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Research article

Effects of experimental warming at the microhabitat scale on oak leaf traits and insect herbivory across a contrasting environmental gradient

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Forest microclimatic variation can result in substantial temperature differences at local scales with concomitant impacts on plant defences and herbivory. Such microclimatic effects, however, may differ across abiotically contrasting sites depending on background environmental differences. To test these cross-scale effects shaping species ecological and evolutionary responses, we experimentally tested the effects of above-ground microhabitat warming on insect leaf herbivory and leaf defences (toughness, phenolic compounds) for saplings of sessile oak *Quercus petraea* across two abiotically contrasting sites spanning 9° latitude. We found higher levels of herbivory at the low-latitude site, but leaf traits showed mixed patterns across sites. Toughness and condensed tannins were higher at the high-latitude site, whereas hydrolysable tannins and hydroxycinnamic acids were higher at the low-latitude site. At the microhabitat scale, experimental warming increased herbivory, but did not affect any of the measured leaf traits. Condensed tannins were negatively correlated with herbivory, suggesting that they drive variation in leaf damage at both scales. Moreover, the effects of microhabitat warming on herbivory and leaf traits were consistent across sites, i.e. effects at the microhabitat scale play out similarly despite variation in factors acting at broader scales. These findings together suggest that herbivory responds to both microhabitat (warming) and broad-scale environmental factors, whereas leaf traits appear to respond more to environmental factors operating at broad scales (e.g. macroclimatic factors) than to warming at the microhabitat scale. In turn, leaf secondary chemistry (tannins) appears to drive both broad-scale and microhabitat-scale variation in herbivory. Further studies

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are needed using reciprocal transplants with more populations across a greater number of sites to tease apart plant plasticity from genetic differences contributing to leaf trait and associated herbivory responses across scales and, in doing so, better understand the potential for dynamics such as local adaptation and range expansion or contraction under shifting climatic regimes.

Keywords: latitudinal variation, microclimate, phenolic compounds, plant–herbivore interactions, *Quercus petraea*, saplings

Introduction

A fundamental goal of global change research is to understand how rising temperatures will affect species interactions which ecological communities and ecosystem functioning depend upon (Parmesan 2006, Kerr et al. 2015). While the threats of climate change are well recognized, previous research has demonstrated that some ecological communities are responding slower than expected to abiotic changes (Bertrand et al. 2011, Devictor et al. 2012, Dullinger et al. 2012, De Frenne et al. 2013). Accordingly, the effects of global change drivers operating at broad scales can sometimes be difficult to predict at local scales given biotic and abiotic heterogeneity commonly found at these finer scales (De Frenne et al. 2021). For example, some authors have argued that local, microhabitat-level abiotic heterogeneity can buffer changes in environmental drivers such as radiation, air mixing, evapotranspiration, local topography and soil properties, which would otherwise have more homogeneous effects across the landscape (Chen et al. 2018, Zellweger et al. 2020, De Frenne et al. 2021).

Herbivory is strongly shaped by variation in the abiotic environment acting at different spatial scales (Karban 1992, Hunter 2016, Moreira and Abdala-Roberts 2020). For instance, herbivory rates at broad spatial scales often correlate with macroclimatic factors such as mean annual temperature and precipitation, as shown repeatedly by studies on latitudinal gradients in species interactions (Schemske et al. 2009, Anstett et al. 2016). Intervening factors associated with climatic variation which are proposed to explain such patterns include higher productivity, longer growing seasons, and more stable species populations favouring higher herbivore pressure and plant defences at lower latitudes (Rasmann and Agrawal 2011, Salazar and Marquis 2012, Moreira et al. 2014, Sanczuk et al. 2021). While predictive of herbivory, these plant traits have also evolved (in some cases perhaps mainly) in response to abiotic forcing (e.g. some types of secondary metabolites, pubescence, etc.), with this in turn driving lower herbivory at high latitudes, i.e. abiotic factors shaping plant traits, and herbivory following the latter (Moreira et al. 2018a). Abiotic variation at small scales, on the other hand, is also highly important in shaping herbivory patterns via multiple pathways. For instance, changes in air temperature at the microhabitat scale have been shown to increase insect metabolic rates, resulting in higher herbivory rates (Lemoine et al. 2013, Zhang et al. 2020) and increased plant defence induction (Karban 2011). Likewise, microhabitat warming can also increase the expression of constitutive levels of secondary metabolites such as phenolic compounds

(reviewed by Holopainen et al. 2018), in turn leading to reduced herbivory (Lemoine et al. 2013). Together, these findings highlight different ecological mechanisms jointly affecting plant–herbivore interactions.

