelerate climate change further. On the other hand, increased concentration of VOCs in the atmosphere could act as nuclei for ice and clouds (Kulmala *et al.*, 2004; Goldstein *et al.*, 2009). Thus, there is potential for both positive and negative feedback loops: positive if insect outbreaks driven by warming climate result in reduced carbon sequestration, and negative in increased VOC emission increases cloud cover and slows down warming.

In addition to positive and negative feedback loops resulting from changes in species interactions, there is a possibility for climate-associated feedbacks arising through evolutionary responses of species. Changes in the environment are likely to change the selection pressures experienced by different species and shape the evolution of their traits. If the traits under selection by climate change affect carbon cycling (e.g. photosynthetic rate of a plant, feeding rate of an insect), changes in the functioning or frequencies of these traits might lead into eco-evolutionary feedbacks with the climate (Monroe *et al.*, 2018). Because photosynthesis is the largest flux of CO_2 on the planet (~ 20 times more than all anthropogenic sources; Beer *et al.*,

2010), even small variation in traits affecting photosynthesis can have large consequences. For example, different selection pressure towards stomatal conductance of plants, a trait linked closely to photosynthetic capacity, can be expected under elevated CO_2 concentration (Grossman & Rice, 2014). There is also evidence for evolution towards increased photosynthesis and respiration rate in plants (Watson-Lazowski *et al.*, 2016), increased rates of consumption of plants by herbivores (Schaum *et al.*, 2018) and altered concentrations of secondary metabolites in plant tissue (Valkama *et al.*, 2007) with warming climate and increasing CO_2. If, for example, warming temperatures result in increased plant respiration or herbivory rate, this could subsequently reduce carbon assimilation and result in accelerated climate change, further influencing trait evolution.

6.3. Suggested directions for future research

There are several areas of future work that would complement and develop the work presented in this thesis. Below, I list some important topics for future studies focusing on the community- and ecosystem-level effects of insect herbivores.

First, there is a need to investigate a wider pool of herbivore and plant species to quantify the herbivore-triggered reductions in photosynthesis and their consequences for canopy- or ecosystem-level carbon cycling, to see how generalisable the results of this study are to other systems. In Chapter 2, I only measured one interaction: the effects of winter moth caterpillars on oak photosynthesis. Since winter moth caterpillars are not restricted to feeding on oak, a useful next step might be to assess its effect on alternative hosts such as sycamore and ash. This would help us to assess if the effects of insect herbivory on ecosystem productivity based on my oak-focused work are representative of the whole forest ecosystem.

Second, since most plant species are attacked by several different enemies over their lifetime, assessing whether the effects of different herbivores on host plant photosynthesis are similar, and whether the effects of multiple herbivores are additive, would be an important topic for future research. For example, does an oak leaf that is simultaneously attacked by a chewing caterpillar and a galler function in the same way as a leaf attacked by just one type of herbivore, and what are the individual and combined effects of different enemy groups such as aphids, leafminers, mildew, and other leaf pathogens on canopy photosynthesis?

Third, considering that populations of primary consumers are often controlled by natural enemies (Schmitz *et al.*, 2000), quantifying the role of predators in controlling herbivores and thus ecosystem productivity would be important. Specific questions to address within this theme are for example how different predator species affect plant function through their interactions with herbivores, whether the effects of individual predator species are additive, and whether potential effects of secondary consumers are mediated by changes in herbivore abundance or behaviour.

Finally, there are several outstanding questions regarding the specific study system used in this thesis. Potential future research topics include for example the differences between sun and shade leaves in their response to insect herbivory; vertical gradients of photosynthesis and the effects of herbivory on it across the oak canopy; photosynthesis of lammas leaves in relation to earlier herbivory; the extent of systemic signals within oak canopy; and temporal trends in the effect of herbivory on photosynthesis and leaf chemistry. Resolving these questions would be important to better describe the effects of the oak-winter moth interaction on community and ecosystem-level processes in Wytham Woods.

6.4. Conclusions

I started this thesis by measuring changes in plant gas exchange and secondary chemistry, continued into looking at the effects of these changes on plant performance and community-level dynamics, and finished by quantifying the effects of herbivore-induced changes in the host plant on ecosystem productivity. My thesis demonstrates the need to combine detailed information on multiple levels of organisation (physiology, community, ecosystem). I show that naturally occurring insect herbivores can affect the primary metabolism of their host plant outside laboratory conditions, with potential implications for canopy-level carbon cycle. I demonstrate that interactions between early and late-season herbivores might be weak in this study system, whereas plant chemistry affected by the environment could be an important factor shaping herbivore communities. I show that, by causing small-scale changes in plant metabolism, insect herbivores might act as important controllers on ecosystem productivity.

Without recognizing the effect of insect herbivores on ecosystem-level carbon sequestration, we might overlook an important controlling factor in forest carbon dynamics and fail to notice possible important feedbacks with climate change. Combining the knowledge on ecosystem-level effects of different species with the knowledge of the interactions these species have with each other will allow us to recognize the controllers of productivity and carbon sequestration. These controllers might be herbivores, predators of the herbivores, other herbivores feeding on the same host plant affecting the first herbivore, or something else (e.g. symbionts; Sanders *et al.*, 2016). We need to include these biotic interactions in models on climate and atmospheric chemistry in order to better predict environmental changes and their consequences.

Supplementary material for chapter 2

Appendix 2.1. Detailed methods

Appendix 2.2. Additional figures and tables

Appendix 2.3. iDirac overview and operation

Appendix 2.1. Detailed methods

Details on experimental methods

Mesh bags were sown from white "tutu mesh", with a mesh size << 1 mm. Bags were approximately 20 cm x 10 cm in size and were attached around the shoots either with a piece of thin metal wire (2015, Figure S1), or with a piece of thread sown around the mouthpiece of the bag (2016). This setup allowed bags to be attached tightly around the shoots without causing damage to the branch while preventing herbivores from leaving or entering the mesh bag. Since the bags were in place only during the time when the caterpillars were active (11th May – 25th June 2015 and 9th May – 28th June 2016), we estimate that any effects of altered light penetration or microclimate on leaf functioning by the time of the measurements (from 28th July 2015 and 11th July 2016) was small, and consistent across the treatments. After removing the bags, the shoots were marked with a small piece of red (2015) or orange (2016) tape.

To create the *herbivory addition* treatment, we collected caterpillars of winter moth on different oak trees around Wytham Wood during early May in 2015 and 2016. Different larval instars were used for creating the treatments, and specific instar used varied randomly between the mesh bags. For each mesh bag belonging to the *herbivory addition* treatment, we carefully placed one caterpillar on a haphazardly chosen leaf inside the bag and waited until the caterpillar attached itself to the leaf before closing the bag. We checked each bag three days after the caterpillar addition. If feeding marks were seen on at least two leaves, caterpillar was removed, otherwise it was left in the bag for another 1-2 days. Dead caterpillars were replaced by fresh ones. All caterpillars were removed after maximum of five days, by which time all shoots in the *herbivory addition* treatment had experienced sufficiently damage.

The *mechanical damage* treatment was created by first pairing up each shoot belonging to that treatment with an *herbivory addition* –shoot (Figure S2) immediately after removing the

caterpillars from the *herbivory addition* -shoots. The amount and type of damage caused by the caterpillar on the leaves in the shoot belonging to the *herbivory addition* -treatment was estimated visually as percentage of leaf area loss from the sides, from the tip, or as holes. The location of the holes was noted (e.g. near the midrib, close to the side). The damage was then carefully replicated on the leaves in the *mechanical damage* -shoot. Damage on the sides and leaf tip was created by tearing, and holes were created by punching with a cork borer. The mesh bags were left around the shoots (including the *control* shoots) until 25th June 2015 or 28th June 2016 to protect the leaves from further herbivory until the amount of insect herbivory had levelled off.

Details on the gas exchange measurements

We constructed photosynthetic light response curves during the period of 28th July - 25th August 2015 for leaves from all the ten study trees (49 leaves). We constructed photosynthesis-CO_2 (A/C_i) -curves during two years, over the periods of 26th August - 10th September 2015 and 11th July - 11th August 2016. During the measuring period of 2015, we measured A/C_i curves for leaves from six trees (28 leaves). During 2016, we measured A/C_i curves for leaves from all the ten experimental trees (51 leaves). Each response curve was measured once per leaf. Leaves on treatment and control shoots not used for gas exchange measurements were later collected for other analyses (K. Visakorpi *et al.*, unpublished.)

For all gas exchange measurements, relative humidity was kept between 60 - 80%, temperature as ambient, and flow rate at 200 ml min^{-1}. Measurements were taken throughout the day between 9:00 h and 20:00 h, except when leaves showed signs of stomatal closure, thus inhibiting photosynthesis (on particularly sunny and dry days). The average daytime air temperature during the measuring period 2015 was 15.7 °C (± 0.1 °C SEM) and 2016 17.1 °C (± 0.2 °C SEM) (Rennie *et al.*, 2017), which are within the range of normal summer temperatures

in the area (Morecroft *et al.*, 2003). Leaf temperatures ranged between 15 °C and 36 °C with a mean of 23 °C (± 0.1 °C SEM).

For the light response curves, we took five point measurements on 15 different light levels (2000, 1500, 1000, 750, 500, 400, 300, 250, 200, 150, 100, 75, 50, 25 and 0 µmol m^{-2} s^{-1} of photosynthetically active radiation (PAR)), starting from the highest level. We allowed the leaf to settle to each new light level for 2 minutes (after testing this was a sufficient time for the leaf to settle to new light conditions) and kept CO_2 concentration at 400 ppm.

For the A/C_i curves, we measured the photosynthetic rate under ten different CO_2 concentrations. Since the gas-analyser was slow to settle to each new exact CO_2 concentration (~15 min with each change in concentration), we set the analyser to find "an approximate" concentration near each target concentration (1500, 1000, 750, 500, 400, 300, 200, 150, 100 and 50 ppm). The realized CO_2 concentrations on average (± SEM) across the different measurements were 1336 ± 4.8, 885 ± 2.9, 702 ± 2.2, 513 ± 1.7, 423 ± 1.3, 328 ± 1.0, 234 ± 0.7, 142 ± 0.5, 63 ± 0.3 and 28 ± 0.1 ppm. This set-up considerably shortened the time it took for the CO_2 concentration to settle (5 min) and allowed the CO_2 concentration to stay stable during each measurement. Since the variation in the realized CO_2 concentrations between the samples was small and random across the treatments, its effect is most likely small. For each leaf, we started at the highest concentration and took three measurements in each concentration. Light intensity was kept at 1000 µmol m^{-2} s^{-1}. For both the light and A/C_i measurements, the leaves were allowed to settle for the highest light or CO_2 concentration until there was no consistent change in the photosynthetic rate (usually after 30 min).

For the isoprene measurements the light was kept at 1000 µmol m^{-2} s^{-1} of PAR, and temperature at the ambient level (leaf temperatures ranging between 18 °C and 35 °C). Since some of the isoprene measurements were taken simultaneously with the A/C_i curves, the CO_2

concentration was again set to approach 400 ppm approximately, in order to keep the measuring conditions similar between the different isoprene samples. The average realized CO_2 concentration was 321 ppm (± 2.6 ppm SEM). Each leaf was measured three times in a row. To record the ambient level of isoprene, we measured the air surrounding the leaf before and after every set of three measurements. The three replicate measurements per leaf were averaged to obtain a single value for each measured leaf. To account for the ambient isoprene concentration, the average of the ambient measurements taken before and after each measured leaf was subtracted from the estimated leaf emission values.

Extracting the gas exchange parameters

To calculate the light-saturated photosynthesis (A_{sat}), we fitted a Michaelis-Menten equation to the light response data for each leaf separately:

$$A(I) = \frac{G_{max} I}{K + I} - R_d$$

(Eq. S1.)

where A is the photosynthetic (assimilation) rate per given light intensity (I). From these variables, the model estimates the parameters for maximum gross photosynthetic rate (G_{max}), the leaf respiration rate (R_d, a model fitted parameter, hence not used in subsequent analyses as the respiration rate) and the light intensity at which the gross photosynthetic rate is half of its maximum (K; Marino *et al.*, 2010). The five point measurements per light level were averaged before fitting the curves.

To analyse the photosynthetic response to experimental treatments under different CO_2 concentrations, we first calculated an average of the three repeated measures per CO_2 concentration per leaf. We then constructed A/C_i response curves for each leaf, where the photosynthetic rate (A) is modelled against the intercellural CO_2 mole fraction (C_i). We fitted the model for photosynthesis as described by Farquhar *et al.* (1980) and Sharkey *et al.* (2007). In this model, the photosynthetic reactions are assumed to be in one of the three steady states: in Rubisco-limited photosynthesis (normally on low C_i), in RuBP regeneration-limited photosynthesis, or in triose phosphate use limited photosynthesis (Farquhar *et al.*, 1980; Sharkey *et al.*, 2007). Fitting this model allowed us to estimate three important parameters describing the photosynthetic efficiency: maximum carboxylation rate, describing the activity of Rubisco (V_{cmax}), rate of photosynthetic electron transport (J_{max}) and triose phosphate use efficiency (TPU).

When photosynthesis is limited by the availability of Rubisco, the response to CO_2 concentrations can be described as:

$$A = V_{cmax}\left[\frac{C_c - \Gamma^*}{C_c + K_c(1 + \frac{O}{K_o})}\right] - R_d$$

(Eq. S2.)

where the parameter V_{cmax} is the maximum velocity of Rubisco for carboxylation, and R_d is the daytime respiration rate. The model variables are as follows: C_c is the CO_2 partial pressure at Rubisco, transformed from C_i (Sharkey *et al.*, 2007). K_C is the Michaelis constant of Rubisco for carbon dioxide (set to 40.4 Pa at 25 °C and then adjusted to the actual leaf temperature) and K_O is the Michaelis constant of Rubisco for oxygen (set to 24.8 kPa at 25 °C and then adjusted to the

actual leaf temperature). These variables describe the kinetic properties of Rubisco, and their values were taken from previous literature on experiments with tobacco leaves (von Caemmerer *et al.*, 1994; Dreyer *et al.*, 2001). O is the partial pressure of oxygen at Rubisco (set to 21 kPa, as defined by the altitude), and Γ * is the CO_2 concentration of the photorespiratory compensation point, i.e. the point where CO_2 uptake by photosynthesis is exactly compensated by the release of CO_2 by photorespiration (set to 3.7 Pa at 25 °C and then adjusted to the actual leaf temperature) (Manter & Kerrigan, 2004; Sharkey *et al.*, 2007). Since the model parameters (K_c, K_o and Γ *) have their own temperature responses, the variables were adjusted to the actual leaf temperature (Sharkey *et al.*, 2007). We assumed the Rubisco-limited state to occur with Ci below 25 Pa, and that the transition between the Rubsico and RuBP limited states occurs between the Ci of 25 Pa and 45 Pa, as these are commonly used and conservative estimates of the upper and lower limits of the transitional stage (Sharkey *et al.*, 2007).

When photosynthesis is limited by the regeneration of RuBP, it can be described with the following equation:

$$A = J\,\frac{C_c - \Gamma^*}{4C_c + 8\Gamma^*} - R_d$$

(Eq. S3.)

where J is the rate of electron transport and other variables and parameters are as above. This equation allows the estimation of the maximum electron transport rate J_{max} that could be obtained in saturating light. We assumed this state to occur with C_i was above 45 Pa.

When the triose phosphate use is the limiting factor, the photosynthetic rate is:

$$A = 3TPU - R_d$$

(Eq. S4.)

where TPU is the rate of use of triose phosphates. TPU-limited photosynthesis describes CO_2 saturated state, where photosynthetic rate (A) stays stable or even decreases with increasing CO_2 concentration. We assumed this state to occur when the CO_2 concentration was at its maximum level.

The temperature correction for isoprene

Since isoprene emission is strongly influenced by temperature and light, the emission values were corrected for temperature with the following equation (Guenther *et al.*, 1993, 1995)

$$I = I_S C_L C_T$$

(Eq. S5.)

where I_S is the standard emission of isoprene (as μg m^{-2} h^{-1}) in standard temperature and light conditions (303 K and 1000 μmol m^{-2} s^{-1} of photosynthetically active radiation). Since the measurements were taken in standard light intensity, the light dependent factor C_L can be ignored. The temperature dependent factor C_T is described as

$$C_T = \frac{\exp(\frac{C_{T1}\ (T - T_S)}{RT_S T}}{C_{T3} + \exp(\frac{C_{T2}\ (T - T_M)}{RT_S T}}$$

(Eq. S6.)

where C_{T1} (95 kJ mol^{-1}), C_{T2} (230 95 kJ mol^{-1}), C_{T3} (0.961) and T_M (314 K) are empirically determined coefficients (Guenther *et al.*, 1993), R is the universal gas constant (8.314 J K^{-1} mol^{-1}), T_S (303 K) is the standard leaf temperature and T is the actual leaf temperature (in K). For the actual leaf temperatures, we averaged the leaf temperature measurements taken with the gas analyser over the period when isoprene was being measured on each leaf.

Appendix 2.2 Additional figures and tables



Figure S1. One of the mesh bags during the spring 2015 used to create the shoot-level experimental manipulations

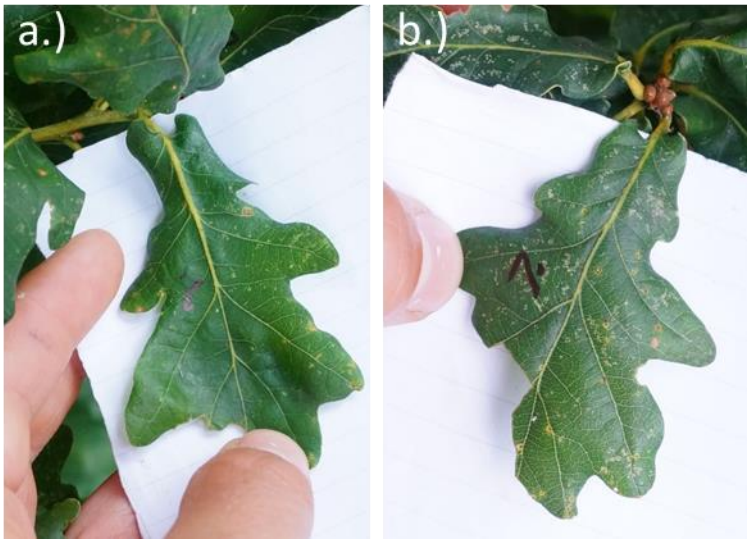


Figure S2. Example of a pair of leaves used in the gas exchange measurements. Panel a.) shows a leaf damaged by herbivores and b.) shows a mechanically damaged leaf.

Table S1. The average level of leaf area lost to herbivory naturally on the two experimental sites, and in the two damage treatments (mechanical damage and herbivore addition). Errors are ±1 SEM.

	Natural herbivory survey	**Experimentally manipulated shoots with herbivore damage**	**Experimentally manipulated shoots with mechanical damage**
Number of shoots surveyed	175	19	19
Leaf area removed per eaten leaf, %	8.45 ± 0.39	14.13 ± 1.91	10.88 ± 1.84
Leaf area lost on the side, %	3.75 ± 0.28	8.22 ± 1.34	6.65 ± 1.63
Leaf area lost on tip, %	2.15 ± 0.28	5.53 ± 1.59	4.09 ± 1.56
Leaf area lost as holes, %	0.17 ± 0.02	0.37 ± 0.34	0.14 ± 0.1

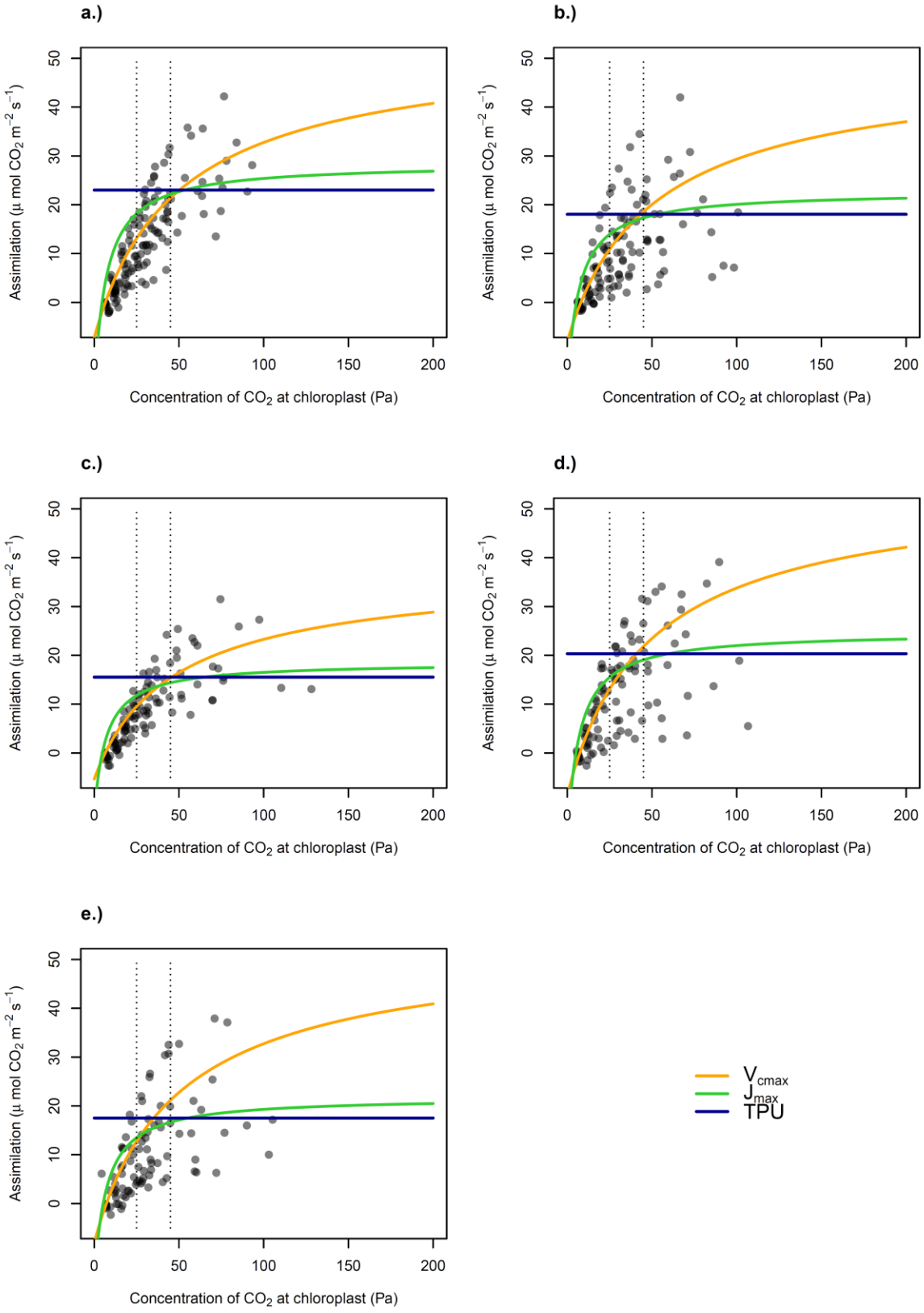


Figure S3. The average A/C_i curves as derived from the Farquhar et al. (1980) model for each leaf type (a – control, b – damaged leaf on an herbivore damaged shoot, c – intact leaf on a herbivore damaged shoot, d – damaged leaf on a mechanically damaged shoot, e – intact leaf on a mechanically damaged shoot). The points show raw measurements of photosynthetic rate (A) plotted against the internal CO_2 concentration (C_i). The

orange line represents photosynthetic rate if carboxylation capacity is limiting ($V_{cmax,}$), the green line represents electron transport limited photosynthesis (J_{max}), and the blue line shows photosynthesis under triose phosphate use limitation (TPU). The dashed vertical lines at C_i concentrations of 25 and 45 Pa represent the points at which the limiting factor of the photosynthetic rate was assumed to change (from Rubisco limited into RuBP limited photosynthesis).