Abiotic effects acting at different spatial scales can also influence each other and further result in cross-scale dependencies (De Frenne et al. 2021). For example, effects of microclimatic variation on plant traits predictive of herbivory might vary across macro-climatically contrasting sites. One potential mechanism could be that trait plasticity is more limited in warmer compared to colder sites because species in warm climates have evolved under a narrower thermal gradient (Gugger et al. 2015, Schmid et al. 2017). In another example, herbivore responses to microhabitat-level climatic variation may vary across broad-scale geographical gradients as in the case of insect herbivores from low latitudes being physiologically less tolerant to warming than those found at higher latitudes (Deutsch et al. 2008). Unfortunately, to date, studies testing for local-scale (e.g. microhabitat-scale) abiotic forcing have been usually performed without considering broad spatial extents (Jamieson et al. 2015, Orians et al. 2019, Moreira et al. 2020a), whereas broader-scale studies (e.g. latitudinal or elevational gradients) have usually ignored microhabitat variability (Anstett et al. 2015, Moreira et al. 2018a). Consequently, our current understanding of commonalities or differences in the mechanisms that shape herbivory at different spatial scales and, especially, cross-scale dependencies, remain quite limited.

We conducted a manipulative field study testing for the effects of experimental warming at the microhabitat-scale on insect leaf herbivory and leaf traits associated with herbivory across two sites with contrasting (e.g. macroclimatic) conditions using sessile oak *Quercus petraea* as model species, a common tree species in many western European broadleaf forests (Supporting information). Specifically, we established two field sites spanning two thirds of this species' latitudinal range (1.3-fold difference in mean temperature from 11.5 to 15.1°C, and two-fold seasonality in precipitation from 809 to 1785 mm), one in Spain (42°N) and one in Belgium (51°N), using three-year-old saplings local to each site. We imposed microhabitat-level warming for half of the saplings at each site using infrared heater arrays. After a full growing season (March–September 2022), we collected leaves to measure insect leaf herbivory and to quantify physical (specific leaf area, correlated with leaf thickness or toughness) and chemical (phenolic compounds) traits known to affect herbivory in oak species. Specifically, we asked: 1) do defensive traits and herbivory in leaves vary in response to warming at the microhabitat scale as well as across sites, the latter

pointing at the influence of broad-scale environmental (e.g. macroclimatic) factors? 2) Do the effects of microhabitat warming vary across sites, therefore providing evidence of an interaction or dependency across scales? And 3) are effects of broad-scale environmental differences, microhabitat-level warming, and any such interactive effect across scales on herbivory related to concomitant variation in oak leaf traits? We hypothesize that if herbivory and leaf defence patterns are related, then effects on each will be interdependent. For example, microhabitat-level warming and warmer conditions at the southern site could lead to elevated defences which then drive lower herbivory, i.e. a bottom-up mechanism. Alternatively, a top-down mechanism could also take place with warming increasing damage and in turn defence levels. It is also possible that broad-scale forcing and microhabitat-level warming lead to independent effects on herbivory and/or defences (responses are uncoupled if traits do not influence damage or vice versa). In addition, we expected interactive effects between micro- and macro-climatic conditions whereby, for example, effects of microhabitat-level warming will be stronger at the most northern site which has the coldest climate because experimental heating would drive the greatest increase in temperature relative to background weather conditions (e.g. reducing within-day temperature fluctuations and buffering against low temperatures at night). Overall, this study provides a unique test of how local warming might interact with regional differences among sites to determine plant traits and patterns of herbivory.

Material and methods

Experimental design, sampling and measurements

The study sites were nearby the town of Pontevedra (northern Spain, 42°40'N, 08°64'W, 39 m a.s.l.) and the other near Ghent (central Belgium, 50°97'N, 03°80'E, 48 m a.s.l.), hereafter low and high-latitude sites, respectively. At each site, we selected an open stand close to an oak-dominated forest. The study sites spanned close to two thirds of the latitudinal range of *Quercus petraea*. Specifically, across sites, oak trees and insect herbivores experience a difference of ca 4°C in mean annual temperature (11.5°C at the high-latitude site while 15.1°C at the low-latitude site) and ca 1000 mm in annual precipitation (809 mm at the high-latitude site and 1785 mm at the low-latitude site) based on 2022 climatic data (Supporting information).

In March 2022, before leaf burst, we purchased three-year-old saplings from nurseries sourced from local populations near experimental sites, measured their height ('initial height') and individually planted them in 2-litre pots containing peat (85%) and coconut fibre (15%). Prior to obtaining them, saplings were grown under greenhouse conditions and did not experience herbivory. After transplanting, we transported saplings to the study sites and assigned half of the plants in each stand to one of two warming treatments: 1) microhabitat-level warming or 2) untreated

control (i.e. ambient temperature). In total, there were 125 saplings (35 control saplings and 34 warmed saplings at the high-latitude site; 28 control saplings and 28 warmed saplings at the low-latitude site). Saplings were subjected exclusively to natural rainfall throughout the experiment. The warming treatment was imposed by means of infrared heaters of 100 W installed at 85 cm height above the saplings which remained in place from April to September 2022. We grouped saplings in blocks ($n=5$ at the high-latitude site and $n=3$ at the low-latitude site), and within each block we paired a group of 6–9 control plants and a group of 6–9 saplings under an infrared heater. Distance between potted saplings within each block was 20 cm and the distance between blocks within each site was 1 m. To assess microclimatic changes in air temperature due to the warming treatment, we installed a climatic-data logger (TMS-4, TOMST) inside the heated area, next to the saplings. These recordings indicated that, from April to August 2022, the warming treatment increased the air temperature, on average, by 1.5°C compared to plants under ambient temperature at both the low-latitude and high-latitude sites (Supporting information). While warming of the northern site never reached temperatures of the southern site, thus setting by default a lower temperature upper limit under warming at the colder site, in both cases we achieved realistic increases in temperature predicted under climate change in each region. Finally, we protected all plants from mammalian herbivores (e.g. deer, rabbits, rodents) by installing fences around the experimental sites.

In late August and early September 2022 (before leaf senescence), we removed the heaters and measured total height of all saplings ('final height'). Shortly after removing the heaters, we haphazardly collected 10 fully expanded leaves of roughly the same age (based on position along branch, color and consistency) for each sapling to assess herbivory and leaf defensive traits. Leaf collection was performed blindly with respect of treatments (Zvereva and Kozlov 2019). We sampled leaves at the end of the growing season at each site, i.e. in early September at the high-latitude site and in late September at the low-latitude site, to 1) minimize phenological differences in herbivory and leaf defensive traits between sites and 2) to provide an assessment of cumulative herbivory over the growing season (given that leaf longevity of *Q. petraea* spans most of the growing season). Most of the damage observed on the collected leaves were due to chewing insects and to a lesser extent by leaf miners or other herbivore guilds (< 2% of sampled leaves). We photographed all leaves with a Samsung Galaxy A30s (25 effective megapixels, 4 × digital zoom) and estimated the percentage of leaf area consumed by chewing insects ('insect herbivory' hereafter) using BioLeaf – Foliar Analysis (Brandoli Machado et al. 2016). We used the average value across leaves per individual sapling for statistical analyses.

For each sapling, we selected four leaves (out of the 10 originally sampled) with little or no herbivore damage and oven-dried them at 40°C for 48 h to quantify physical and chemical defences. We used undamaged leaves to partially

control for variation due to induced defences, namely local induction (Abdala-Roberts et al. 2016). However, variation due to systemic induction is also possible and was not prevented by this sampling approach. Accordingly, measured levels of leaf traits, particularly secondary metabolites, represent constitutive levels, plus variation due to systemic induction (Abdala-Roberts et al. 2016).

Quantification of physical defences

We estimated specific leaf area (SLA) as a proxy for physical defence (Lill et al. 2006, Moreira et al. 2020b). Previous work has shown that low SLA correlates negatively with high leaf toughness (Hanley et al. 2007, Pearse and Hipp 2009) and can therefore be used as a proxy for structural resistance against herbivory. We measured SLA ($\text{cm}^2 \text{g}^{-1}$) by dividing the surface area of the four selected leaves by its dry mass and then averaged these values across leaves to obtain a single (mean) value for statistical analyses.

Quantification of chemical defences

Phenolic compounds have been shown to defend oaks against insect herbivores (Feeny 1970, Roslin and Salminen 2008, Moreira et al. 2018a, b, 2020b), and therefore represent a suitable class of compounds to assess chemical defences in species of this group. For these analyses, we used the same four leaves used to quantify SLA but ground them all together using liquid nitrogen to obtain a single pooled sample. We then extracted phenolic compounds from 20 mg of dry pulverized leaf tissue with 1 ml of 70% methanol in an ultrasonic bath for 15 min, followed by centrifugation (Moreira et al. 2014). We then transferred the extracts to chromatographic vials and performed ultra-high-performance liquid chromatography analyses (Supporting information). We identified four groups of phenolic compounds: 1) flavonoids; 2) ellagitannins and gallic acid derivatives ('hydrolysable tannins' hereafter); 3) proanthocyanidins ('condensed tannins' hereafter); and 4) hydroxycinnamic acids, based on the comparison of their parent ion mass and fragmentation pattern, UV spectra and retention time with commercial standards and literature data. We quantified flavonoids as rutin equivalents, condensed tannins as catechin equivalents, hydrolysable tannins as gallic acid equivalents, and hydroxycinnamic acids as ferulic acid equivalents (Moreira et al. 2018b, Galmán et al. 2019). We achieved the quantification of these phenolic compounds by external calibration using calibration curves at 0.16, 0.8, 4, 20, 100 and 500 $\mu\text{g ml}^{-1}$. We expressed phenolic compound concentrations in mg g^{-1} dry tissue.