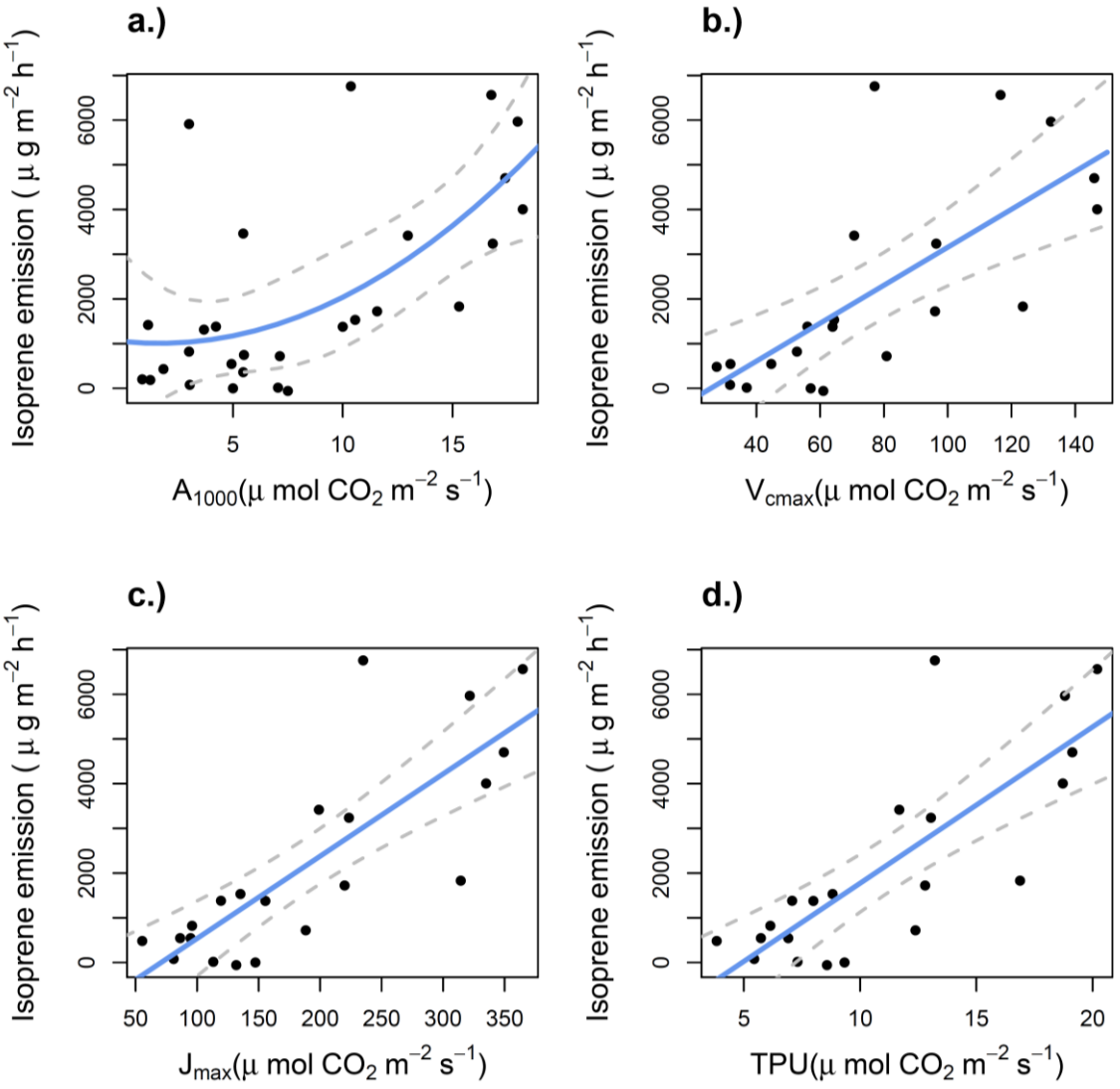


Figure S4. Isoprene emission rate correlated positively and significantly with the photosynthetic parameters A_{1000} ($r^2 = 0.37$, *p = 0.001*), V_{cmax} ($r^2 = 0.49$, *p < 0.001*), J_{max} ($r^2 = 0.64$, *p < 0.001*) and TPU ($r^2 = 0.62$, *p < 0.001*) Panel a) shows relationship between the isoprene standard emission factor and A_{1000} (photosynthetic rate at 400 ppm of CO_2 and 1000 µmol m^{-2} s^{-1} of photosynthetically active radiation), b) the relationship between isoprene emission and carboxylation rate $V_{cmax,}$ c) the relationship between isoprene emission and the mean electron transport rate J_{max}, and d) the relationship between isoprene emission and triose phosphate use efficiency TPU. All the parameters except A_{1000} are corrected for standard temperature. The raw data are shown as points.

The solid line shows model estimated mean for a quadratic linear (a) and linear (b, c, d) model, and dotted lines represent 95% confidence intervals. Note that the three parameters extracted from the A/Ci curves (V_{cmax}, J_{max} and TPU) correlate with each other and thus show almost identical relationship with isoprene.

Table S2. Coefficient estimates for fixed effects of the linear mixed effects models assessing the relationships between variables reflecting photosynthetic capacity, leaf respiration, stomatal conductance or isoprene emissions and explanatory variables of the final model. Shown are estimates from the minimum adequate models for each response variable. Note that the negative slope of leaf temperature on respiration means that temperature has a positive effect on the rate of respiration, which has been measured as negative assimilation rates. For each model, the intercept indicates the mean value of the response variable for a given level of the fixed effect(s) and for a covariate value = 0 (stated in parentheses), and the other effects show the mean change, compared with the intercept, caused by the other fixed factor levels and by a unit change in covariate value

Response variable	**Final model**	**Effects**	**Estimate**	**SD error**	**t-value**
A_{sat}	~ leaf treatment	Intercept (Control)	19.82	1.80	11.03
		Herbivore damaged	−9.03	2.12	−4.26
		Herbivore undamaged	−7.35	2.12	−3.47
		Mechanical damaged	−2.12	2.19	−0.97
		Mechanical undamaged	−3.90	2.12	−1.84
K	~ 1	Intercept	171.19	18.38	9.32
Respiration	~ leaf temperature	Intercept (leafT = 0)	0.2	0.25	1.06
		Leaf temperature	−0.035	0.012	−2.95
V_{cmax}	~ leaf treatment + site + leaf temperature + year	Intercept (Control, Site John Krebs, leafT = 0, year = 2015)	133.56	23.92	5.58
		Herbivore damaged	−20.99	10.29	−2.04
		Herbivore undamaged	−24.20	10.23	−2.37
		Mechanical damaged	3.30	10.46	0.32
		Mechanical undamaged	−5.00	10.64	−0.47
		Site (Wytham Woods)	32.43	14.46	2.24

		Leaf temperature	−2.37	1.07	−2.21
		Year (2016)	−26.15	8.69	−3.01
J_{max}	~ leaf treatment + site + leaf temperature	Intercept (Control, Site John Krebs, leafT = 0)	352.42	55.50	6.35
		Herbivore damaged	−74.89	26.34	−2.84
		Herbivore undamaged	−84.97	26.26	−3.24
		Mechanical damaged	−23.03	26.32	−0.88
		Mechanical undamaged	−39.75	26.47	−1.50
		Site (Wytham Woods)	77.59	32.70	2.37
		Leaf temperature	−8.51	2.40	−3.55
TPU	~ site + leaf temperature	Intercept (Site John Krebs, leafT = 0)	17.83	2.74	6.50
		Site (Wytham Woods)	3.61	1.56	2.32
		Leaf temperature	−0.43	0.13	−3.39
Stomatal conductance	~ site + leaf temperature + leaf treatment	Intercept (Site John Krebs, leafT = 0, Control)	270.94	46.68	5.80
		Herbivore damaged	−59.82	22.94	−2.61
		Herbivore undamaged	−63.88	23.34	−2.74
		Mechanical damaged	−16.95	22.76	−0.74
		Mechanical undamaged	−45.92	22.64	−2.03
		Site (Wytham Woods)	70.29	27.92	2.52
		Leaf temperature	−5.12	1.98	−2.58
Isoprene	~ leaf treatment + Damage %	Intercept (Control, Damage = 0)	912.8	289.9	3.15
		Herbivore damaged	3707.0	1254.8	2.95
		Herbivore undamaged	−285.6	201.3	−1.42
		Mechanical damaged	5383.2	844.0	6.38
		Mechanical undamaged	1582.2	838.1	1.89
		Damage %	−176.8	48.2	−3.67

Table S3. Canopy- and leaf-level effects of herbivory on photosynthesis in 1000 µmol m^{-2} s^{-1} of photosynthetically active radiation (A_{1000}) The effects are expressed relative to the control treatment values (intact leaves in intact shoots). Errors are ±1 SEM derived through error propagation.

Appendix 2.2

	Intact leaf, intact shoot (1)	Intact leaf, damaged shoot (2)	Damaged leaf, damaged shoot (3)	Canopy scale total effect
A_{1000}				
Rate (μmol CO_2 m^{-2} s^{-1})	10.8 ± 1.35	7.1 ± 0.9	7.9 ± 1.1	
Rate (% of intact)	100	65.7 ± 11.7	73.1 ± 13.7	
Indirect effect per unit leaf area %	0	−34.3 ± 11.7	−26.9 ± 13.7	
Leaf scale effect % (direct + indirect, Eq. 1)	0	−34.3 ± 11.7	−33.1 ± 12.9	
Canopy scale effect % (direct + indirect, Eq. 2)				−32.8 ± 9.7

Appendix 2.3. iDirac overview and operation

iDirac is a simple gas chromatograph with a photo-ionisation detector (GC-PID) which was designed to make continuous measurements of isoprene away from the laboratory environment (C. Bolas *et al.*, unpublished). Samples are collected on a Carboxen absorbent trap which is then heated in a flow of nitrogen and passed into a short pre-column. Once isoprene has passed through onto the main column, the flow is reversed, and the rest of the sample vented to minimise contamination. A prepared isoprene mixture is used at regular intervals to provide calibration linked to the scale provided by the National Physical Laboratory. Different volumes of this calibration gas are collected on the absorbent trap to provide calibration across a range of mixing ratios. Blank runs were run before each calibration run to ensure to keep the absorbent trap free of contaminants. Calibration programme was run before (15th - 19th July), in the middle (29th July), and after the campaign (4th - 6th November). For the leaf measurements presented here, the air being analysed is the exhaust from a CIRAS-2 (PP-Systems, Hitchin, UK) photosynthesis measurement leaf chamber. Analysis of the chromatograms is done by Igor (IGOR Pro Version 6.3.7.2, WaveMetrics Inc, 1988-2014, www.wavemetrics.com) fitting a linear baseline and a Gaussian curve to the isoprene peak.

The overall sampling time for the high isoprene mixing ratios encountered here is 3 minutes. The detection limit of the instrument depends on the volume of air sampled for a particular experiment. Full uncertainty characterisation is being carried out. However, our preliminary estimate for the samples volumes used here is a few 10s of ppt. This means that the observed variability in the emission rates (see sentence below) is dominated by the variation between experiments and not by instrumental uncertainty. We estimate a precision of ±7.80% based on repetitive measurements of the calibration gas before, during and after the experiment.

Supplementary material for chapter 3

Appendix 3.1: Additional tables and figures

Appendix 3.2: Additional analyses

Table S1. Summary of the investigated polyphenol compounds and their mean concentrations (mg/g dry leaf mass) overall and across different experimental treatments. Compounds are listed from highest to lowest mean concentration. HT = hydrolysable tannin, PR = proanthocyanidin, FL = flavonol, PA = phenolic acid.

Compound	Type	Mean	Control	Herbivore damage	Herbivore intact	Mechanical damage	Mechanical intact
Cocciferin D2	HT	9.22	9.09 ± 1.28	10.1 ± 1.34	8.36 ± 1.65	8.84 ± 1.28	9.60 ± 1.58
Vescalagin	HT	7.92	7.92 ± 0.98	8.38 ± 0.73	7.10 ± 0.83	7.77 ± 0.68	8.28 ± 0.83
Catechin	PR	3.4	3.68 ± 0.40	3.07 ± 0.36	3.52 ± 0.43	3.37 ± 0.29	3.40 ± 0.34
Vescavaloninic acid	HT	2.82	2.78 ± 0.47	3.01 ± 0.48	2.62 ± 0.52	2.83 ± 0.43	2.83 ± 0.53
Castalagin	HT	1.63	1.67 ± 0.20	1.72 ± 0.14	1.49 ± 0.17	1.58 ± 0.15	1.64 ± 0.18
Quercetin glucoside	FL	1.53	1.44 ± 0.13	1.47 ± 0.16	1.63 ± 0.18	1.37 ± 0.14	1.80 ± 0.31
Quercetin glucuronide	FL	1.42	1.40 ± 0.13	1.50 ± 0.19	1.62 ± 0.21	1.20 ± 0.11	1.41 ± 0.17
Kaempferol glucoside	FL	1.2	1.25 ± 0.26	1.25 ± 0.27	1.47 ± 0.25	0.92 ± 0.16	1.17 ± 0.26
Pedunculagin	HT	1.12	1.06 ± 0.08	1.19 ± 0.08	1.05 ± 0.08	1.15 ± 0.07	1.14 ± 0.10
Castavaloninic acid	HT	1.09	1.07 ± 0.09	1.14 ± 0.08	1.00 ± 0.11	1.14 ± 0.10	1.10 ± 0.13
PC dimer	PR	0.81	0.95 ± 0.13	0.70 ± 0.09	0.89 ± 0.14	0.77 ± 0.09	0.79 ± 0.11
Quercetin diglycoside	FL	0.74	0.74 ± 0.09	0.74 ± 0.10	0.80 ± 0.12	0.73 ± 0.08	0.70 ± 0.10
Chlorogenic acid	PA	0.22	0.21 ± 0.07	0.26 ± 0.11	0.29 ± 0.13	0.17 ± 0.06	0.21 ± 0.07
Casuarictin	HT	0.17	0.16 ± 0.03	0.15 ± 0.04	0.28 ± 0.17	0.13 ± 0.03	0.13 ± 0.02
HHDP-glucose	HT	0.14	0.14 ± 0.01	0.14 ± 0.008	0.13 ± 0.01	0.14 ± 0.008	0.13 ± 0.01
Monogalloylglucose	HT	0.13	0.14 ± 0.02	0.11 ± 0.02	0.15 ± 0.04	0.12 ± 0.02	0.13 ± 0.02
Kaempferol glucuronide	FL	0.12	0.12 ± 0.01	0.13+ ± 0.02	0.14 ± 0.02	0.10 ± 0.01	0.11 ± 0.02
Quercetin malonylglucoside	FL	0.08	0.07 ± 0.01	0.08 ± 0.02	0.09 ± 0.03	0.07 ± 0.02	0.08 ± 0.02
Tellimagrandin 1	HT	0.07	0.08 ± 0.01	0.07 ± 0.01	0.06 ± 0.01	0.07 ± 0.01	0.07 ± 0.01
Kaempferol malonylglucoside	FL	0.04	0.04±0.01	0.05 ± 0.01	0.05 ± 0.01	0.04 ± 0.008	0.04 ± 0.009
Galloyl-HHDP-glucose	HT	0.03	0.03 ± 0.003	0.03 ± 0.002	0.03 ± 0.003	0.03 ± 0.003	0.03 ± 0.003

Casuarinin	HT	0.03	0.02 ± 0.002	0.04 ± 0.02	0.05 ± 0.03	0.02 ± 0.003	0.02 ± 0.002
Kaempferol diglycoside	FL	0.03	0.03 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.01 ± 0.003	0.02 ± 0.01
Tellimagrandin 2	HT	0.02	0.02 ± 0.004	0.02 ± 0.01	0.02 ± 0.008	0.02 ± 0.004	0.02 ± 0.01
Stachyurin	HT	0.01	0.01 ± 0.001	0.02 ± 0.01	0.02 ± 0.01	0.01 ± 0.001	0.01 ± 0.001
Tetragalloylglucose	HT	0.001	0.0004 ± 0.0004	0.001 ± 0.001	0.002 ± 0.002	0.0004 ± 0.0004	0.0004 ± 0.0004
Pentagalloylglucose	HT	0.0003	0	0.0004 ± 0.004	0.001 ± 0.001	0	0

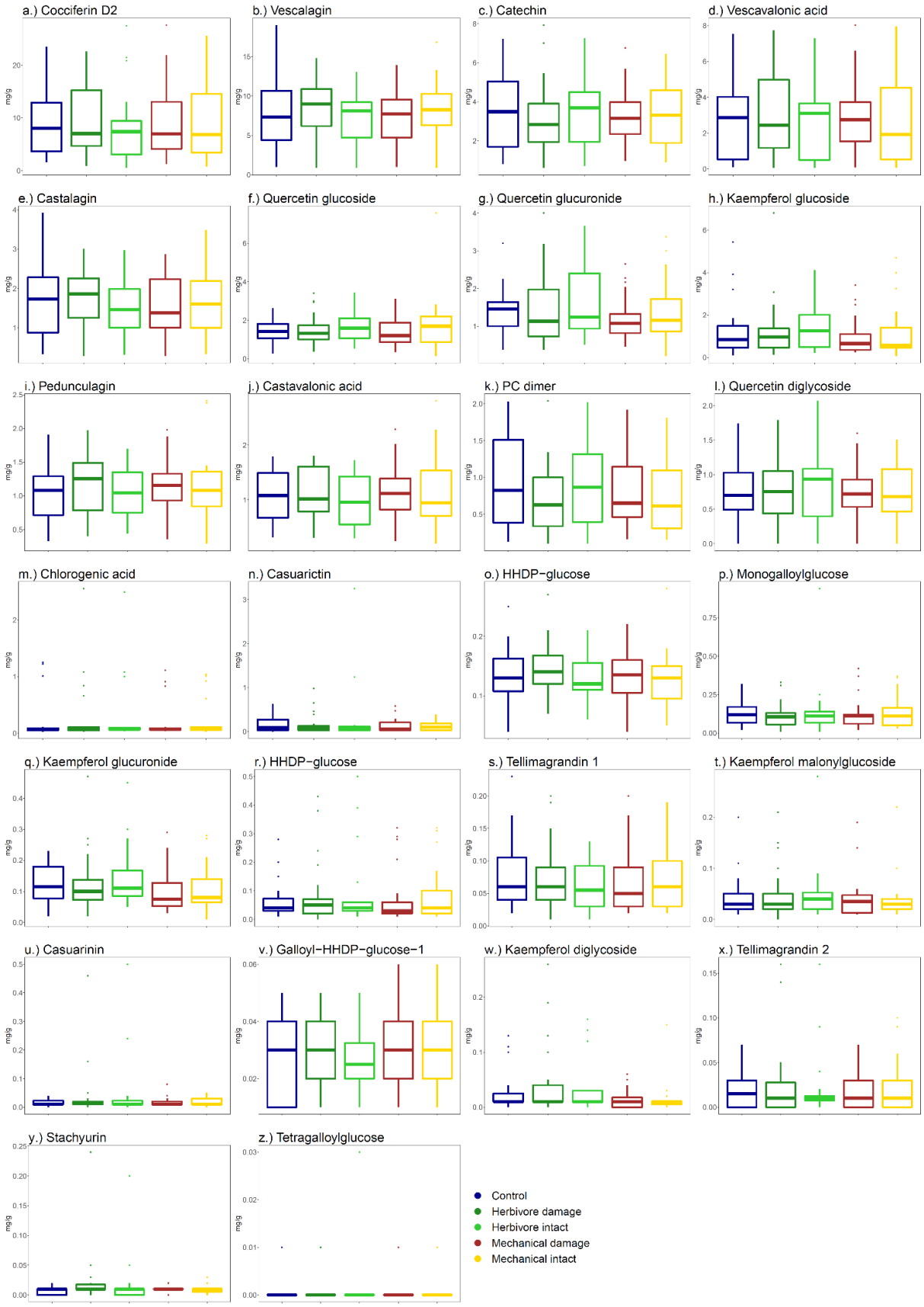


Figure S1. Concentrations of the different polyphenol compounds by leaf-level treatment. The differences between treatments are not significant (see Table S3).

Table S2. Summary of statistical models assessing the effect of treatment on the studied leaf traits (mixed effect models), and the relationship between photosynthesis and leaf nitrogen content (linear models). Shown are coefficient estimates for fixed effects and the explanatory variables included in the final model (i.e. variables remaining after model selection; see methods). For each model, the intercept indicates the mean value of the response variable for a given level of the fixed effect(s) and for a given covariate value (0); as stated in parentheses. The other estimates indicate the mean change from the intercept, caused by the other fixed factor levels and by a unit change in covariate value. Date was expressed as Julian day, so that day = 1 is January 1st.

Response	Final model	Effects	Estimate	STD error	t value
The effect of treatment (mixed effect models)					
LMA	~ Proportion of damage + Dry mass	Intercept	52.0	2.99	17.4
		Proportion of damage	14.0	6.19	2.25
		Leaf mass	0.06	0.008	8.15
Leaf area	~ 1	Intercept	19.5	2.63	7.40
C:N	~ Leaf mass	Intercept (mass = 0)	1.36	0.017	80.6
		Leaf mass	−0.19	0.04	−4.48
C	~ Leaf mass	Intercept	46.5	0.8	61.6
		Leaf mass	−0.23	0.05	−4.08
N	~ Site + Leaf mass	Intercept (Site=JK, Leaf mass=0)	2.02	0.09	21.4
		Site WW	0.23	0.11	2.03
		Leaf mass	0.61	0.19	3.18
Total polyphenols	~ Site + Date	Intercept (Site=JK, Date=0,)	81.5	22.4	3.64
		Site WW	9.62	4.81	2.00
		Date	−0.24	0.10	−2.43
Total flavonols	~ Date + Treatment + Site + Date : Treatment + Site : Treatment	Intercept (Date = 0, Site=JK, Control)	-23.3	10.6	-2.20
		Date	0.12	0.05	2.62
		Herbivore damage	29.2	14.6	2.00
		Herbivore intact	23.74	15.1	1.57
		Mechanical damage	27.0	14.5	1.86
		Mechanical intact	35.6	14.5	2.45
		Site (WW)	2.65	1.08	2.45
		Date : Herb dmg	−0.13	0.07	−1.97
		Date : Herb int	−0.11	0.07	−1.56
		Date : Mec dmg	−0.12	0.06	−1.86
		Date : Mec int	−0.16	0.07	−2.44
		Site : Herb dmg	−2.39	1.19	−2.00
		Site : Herb int	−1.16	1.24	−0.94
		Site : Mec dmg	−2.51	1.15	−2.17
		Site : Mec int	−2.77	1.17	−2.37
	~ Date + Treatment +	Intercept (Site=JK, Date=0, Control)	129.7	36.4	3.56

Total hydrolysable tannins	Site + Date : Treatment + Site : Treatment				
		Site WW	1.97	7.19	0.27
		Date	−0.48	0.16	−2.99
		Herbivore damage	−185.2	48.9	−3.79
		Herbivore intact	−63.8	48.9	−1.30
		Mechanical damage	40.8	48.7	0.84
		Mechanical intact	−111.3	48.7	−2.29
		Site : Herb dmg	10.2	3.74	2.73
		Site : Herb int	5.99	3.75	1.60
		Site : Mec dmg	6.53	3.66	1.78
		Site : Mec int	11.6	3.67	3.16
		Date : Herb dmg	0.83	0.22	3.78
		Date : Herb int	0.27	0.22	1.22
		Date : Mec dmg	−0.21	0.22	−0.98
		Date : Mec int	0.47	0.22	2.17
Diversity of all polyphenols	~ Date	Intercept (Date =0)	1.43	0.20	7.03
		Date	0.003	0.001	3.65
Diversity of flavonols	~ 1	Intercept	1.45	0.04	38.2
Diversity of hydrolysable tannins	~ Date : Treatment	Intercept (Date=0, Control)	1.11	0.25	4.44
		Date	0.002	0.001	1.82
		Herbivore damage	0.08	0.33	0.24
		Herbivore intact	-0.30	0.33	−0.90
		Mechanical damage	-0.38	0.34	−1.14
		Mechanical intact	0.04	0.34	−1.14
		Date : Herb dmg	−0.0004	0.002	−0.30
		Date : Herb int	0.001	0.001	0.90
		Date : Mec dmg	0.002	0.002	1.19
		Date : Mec int	−0.0002	0.002	−0.11

The relationships between photosynthesis and other leaf traits (linear models)					
A_{1000}	~ site + N	Intercept (Site = JK)	−5.9	4.8	-1.2
		Site WW	1.3	1.3	1.0
		N	6.6	2.9	2.9

Abbreviations: Leaf dmg = proprotion of damage per leaf, JK = John Krebs field station, WW = Wytham Woods, Herb dmg = damaged leaf in the herbivory treatment, Herb int = intact leaf in the herbviory treatment, Mec dmg = mechanically damaged leaf, Mec int = intact leaf in the mechnical treatment.