Statistical analysis

We ran general linear mixed models (GLMMs) testing for the effect of site (two levels: high- versus low-latitude), microhabitat-level warming (two levels: control or ambient temperature versus experimental warming) and their interaction on four different response variables: plant relative growth rate

(estimated as: $[\text{final height} - \text{initial height}]/\text{initial height}$), phenolic compounds (separately for each group of compounds), SLA and insect leaf herbivory. The site by warming interaction tested whether the effects of microhabitat-level warming varied across sites, i.e. a cross-scale dependency. In all models, we also included the three-way interaction between block, site and warming treatment as a random effect in order to analyse the main factors (i.e. site and warming) with the appropriate error terms.

To assess which leaf traits were associated with herbivory, we firstly ran a stepwise multiple regression with herbivory as a response and all leaf traits as predictors. We used a backward model selection based on the difference in the values of the Akaike information criterion (AIC) between models (AIC; delta AIC values between two competing models being higher than 2) to identify the most important traits predictive of herbivory (Supporting information). We tested for leaf trait effects on herbivory rather than the reverse as preliminary analyses indicated that traits, namely phenolic compounds, significantly negatively correlated with leaf damage suggesting that these compounds predict leaf damage, i.e. confer plant biotic resistance. While this interpretation is not definitive as other factors correlated with phenolics could underlie this correlation, it is the best evidence we have suggestive of causality based on available data. Second, to assess whether environmental conditions (broad-scale versus microhabitat scale due to warming) influenced herbivory via effects on leaf traits, namely broad-scale (e.g. abiotically-driven) differences versus plasticity responses to local warming, respectively, traits retained in the above stepwise regression were included as predictors (covariates) of herbivory in the initial leaf damage model testing for main effects (site, microhabitat-level warming) and their interaction. For this regression analysis, we standardized herbivory data (mean = 0, SE = 1) by site-by-warming treatment combination. We expected that if a given trait explains site or microhabitat-level warming effects, then any such effects on leaf damage in the initial model without covariates should turn non-significant after its inclusion (Abdala-Roberts et al. 2016, Moreira et al. 2018a). By the same token, if effects remain significant this would suggest that site or microhabitat-scale warming effects on herbivory act via other (unmeasured) leaf traits or through some other mechanism, e.g. direct effect of warming on insects influencing leaf damage (e.g. mobility, physiological state) independently of leaf defensive traits.

We performed all analyses with PROC MIXED in SAS ver. 9.4 (SAS Inst.) (Littell et al. 2006). For standardisation of herbivory data, we used PROC STANDARD in SAS 9.4. We log-transformed herbivory, condensed and hydrolysable tannins, flavonoids and plant relative growth rate to achieve normality of the residuals, and report model least-squares means and SEs as descriptive statistics. We calculated the post hoc statistical power of our analyses by using the online tool at <https://clincalc.com/stats/Power.aspx>, using the experimental unit (group of control or heated plants) as the level of replication (consistent with the statistical models above).

The statistical power always exceeded 65%, indicating that it was sufficient to detect an effect of site and warming treatment on herbivory and plant defences if that effect did exist (Kozlov et al. 2022).

Results

Effects of site and microhabitat-level warming on oak traits

There were significant effects of site on most groups of phenolic compounds, except flavonoids (Table 1, Fig. 1A–D), though the latitudinal direction was contingent on the type of compound. For example, the concentration of condensed tannins was, on average, 1.2-fold higher for saplings growing at the high-latitude site (mean \pm SE of back-transformed log data: 5.11 ± 1.05 mg g⁻¹ dw) than for those growing at the low-latitude site (4.32 ± 1.06 mg g⁻¹ dw) (Fig. 1A). In contrast, hydrolysable tannins and hydroxycinnamic acids were, on average, 2.0-fold and 1.2-fold higher for saplings growing at the low-latitude site than for those growing at the high-latitude site (mean \pm SE of back-transformed log data for hydrolysable tannins: 4.56 ± 1.04 versus 2.32 ± 1.03 mg g⁻¹ dw; mean \pm SE for hydroxycinnamic acids: 15.81 ± 0.57 versus 13.52 ± 0.51 mg g⁻¹ dw) (Fig. 1B, D). Microhabitat warming and the site by warming interaction were not significant in any case (Table 1).