Appendix 3.1

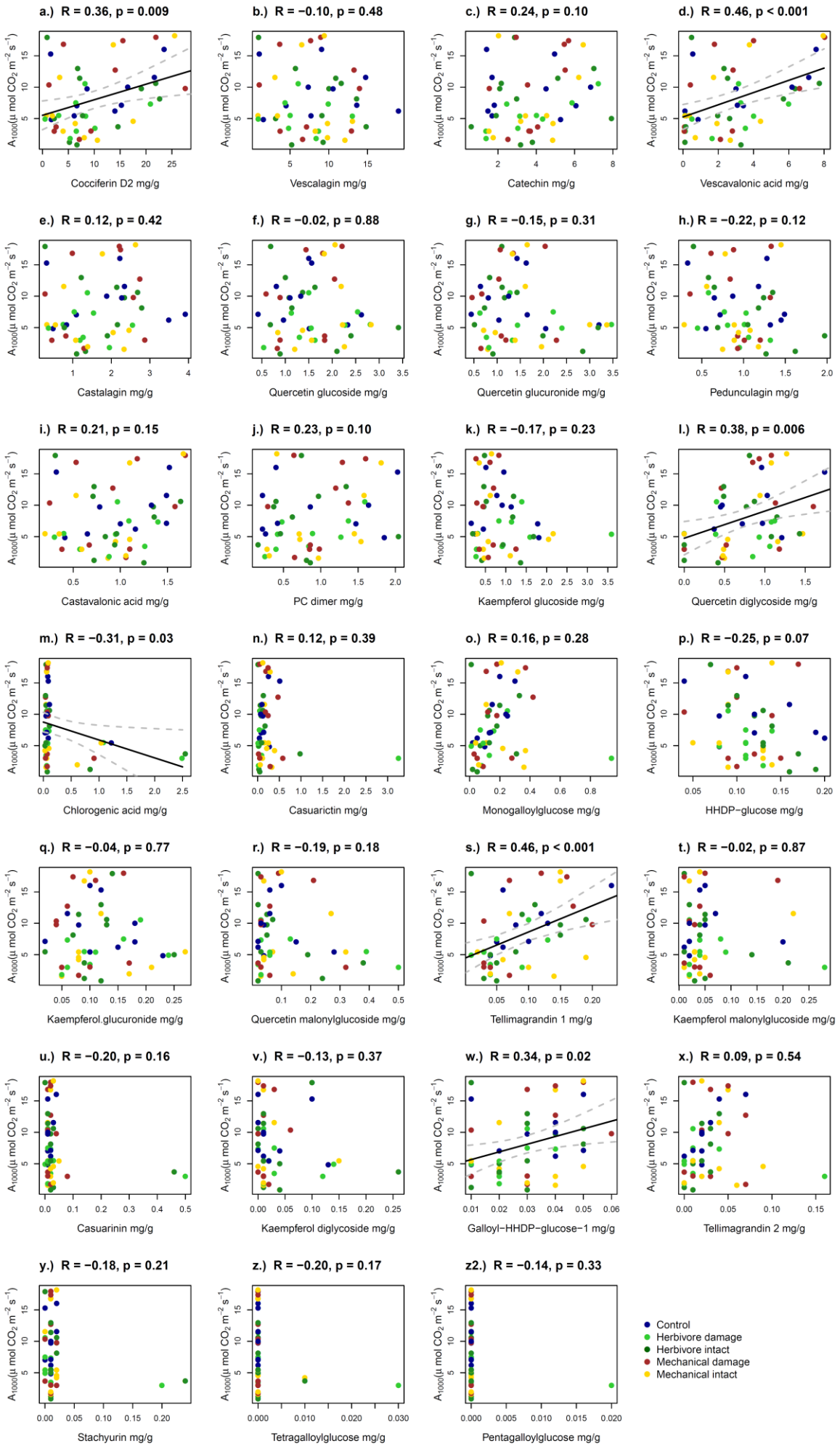

a.) R = 0.36, p = 0.009
b.) R = −0.10, p = 0.48
c.) R = 0.24, p = 0.10
d.) R = 0.46, p < 0.001
e.) R = 0.12, p = 0.42
f.) R = −0.02, p = 0.88
g.) R = −0.15, p = 0.31
h.) R = −0.22, p = 0.12
i.) R = 0.21, p = 0.15
j.) R = 0.23, p = 0.10
k.) R = −0.17, p = 0.23
l.) R = 0.38, p = 0.006
m.) R = −0.31, p = 0.03
n.) R = 0.12, p = 0.39
o.) R = 0.16, p = 0.28
p.) R = −0.25, p = 0.07
q.) R = −0.04, p = 0.77
r.) R = −0.19, p = 0.18
s.) R = 0.46, p < 0.001
t.) R = −0.02, p = 0.87
u.) R = −0.20, p = 0.16
v.) R = −0.13, p = 0.37
w.) R = 0.34, p = 0.02
x.) R = 0.09, p = 0.54
y.) R = −0.18, p = 0.21
z.) R = −0.20, p = 0.17
z2.) R = −0.14, p = 0.33
A1000(μ mol CO2 m−2 s−1)
Cocciferin D2 mg/g
Vescalagin mg/g
Catechin mg/g
Vescavalonic acid mg/g
Castalagin mg/g
Quercetin glucoside mg/g
Quercetin glucuronide mg/g
Pedunculagin mg/g
Castavalonic acid mg/g
PC dimer mg/g
Kaempferol glucoside mg/g
Quercetin diglycoside mg/g
Chlorogenic acid mg/g
Casuarictin mg/g
Monogalloylglucose mg/g
HHDP−glucose mg/g
Kaempferol glucuronide mg/g
Quercetin malonylglucoside mg/g
Tellimagrandin 1 mg/g
Kaempferol malonylglucoside mg/g
Casuarinin mg/g
Kaempferol diglycoside mg/g
Galloyl−HHDP−glucose−1 mg/g
Tellimagrandin 2 mg/g
Stachyurin mg/g
Tetragalloylglucose mg/g
Pentagalloylglucose mg/g
Control
Herbivore damage
Herbivore intact
Mechanical damage
Mechanical intact

Figure S2. Relationship between concentrations of individual polyphenol compounds and photosynthetic rate at 1000 µmol m^{-2} s^{-1} photosynthetically active radiation (parameter A_{1000}). Pearson's correlation coefficient ("R") and the associated p-value are shown on each panel. For statistically significant relationships ($p < 0.05$), fitted values and 95% confidence intervals from a linear model describing the specific relationship are shown.

Table S3. The unadjusted F and p-values for each polyphenol compound separately from the multivariate analysis. Shown are the effects of the five experimental treatments (*herbivore damage, herbivore intact, mechanical damage, mechanical intact and control*), the two sites, the individual trees and the collection date. Statistically significant effects ($p < 0.05$) are highlighted in bold.

Compound	**Site**		**Tree**		**Collection date**		**Leaf treatment**	
	F	p	F	p	F	p	F	P
Cocciferin D2	35.5	**0.002**	-11.8	0.38	248	**0.002**	2.34	0.06
Vescalagin	7.90	**0.01**	-11.8	0.46	245	**0.002**	2.23	0.08
Catechin	3.97	0.06	-11.7	0.76	241	**0.002**	2.13	0.09
Vescavaloninic acid	95.6	**0.002**	-11.0	0.30	244	**0.002**	2.36	0.06
Castalagin	27.7	**0.002**	-10.7	0.25	226	**0.002**	2.27	0.07
Quercetin glucoside	3.14	0.08	-8.90	0.45	166	**0.002**	1.67	0.17
Quercetin glucuronide	1.81	0.20	0.35	**0.02**	64.2	**0.049**	1.18	0.32
Kaempferol glucoside	8.72	**0.008**	3.27	**0.002**	6.41	0.30	0.83	0.48
Pedunculagin	0.33	0.57	12.1	**0.002**	48.4	**0.02**	1.17	0.18
Castavaloninic acid	24.2	**0.002**	-3.77	0.06	128	**0.002**	2.25	0.06
PC-dimer	0.95	0.32	-6.35	0.12	108	**0.003**	1.31	0.19
Quercetin diglycoside	25.1	**0.002**	25.3	**0.002**	82.1	0.05	1.14	0.32
Chlorogenic acid	17.6	**0.002**	-11.8	0.59	247	**0.002**	2.26	0.08
Casuarictin	0.02	0.89	-11.7	0.57	238	**0.002**	2.16	0.11
HHDP-glucose	1.06	0.29	-12.0	0.71	249	**0.002**	2.28	0.08
Monogalloylglucose	29.5	**0.002**	-11.8	0.57	241	**0.002**	2.21	0.09
Kaempferol glucuronide	0.34	0.56	-11.9	0.66	239	**0.002**	2.16	0.09
Quercetin malonylglucoside	2.11	0.16	-11.9	0.55	2489	**0.002**	2.24	0.08
Tellimagrandin 1	69.1	**0.002**	-11.9	0.76	246	**0.002**	2.22	0.09
Kaempferol malonylglucoside	8.64	**0.005**	-11.9	0.60	252	**0.002**	2.29	0.08
Galloyl-HHDP-glucose	84.2	**0.002**	-12.0	0.83	248	**0.002**	2.27	0.08
Casuarinin	3.01	0.10	-12.0	0.78	248	**0.002**	2.25	0.08
Kaempferol diglycoside	31.9	**0.002**	-12.0	0.46	249	**0.002**	2.29	0.08
Tellimagrandin 2	11.4	**0.003**	-11.9	0.62	249	**0.002**	2.28	0.08
Stachyurin	0.02	0.91	-12.0	0.80	249	**0.002**	2.28	0.08
Tetragalloylglucose	1.99	0.21	-12.0	0.81	250	**0.002**	2.29	0.07
Pentagalloylglucose	0.14	0.69	-12.0	0.78	250	**0.002**	2.29	0.07

Appendix 3.1

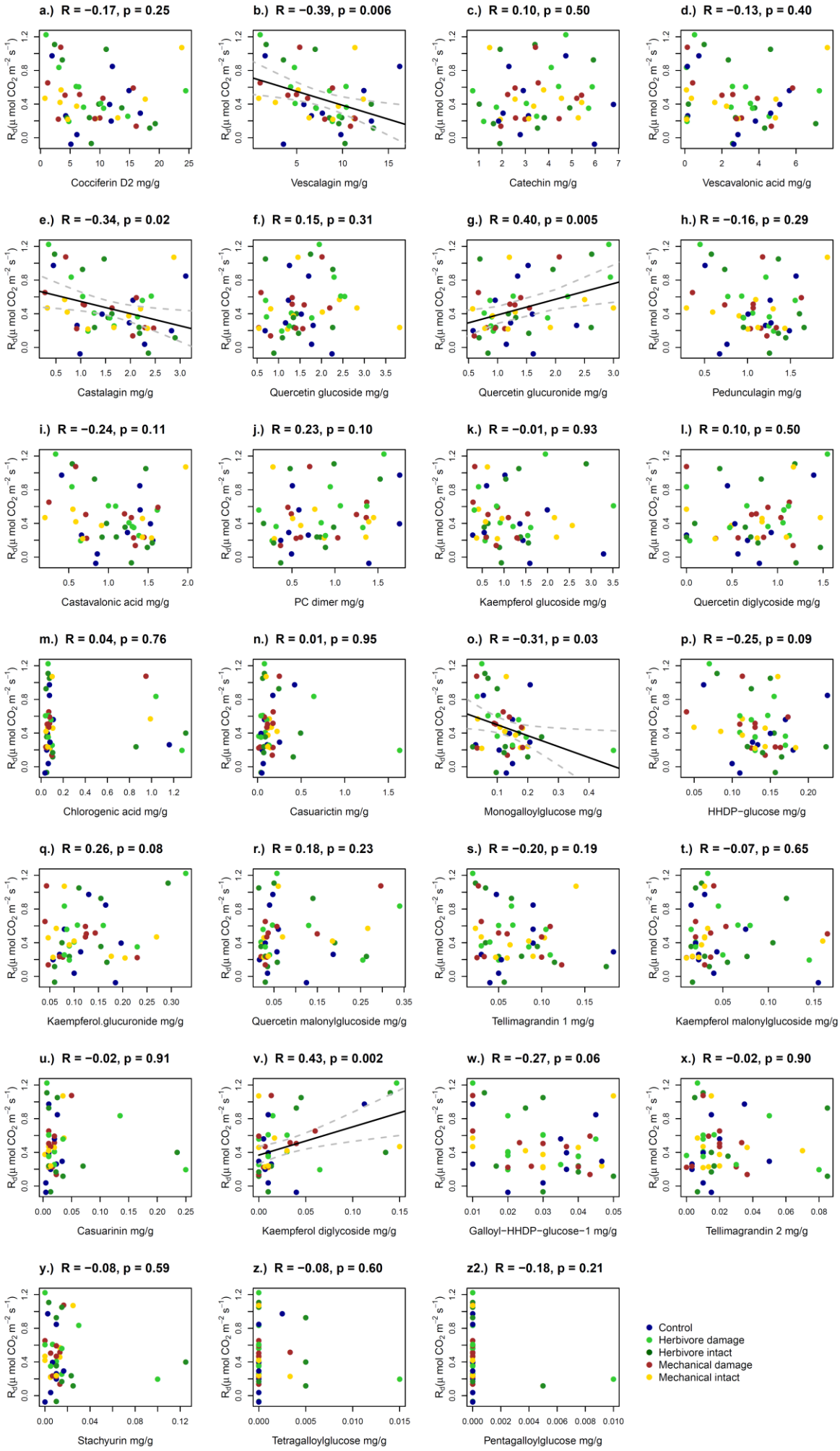

a.) R = −0.17, p = 0.25
Cocciferin D2 mg/g
b.) R = −0.39, p = 0.006
Vescalagin mg/g
c.) R = 0.10, p = 0.50
Catechin mg/g
d.) R = −0.13, p = 0.40
Vescavalonic acid mg/g
e.) R = −0.34, p = 0.02
Castalagin mg/g
f.) R = 0.15, p = 0.31
Quercetin glucoside mg/g
g.) R = 0.40, p = 0.005
Quercetin glucuronide mg/g
h.) R = −0.16, p = 0.29
Pedunculagin mg/g
i.) R = −0.24, p = 0.11
Castavalonic acid mg/g
j.) R = 0.23, p = 0.10
PC dimer mg/g
k.) R = −0.01, p = 0.93
Kaempferol glucoside mg/g
l.) R = 0.10, p = 0.50
Quercetin diglycoside mg/g
m.) R = 0.04, p = 0.76
Chlorogenic acid mg/g
n.) R = 0.01, p = 0.95
Casuarictin mg/g
o.) R = −0.31, p = 0.03
Monogalloylglucose mg/g
p.) R = −0.25, p = 0.09
HHDP−glucose mg/g
q.) R = 0.26, p = 0.08
Kaempferol.glucuronide mg/g
r.) R = 0.18, p = 0.23
Quercetin malonylglucoside mg/g
s.) R = −0.20, p = 0.19
Tellimagrandin 1 mg/g
t.) R = −0.07, p = 0.65
Kaempferol malonylglucoside mg/g
u.) R = −0.02, p = 0.91
Casuarinin mg/g
v.) R = 0.43, p = 0.002
Kaempferol diglycoside mg/g
w.) R = −0.27, p = 0.06
Galloyl−HHDP−glucose−1 mg/g
x.) R = −0.02, p = 0.90
Tellimagrandin 2 mg/g
y.) R = −0.08, p = 0.59
Stachyurin mg/g
z.) R = −0.08, p = 0.60
Tetragalloylglucose mg/g
z2.) R = −0.18, p = 0.21
Pentagalloylglucose mg/g
Control
Herbivore damage
Herbivore intact
Mechanical damage
Mechanical intact

Figure S3. Relationship between concentrations of individual polyphenol compounds and leaf respiration rate (parameter R_d). Since leaf respiration and polyphenol concentrations were measured on different leaves and during different years, the data points represent averages over trees and leaf-level treatments per tree. Pearson's correlation coefficient ("R") and the p-value for the correlation are shown. For statistically significant relationships ($p < 0.05$), fitted values and 95% confidence intervals from a linear model describing the specific relationship are shown. Note that the respiration values on y-axis are shown as positive to make the graph more intuitive (i.e. so that larger respiration values correspond to higher respiration rate).

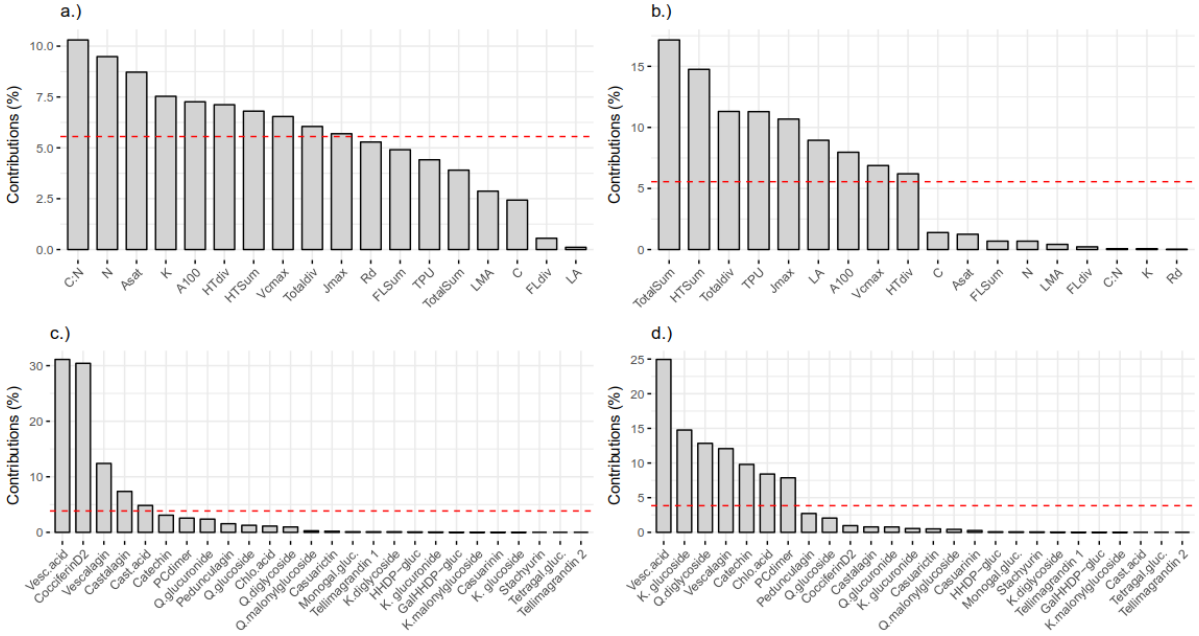


Figure S4. Contributions of the different leaf traits to the PCA axes in Figure 3.3 in the main text. Panel a) PC1 and b) PC2 for all traits except individual polyphenols. Panel c) PC1 and d) PC2 for the different polyphenol groups. The red reference line corresponds to the expected value if the contributions were uniform.

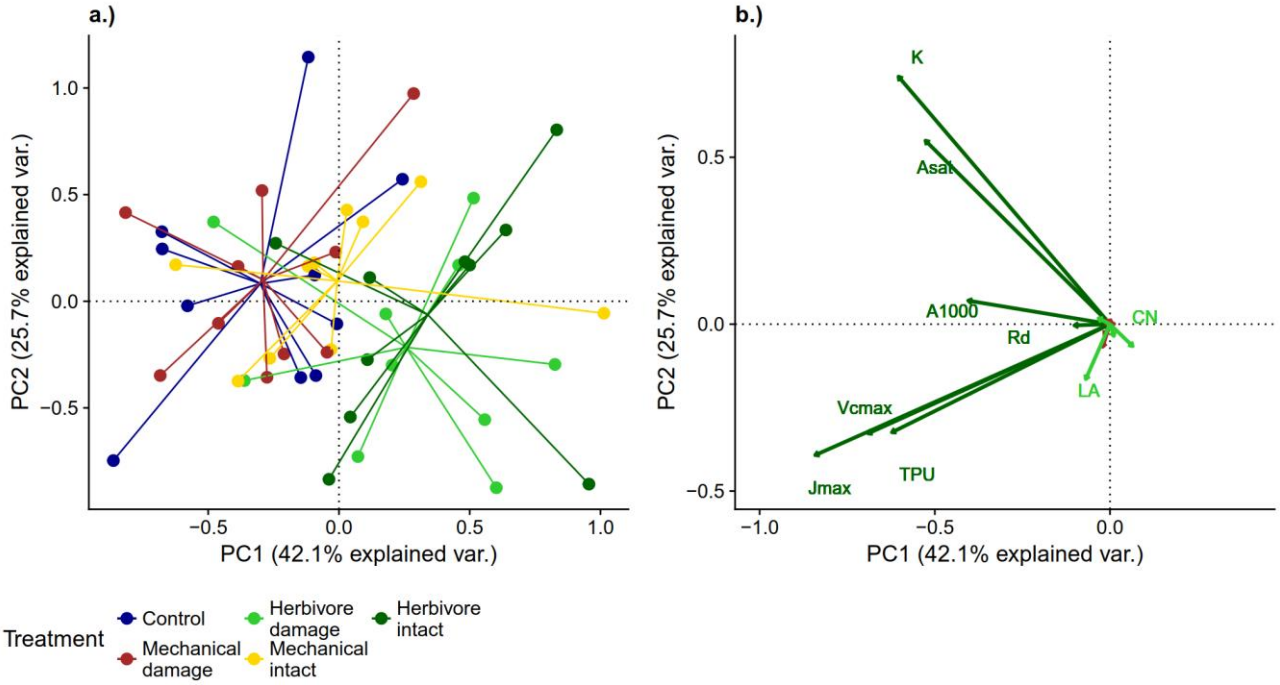


Figure S5. Partial RDA analysis on all leaf traits except the individual polyphenol compounds, in which the variation caused by tree has been removed. Data are shown for the unconstrained analyses. Panel a) shows ordination with grouping by the experimental treatment, and panel b) shows the effect of different leaf traits to the ordination results. Data points represent averages over tree-treatment combinations and use imputed values for missing values of nitrogen and carbon content. In panel b) dark green arrows represent different photosynthetic parameters, brown arrows (partially obscured by the light green arrows) represent defensive traits, and light green arrows represent other traits.

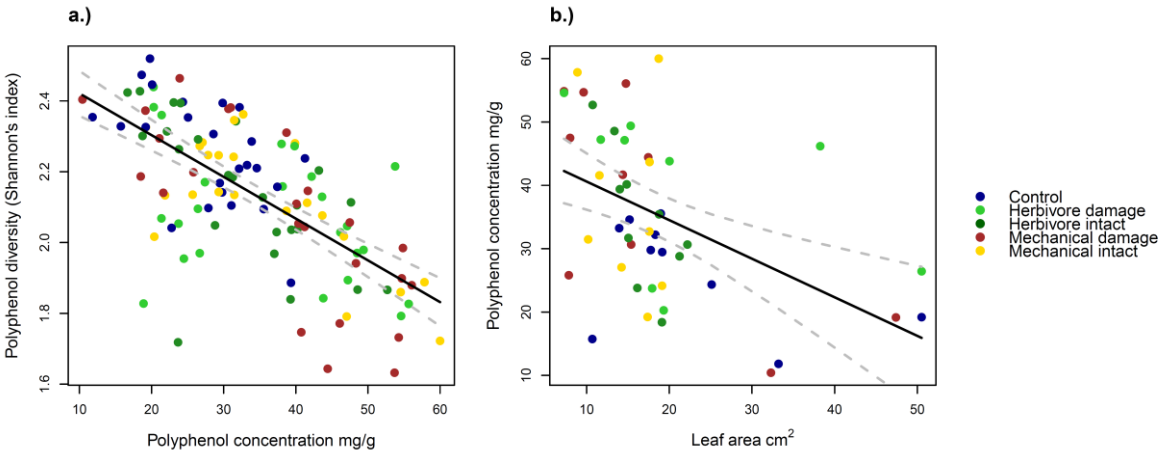


Figure S6. Panel a) shows the relationship between concentration and diversity of polyphenols (Pearson's r = −0.67, $p < 0.001$). Panel b) shows the relationship between concentration of polyphenols and estimated leaf area without herbivory (Pearson's −0.47, $p < 0.001$).

Appendix 3.2: Additional analyses

The interaction between treatment and collection date

To study the significant interaction between treatment and collection date affecting leaf chemistry, we built LMMs for early and late-season leaves separately with concentration of hydrolysable tannins, diversity of hydrolysable tannins, or concentration of flavonols (i.e. response variables that showed significant date : treatment interaction, Table 3.3, Table S4) as a function of the study site, collection date (for early season model only) and the experimental treatment. Interactions were not included in the early-season model to avoid spurious interactions due to small sample size. Interaction between site and treatment was included in the model for late-season leaves. For the late-season model on hydrolysable tannin concentration, the variance was allowed be different between the two sites. For early-season model on hydrolysable tannin diversity, the variance varied exponentially with the collection date. For late-season model on flavonol concentration, the variance varied between the experimental treatments. When treatment turned out significant, we performed Tukey's test to find out which treatments differed from each other. Model selection and diagnostics checks were performed as described in the main text (see "Statistical analyses").

Hydrolysable tannin concentration

For late-season leaves, the hydrolysable tannin concentration was affected by the experimental treatment (Lratio = 17.3, p = 0.002, df = 4, 9). The significant differences were between control leaves and leaves damages by herbivores (t.ratio = −3.16, p = 0.03) and between intact and damaged leaves in the herbivory treatment (t.ratio = 3.93, p = 0.006). There were no differences between the two sites (Lratio = 1.80, p = 0.18, df = 1, 10) in hydrolysable tannin concentration on late-season leaves. For leaves collected throughout the season, only collection date had a significant effect (χ^2 = 5.65, p = 0.02, df = 1, 5), with no

differences between sites (χ^2 = 0.03, p = 0.85, df = 1, 6) or treatments (χ^2 = 4.52, p = 0.34, df = 4, 9).

Hydrolysable tannin diversity

There was no effect of treatment (Lratio = 7.31, p = 0.12, df = 4, 9), site (Lratio = 0.59, p = 0.44, df = 1, 6) or date (Lratio = 1.08, p = 0.30. df = 1, 6) on the diversity of hydrolysable tannins on leaves collected throughout the season. Similarly, there was no effect of treatment (χ^2 = 7.56, p = 0.11, df = 4, 8), site (χ^2 = 0.03, p = 0.87, df = 1, 5) or their interaction (χ^2 = 6.58, p = 0.16, df = 4, 13) on the hydrolysable tannin diversity on leaves collected at the end of the growing season.

Flavonol concentration

There was no effect of treatment (χ^2= 1.32, p = 0.86, df = 4, 8), site (χ^2 = 0.88, p = 0.35 df = 1, 6) or date (χ^2 = 0.42, p = 0.52. df = 1, 5) on the concentration of flavonols on leaves collected throughout the season. Similarly, treatment (Lratio = 3.77, p = 0.44, df = 4, 12) or site (Lratio = 0.32, p = 0.57, df = 1, 9) or their interaction (Lratio = 2.94, p = 0.57, df = 4, 17) had no effect on late-season flavonol concentration.

The interaction between treatment and site

To study the interaction between the experimental treatments and site affecting leaf chemistry, we built linear mixed effect models for both sites (Wytham Woods and John Krebs field station) separately with the summed concentration of flavonols or hydrolysable tannins as a function of the collection date, treatment, and their interactions (Table S4). Variance was set to vary between the trees for both models on flavonol concentration, and on the model on hydrolysable tannin concentration for Wytham Woods.

Flavonol concentration

In Wytham Woods, the flavonol concentration was significantly affected by the interaction between date and treatment (Lratio = 22.8, $p < 0.001$, df = 17, 4, Figure S7a). By the field station, there were no significant effect of the date (Lratio = 0.03, p = 0.87, df = 13, 1), the treatment (Lratio = 1.31, p = 0.86, df = 13, 4) or their interaction (Lratio = 2.10, p = 0.72, df = 17, 4, Figure S7b) on flavonol concentration.

Hydrolysable tannin concentration

In Wytham Woods, the interaction between date and treatment had a significant effect on hydrolysable tannin concentration (Lratio = 39.8, $p < 0.001$, df = 4, 17, Figure S7c). On leaves collected by the field station, the hydrolysable tannin concentration was significantly different between the treatments ($\chi^2 = 13.6$, p = 0.008, df = 4, 8, Figure S7d). The significant differences were between intact and damaged leaf in the herbivory treatment (t.ratio = 3.21, p = 0.03). Collection date had no effect on hydrolysable tannin concentration at the field site ($\chi^2 = 0.02$, p = 0.88, df = 1, 9), nor did the interaction between date and treatment ($\chi^2 = 9.18$, p = 0.06, df = 4, 13, Figure S7d).

Table S4. The significant explanatory factors for the linear mixed effect models testing the effect of the leaf level treatments on leaf chemistry at two sites and at two temporal scales separately. The response variables had previously been identified to have been significantly affected by the interaction between collection date and treatment and between site and treatment (concentration of hydrolysable tannins and flavonols). ~1 refers to a null model, i.e. none of the explanatory factors were significant. HT = hydrolysable tannins, FL= flavonols.

Response	Wytham Woods	Field station	During the season	At the end of the season
Concentration of HT	Date * Treatment	Treatment	Date	Treatment
Concentration of FL	Date * Treatment	~ 1	~ 1	~ 1
Diversity of HT	-	-	~ 1	~ 1

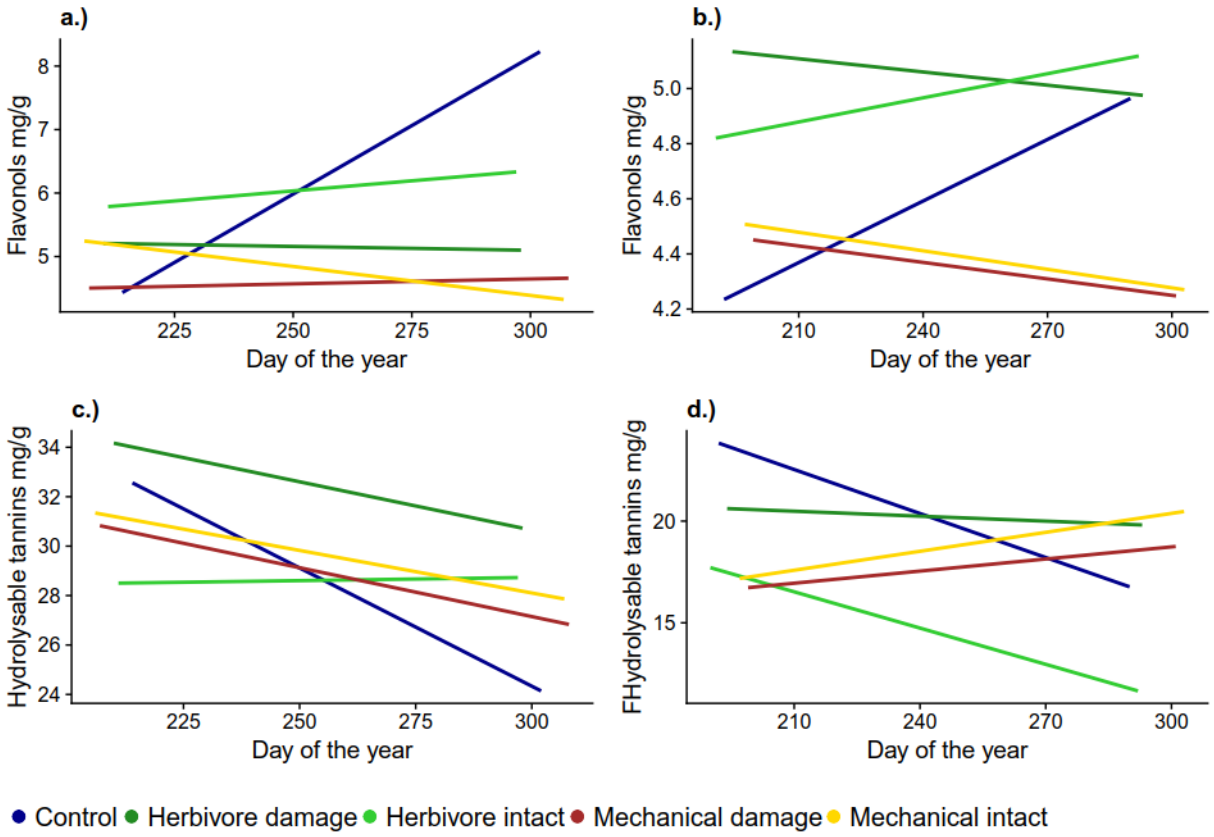


Figure S7. Predicted values for the response variable which showed significant site : treatment or date : treatment interactions over the sampling period. Panel a) shows flavonol concentration in Wytham Woods, b) flavonol concentration by the John Krebs field station, c) hydrolysable tannin concentration in Wytham Woods and d) hydrolysable tannin concentration by the John Krebs field station.

Supplementary material for chapter 4

Appendix 4.1: Supplementary figures

Appendix 4.2: Supplementary tables

Appendix 4.3: Detailed statistical methods and additional results

Appendix 4.1: Supplementary figures

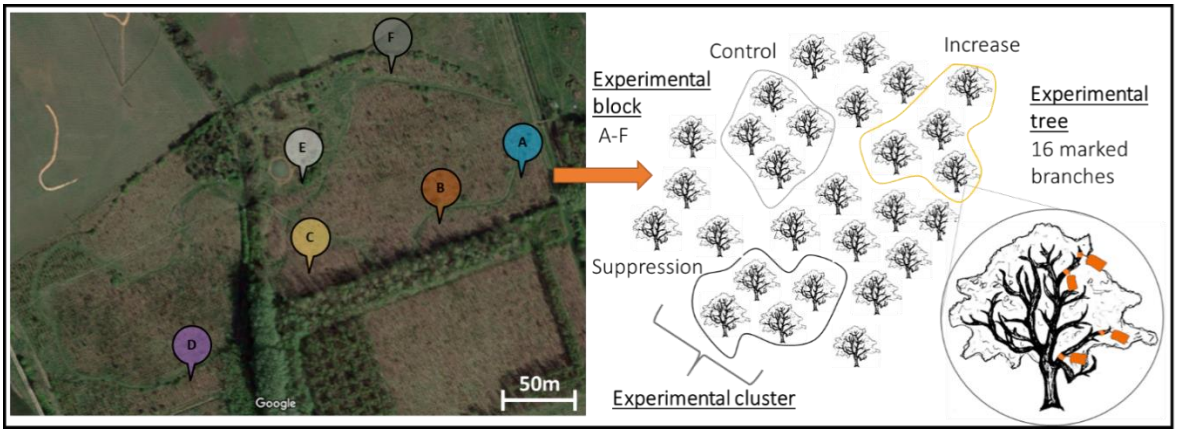


Figure S1. The experimental setup consisted of six experimental blocks (A-F), three clusters within each block (n = 18) and four trees within each cluster (n = 72). Each block had one cluster representing each of the three treatments: suppression of herbivory, increased herbivory and control. On each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

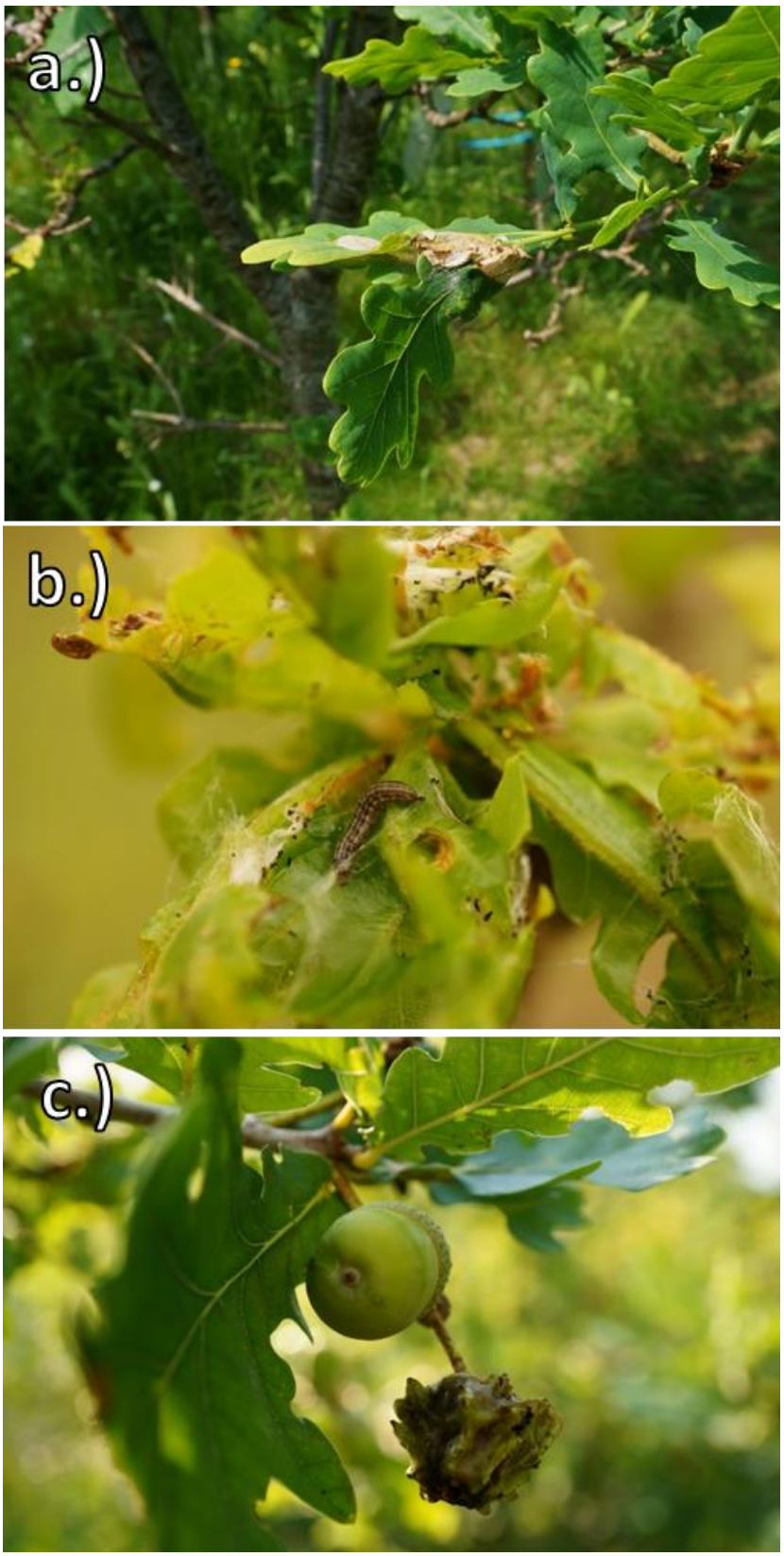


Figure S2. a.) and b.) *Acrobasis consociella* (”Broad-barred Knot-horn”) caterpillar shelter on oak. c.) Acorn deformed by the seed galler *Andricus quercuscalicis* (“Knopper gall”) next to a healthy acorn.

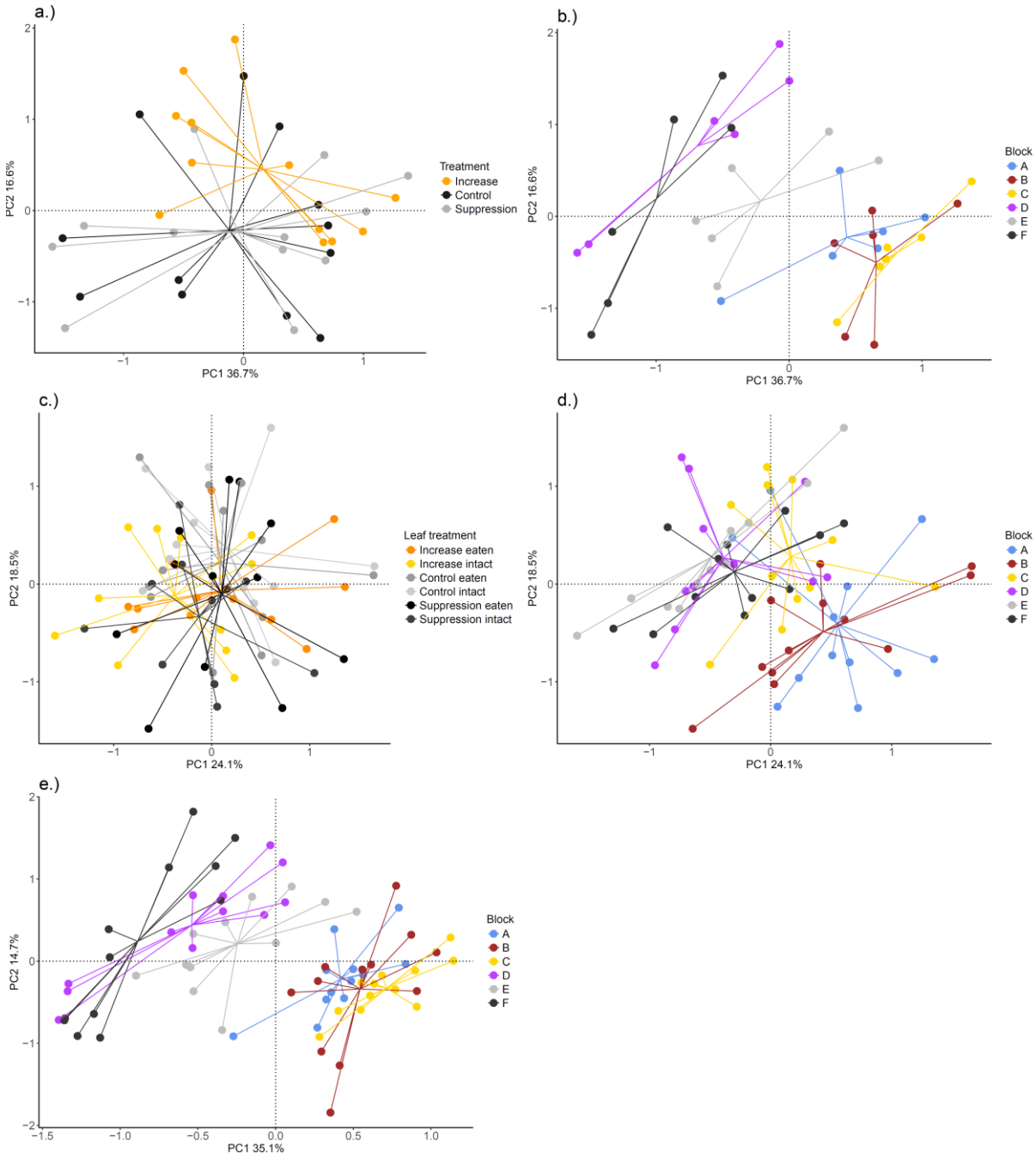


Figure S3. Ordination plots showing different data sets grouped by treatment or the experimental block. Panel a) shows PCA of the late-season insect community for those experimental trees that had their chemistry analysed (n = 36) grouped by the treatment and panel b) shows the same ordination grouped by experimental block. Panel c) shows the leaf-level composition of polyphenols grouped by the leaf type (n = 72 leaves, n = 36 trees), and panel d) shows the same ordination grouped by the experimental block. Finally, panel e) shows the PCA of the late-season insect community structure for all experimental trees (n = 72), grouped by the experimental block, comparable to Figure 4.2b in the main text. In panels b, d and e, the different colours and letter codes correspond to the different experimental blocks (significant, model comparison p = 0.001) shown in Figure S1.

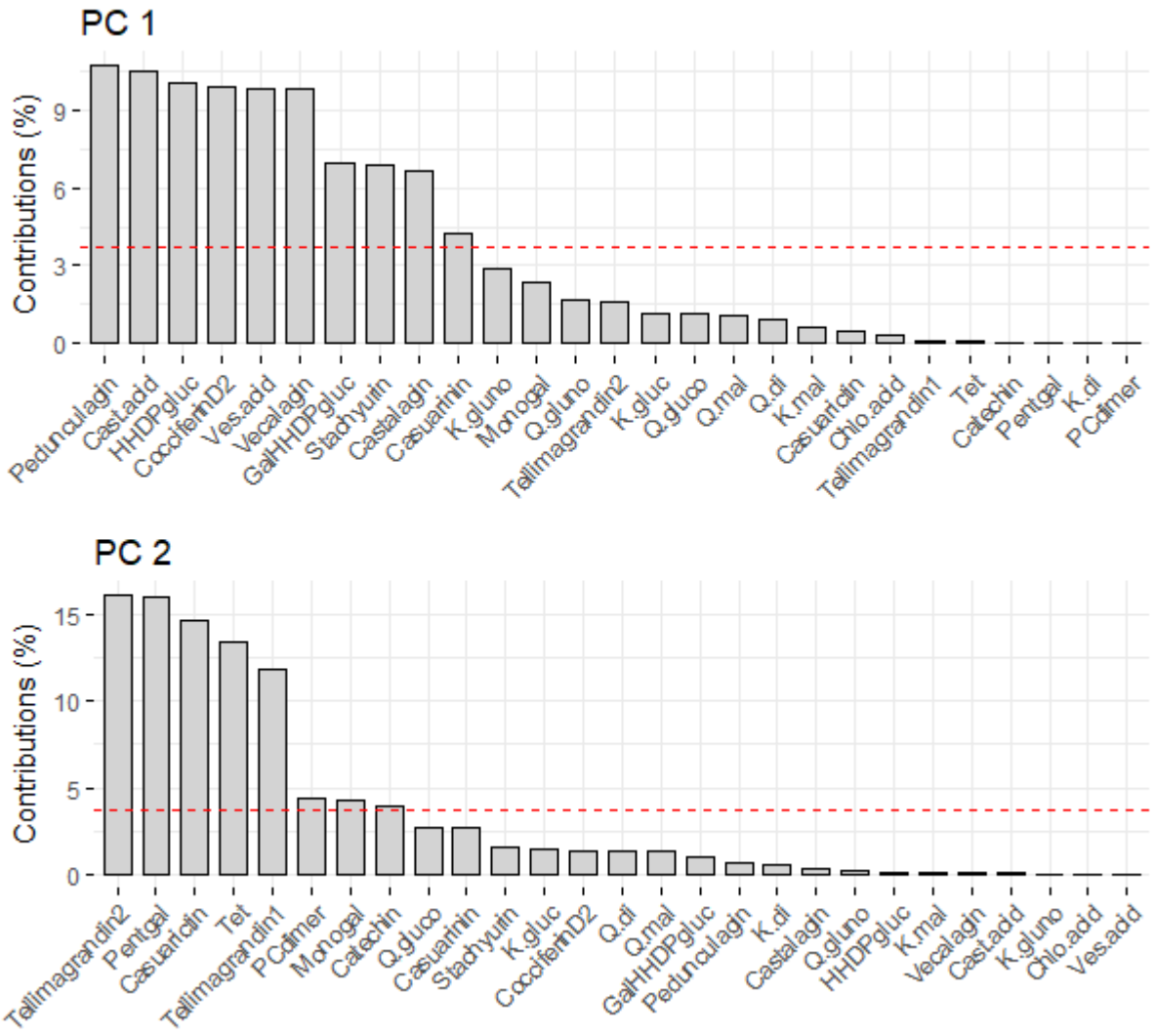


Figure S4. The contribution of different compounds to the two PC-axis describing the chemical composition of oak leaves in three treatments (increase of herbivory, suppression of herbivory, control) and two leaf types (eaten and intact). The red dashed line corresponds to the expected value if contributions were uniform, hence compounds above the line can be considered important contributors to the axis values.