We also found a significant effect of site on SLA (Table 1), whereby the mean value was 1.3-fold higher for saplings growing at the low-latitude site (mean \pm SE: 136.59 ± 3.38 cm² g⁻¹) relative to those growing at the high latitude site (104.78 ± 2.90 cm² g⁻¹) (Fig. 2). We found no significant effects of microhabitat warming and the site by warming interaction on SLA (Table 1).

There were no significant effects of site, microhabitat warming, or their interaction on sapling relative growth rate (Table 1, Supporting information).

Effects of site and microhabitat warming on herbivory

Levels of insect leaf damage were somewhat low (mean \pm SE: $4.35 \pm 0.32\%$, range: 0–15.93%, data based on individual

plants). There was a significant effect of site (Table 1), whereby herbivory was, on average, 1.4-fold higher for saplings growing at the low-latitude site (mean \pm SE of back-transformed log data: $5.05 \pm 1.11\%$) relative to those growing at the high-latitude site ($3.65 \pm 1.10\%$) (Fig. 3). At the microhabitat scale, there was a significant effect of warming on herbivory (Table 1), leaf damage being, on average, 1.4-fold higher for saplings subjected to warming (mean \pm SE of back-transformed log data: $5.03 \pm 1.10\%$) than for control saplings ($3.66 \pm 1.10\%$) (Fig. 3). We found no significant interaction site by warming interaction (Table 1), i.e. microhabitat warming effects were similar across sites (Fig. 3).

Leaf traits underlying site and microhabitat-level warming effects on herbivory

We found that condensed tannins and hydrolysable tannins were the only traits retained after multiple regression backward elimination, but only the former had a significant negative effect (condensed tannins: mean \pm SE of slope estimator $[\beta] = -0.067 \pm 0.031$, $p = 0.033$; hydrolysable tannins: slope estimator $[\beta] = 0.075 \pm 0.040$, $p = 0.066$). Following from this, we ran again the herbivory model with main effects but now including also condensed and hydrolysable tannins as covariates. Results from this model indicated that both the site and warming effects turned non-significant (Table 2 versus 1).

Discussion

We found significant effects of broad-scale site differences and microhabitat-scale warming on leaf defensive traits and insect herbivory on *Q. petraea* saplings. Across sites, herbivory was highest on saplings growing at the low-latitude site (Spain), whereas leaf traits showed mixed responses, i.e. both increases and decreases at low latitude depending on the focal trait. At the microhabitat scale, warming increased herbivory but did not affect leaf traits. Condensed tannin concentrations, the only trait significantly predicting leaf damage (negative effect), were lower at the low latitude site and seemingly accounted for site and warming differences in herbivory. Together, these findings indicate different responses acting

Table 1. Effects of the site (two levels: low-latitude site in Spain, and high-latitude site in Belgium), microhabitat-level warming treatment (two levels: control or ambient temperature versus experimental warming), and their interaction on the concentration of leaf chemical defences (condensed and hydrolysable tannins, flavonoids and hydroxycinnamic acids), specific leaf area, leaf herbivory by chewing insects and plant relative growth rate in *Q. petraea* saplings. We also included the three-way interaction between block, site and warming treatment as a random effect. *F*-values with the degrees of freedom (numerator, denominator) and associated significance levels (*p*-values) are shown. Significant *p*-values ($p < 0.05$) are in bold

	Site (S)		Warming (W)		S \times W	
	<i>F</i> _{1,12}	<i>p</i>	<i>F</i> _{1,12}	<i>p</i>	<i>F</i> _{1,12}	<i>p</i>
Condensed tannins	4.64	0.033	0.23	0.634	0.03	0.873
Hydrolysable tannins	194.06	<0.001	1.20	0.294	0.80	0.388
Flavonoids	0.51	0.489	0.07	0.803	0.01	0.929
Hydroxycinnamic acids	9.08	0.011	0.05	0.829	0.81	0.386
Specific leaf area	51.07	<0.001	2.85	0.117	4.37	0.059
Insect leaf herbivory	5.40	0.039	5.23	0.041	1.20	0.296
Relative growth rate	0.84	0.377	0.32	0.583	0.37	0.555

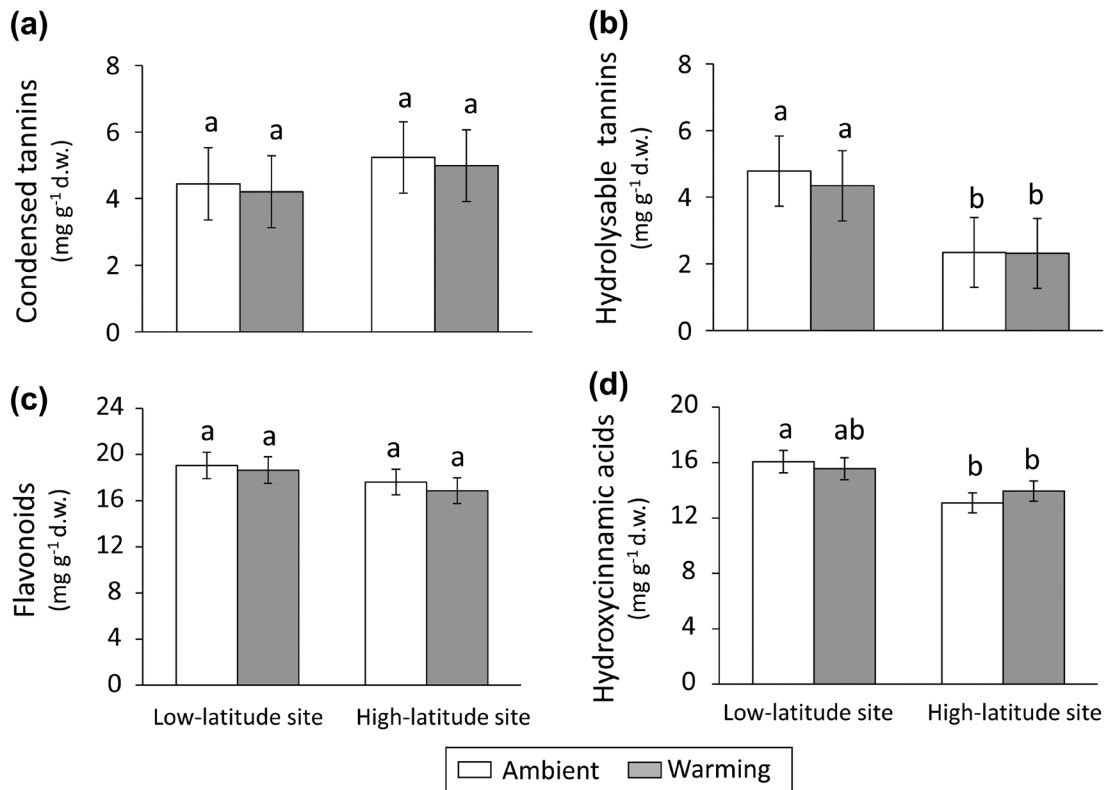


Figure 1. Concentration of leaf (A) condensed tannins, (B) hydrolysable tannins, (C) flavonoids, and (D) hydroxycinnamic acids in *Quercus petraea* saplings subjected to experimental warming (gray bars) and ambient temperature (white bars) at two macro-climatically contrasted sites (low-latitude site in Spain, and high-latitude site in Belgium). Bars are back-transformed least square means \pm SE from the linear mixed models (except for hydroxycinnamic acids). Statistical results are shown in Table 1. Different letters above the bars indicate significant differences (at $p < 0.05$) between treatments.

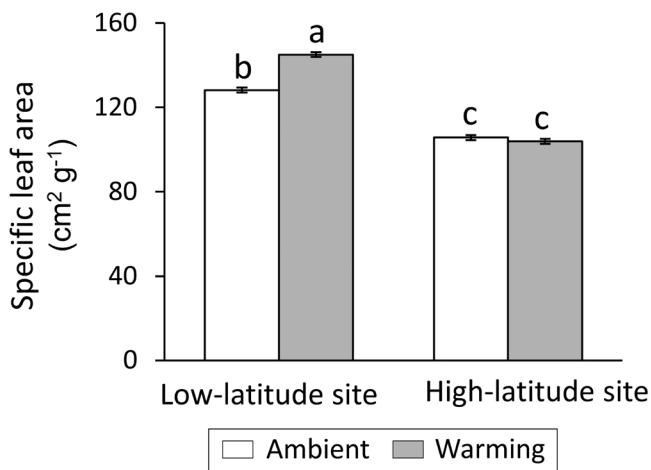


Figure 2. Specific leaf area in *Q. petraea* saplings subjected to experimental warming (gray bars) and control or ambient temperature (white bars) at two macro-climatically contrasted sites (low-latitude site in Spain, and high-latitude site in Belgium). Bars are least square means \pm SE. Statistical results are shown in Table 1. Different letters above the bars indicate significant differences (at $p < 0.05$) between treatments.

at each scale, namely: leaf defences were more responsive to broad-scale environmental differences than to microhabitat warming whereas herbivory was affected at both scales. Furthermore, our findings also indicated no evidence of cross-scale dependencies, namely that warming effects at the microhabitat scale played out similarly across latitudes.