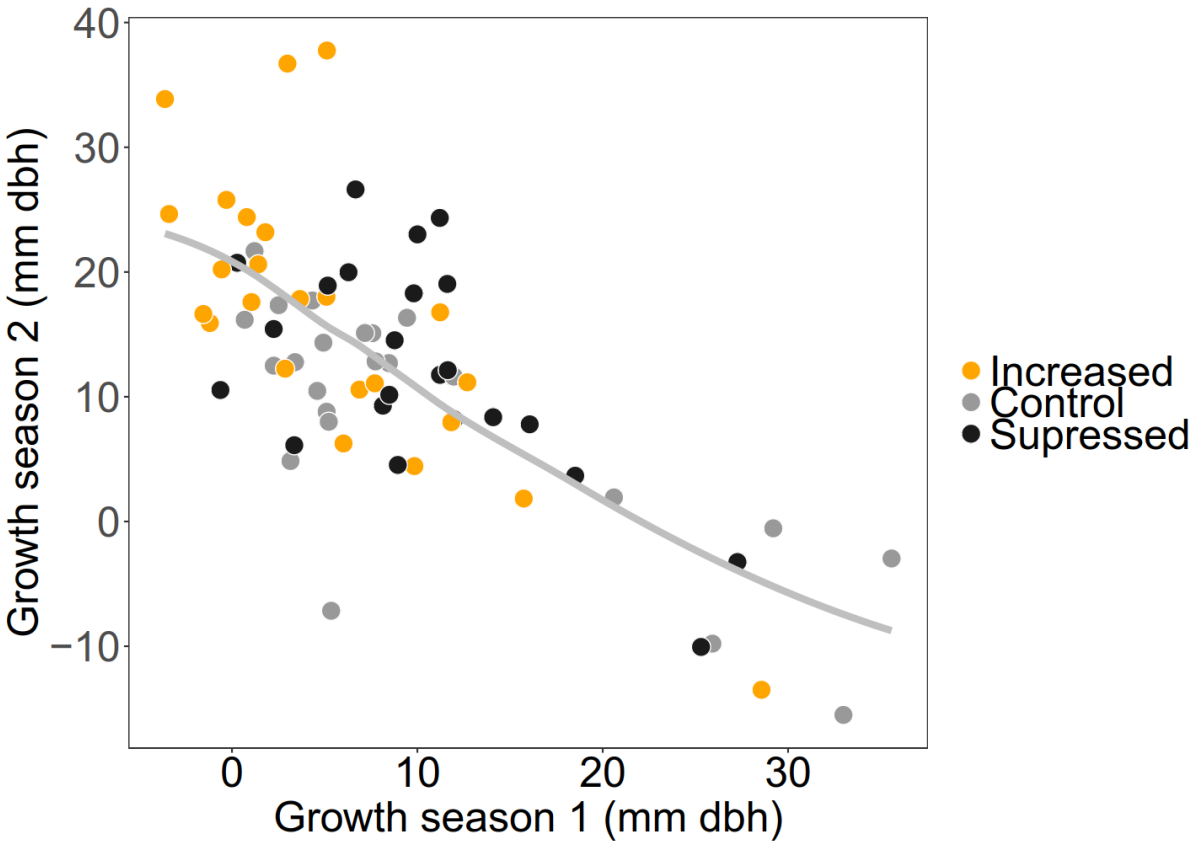


Figure S5. The correlation between tree growth during the first season and growth during the second (as mm increase in dbh).

Appendix 4.2: Supplementary tables

Table S1. The mean concentrations (mg/g) of the different polyphenol compounds based on all analysed samples and abbreviations of compound names used in the figures. HT = hydrolysable tannin.

Compound	Abbreviation	Type	Mean mg/g
Cocciferin D2		Ellagitannin (HT)	6.791
Vescalagin		Ellagitannin (HT)	5.573
Catechin		Flavan-3-ol	4.175
Quercetin glucoside	G.gluco	Flavonol	3.562
Pedunculagin		Ellagitannin (HT)	3.544
Castalagin		Ellagitannin (HT)	3.04
Casuarictin		Ellagitannin (HT)	2.494
PC dimer		Proanthocyanidin	2.376
Kaempferol glucoside	K.gluc	Flavonol	2.218
Quercetin diglycoside	Q.di	Flavonol	2.078
Vescavaloninic acid	Ves.acid	Ellagitannin (HT)	1.892
Quercetin glucuronide	Q.gluno	Flavonol	1.592
Castavaloninic acid	Cast.acid	Ellagitannin (HT)	1.588
Tellimagrandin I		Ellagitannin (HT)	0.498
Tellimagrandin II		Ellagitannin (HT)	0.363
Monogalloylglucose	Monogal	Simple gallic acid derivative (HT)	0.325
Chlorogenic acid	Chlo.acid	Phenolic acid	0.119
HHDP-glucose	HHDPgluc	Ellagitannin (HT)	0.116
Casuarinin		Ellagitannin (HT)	0.104
Kaempferol diglycoside	K.di	Flavonol	0.103
Kaempferol glucuronide	K.gluno	Flavonol	0.093
Kaempferol malonylglucoside	K.mal	Flavonol	0.08
Galloyl-HHDP-glucose	GalHHDPgluc	Ellagitannin (HT)	0.066
Stachyurin		Ellagitannin (HT)	0.035
Tetragalloylglucose	Tetgal	Simple gallic acid derivative (HT)	0.026
Quercetin malonylglucoside	Q.mal	Flavonol	0.025
Pentagalloylglucose	Pentgal	Simple gallic acid derivative (HT)	0.013

Table S2. Coefficient estimates for the fixed effects of the different models described in the main text. For the models in which some of the continuous variables were scaled, the coefficient estimates are presented for the scaled data. Shown are estimates from the minimum adequate models for each response variable. For each model, the intercept indicates the mean value of the response variable for a given level of the fixed effect(s) and for a covariate value = 0 (stated in parentheses), and the other effects show the mean change, compared with the intercept, caused by the other fixed factor levels and by a unit change in covariate value

Response	Final Model	Effect	Estimate	SD Error	Test statistic[*1.]	Model type
All trees (n = 72)						
Caterpillar shelters	~ Dbh + Treatment	Intercept (Increase, dbh=0)	5.31	0.14	39.21	Negative Binomial GLMM
		Dbh	0.20	0.07	2.91	
		Control	−0.72	0.15	−4.86	
		Suppression	−1.7	0.15	−11.25	
Tree growth year 1	~ Dbh	Intercept (dbh=0)	22.57	3.51	6.42	LMM
		Dbh	−0.31	0.07	−4.66	
Tree growth year 2	~ Leaf area loss + Growth season 1	Intercept (Leaf area loss =0, Growth=0)	28.63	3.59	7.97	LMM
		Leaf area loss	−1.26	0.51	−2.46	
		Growth	−0.99	0.11	−9.46	
Tree growth years 1 & 2	~ Leaf area loss	Intercept	28.10	2.87	9.79	LMM
		Leaf area loss	−1.18	0.45	−2.60	
Acorn production	~ Dbh	Intercept (dbh=0)	2.76	0.31	9.05	Negative Binomial GLMM
		Scaled dbh	0.77	0.21	3.65	
Seed galling rate	~1	Intercept	−0.37	0.24	−1.56	Betabinomial GLMM
Mine abundance	~ Leaf area loss	Intercept (Leaf area loss =0)	−1.62	0.06	−26.15	Negative binomial GLMM
		Scaled leaf area loss	0.14	0.06	2.35	
Gall abundance	~1	Intercept	−0.29	0.07	−4.02	Negative binomial GLMM
		Dbh : Shelters	−0.05	0.02	−3.05	
Diversity of mines	~1	Intercept	0.86	0.08	10.54	LMM
Diversity of galls	~ Dbh * Shelters + Date	Intercept (Date=0, Dbh=0, Shelters =0)	−0.09	0.43	−0.22	LMM
		Scaled dbh	0.05	0.02	1.93	
		Scaled shelters	−0.0003	0.02	−0.014	
		Date	0.005	0.002	3.02	

Leaf area loss (n = 39)						
Leaf area loss	~ Block	Intercept (Block A)	−2.78	0.13	−21.77	Beta regression
		Block B	−0.62	0.24	−2.63	
		Block C	−0.24	0.21	−1.13	
		Block D	0.07	0.21	0.32	
		Block E	0.25	0.19	1.30	
		Block F	0.25	0.19	1.32	
Leaf chemistry (n = 36)						
Total concentration of all polyphenols	~ Leaf type	Intercept (Elevation, dmg)	41.32	5.33	7.75	LMM
		Elevation, intact	11.54	2.79	4.14	
		Control, damaged	9.99	6.17	1.62	
		Control, intact	7.86	6.17	1.28	
		Suppression, damaged	−0.69	6.17	−0.11	
		Suppression, intact	5.02	6.21	0.8	
Concentration of hydrolysable tannins	~ Leaf type	Intercept	25.85	2.97	8.69	LMM
		Elevation, intact	4.12	1.37	3.00	
		Control, damaged	2.06	3.32	0.62	
		Control, intact	1.43	3.32	0.43	
		Suppression, damaged	−3.04	3.32	−0.92	
		Suppression, intact	−0.91	3.34	−0.27	
Concentration of flavonols	~ Leaf type	Intercept	6.01	2.14	2.83	LMM
		Elevation, intact	4.09	1.23	3.32	
		Control, damaged	6.38	2.80	2.28	
		Control, intact	5.36	2.80	1.91	
		Suppression, damaged	2.17	2.80	0.77	
		Suppression, intact	4.70	2.82	1.67	
Diversity of polyphenols	~1	Intercept	2.48	0.017	144.9	LMM
Diversity of hydrolysable tannins	~1	Intercept	1.96	0.02	127.4	LMM
Diversity of flavonols	~1	Intercept	1.35	0.04	33.97	LMM

***1. z value if GLMM or Beta regression, t-value if LMM**

Table S3. The late-season lefminers and gallers in order of abundance and the abbreviations used for the species names in figures, their lifeform, taxon and feeding type and known host plants. Weighted abundance has been scaled with the number of leaves surveyed. *Phyllonorycter* species are sap-suckers during the first three larval instars, after which they feed on the leaf tissue (Askew & Shaw, 1979).

Species + generation	Abbreviation	Weighted abundance	Lifeform	Taxon (family)	Feeding type	Host plants
Neuroterus anthracinus, asex.	Neu.ant	372.3	Gall	Gall wasp (Cynipidae)	Chewing	*Quercus* spp.
Neuroterus albipes, asex.	Neu.alb	262.1	Gall	Gall wasp (Cynipidae)	Chewing	*Quercus* spp.
Phyllonorycter spp.	Phy.	184	Mine	Moth (Gracillariidae)	Sap-sucking/Chewing	*Quercus* spp.[1.)]
Macrodiplosis pustularis	Mac.pus	135	Gall	Gall midge (Cecidomyiidae)	Chewing	*Quercus* spp.
Neuroterus quercusbaccarum, asex.	Neu.que	62.12	Gall	Gall wasp (Cynipidae)	Chewing	*Quercus* spp.
Trioza remota	Tri.re	46.22	Gall	Jumping plant lice (Triozidae)	Sap-sucking	*Quercus* spp.
Neuroterus numismalis, asex.	Neu.num	37.99	Gall	Gall wasp (Cynipidae)	Chewing	*Quercus* spp.
Stigmella spp.	Sti.	25.92	Mine	Moth (Nepticulidae)	Chewing	*Quercus* spp.[2.)]
Bucculatrix ulmella	Buc.ulm	11.53	Mine	Moth (Bucculatricidae)	Chewing	*Quercus* spp.
Gypsonoma dealbana	Gyp.dea	4.94	Mine	Moth (Tortricidae)	Chewing	Many trees and shrubs
Ectoedemia sp. 1	Ect.1	4.38	Mine	Moth (Nepticulidae)	Chewing	*Quercus* spp
Andricus curvator, sex.	And.curv	3.85	Gall	Gall wasp (Cynipidae)	Chewing	Quercus spp
Tischeria spp.	Tis.	3.71	Mine	Moth (Tischeriidae)	Chewing	*Quercus* spp., *Castanea* spp.
Coleophora spp.	Col.	3.17	Mine	Moth (Coleophoridae)	Chewing	*Quercus* spp.[3.)]
Cynips longiventris, asex.	Cyn.lon	2.72	Gall	Gall wasp (Cynipidae)	Chewing	*Quercus* spp
Ectoedemia sp. 2	Ect.2	2.04	Mine	Moth (Nepticulidae)	Chewing	*Quercus* spp
Profenusa pygmyea	Pro.pyg	1.94	Mine	Sawfly (Tenthredinidae)	Chewing	*Quercus* spp

Ectoedemia sp. 3	Ect.3	1.6	Mine	Moth (Nepticulidae)	Chewing	*Quercus* spp.
Macrodiplosis roboris	Mac.rob	0.577	Gall	Gall midge (Cecidomyiidae)	Chewing	*Quercus* spp.
Cynips quercusfolii, asex.	Cyn.que	0.067	Gall	Gall wasp (Cynipidae)	Chewing	*Quercus* spp.
Cynips divisa, asex.	Cyn.di	0.03	Gall	Gall wasp (Cynipidae)	Chewing	*Quercus* spp.

1.) Of the nine *Phyllonorycter* species feeding on *Q. robur*, one (*P. messaniella*) feeds on several tree genera (*Quercus, Fagus, Castanea*, and *Ostrya*).

2.) Of the six *Stigmella* species feeding on *Q. robur*, one (*S. samiatella*) feeds also on *Castanea*.

3.) Of the five *Coleophora* species feeding on *Q. robur*, one (*C. currucipennella*) feeds on several tree genera (*Quercus, Carpinus, Salix, Corylus*)

Table S4. The analyses of deviance table for fixed effects of the multivariate models describing the late-season herbivore community composition. Shown the explanatory variables of the full model for each response variable. Statistically significant ($p < 0.05$) variables have been bolded.

Response	Full model	LRT	p-value
Community composition of galls and mines per tree	**Block**	**333.9**	**0.001**
	Cluster	**490.1**	**0.001**
	Dbh	27.5	0.33
	Date	**59.3**	**0.01**
	Shelters	26.0	0.46
	Date : Shelters	18.9	0.71
	Dbh : Shelters	37.0	0.14
Community composition of galls and mines per tree	**Block**	**282.9**	**0.001**
	Total polyphenol composition per tree	**553.4**	**0.020**

Table S5. The unadjusted F and p-values for each late-season species separately from the multivariate analysis describing the late-season community structure. Statistically significant effects ($p < 0.05$) have been bolded. The first seven variables (block, cluster, dbh, date, shelters, date : shelters and dbh:shelters) have been derived from multivariate analysis including all the experimental trees (n = 72). The last variable (polyphenols) is from an analysis with only those trees that were part of the chemical analyses (n = 36). The analysis including tree chemistry did not include the species *M. roboris*, because in the smaller data set it only occurred on one tree.

Species	Test statistic	Block	Cluster	Dbh	Date	Shelters	Date: Shelters	Dbh: Shelters	Poly-phenols
Neuroterus anthracinus	F	15.5	25.3	0.66	7.74	1.27	10.28	3.02	43.3
	p	**0.01**	**0.04**	0.49	**0.01**	0.36	**0.006**	0.18	**0.03**
Neuroterus albipes	F	10.3	25.1	0.06	0.63	4.55	0.23	2.69	28.0
	p	**0.03**	**0.03**	0.81	0.46	0.06	0.67	0.18	0.18
Phyllonorycter spp.	F	16.6	28.6	0.58	0.11	0.47	0.83	4.71	18.6
	p	**0.02**	**0.048**	0.50	0.78	0.58	0.45	0.08	0.69
Macrodiplosis pustularis	F	62.4	103	0.48	0.03	0.77	0.97	0.10	45.7
	p	**0.001**	**0.001**	0.55	0.87	0.47	0.41	0.78	0.06
Neuroterus quercusbaccarum	F	5.28	5.46	0.01	0.58	0.85	1.94	0.67	37.8
	p	0.31	0.96	0.76	0.46	0.42	0.22	0.47	0.06
Trioza remota	F	16.3	22.1	2.51	0.004	0.66	0.008	1.34	15.1
	p	0.09	0.17	0.16	0.95	0.45	0.93	0.31	0.80
Neuroterus numismalis	F	19.8	11.7	0.10	2.77	0.54	0	0.54	27.2
	p	**0.006**	0.65	0.77	0.13	0.53	0.99	0.55	0.45
Stigmella spp.	F	24.0	46.0	6.80	0.39	0.08	0.55	1.74	29.44
	p	**0.002**	**0.001**	**0.02**	0.61	0.83	0.55	0.28	0.21
Bucculatrix ulmella	F	29.5	29.7	0.20	0.57	0.06	1.19	0.02	54.1
	p	**0.001**	**0.03**	0.67	0.51	0.85	0.40	0.94	**0.02**
Gypsonoma dealbana	F	3.50	17.4	0.04	1.24	**0.03**	0.71	2.41	19.5
	p	0.53	0.31	0.86	0.33	0.89	0.48	0.22	0.69

Ectoedemia sp. 1	F	34.4	11.8	0.26	0.04	1.40	0.02	0.14	24.1
	p	**0.001**	0.69	0.67	0.87	0.32	0.88	0.77	0.26
Andricus curvator	F	10.2	29.2	1.78	7.02	0.67	0.02	0.45	31.7
	p	0.09	**0.04**	0.23	**0.03**	0.50	0.91	0.60	0.18
Tischeria spp.	F	14.8	18.4	0.55	5.87	0.06	0.81	8.12	20.8
	p	**0.007**	0.28	0.51	**0.03**	0.80	0.41	**0.006**	0.30
Coleophora spp.	F	7.79	27.5	0.88	11.1	8.65	0.76	0.62	27.9
	p	0.42	0.07	0.45	**0.009**	**0.04**	0.42	0.52	0.27
Cynips longiventris	F	25.28	23.7	0.09	12.9	0.006	0.09	0.04	34.1
	p	**0.001**	**0.03**	0.80	**0.006**	0.96	0.80	0.88	**0.047**
Ectoedemia sp. 2	F	6.15	12.4	4.90	0.005	0.001	0.40	3.77	34.4
	p	0.33	0.79	**0.05**	0.96	0.98	0.65	0.13	0.23
Profenusa pygmyea	F	6.96	22.7	0.33	0.21	0.19	0.07	2.70	25.0
	p	0.25	0.12	0.54	0.65	0.66	0.80	0.11	0.24
Ectoedemia sp. 3	F	11.10	15.3	7.13	0.01	1.76	0.03	2.13	36.9
	p	0.08	0.49	**0.01**	0.92	0.27	0.87	0.16	0.37
Macrodiplosis roboris	F	14.1	14.1	0.03	8.06	3.98	0	1.79	NA
	p	**0.01**	**0.04**	0.89	**0.02**	**0.048**	0.52	0.20	NA

Appendix 4.3. Detailed statistical methods and additional results

Detailed statistical methods

Our data formed two main data sets: the larger of these included all the experimental trees (n = 72) and measures on the number of early-season caterpillar shelters, tree performance (growth, acorn production, acorn health), leaf area loss on the leaves outside the caterpillar shelters, and the late-season insect community structure. Since, due to time constrains, leaf area loss was not estimated for all the individual trees (n = 39), we used average values for each experimental cluster as an estimate for leaf area loss. The second data set included half of the trees (n = 36) and data on polyphenol concentrations in two different leaf types per tree (72 leaf samples). Below, we describe model structures per dataset in detail:

Tree and insect data set

The effect of treatment on the number of caterpillar shelters

To test whether the experimental treatments yielded detectable differences in the density of early-season herbivores, we built a generalized linear mixed effect model (hereafter "GLMM") with the number of early-season caterpillar shelters per tree as a function of the treatment (*increase*, *suppression* or *control*), the size of the tree (as dbh), and their interaction as fixed effects. We used negative binomial distribution to account for overdispersion. Tukey's test was performed to investigate whether the differences between the treatments were statistically significant. Random effects were cluster nested within block to account for the hierarchical study design

Does the abundance of *A. consociella* covary with leaf area loss on leaves outside the shelters?

To test whether early-season herbivory by the shelter-building *A. consociella* covaried with leaf area loss on leaves outside the shelters, we built a beta-regression model of the average proportion of the leaf area missing per tree as a function of the experimental block, tree size and the number of early-season caterpillar shelters. For this model, we used tree-level data on those trees from which the leaf area loss was estimated (n = 39).

The effects of *A. consociella* shelters on tree performance

To test the effect of early-season *A. consociella* caterpillar shelters on tree growth, we built linear mixed effect models (hereafter "LMM") with tree growth as mm of dbh as a function of the tree size, the number of caterpillar shelters, and leaf area loss on a level of a cluster. Because of the large number of possible interactions, only two-way interactions judged to be ecologically meaningful (between caterpillar shelters and the other variables) were included. We used separate models for growth during the first and second growing season, and for the cumulative growth after the two seasons. For the growth during the second season, we also included growth rate during the first season as an explanatory variable to improve the spread of residuals against fitted values. For the growth during the first season, variance structure was set to vary exponentially with tree size to improve the spread of residuals against fitted values (Zuur *et al.*, 2009). Random effects were cluster nested within block.

To test the effects of early-season *A. consociella* caterpillars on the total acorn production, we built a GLMM with the number of all acorns (healthy + galled), as a function of tree size, number of caterpillar shelters, leaf area loss and the two-way interactions between caterpillar shelters and the other variables with negative binomial distribution to account for overdispersion. Random effects were cluster nested within block.

To test the effects of early-season *A. consociella* caterpillars on the proportion of acorns deformed by the seed galler *Andricus quercuscalicis*, we built a GLMM with the number of healthy and infested acorns (as a two-column matrix of "successes" and "failures")

as a function of the tree size, the amount of early-season caterpillar shelters, leaf area loss and the two-way interactions between caterpillar shelters and the other variables. To account for overdispersion, we used betabinomial distribution (Harrison, 2015). Random effects were cluster nested within block.

The effects of *A. consociella* shelters on the late-season insect abundance and diversity

To analyse the effects of early-season *A. consociella* caterpillars on the abundance of leafminers and gallers, we built a GLMM for the abundance of leafminers and gallers separately as a function of tree size, survey date, number of early-season caterpillar shelters, leaf area loss and the two-way interactions between caterpillar shelters and the other variables with negative binomial error distribution to account for overdispersion. We used "bobyqa" optimising algorithm (Powell, 2009) to help with model convergence. Since the number of leaves surveyed for the late-season insects varied depending on size of the surveyed branch (some having < 50 leaves), differences in search effort were taken into account in the analyses: the number of surveyed leaves per tree was log-transformed and set as an offset to account for differences in search effort between trees. Random effects were cluster nested within block.

To study the effects of early-season caterpillars on the late-season leafminer and galler diversity, we calculated Shannon's diversity index for the late-season insect communities on each tree. To account for different search efforts due to the number of leaves surveyed differing between the searched branches, the branch-specific insect counts were weighted with the number of leaves surveyed before calculating the index. We then built LMMs with Shannon's diversity index for leafminers and gallers separately as a function of tree size, survey date, the number of early–season caterpillar shelters, leaf area loss and the two-way interactions between caterpillar shelters and the other variables. Random effects were cluster nested within block.