Insect leaf damage was highest at the low-latitude site, supporting predictions of latitudinal variation in species interactions (reviewed by Schemske et al. 2009), including herbivory (Coley and Barone 1996, Rasmann and Agrawal 2011, Pearse and Hipp 2012, Salazar and Marquis 2012, Moreira et al. 2014). This result is also consistent with our previous work on pedunculate oak *Q. robur*, another common European oak, showing higher insect leaf herbivory at lower latitudes (Moreira et al. 2018a). In addition, we found that some defensive traits such as condensed tannins and SLA (proxy for leaf thickness or toughness) were higher at the high-latitude site, whereas hydrolysable tannins and hydroxycinnamic acids were higher at the low-latitude site. Further analyses indicated that herbivory significantly correlated (negatively) only with condensed tannins and that these compounds accounted for site differences in herbivory. These results are also consistent with our previous work on *Q. robur* suggesting condensed tannins drive latitudinal variation in herbivory (Moreira et al. 2018b). We argue

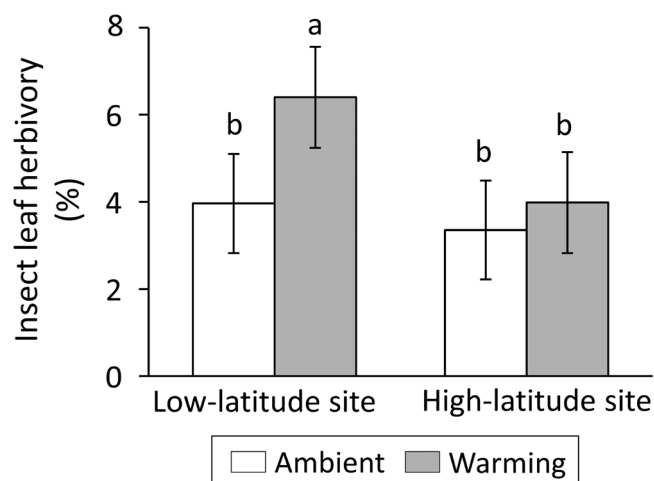


Figure 3. Percent leaf herbivory by insect chewers on *Q. petraea* saplings subjected to experimental warming (gray bars) and control or ambient temperature (white bars) at two macro-climatically contrasted sites (low-latitude site in Spain, and high-latitude site in Belgium). Bars are back-transformed least square means \pm SE from the linear mixed model. Statistical results are shown in Table 1. Different letters above the bars indicate significant differences (at $p < 0.05$) between treatments.

that for this species, and possibly also for sessile oak, higher temperature and precipitation drive lower tannin concentrations at lower latitudes and this in turn results in higher herbivory, i.e. a bottom-up causality that differs from classical theory which postulates that higher herbivore pressure drives higher defences at low latitude, a top-down mechanism (Moreira et al. 2018a). It is also plausible that higher herbivory at the low-latitude site is shaped by direct effects of climate on insect performance and resulting impacts on population growth or life cycle dynamics (e.g. voltinism) leading to greater leaf damage. For example, previous studies have reported that higher temperatures (as at our low-latitude

Table 2. Effects of the site (two levels: low-latitude site in Spain, and high-latitude site in Belgium), microhabitat-level warming treatment (two levels: control or ambient temperature vs experimental warming), and their interaction on leaf herbivory by chewing insects in *Q. petraea* saplings. In this model, we included selected leaf defensive traits (statistical analysis in Material and methods) as covariates to test whether the effects of the site and microhabitat-level warming treatment on insect leaf herbivory were explained by changes in leaf traits. We also included the three-way interaction between block, site and warming treatment as a random effect. For these analyses, we standardized herbivory data (mean=0, SE=1) by site-by-warming treatment combination. Degrees of freedom (numerator, denominator), *F*-values and associated significance levels (*p*-values) are shown

	Insect leaf herbivory		
	DF _{num,den}	<i>f</i>	<i>p</i>
Site	1, 12	1.42	0.256
Warming	1, 12	0.00	0.996
Site \times Warming	1, 12	0.02	0.887
Condensed tannins	1, 107	3.79	0.054
Hydrolysable tannins	1, 107	1.51	0.221

site) increase physiological activity and metabolic rates of insect herbivores which in turn consume more plant tissues and grow faster (Bale et al. 2002).