The effects of *A. consociella* shelters on the late-season insect community composition

To analyse the effect of early-season caterpillars on herbivore community composition, we built generalised linear models for multivariate data (Wang *et al.*, 2012). These types of models have recently been suggested as a better alternative for distance-based ordination techniques for multivariate abundance data (Warton *et al.*, 2012, 2015). We modelled tree-level insect abundance (leafminers and gallers combined) as a function of the experimental block and cluster, tree size, survey date and the number of early-season caterpillar shelters with negative binomial error distribution. Only interactions between caterpillar shelters and date and shelters and tree size were included. Species which were found only on one tree (*Cynips divisa* and *Cynips quercusfolii*) were removed from all multivariate analyses.

Chemistry data set

The differences in polyphenol concentration and diversity between leaf types

We studied the differences in polyphenol concentration and diversity between the three tree-level treatments (*increased herbivory*, *suppressed herbivory*, *control*) and the two leaf damage classes (intact and eaten). As response variables, we used total concentration of all compounds (27 compounds), diversity of all compounds (as Shannon's diversity index), and concentration and diversity of the two polyphenol subgroups focused: hydrolysable tannins (16 compounds) and flavonols (8 compounds, Appendix 4.2, Table S1). Since most compounds were present in every leaf, the diversity index here describes how evenly the different compounds were represented per sample. We built LMMs with each response variables as a function of tree size, and the leaf type (intact leaf in *suppressed herbivory* treatment, damaged leaf in *suppressed herbivory* treatment, intact leaf in *control* treatment, damaged leaf in *control* treatment, intact leaf in *increased herbivory* treatment, and damaged leaf in *increased herbivory* treatment). Interactions were not included to avoid spurious

interactions (Zuur *et al.*, 2009) due to the small sample size (36 trees). Where the leaf type turned out significant, we performed post-hoc Tukey's test to assess which leaf types were significantly different from each other. For the model on the diversity of hydrolysable tannins, the variance was let to vary with tree size to account for the unequal variances between trees of different sizes. Random effects were tree nested within cluster nested within a block.

The differences in polyphenol composition between leaf types. To study the differences in polyphenol composition between the leaf types, we performed a principal component analysis (PCA), and extracted values for the two first PC axes. We then built LMMs with each of the axis as a response separately. Fixed effects included tree size and leaf type, and random effects included tree nested within a cluster nested within the block. When leaf type turned out significant, we performed a post-hoc Tukey's test yielding pairwise tests between the six leaf types. This set-up allowed us to replicate the nested random structure describing the experimental set-up (by using LMMs), analyse changes in composition of multiple compounds simultaneously (by analysing the PCA-axis) and perform post-hoc pair-wise comparisons between the leaf types (by using Tukey's test). Random effects were tree nested within cluster nested within a block.

The correlation between insect community structure and polyphenol composition. To study whether the tree-level composition of polyphenols correlated with the tree-level late-season insect community structure, we built a multivariate GLM with insect community composition per tree as a response variable and experimental block and the average concentration of the different polyphenol compounds per tree as explanatory variables. Since the galler *Macrodiplosis roboris* occurred only on one tree in this data set (leaf chemistry was only measured for half of the trees), this species was additionally removed from the analysis. To estimate the average polyphenol concentration per tree, we weighted the concentration of each

compound per leaf damage class (eaten or intact) with the proportion of that leaf class on average in that experimental cluster and summed the two proportional concentrations. The proportions of intact and eaten leaves per cluster were derived from the surveys of early-season herbivory damage (see "Early-season herbivory" in the methods in the main text). We examined multicollinearity between the different polyphenol compounds by calculating variance inflation factors, and dropped variables sequentially with the highest vif-factor until all the remaining variables had a vif-factor < 5 (Zuur *et al.*, 2010). Matrix of these remaining compounds (n = 14) was then set as an explanatory variable in the model. To partition the variance into components explained by the different explanatory and random effects, we built a hierarchical Bayesian joint species distribution model with the HMSC –package (Ovaskainen *et al.*, 2017), in which the late-season species abundances was set as the response matrix and concentrations of all individual polyphenol compounds and the number of early-season caterpillar shelters as the environmental covariate matrix, and experimental block and cluster as random effects. We used overdispersed Poisson –error distribution, because the previous models on late-season insect abundance had showed signs of overdispersion.

To visualise the results of the multivariate models, we performed additional PCAs with the abundance of the all the late-season species as a response. We performed one PCA for species abundance data from all trees, and one for those trees from which leaf chemistry was measured (n = 36). For the latter ordination, we overlaid the concentrations of the different polyphenol compounds as vectors. Both data sets were log-transformed (log +1 for insect abundance and log + 0.01 for polyphenol concentration) to improve the visualization.

In all GLMMs, and LMMs with Shannon's diversity index as response variable, all the continuous explanatory variables (the number of shelters, dbh, survey date as day of the year and leaf area loss) were centred and scaled to help model convergence. For each response, the full model was simplified by dropping one explanatory variable at a time. The change in the

model fit was assessed using likelihood ratio tests. Fixed factors that did not improve model fit were dropped from the final model (Crawley, 2007). Overdispersion was examined by comparing the sum of squared Pearson's residuals with the residual degrees of freedom. The significance of fixed factors in the multivariate models was assessed by performing an analysis of deviance on the full model and assessing the significance of the individual terms with likelihood ratio tests and resampled p-values. To examine which individual species or compounds (as part of the multivariate response variable) expressed significant effects, we used univariate likelihood ratio tests with unadjusted p-values.

The model assumptions were tested by visually examining plots of residuals against fitted values for the homoscedasticity of residuals. For LMMs and GLMMs, we also examined plots of residuals against the explanatory variables, and a Quantile-Quantile plot for the normal distribution of the residuals. The latest version of R (3.5.0) and packages nlme (for LMMs and GLMMs; Pinheiro *et al.*, 2017), multcomp (for Tukey's tests; Hothorn *et al.*, 2008), DHARMa (for model assumption testing for GLMMs; Hartig, 2017), betareg (for beta regression models; Cribari-Neto & Zeileis, 2010), glmmADMB (for betabinomial models; Fournier *et al.*, 2012), vegan (for community and diversity metrics; Oksanen *et al.*, 2017), mvabund (for multivariate testing; Wang *et al.*, 2017a), HH (for collinearity testing; Heiberger, 2017) and HMSC (for variance partitioning; Ovaskainen *et al.*, 2017) were used in the analyses.

The effects of tree size and collection date

Tree size had a negative effect on tree growth during the first season (Lratio = 18.1, $p < 0.001$, df = 1, 5) and positive effect on the number of caterpillar shelters ($\chi^2 = 7.81$, $p = 0.005$, df = 1, 7) and acorns ($\chi^2 = 14.1$, $p < 0.001$, df = 1, 5). Tree size did not affect leaf area loss ($z = 0.19$, $p = 0.85$), polyphenol concentration or diversity ($\chi^2 = 1.06$, $p = 0.30$, df = 1, 11 all polyphenols, $\chi^2 = 1.63$, $p = 0.20$, df = 1, 11 hydrolysable tannins, $\chi^2 = 0.06$, $p = 0.81$, df = 1,

11 flavonols, $\chi^2 = 0.88$, p = 0.35, df = 1, 6, total diversity, $\chi^2 = 2.58$, p = 0.11, df = 1, 6 hydrolysable tannin diversity, $\chi^2 = 0.05$, p = 0.82, df = 1, 6 flavonol diversity), polyphenol composition ($\chi^2 = 0.06$, p = 0.81, df = 1, 11, axis 1, $\chi^2 = 0.70$, p = 0.40, df = 1, 6 axis 2), tree growth during the second season ($\chi^2 = 1.78$, p = 0.18, df = 1, 7), tree growth over two seasons ($\chi^2 = 1.78$, p = 0.18, df = 1, 7), proportion of healthy acorns ($\chi^2 = 0.01$, p = 0.91, df = 1, 5), late-season galler ($\chi^2 = 1.28$, p = 0.26, df = 1,5) or leafminer abundance ($\chi^2 = 0.43$, p = 0.51, df = 1, 6) or leafminer diversity ($\chi^2 = 0.14$, p = 0.70, df = 1, 5). Collection date affected the diversity of gallers ($\chi^2 = 6.46$, p = 0.01, df = 1, 8) and the community composition of late-season herbivores (LRT = 59.3, p = 0.01, Appendix 4.2, Table S4), but had no effect on the abundance of gallers ($\chi^2 = 0.21$, p = 0.64, df = 1, 5) or leafminers ($\chi^2 = 1.86$, p = 0.17, df = 1, 6) or on the diversity of leafminers ($\chi^2 = 0.02$, p = 0.88, df = 1, 5).

Patterns of tree resource allocation

To study if the experimental trees allocated their resources on the growth/reproduction/defence –axes as predicted by plant resource allocation hypothesis, we carried out three tests: 1.) Is growth rate affected by total concentration or diversity of polyphenols, 2.) Is reproduction affected by total concentration or diversity of polyphenols and 3.) Do growth rate and reproduction rate correlate? For measure of growth, we used the cumulative growth rate over two seasons and for measure of reproduction we used the total number of acorns produced (both galled and healthy). To answer question 1, we a built LMM with growth rate as a function of tree size, total concentration of all polyphenols and Shannon's diversity index for all polyphenols. To answer question 2, we built a zero-inflated GLMM with number of acorns as a function of the same explanatory variables and negative binomial -distributed errors. To help with model convergence, zero-inflation factor was set to vary with tree size. To answer question 3, we built a zero-inflated GLMM with number of acorns as a function of the cumulative growth rate over the two seasons. Since growth rate

correlates with tree size, tree size was not included in the model. For questions 1 and 2, we used data on trees that were part of the chemical analyses (n = 36 trees) and for question 3 we used data on all trees (n = 72 trees). For all models, we included an interaction term with the number of early-season caterpillars and the main explanatory variable (polyphenol metrics in models 1 and 2 and the growth rate in model 3) to test if early-season herbivory changed relationships between the three life history traits. Model selection and diagnostics were performed as described above.

Total concentration of polyphenols ($\chi^2 = 0.52$, p = 0.47, df = 1, 5), diversity of all polyphenols ($\chi^2 = 2.41$, p = 0.12, df = 1, 5) and their interactions with early-season caterpillars ($\chi^2 = 0.29$, p = 0.59, df = 1, 7 concentration, $\chi^2 = 0.01$, p = 0.92, df = 1, 7 diversity) did not affect tree growth. Similarly, acorn production was not affected by concentration ($\chi^2 = 2.86$, p = 0.09, df = 1, 7) or diversity ($\chi^2 = 0.17$, p = 0.68, df = 1, 7) of polyphenols or their interaction with caterpillar shelters ($\chi^2 = 0.12$, p = 0.73, df = 1, 9 concentration, $\chi^2 = 0.16$, p = 0.69, df = 1, 9 diversity). Number of acorns and tree growth did not correlate ($\chi^2 = 1.31$, p = 0.52, df = 2, 8) and the interactions between tree growth and early-season caterpillar shelters was not significant ($\chi^2 = 1.02$, p = 0.31, df = 1, 9).

The interaction between tree size and the diversity of late-season gallers

To explore the significant interaction between tree size and the galler diversity, we divided our data set in two parts depending on the size of the experimental tree: smaller (dbh < average dbh 43.1 mm) and larger (dbh > average dbh) trees. We built a linear mixed effect model for both data sets. In both models, galler diversity was set as a response variable, and tree size, collection date, the number of early-season caterpillar shelters, and the interaction between tree size and caterpillar shelters as explanatory variables. Random effects included cluster nested within a block. Model selection and diagnostics were performed as described above.

For smaller trees, the effect of caterpillar shelters ($\chi^2 = 2.23$, p = 0.14, df = 1, 6) and tree size ($\chi^2 = 0.48$, p = 0.49, df = 1, 6) were not significant. The collection date had a positive effect on galler diversity on smaller trees ($\chi^2 = 6.43$, p = 0.01, df = 1, 5). On larger trees, the amount of caterpillar shelters had a significant negative effect on galler diversity ($\chi^2 = 6.01$, p = 0.01, df = 1, 6, Appendix 4.3, Figure S1a) when the marginally non-significant effect of tree size ($\chi^2 = 3.53$, p = 0.06, df = 1, 6) was kept in the model. See Appendix 4.3, Figure S1b for the relationship between tree size and galler diversity across all trees.

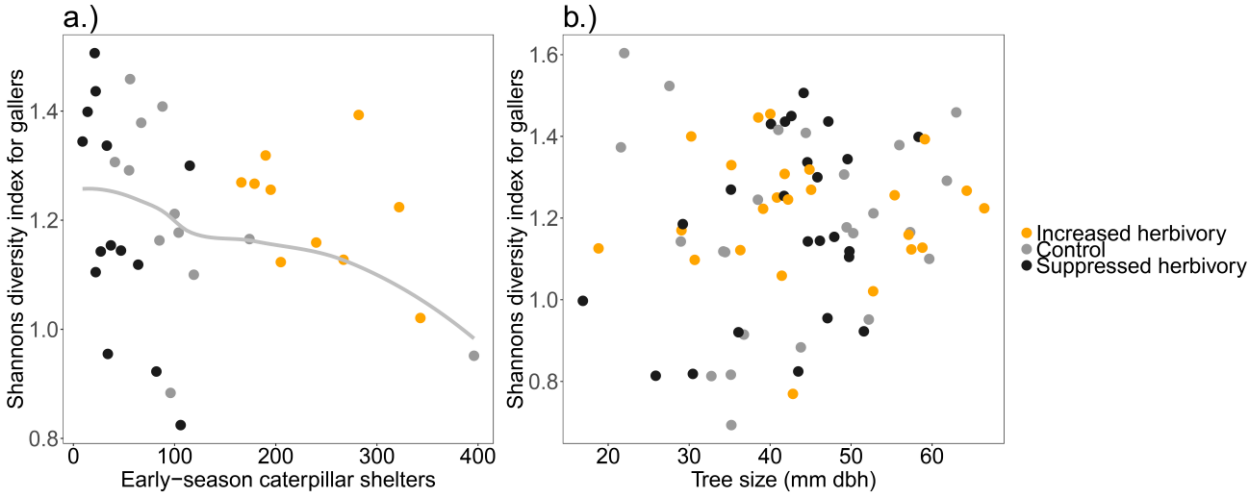


Figure S1. Panel a) shows the effect of early-season caterpillar shelters on galler diversity only on larger trees (dbh > average dbh 43.1 mm, n = 36). Panel b) shows the relationship between tree size and galler diversity across all trees (n = 72).

Supplementary material for chapter 5

Appendix 5.1. Detailed methods

Appendix 5.2. Meteorological conditions at the site

Appendix 5.3. Additional figures and tables

Appendix 5.1. Detailed methods

Meteorological data. To describe the meteorological conditions at our study site, and to reconstruct photosynthetic and respiration rates for the whole growing season, we used hourly temperature, rainfall and irradiance data recorded in Wytham Woods (Rennie *et al.*, 2017). These measurements are taken from an area of semi-natural grassland at an altitude of 160 m., known as Upper Seeds (51° 46' 14.31" N, 1° 19' 56.53" W) –an open site with the nearest trees 100 m from the Automatic Weather Station (AWS). The AWS is to a standard specification adopted for the UK Environmental Change Network, using a system supplied by Didcot Instruments (now ELE International Ltd., Hemel Hempstead, UK) all of which are based around Campbell CR10 dataloggers recording hourly integrals (Logan, Utah, USA).

Crown and stem area. To describe the size of the studied trees, and to scale gas exchange measurements per unit area to whole tree, we measured tree height, canopy height and canopy area of 40 oaks in Wytham Woods with a clinometer (TruPulse 200™ laser hypsometer) on 5th April 2017. The oaks were chosen so that they would represent the size class distribution of the oaks in the plot, and the exact tree was chosen randomly by drawing from an existing database of all oak trees at the plot (384 trees).

To estimate stem area for each of the five intensively studied trees, we used the following equation (Whittaker & Woodwell, 1967):

$$log_{10} S = 1.2980 + 0.8259\ log_{10}\ ABW$$

(Eq. 1)

where ABW is aboveground woody biomass (in g) and S is stem area (in cm^2). This equation is derived using woody species (e.g. *Q. alba* and *Q. coccinea*) in a temperate forest. To

estimate ABW, we used data on tree dbh of the five studied oak trees measured in 2016 (Y. Malhi, unpublished analysis). We then used the following equation:

$$ABW = e^{-5.284602+(2.4682 \ln c)}$$

(Eq. 2)

where ABW is the aboveground woody biomass of oak (as kg) and c is the circumference on the tree (in cm) at breast height (Bunce 1968).

Leaf area loss. To estimate the level of insect herbivory throughout the growing season, we used earlier estimates of herbivory on the five oak trees (Visakorpi *et al.*, 2018). These estimates were acquired by collecting 15 shoots from each of the five oaks at four time points (16-28th May, 25th June, 14th July - 10th August and 18th August 2015). The leaves were pressed and scanned, and the remaining leaf area and the area lost to herbivory were estimated using ImageJ (NIH, MD, USA) software. Because most of the leaf damage occurred at the beginning of the season, and there were no seasonal trends in the amount of leaf area loss, we used an average leaf area loss value per tree. Using the same survey data, we also estimated the proportion of three different leaf types on each tree: completely intact leaves which are growing on shoots with only other intact leaves ("completely intact"), intact leaves growing on shoots with leaves damaged by herbivores on them ("systemically affected"), and leaves damaged by herbivores ("damaged").

To estimate the amount of carbon lost through the eaten leaf tissue, we used estimates for leaf mass per area ("LMA", gm^{-2}) and leaf carbon content (% of dry mass), which were measured for the same three leaf types from the same five trees (Visakorpi et al. unpublished). To calculate the leaf area loss as m^2, the percentage of leaf area loss per tree was multiplied

with the estimated leaf area of that tree. Leaf area was estimated by multiplying the full LAI of the site (6.5 m^2m^{-2}, Fenn 2010) with the estimated crown area of each tree. Crown area was estimated from a relationship between tree dbh and crown area (see above "Crown and stem area"). The estimate for leaf area loss was then multiplied with leaf mass per area, and with leaf carbon content (corrected with ratio between leaf dry mass and fresh mass from the same samples) to achieve the amount of carbon lost to herbivory due to direct leaf area loss. The measuring errors were propagated using the sampling errors in leaf area loss, LMA, leaf carbon content and the standard error of the crown area estimate (see Table S2 for sample sizes per tree).

Oak NPP. We used three methods to estimate oak NPP. First, NPP was calculated as the difference between canopy photosynthesis and autotrophic respiration (sum of leaf, stem and root respiration). We upscaled leaf-level photosynthesis and respiration measures to canopy-level values and combined these with estimates on oak stem and root respiration at the site obtained from previous studies ("*NPP through canopy upscaling*"). Second, we used census data on tree growth at the site to estimate woody growth, and allometric equations for oak to estimate leaf production and belowground production ("*NPP through tree growth census*"). Third, we used earlier estimates of oak above- and belowground NPP at the site ("*NPP through biometric measurements*") measured during 2007 and 2008 (Fenn, 2010).

1.) NPP through canopy upscaling

Daytime canopy photosynthesis and respiration. To estimate daytime canopy photosynthesis and respiration, we used light-saturated photosynthetic rates measured on the five focal study trees (Visakorpi *et al.*, 2018). In brief, we measured photosynthesis - light response curves for the five oak trees using three different leaf types (completely intact, systemically affected, damaged). The three leaf types were a result of an experimental manipulation of herbivory,

and thus represent the effect of herbivory on the physiology of a random leaf (rather than correlation between herbivore feeding preferences and photosynthetic rate). One leaf of each leaf type was measured on each tree. All measurements took place between 9am and 8pm and from 28th July to 7th August 2015.

We estimated canopy photosynthesis for each tree separately using the photosynthetic light response data and the following equation (Visakorpi *et al.*, 2018):

$$NPC = \int_{0}^{LAI} A_{sat} * \left(\frac{PAR}{K + PAR}\right) * (e^{-k*LAI}) - (0.5 - 0.05 * \ln(PAR * e^{-k*LAI})) * R_d$$

(Eq. 3)

where NPC is canopy net photosynthesis (as μmol m^{-2} s^{-1} of ground area), A_{sat} is the light-saturated photosynthetic rate, k is a light extinction coefficient, LAI is the canopy leaf area index, PAR is the light intensity at the top of the canopy and R_d is the dark respiration rate estimated from Michaelis-Menten equation. The light extinction coefficient (k) was set to 0.5 as a previously used estimate for broadleaf forests (Clark *et al.*, 2011) and PAR was set to 1000 μmol m^{-2} s^{-1} as a standard daytime light intensity at the top of the canopy. We estimated canopy net photosynthesis for each leaf type separately, i.e. for canopies consisting of only intact, damaged or systematically affected leaves. LAI for the canopy estimate based on damaged leaves was set to 6.5 as an earlier estimate of the peak LAI of the plot (Fenn 2010). LAI for the intact canopy and the canopy consisting of systemically affected leaves was set to be 6% higher reflecting the canopy-level leaf area loss (Table 5.1 in the main text, Visakorpi *et al.*, 2018). The canopy estimate based on the intact leaves only was used for the estimate of a canopy in the absence of any herbivory (hereafter "*Intact canopy*"). For estimating photosynthesis for a canopy with the observed amount of herbivory (hereafter "*Normal*

herbivory"), we multiplied the estimates with the proportion of the respective leaf type observed in the canopy, and then summed these values over the three leaf types:

$$CP_{herb} = (\sum_{t=1}^{3} \mathrm{NPC_t} * \mathrm{l_t})$$

(Eq. 4)

where CP_{herb} is canopy photosynthesis under normal herbivory, t denotes the three different leaf types (1 = completely intact, 2 = systemically affected, 3 = damaged), l_t is the proportion of leaf type t in the canopy. This estimate takes into account the different proportions of the three leaf types in the canopy, the respective leaf-level photosynthetic rates, the reduction in photosynthesising leaf area caused by herbivory, the effect of reduced light through the different canopy layers, and daytime respiration rate (as part of the gas exchange measurements).

Seasonality of photosynthesis. The canopy photosynthesis is seasonally affected by three different phenomena. First, the leaf expansion in the spring after the budburst affects the area of photosynthesising tissue. Second, the oak photosynthetic rate per unit leaf area changes throughout the season (Morecroft *et al.*, 2003). This change consists of a slow increase from budburst until late June, after which photosynthesis decreases slowly until late October, and then more rapidly until leaf fall (Appendix 5.3, Figure S2a). Third, the leaf fall in the autumn reduces the area of photosynthesising tissue.

To estimate the effect of the spring leaf expansion on canopy photosynthesis, we used measures on the sizes of expanding oak leaves in Wytham Woods during the spring of 2000 (Stokes, 2000). The leaves were measured from the moment when half of the buds had burst until full leaf expansion (from 2nd May until 14th June). We measured the proportional change

in leaf area over the time leaves were expanding and used these values to scale the leaf area index for that period.

To estimate reduction in LAI at any particular date in the autumn due to leaf fall, we used records of the proportion of oak leaves collected from leaf litter traps in Wytham Woods during autumn 2007 (Fenn, 2010). We again calculated the proportional change in leaf area over the autumn leaf fall period and used these values to scale the leaf area index for that period. Since the autumn leaf fall was estimated only from one season, we used the average error in the change of LAI during the spring to express the uncertainty in autumn LAI (Appendix 5.3, Figure S2b).

To estimate the seasonal changes in photosynthetic rate per unit leaf area, we used measurements by Morecroft *et al.* (2003) in Wytham Woods for field seasons 1996, 1999 and 2000. These data consisted of photosynthetic rates (measured at 1000 $\mu mol\, m^{-2}\, s^{-1}$ of photosynthetically active radiation, "PAR") measured at different times over the season. We set day 185 (4th July or 3rd July 2000) as the seasonal photosynthetic peak based on these earlier measurements and calculated proportional change from that peak for each day separately. We then scaled our canopy photosynthesis estimates (measured during days 209 - 219, 28th July - 7th August 2015) with the proportional change to achieve an estimate of the photosynthetic rate for each day in the season. Finally, we scaled these seasonal photosynthetic estimates with the reduced leaf area in spring and autumn. These values represent instantaneous canopy photosynthetic rates per m^2 of ground area, when the top of the canopy is exposed to maximum daylight (1000 PAR $\mu mol\, m^{-2}\, s^{-1}$).