Microhabitat-level warming, on the other hand, increased leaf herbivory, consistent with previous studies pointing at temperature-driven increases in insect metabolic rates or changes in feeding behaviour as potential mechanisms (Bale et al. 2002, Hamann et al. 2021). For instance, Nakamura et al. (2022) recently reported that microhabitat-scale warming of 5°C above ambient temperature using heat resistance cables (in the soil and branches) markedly increased herbivory by chewing insects on birch trees. Similarly, Lemoine et al. (2013) found that consumption rates by the generalist herbivore *Popillia japonica* on several plant species increased under microhabitat warming. Contrarily to leaf damage, microhabitat warming did not affect SLA and the concentration of any of the analysed phenolic compounds. Such lack of warming effects does not agree with results from other studies reporting increases in chemical defences with experimental warming in *Q. rubra* (Top et al. 2017), *Betula nana* (Graglia et al. 2001) and *Populus tremuloides* (Li et al. 2021). Differences between studies in the timing of leaf collection could explain these differences in results, provided seasonal changes in plasticity responses to warming in phenolic compound concentrations (Hunter and Price 1992, Forkner and Hunter 2000) as well as changes in secondary chemistry with leaf ontogeny or between leaf flushes early versus later in the growing season which overrun warming effects (Matsuki et al. 2004, van Asch and Visser 2007, Gaytán et al. 2022).

The fact that most leaf traits were not affected by microhabitat warming would have precluded trait plasticity-mediated effects on herbivory. Despite this, once having accounted for hydrolysable tannins and condensed tannins (retained leaf traits after stepwise procedure), the warming effect on herbivory turned non-significant, suggesting that the effects of microhabitat temperature manipulation on damage were underlain by warming-induced changes in these compounds. However, the fact that warming did not significantly influence these compounds casts doubt on this trait-mediated interpretation. Further work assessing warming-induced changes in additional leaf traits such as terpenoids and fiber content is needed, including diurnal and nocturnal plant secondary chemistry-related metabolism as well as insect behavioural changes under warming. In addition, while the achieved temperature increase is within the range of predicted increases due to climate change, our experiment did not test for temporal variability in temperature increases, including peaks in temperature which have been shown to have especially strong impacts on plant physiology and defensive responses (e.g. heat waves; Tian et al. 2022).

The warming effect on herbivory and leaf traits was consistently non-significant across the studied distant sites despite substantial differences in abiotic (e.g. climatic) conditions. Few studies have experimentally tested for microhabitat-level warming effects across abiotically-contrasting sites (Birkemoe et al. 2016, Pepi and Karban 2021,

Zvereva et al. 2022). Consistent with our findings, Pepi and Karban (2021) reported that microhabitat warming drove an increase in herbivory by woolly bear caterpillars *Arctia virginialis* on pacific silverweed *Argentina anserina* ssp. *pacifica*, with such effect being similar in magnitude across two sites located at the two extremes of a precipitation gradient in California. In contrast, Birkemoe et al. (2016) found contrasting effects of microhabitat warming on herbivory for two plant species growing at two sites differing in elevation. Namely, warming increased herbivory by leaf-chewing insects on the mountain avens *Dryas octopetala* but only at low elevation, whereas for the alpine bistort *Bistorta vivipara* it increased herbivory only at high elevation (Birkemoe et al. 2016). These authors argued that different herbivores appear to be responsible for damage on the studied plant species, pointing at herbivore-specific responses to warming (Birkemoe et al. 2016). At our field sites, *Q. petraea* is mainly attacked by lepidopteran species such as *Tortrix viridana*, *Lymantria dispar* and *Malacosoma neustria* (Moreira et al. 2017, 2018a, 2020b). Unfortunately, we were unable to record the identity of the herbivores based on observed leaf damage patterns to inform site differences in herbivore composition to assess whether the same or different herbivores responded similarly to warming across sites.

Study limitations and future work

The first limitation of our study is that by using only one source of saplings at each site we cannot disentangle plant genetically based from plasticity effects on species traits which is essential for predicting species responses to climatic variation and its consequences for species interactions. To this end, follow-up studies should make use of plants from multiple source sites located at each latitude and sampling at multiple points along the studied gradient (including expanding beyond the latitudinal range used). Another limitation is that by not including a detailed inspection of the biotic and abiotic drivers of broad-scale effects we cannot identify mechanisms operating at this scale, tease apart their relative effects, as well as their potential to interact locally with microclimatic effects. To this end, further studies should include direct measurements of target factors across sites (e.g. using meteorological stations, soil samples, herbivore community sampling, etc.) or using available databases (e.g. climate). An additional limitation worth emphasizing is that by including a single time point of leaf collection over the same growing season we cannot provide a fine-tuned picture of within- or among-season variation in leaf chemistry, and a better understanding of its driving factors and cause–effect linkages to herbivory. To this end, further studies should sample leaves across different moments of the growing season and during multiple growing seasons. Broadly, research aiming to address one or several of these features will lead to a better understanding of the drivers of plant–herbivore interactions and how abiotic factors acting at different spatial scales shape species traits and interactions under climate change.

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Data availability statement

Data are available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.8931zcrx4> (Moreira et al. 2023).

Supporting information

The Supporting information associated with this article is available with the online version.

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