To estimate the proportional change in photosynthesis and LAI for each day, we built a general additive model (GAM), in which the proportional change in photosynthesis due to seasonal changes and reduced LAI was modelled against the day of the year. We then used this model to predict the proportional change in photosynthesis for each day of the growing season. We built another model for the uncertainty associated with seasonal change as a

function of the day and used the fitted values for this models as errors for the daily photosynthesis values. We did not include any estimates of second leaf flush on oak (the "lammas" shoots), since this type of leaves has not been sampled before at our study site. Since the seasonal changes in photosynthesis include the effect of temperature, we did not apply any temperature corrections.

Diurnal pattern in photosynthesis. Since photosynthetic rate is strongly affected by changes in light intensity throughout the day and season, we estimated the effect of changing daylight on photosynthetic rate for each hour for each of the days during the growing season (Appendix 5.3, Figure S3a). We set the values calculated above as the photosynthetic rate (as μmol CO_2 m^{-2} s^{-1} of ground area) when the light intensity at the top of the canopy is 1000 μmol m^{-2} s^{-1} PAR. We then used hourly solar irradiation data measured 2014 (Rennie *et al.*, 2017, see above "Meteorological data"). We used data from year 2014, because the data set on 2015 was not complete for the whole growing season. The irradiance was converted into PAR by multiplying the values with 4.6 (transformation from W m^{-2} into photons μmol m^{-2} s^{-1}; Sager & McFarlane, 1997) and with 0.45 (the proportion of solar irradiance at the visible light, 400-700 nm range).

To scale the canopy photosynthesis for the hourly light data, we used data on photosynthetic light response described above (Appendix 5.3, Figure S3c). Since there were no differences in the proportional change in photosynthetic rate with decreasing light levels (Appendix 5.3, Figure S3d) between the three different leaf types, we used an average light response curve for scaling. We modelled the percentage change in photosynthesis for each observed light intensity with the Michaelis - Menten equation:

$$X_I = \frac{G_{max} I}{K + I} - R_d$$

(Eq. 5)

where X_I is the average proportional change in photosynthetic rate per light intensity I compared to photosynthetic rate at 1000 µmol m^{-2} s^{-1} of PAR (i.e. $X_{1000} = 1$), estimated from the light response curves. The maximum gross photosynthetic rate (G_{max}), the leaf respiration rate (R_d), and the light intensity at which the gross photosynthetic rate is half of its maximum (K, Marino *et al.*, 2010) are model estimated parameters. We used this model to predict values for each hourly light intensity, yielding estimates for the change in photosynthesis compared to the reference level (photosynthetic rate in 1000 µmol m^{-2} s^{-1} PAR) for each light intensity. Since there is little evidence for mid-day water stress and stomatal closure at our study site (Morecroft & Roberts, 1999), we did not include this in our estimate of the diurnal change in photosynthesis. The uncertainty in the hourly photosynthesis estimate consists of the sampling errors in the original light response curves (5 point measurement per light intensity per leaf type). Because each leaf type showed a similar proportional error at the different light intensities, we used an average error over the three different leaf types for each light intensity. The errors were extrapolated for each observed hourly light intensity by building a GAM on the proportional error as the function of light intensity and extracting the fitted values of this model. These hourly estimates for the proportional change in photosynthesis and the associated error were then multiplied with the daily canopy photosynthesis estimates. This output describes instantaneous canopy photosynthetic rate as µmol CO_2 m^{-2} s^{-1} of ground area for each hour of the day. After obtaining the canopy photosynthetic rates for each hour for each day, we calculated the summed rate for each day (as "area under curve") as µmol CO_2 m^{-2} d^{-1} of ground area. We then summed these daily values to get an estimate for CO_2 assimilated over the whole growing season. The errors in the estimates for canopy photosynthesis per tree include the variation in photosynthetic rates between the five trees, sampling errors on the proportions of the three different leaf types in the canopy, the

uncertainty on the seasonal changes in photosynthetic rate and the sampling error on the light response curves.

Night-time canopy respiration. To estimate night-time canopy respiration, we measured leaf respiration on the five oaks during two nights in July 2015 (8th - 9th July and 22nd - 23rd July). Measurements were taken after sunset and before sunrise (10pm - 2am) with an infra-red gas analyser (CIRAS-2, PPsystems, Hitchin, UK). Temperature was kept as ambient, flowrate at 200 ml min^{-1}, and CO_2 concentration at 400 ppm. From each tree, we measured one completely intact leaf, one leaf damaged by herbivores and one intact leaf growing next to the damaged leaves, using the same experimental setup as for the photosynthetic measurements (Visakorpi *et al.*, 2018). Since nightly respiration rates are usually very low, and therefore affected by possible leaks in the measuring chamber of the gas analyser, we measured a piece of paper at the beginning and at the end of each measuring night. If the rate measured from the paper was different from zero, this value was subtracted from the leaf respiration rates. All positive respiration values (apparent net CO_2 uptake) were discarded as measurement errors. Because the respiration rates did not show any trends throughout the night (Appendix 5.3, Figure S4a), and because previous studies (Stokes, 2002) have shown that the respiration rate does not change throughout the night, we used mean respiration values per leaf type per tree for upscaling canopy respiration.

To estimate respiration under normal level of herbivory, the rates per unit leaf area were scaled with the proportion of the different leaf types in the canopy using the following equation (Visakorpi *et al.*, 2018):

$$CR_{herb} = \sum_{t=1}^{3} R_t * (1 - D_t) * l_t$$

(Eq. 6)

where CR_{herb} is canopy respiration under herbivory, t denotes the three different leaf types (1 = completely intact, 2 = systemically affected, 3 = damaged), D_t is the proportion of leaf area loss per leaf type, R_t is the mean night-time respiration rate of that leaf type as µmol CO_2 m^{-2} d^{-1} s^{-1}, and l_t is the proportion of leaf type t in the canopy. The output is instantaneous canopy respiration as µmol CO_2 m^{-2} s^{-1} of leaf area at the top of the canopy. For an estimate of respiration for a completely intact canopy, we used the average value measured for the completely intact leaves.

Because night respiration often follows the total photosynthetic rate of the day before (Whitehead *et al.*, 2004), we scaled the night-time respiration rates with a previously identified relationship between daytime canopy photosynthesis and respiration the following night (Whitehead *et al.*, 2004):

$$R_t = 0.074\, A_t + 0.03$$

(Eq. 7)

where R_t (mol CO2 m^{-2} d^{-1}) is total night time canopy respiration and A_t (mol CO_2 m^{-2} d^{-1}) is total daytime canopy photosynthesis. We estimated R_t for each night based on the canopy photosynthesis of the previous day. These values were turned into proportional change in respiration from the reference night when the respiration measurements were taken (8^{th} - 9^{th} July and 22^{nd} - 23^{rd} July). The canopy respiration rates estimated above (CR) were then scaled with this estimated proportional change over the season.

To take into account the change in respiration rate though the different canopy layers, we used formula by (Griffin *et al.*, 2001), who studied the change in nightly respiration rates through different canopy layers on red beech (*Nothofagus fusca*, Hook. F.). We calculated respiration rate per unit leaf area for each one meter layer in the canopy, using the average canopy height for oaks measured at our study site (7.8 m, see above "Crown and stem area") and the following equation (Appendix 5.3, Figure S4b):

$$CR_L = -0.0028 * height + CR$$

(Eq. 8)

where CR_L is canopy respiration per canopy layer L, height is the height of the layer as meters from the top of the canopy and CR is the canopy respiration measured at the top of the canopy (μmol CO_2 m^{-2} d^{-1} s^{-1}). We divided each value with the average canopy height and summed these estimates together to yield an estimate of the average respiration rate per unit leaf area of the whole canopy.

Since variation in respiration rate is mainly controlled by temperature (Ryan, 1991; Tjoelker *et al.*, 2001), we scaled the canopy respiration rates with the ambient air temperatures measured in Wytham Woods throughout the season of 2015 (Appendix 5.2, Figure S1a and Appendix 5.3, Figure S4c) with the following equation (Bolstad *et al.*, 2003),

$$R_T = R_{Tref} * Q_{10}^{\frac{(T-T_{ref})}{10}}$$

(Eq. 9)

where R_T is respiration rate at a given temperature T (ºC), R_{Tref} is the respiration rate at reference temperature T_{ref}, and Q_{10} is a coefficient describing ratio of respiration measured over a 10-degree span. As a reference temperature for the intact canopy, we used the average measuring temperature of the completely intact leaf on each tree. As a reference temperature for the canopy under normal levels of herbivory, we weighted the measuring temperatures of the three leaf types with their average proportion in the canopy and used the summed temperature as the reference temperature. Night-time hours were defined as those when light level was zero.

Since the temperature response of respiration differs between different canopy layers, we applied temperature correction for three canopy layers separately (top of the canopy 0-2 m, middle of the canopy 3-5 m and bottom of the canopy 6-8 m). We used average Q_{10} values per canopy layer measured for oaks *Q. rubra* and *Q. prinus* (bottom layer $Q_{10} = 2.15 \pm 0.12$, middle layer $Q_{10} = 1.89 \pm 0.1$, top layer $Q_{10} = 1.87 \pm 0.19$; Turnbull *et al.*, 2003), Appendix 5.3, Figure S4d), divided each estimate by three and summed these proportional estimates. This gave us respiration estimates for each hour for each night during the growing season for the whole canopy. We then summed the hourly estimates for each night (as area under curve) to achieve the respiration per night as µmol $CO_2\, m^{-2}\, d^{-1}$ of leaf area. Finally, this rate was multiplied with the LAI to achieve µmol $CO_2\, m^{-2}\, d^{-1}$ of ground area and scaled with the reduced LAI in the autumn and in the spring. The values for each night were summed to achieve the yearly CO_2 loss to night-time respiration per m^2 of ground area. Errors for the final canopy respiration estimates were propagated by using variation in respiration rate over the five trees (standard error of the mean), in the scaling function from daytime photosynthesis to night-time respiration (estimated 15% based on the original data, Whitehead et al. 2004) and in the change of LAI in the spring and autumn.

Stem and root respiration. To estimate stem respiration for each of the five oaks, we used the average stem respiration rate measured in Wytham Woods during the summer 2017 from 25 mature oak trees at 1.3m (Walker, 2017). Stem respiration was corrected for hourly temperature of the whole year, both day and night with Equation 9 (see "Leaf respiration" above, Appendix 5.3, Figure 5a). We assumed a Q_{10} value of 2 (describing change in rate by 2 units for every 10 degrees), which is commonly used to describe the temperature response of stem respiration of temperate deciduous trees (e.g. $Q_{10} = 1.7$ for beech; Damesin *et al.*, 2002) $Q_{10} = 2.3$ for *Q. rubra*; Bolstad *et al.*, 2004). Since the differences between stem and air temperatures were small (Appendix 5.3, Figure S5b), we assumed stem temperature to equal

air temperature. From the hourly data, we estimated carbon loss per day per unit stem area and scaled the daily rates for the stem area of each of the measured trees (Appendix 5.3, Figure S5c). Previous measurements at the site have revealed that stem respiration measured at 1.3m and scaled for the whole stem tends to underestimate the respiration rate of the whole tree by on average 12% compared to measurements taken at different heights on the trunk (Walker, 2017). Thus, we multiplied our per-stem respiration rates with 1.12 to correct for this. The temperature corrected rates per day were then summed to achieve the amount of CO_2 respired by each stem in 24h over the season. Since stem respiration shows a positive relationship with leaf photosynthesis similar to leaf respiration (Wertin & Teskey, 2008), we assumed that increased photosynthesis in the absence of herbivores would also increase stem respiration rates. To model this, we calculated the ratio between leaf respiration between intact and normal canopies and used this to scale respiration measures for the intact canopy (Appendix 5.3, Table S3). The errors associated with the stem respiration estimates consist of the sampling error in stem respiration rates (n = 25), standard error over the five stem area estimates, and the error scaling from normal rate to rate of an intact tree (Appendix 5.3, Table S3).

To estimate root respiration, we calculated the ratio between stem and root respiration based on earlier measurements at the plot (Fenn *et al.*, 2015). This ratio (root respiration 13.0 ± 4.0 % of stem respiration) was used to scale the estimated stem respiration values into root respiration values. The errors here include the error associated with stem respiration estimate and the uncertainty in scaling from stem respiration to root respiration.

Combining stem, root and canopy estimates. To combine estimates of tree-level stem and root respiration with the canopy gas exchange, we multiplied the canopy photosynthesis and respiration estimates (expressed as m^2 of ground area under a canopy of 6.5 LAI) with the average oak crown area (see above "Crown and stem area"). We then combined the estimates

of stem respiration (per stem), root respiration (per tree) and leaf photosynthesis and respiration (per tree crown). Finally, the amount of CO_2 photosynthesised or respired were transformed into grams of carbon by multiplying the rate as moles with the molar mass of CO_2 (44.01) and with the ratio of carbon molar mass to CO_2 molar mass (0.27). To estimate values per ha, we used two different methods. Firstly, to estimate NPP of a forest, which was assumed to consist of oak trees only (although in reality the site is a mixed species forest), we calculated the rates of photosynthesis and respiration per m^2 of oak basal are and multiplied these values with the total basal area of the plot (33.3 m^2 ha^{-1}, Table 5.1 in the main text). To estimate oak NPP per hectare using the actual density of the oak trees within the plot (20% of the total plot basal area, Table 5.1 in the main text), we multiplied the values per m^2 of basal area with the oak basal area of the plot.

2.) NPP through tree growth census

To compare the estimate of oak NPP derived through canopy upscaling with NPP estimates obtained through other methods, we used data on tree dbh measures on all oak trees (355 stems) within the 18-ha plot (see "Study site" in the main text) collected in 2010 and 2016 (Y. Malhi, unpublished analysis). We then used Equation 2 to estimate the aboveground oak woody biomass for both censuses, converted biomass into carbon by multiplying with the carbon content of oak wood (47% ± 1%; Butt *et al.*, 2009) and used the change in above-ground woody carbon stock between 2010 and 2016 as an estimate of woody NPP. We then used oak-specific allometric relationships measured previously at the plot to estimate oak canopy NPP and belowground NPP (Fenn, 2010; Appendix 5.3, Table S3). To estimate the effect of herbivory on oak NPP, we estimated NPP in the absence of herbivory assuming that the total NPP was reduced to the same extent (ca. 55%, Appendix 5.3, Table S3) as canopy gas exchange in the canopy upscaling calculations described above. To present the values as per hectare in a plot assumed to comprise only of oak trees, we divided the mean NPP per tree

with the mean oak basal area and multiplied this values with the total basal area of the plot (33.3 m^2 ha^{-1}). To estimate oak NPP per ha of the actual study site, we summed NPP estimates of each tree in the study area and divided this with the size of the surveyed area (18 ha). Trees with extreme negative growth rates (>-2cm per year, n = 3) were removed as measurement errors. The final errors represent sampling error in tree circumference change (n = 355), the error in the oak wood C content and the uncertainties in the relationship between aboveground to belowground NPP, wood NPP to leaf NPP and intact to normal canopy gas exchange (Appendix 5.3, Table S3).

3.) NPP through earlier biometric measurements

To obtain a third estimate on the effect of herbivory on oak NPP, we used an earlier estimate for oak NPP at the plot based on biometric measures (Fenn, 2010). These measures include aboveground oak NPP for an area of one hectare within the 18-ha plot, oak-specific ratio of wood NPP to leaf NPP and oak-specific ratio of aboveground biomass to belowground biomass (see Appendix 5.3, Table S3). We combined these relationships to obtain an estimate of oak NPP as Mg C yr^{-1} per ha of the real plot area. We then divided this estimate with the oak basal area on the plot and multiplied that value with the basal area of the five studied oaks to achieve an estimate for each tree. To estimate the effect of herbivory on oak NPP, we again assumed that the total NPP was reduced to the same extent as canopy gas exchange in the canopy upscaling calculations above. To scale the values for plot level assuming the plot only has oak trees, we again divided the original estimate (per ha of the real plot) with the average oak basal area on that study site, and then multiplied this values with the full basal area of the site. The error here represents the propagated uncertainties with the original oak NPP estimate, the ratio of aboveground to belowground NPP, wood NPP to leaf NPP and intact to normal canopy gas exchange (Appendix 5.3, Table S3).

Error propagation

Errors were propagated using standard error propagation rules: when summing values that each had an associated error, the errors were squared and summed, and from the resulting sum we took a square root. When values with errors were multiplied or divided, the proportional errors were summed, squared, and from the resulting sum we took a square root.

All analyses were carried out using the latest version of R 3.5.0, (R Core Team, 2018) and packages geosphere (for daylength estimation; Hijmans, 2017), MESS (for calculating area under curve, Ekstrøm, 2018), and mgcv (for GAM models; Wood, 2011).

Appendix 5.2: Meteorological conditions at the site

The average temperature over the growing season in Wytham Woods in 2015 was 13.8 ºC during the day and 10.7 ºC during the night (Figure S1a). The highest daytime temperature occurred 1st July (32 ºC) and warmest night-time temperature was measured on 22nd August (23 ºC). Rainfall was evenly distributed over the summer with no indication of longer periods of drought (Figure S1c). The daily amount of solar radiation (Figure S1b) was mainly affected by the length of the day (Figure S3b). The mean annual temperature 2015 (10.2 ℃) was similar to previous years (1993–2011 mean of 10.0 ± 0.1 ℃, Fenn et al. 2015). The annual rainfall (637 mm) was slightly less than the 1993–2010 average of 714 mm (Fenn et al. 2015). We estimated LAI to increase from budburst until 14th June and start decreasing from 3rd September until 12th December (Appendix 5.3, Table 2S, Figure S2b).

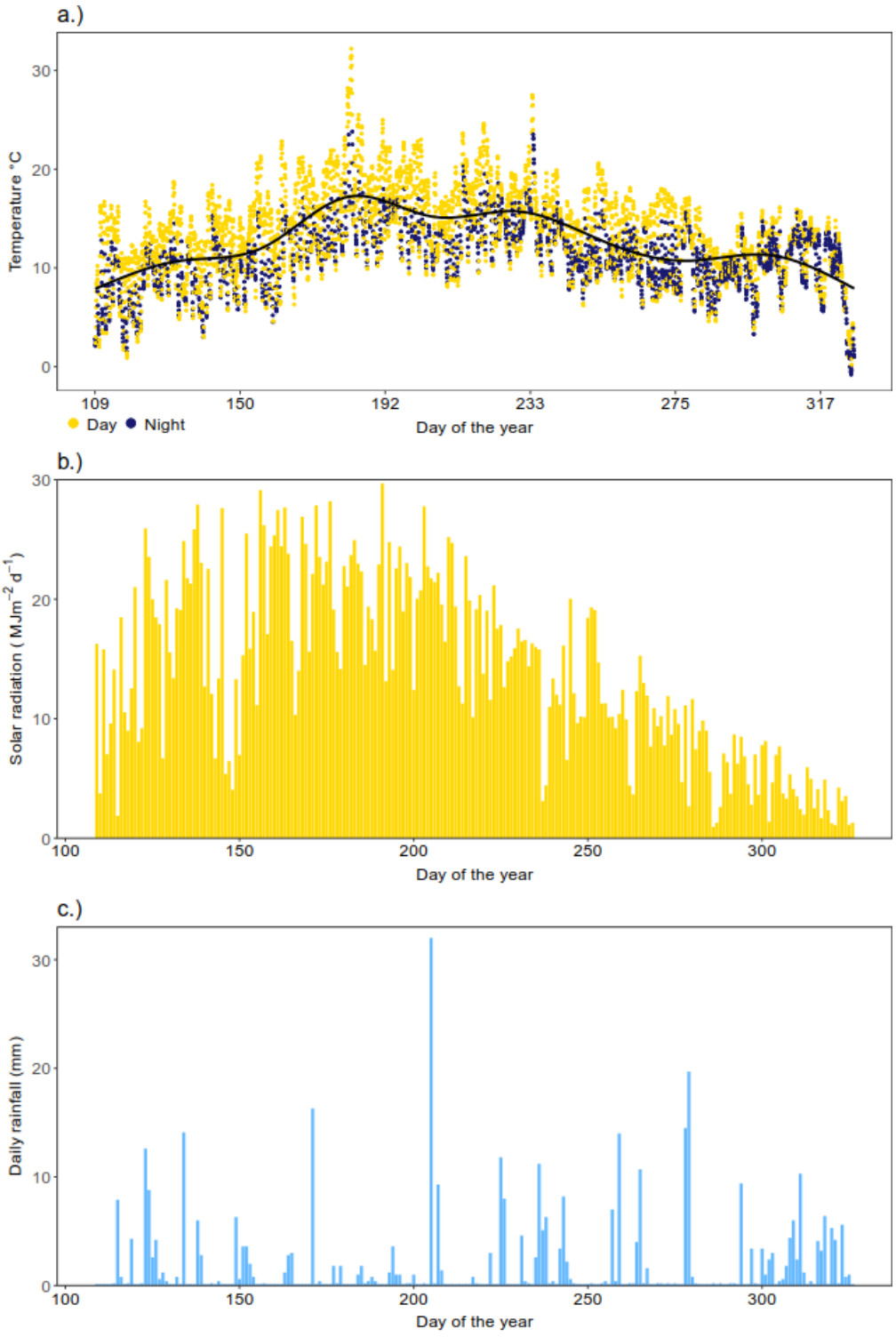


Figure S1. Meteorological data from Wytham Woods. a) Hourly temperature in Wytham Woods for the growing season 2015, which were used for upscaling all respiration rates. Yellow dots show daytime temperatures and dark blue dots night-time temperatures. b) Solar radiation per day over the season 2014, which was used to upscale the canopy photosynthesis measurements. Solar radiation data were not available for the growing season 2015. c) Amount of rainfall per day over the growing season 2015.

Appendix 5.3: Additional figures and tables

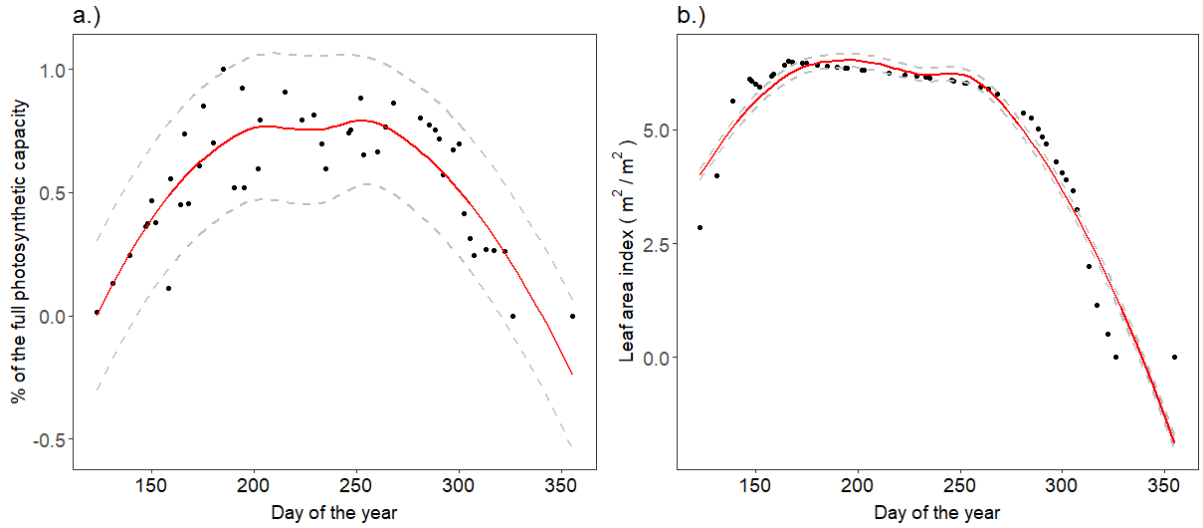


Figure S2. Model assumptions on the seasonal change in photosynthesis and leaf are index: a) seasonal change in photosynthetic rate per unit leaf area over the season (Morecroft *et al.*, 2003), representing measurements over three years (1996, 1999, 2000) and b) the change in the total LAI in Wytham Woods, assuming the seasonality of oak. The proportional change from full LAI (6.5) is based on changes in leaf size during spring (Stokes, 2000) and on rates of litter fall in the autumn (Fenn, 2010). The dashed grey lines represent ± 1 SEM.

Table S1. The change in leaf size in the spring, the change in leaf cover in the autumn, and the corresponding leaf area index (LAI, m^2/m^2). Leaf lengths are averages over sun and shade leaves and errors are ± 1 SEM (Stokes, 2000). Leaf cover has been estimated from litter fall traps (Fenn, 2010). LAI-values are estimated based on leaf size during the spring and leaf cover percentage compared to the full LAI 6.5 (Fenn, 2010).

Date	Julian date	Average leaf length (mm)	% leaf size	% leaf cover of the peak	LAI, m^2/ m^2
2nd May	123	33 ± 1	44.0 ± 1	-	2.86 ± 0.03
10th May	131	46 ± 4	61.3 ± 5	-	3.99 ± 0.20
18th May	139	65 ± 0	86.7 ± 0	-	5.63 ± 0
26th May	147	71 ± 1.5	94.0 ± 2	-	6.11 ± 0.12
31st May	152	69 ± 6.5	91.3 ± 9	-	5.94 ± 0.53
14th June	166	75 ± 8	100	-	6.50 ± 0
3rd Sept	246	-	-	93.6	6.08 ± 0.15
21st Sept	264	-	-	90.8	5.90 ± 0.15
12th Oct	285	-	-	80.8	5.25 ± 9.15
1st Nov	305	-	-	56.2	3.66 ± 0.15
13 Nov	317	-	-	17.8	1.16 ± 0.15
22nd Nov	326	-	-	0	0.00 ± 0.15
12th Dec	355	-	-	0	0.00 ± 0.15

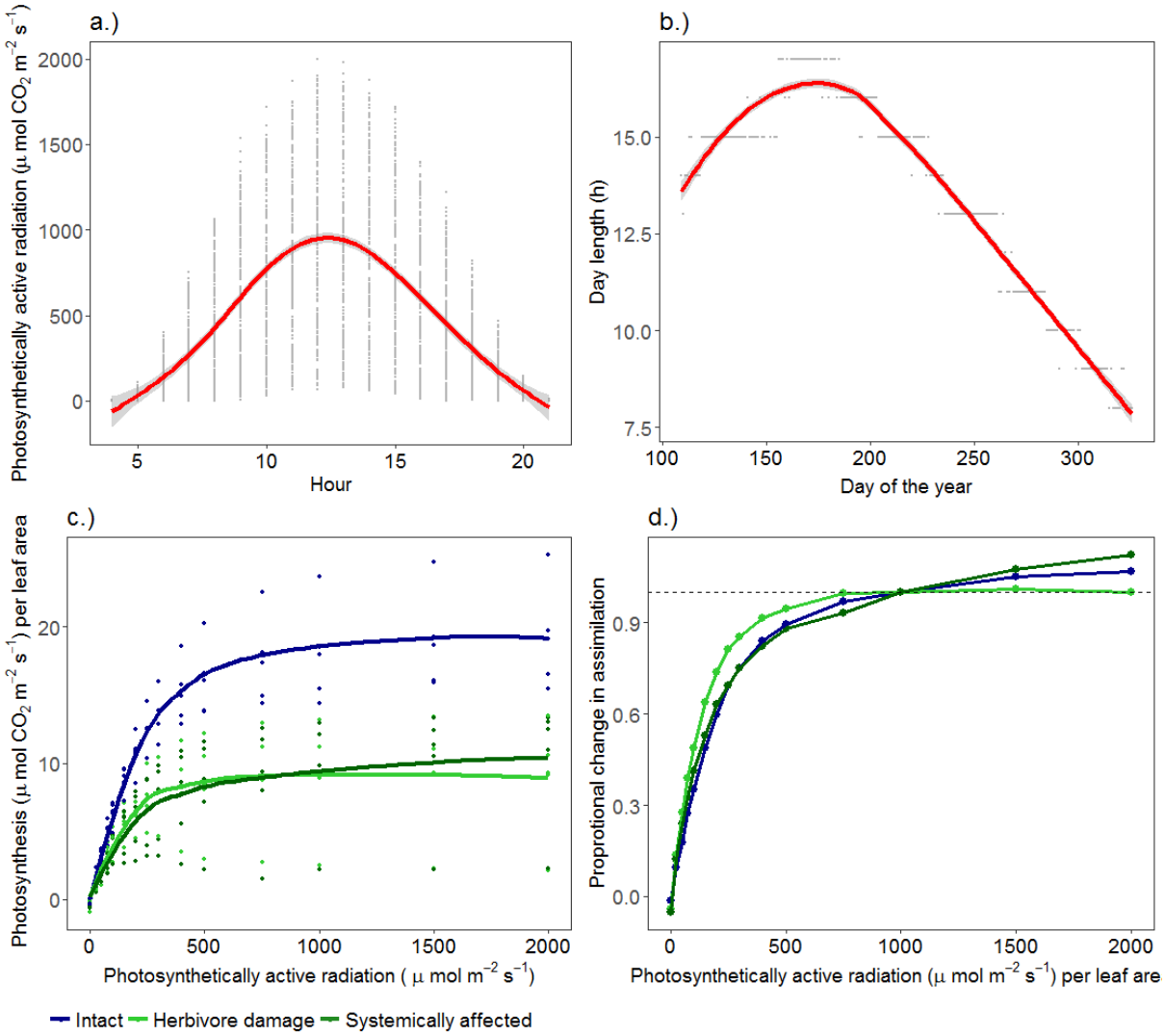


Figure S3. The underlying data and assumptions for the model on the canopy photosynthesis. a) The diurnal change in solar radiation for each day during the growing season (Rennie *et al.*, 2017). The hourly measurements are shown as grey dots, and the red line represents a smoothed average. b) The length of the day (h) over the growing season. c) The photosynthetic response of oak leaves to light (Visakorpi *et al.*, 2018) for each of the three leaf types separately. d) The proportional change in photosynthesis when compared to the photosynthetic rate at light intensity 1000 µmol m^{-2} s^{-1} of photosynthetically active radiation. The dashed line shows the rate at light intensity 1000 µmol m^{-2} s^{-1} PAR, which was se as the level for 100% photosynthesis.

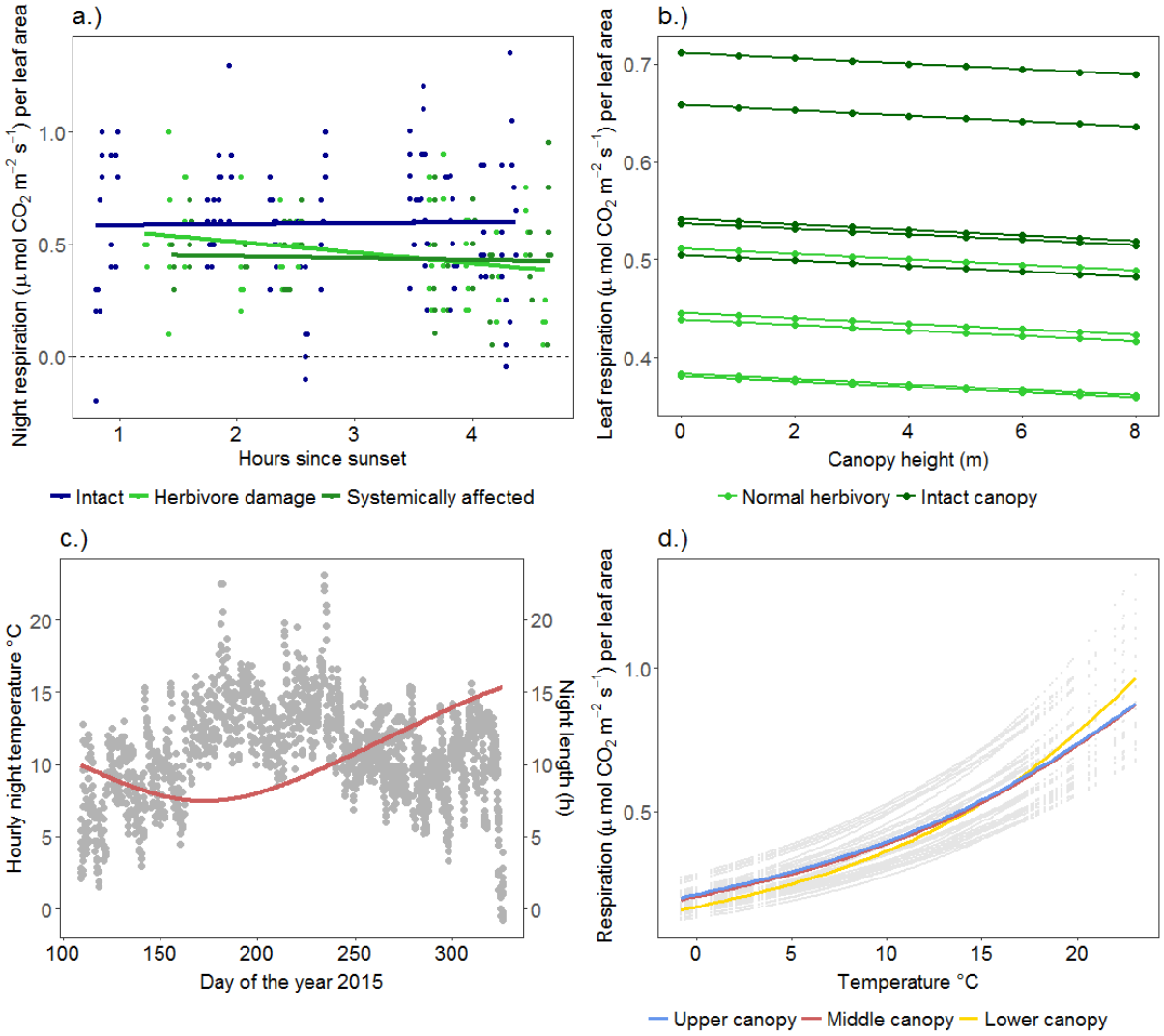


Figure S4. The underlying data and assumptions for the model on nightly canopy respiration. a) The measurements of night-time leaf respiration rates during summer 2015 at the upper canopy leaves in Wytham Woods. Data have been cleaned for values < 0. b) The relationship between canopy height (0 indicates top of the canopy) and leaf respiration rate (per unit leaf area) for the five trees under the two studied scenarios (intact canopy or normal herbivory) separately. c) Hourly night temperatures during the summer 2015 in Wytham Woods (grey dots), and the length of the night in hours (red line). d) The response of leaf respiration rate to temperature for the three canopy layers separately. Grey dots represent leaf respiration rates scaled for the hourly temperature data, and the colourful lines show smoothed average per canopy layer. Note that the values have not been corrected to daytime canopy photosynthesis to make the relationship between temperature and respiration clearer.

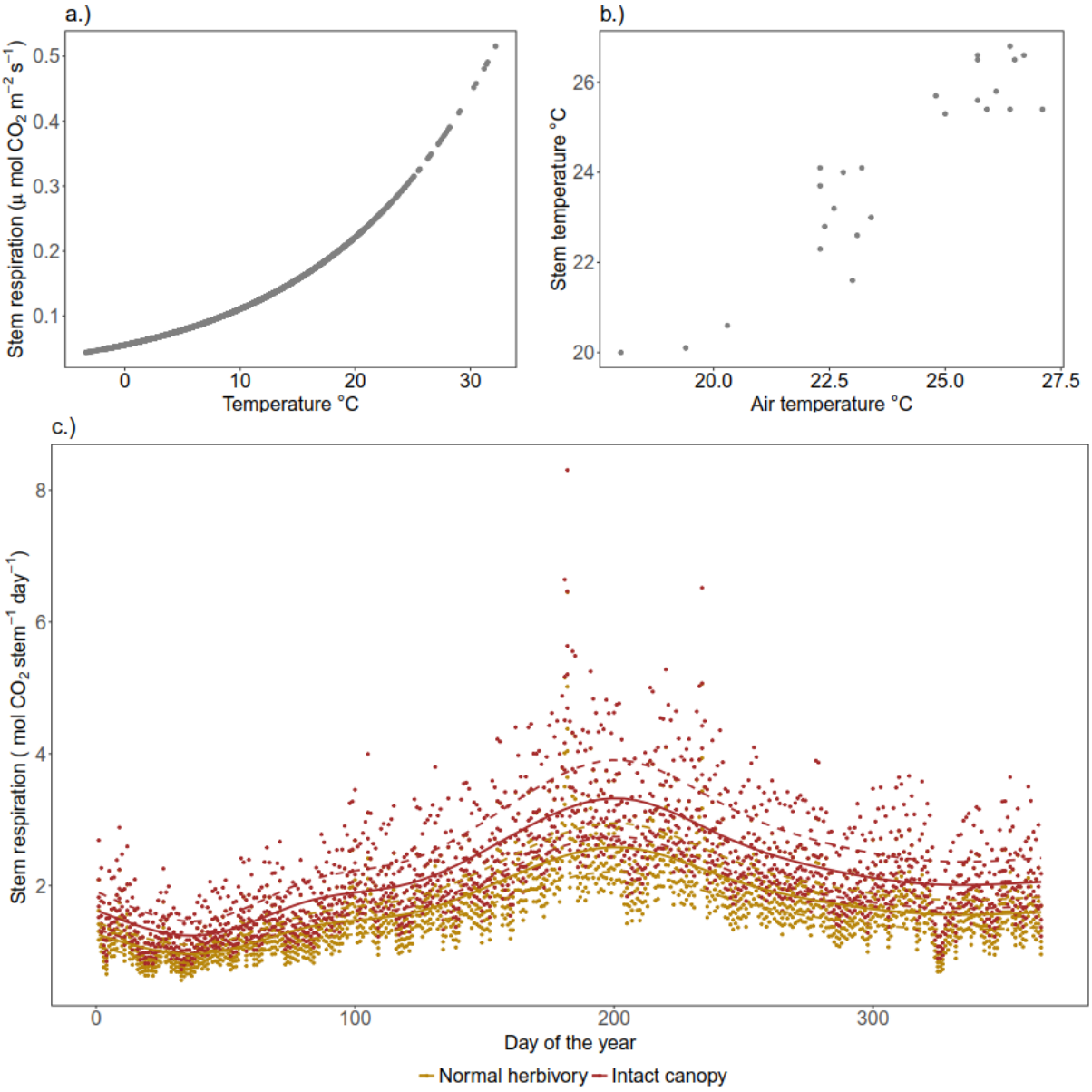


Figure S5. The model assumptions for stem respiration measurements. a) The response of stem respiration rate to temperature, based Equation 9 in Appendix 5.1 (used for scaling both the leaf and stem respiration rates to hourly temperature). b) The relationship between stem temperature and ambient temperature (Walker, 2017). c) The stem respiration rate throughout the whole year 2015 for stems of intact trees and of trees experiencing normal level of herbivory. The dashed lines are ± 1 SEM derived through error propagation.

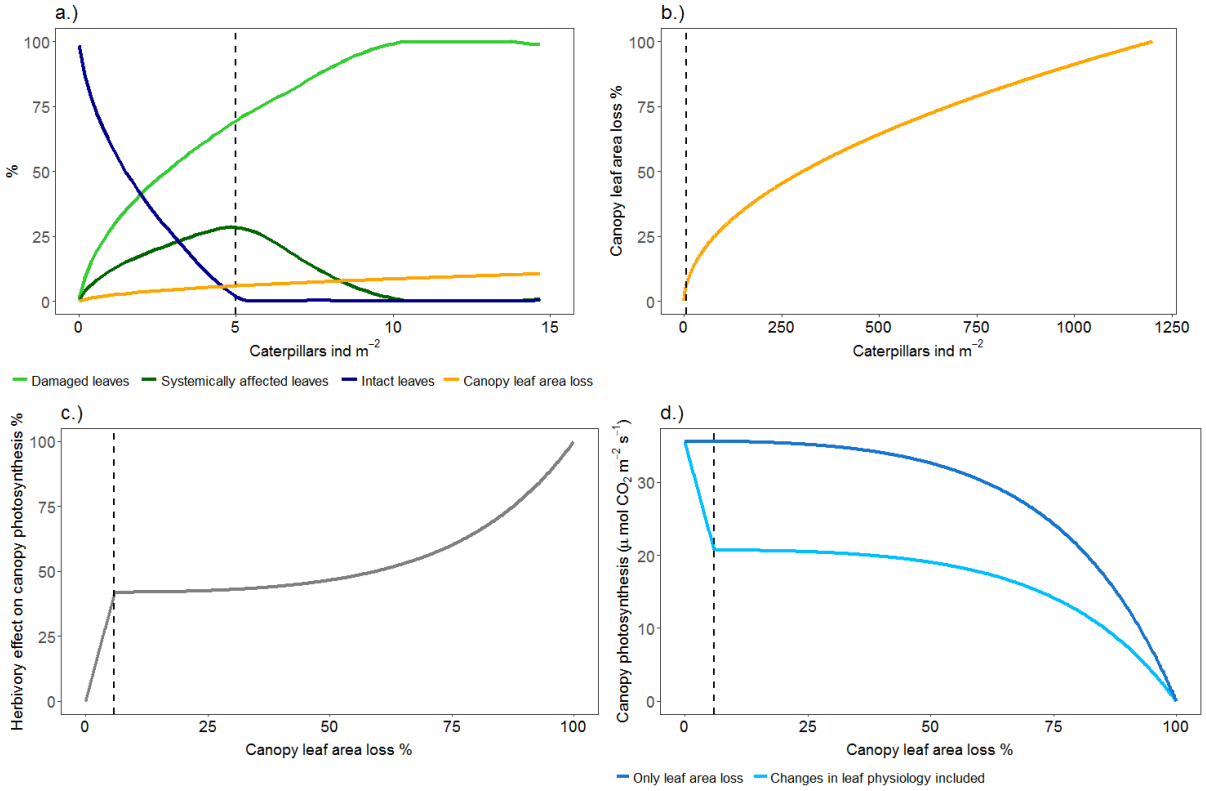


Figure S6. Simulated proportions of the three different leaf types (intact, systemically affected and damaged) and leaf area loss in the canopy with increasing winter moth caterpillar density and the effect of increasing herbivory on canopy photosynthesis. The dashed line shows the point of 5.92% leaf area loss which represents our sampling year (2015). a) Caterpillar density up to 15 individuals m^{-2} and the proportion of the three different leaf types (intact, systemically affected, damaged) and leaf area loss. b) The relationship between caterpillar density and leaf area loss until complete defoliation. c) The effect of increasing herbivory on canopy photosynthesis (% of reduction from the full potential rate), and d) canopy photosynthesis with increasing leaf area loss in two scenarios: when only the effect of leaf area loss is taken into account, and when the herbivore-induced changes in leaf physiology are included in the canopy photosynthesis estimate.

Table S2. Leaf area loss to herbivory, mean leaf mass per area (LMA) and leaf carbon content in the five studied oak trees. The errors are ± 1 SEM representing the error over several leaf samples per tree. N is sample size per tree

Oak ID	% of herbivory	n	Mean LMA (gm^{-2})	n	Mean C (%) of leaf	n
F	4.58 ± 0.8	164	58.1 ± 4.5	11	44.6 ± 1.2	4
G	7.02 ± 0.8	166	4. 8± 2.8	43	50.9 ± 1.4	3
H	7.54 ± 0.9	199	73.0 ± 1.5	41	50.6 ± 1.0	4
I	4.84 ± 0.8	191	63.6 ± 1.4	100	46.3 ± 1.0	6
J	5.34 ± 0.9	126	60.1 ± 1.5	40	50.2	1

Table S3. Allometric relationships on oak at the study site, used for estimating total oak NPP: NPP of leaves and wood, above-ground NPP (ANPP) as their sum, the proportional allocation of ANPP into wood production, ratio of below-ground and above-ground NPP and ratios between leaf and wood production, intact to normal canopy gas exchange and respiration and root to stem respiration.

Measure	Estimate	SEM	Reference
Oak leaf NPP, Mg C ha^{-1} yr^{-1}	0.25	0.14	Fenn 2010
Oak wood NPP, Mg C ha^{-1} yr^{-1}	0.3	0.07	Fenn 2010
Oak ANPP, Mg C ha^{-1} yr^{-1}	0.55	0.1	Fenn 2010
Allocation of NPP into wood	55%	20%	Fenn 2010
Ratio of leaf to wood production	0.85	0.31	Fenn 2010
NPP below/NPP above	0.59	0.18	Fenn 2010
Ratio of intact to normal canopy gas exchange	2.25	0.22	This study
Ratio of intact to normal respiration	1.28	0.13	This study
Ratio of root respiration to stem respiration	0.13	0.04	Fenn *et al.* 2015

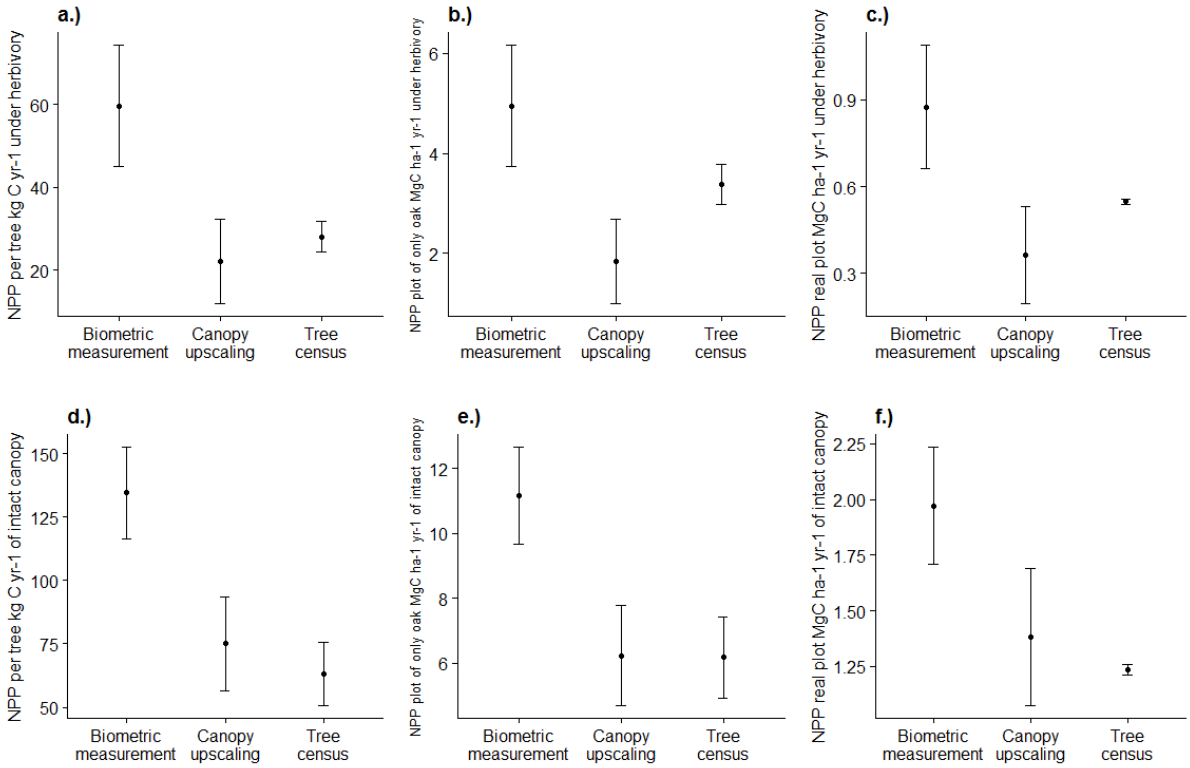


Figure S7. The oak NPP under observed level of herbivory (panels a, b, c) and of an intact canopy (panel d, e, f) estimated with three different methods. Panels a and d show NPP per tree, panels b and e show NPP per ha of a plot of only oak trees, and panels c and f show oak NPP per ha at our study site.

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