



Latitudinal variation in plant chemical defences drives latitudinal patterns of leaf herbivory

Xoaquín Moreira, Bastien Castagneyrol, Luis Abdala-Roberts, Jorge C. Berny-Mier y Teran, Bart G. H. Timmermans, Hans Henrik Bruun, Felisa Covelo, Gaëtan Glauser, Sergio Rasmann* and Ayco J. M. Tack*

X. Moreira (<http://orcid.org/0000-0003-0166-838X>) (xmoreira1@gmail.com), Misión Biológica de Galicia (MBG-CSIC), Pontevedra, Galicia, Spain. – B. Castagneyrol, BIOGECO, INRA, Univ. Bordeaux, Cestas, France. – L. Abdala-Roberts (<http://orcid.org/0000-0003-1394-3043>), Depto de Ecología Tropical, Campus de Ciencias Biológicas y Agropecuarias, Univ. Autónoma de Yucatán, Itzimmá, Mérida, Yucatán, México. – J. C. Berny-Mier y Teran, Dept of Plant Sciences, Univ. of California-Davis, Davis, California, USA. – B. G. H. Timmermans, Dept of Agriculture, Louis Bolk Inst., Driebergen, the Netherlands. – H. H. Bruun, Dept of Biology, Univ. of Copenhagen, Copenhagen, Denmark. – F. Covelo, Depto de Sistemas Físicos, Químicos y Naturales, Univ. Pablo de Olavide, Sevilla, Spain. – G. Glauser, Neuchâtel Platform of Analytical Chemistry, Univ. of Neuchâtel, Neuchâtel, Switzerland. – S. Rasmann, Inst. of Biology, Laboratory of Functional Ecology, Univ. of Neuchâtel, Neuchâtel, Switzerland. – A. J. M. Tack (<http://orcid.org/0000-0002-3550-1070>), Dept of Ecology, Environment and Plant Sciences, Stockholm Univ., Stockholm, Sweden.

A long-standing paradigm in ecology holds that herbivore pressure and thus plant defences increase towards lower latitudes. However, recent work has challenged this prediction where studies have found no relationship or opposite trends where herbivory or plant defences increase at higher latitudes. Here we tested for latitudinal variation in herbivory, chemical defences (phenolic compounds), and nutritional traits (phosphorus and nitrogen) in leaves of a long-lived tree species, the English oak *Quercus robur*. We further investigated the underlying climatic and soil factors associated with such variation. Across 38 populations of *Q. robur* distributed along an 18° latitudinal gradient, covering almost the entire latitudinal and climatic range of this species, we observed strong but divergent latitudinal gradients in leaf herbivory and leaf chemical defences and nutrients. As expected, there was a negative relationship between latitude and leaf herbivory where oak populations from lower latitudes exhibited higher levels of leaf herbivory. However, counter to predictions there was a positive relationship between leaf chemical defences and latitude where populations at higher latitudes were better defended. Similarly, leaf phosphorus and nitrogen increased with latitude. Path analysis indicated a significant (negative) effect of plant chemical defences (condensed tannins) on leaf herbivory, suggesting that the latitudinal gradient in leaf herbivory was driven by an inverse gradient in defensive investment. Leaf nutrients had no independent influence on herbivory. Further, we found significant indirect effects of precipitation and soil porosity on leaf herbivory, which were mediated by plant chemical defences. These findings suggest that abiotic factors shape latitudinal variation in plant defences and that these defences in turn underlie latitudinal variation in leaf herbivory. Overall, this study contributes to a better understanding of latitudinal variation in plant–herbivore interactions by determining the identity and modus operandi of abiotic factors concurrently shaping plant defences and herbivory.

A broadly recognized pattern in ecology is that geographic variation in species' traits tends to vary clinally in relation to biotic (i.e. species interaction intensity) and abiotic (e.g. climate) factors (Linhart and Grant 1996). In particular, due to higher temperatures, longer growing seasons, and a greater diversity of animal and plant species at lower latitudes, it is assumed that the strength of species interactions increases towards the Equator and that these factors drive concomitant patterns of latitudinal variation in species traits associated with such interactions (Dobzhansky 1950, Janzen 1970, Schemske et al. 2009). In the case of plant–herbivore

interactions, studies have shown that plant species growing at lower latitudes frequently experience higher amounts of herbivory than their counterparts at higher latitudes. This has been shown by studies comparing temperate vs. tropical species (Schemske et al. 2009, Marquis et al. 2012, Lim et al. 2015, Zhang et al. 2016), as well as by studies comparing species restricted to tropical zones (Salazar and Marquis 2012, Moreira et al. 2015a) or temperate zones (Pennings et al. 2009, Anstett et al. 2015). It is therefore expected that plant species have evolved higher levels of anti-herbivore defences at lower latitudes, where herbivore pressure is higher (Rasmann and Agrawal 2011, Pearse and Hipp 2012, Moreira et al. 2014, Abdala-Roberts et al. 2016a). In addition, some studies have suggested that leaf nutrient

*Joint last authorship.

concentrations, another proxy of plant quality to herbivores, could also be associated with latitudinal gradients in herbivory and exhibit latitudinal clines (Marquis et al. 2012). However, the generality of these plant–herbivore latitudinal trends has recently been called into question by work showing that latitudinal trends in herbivory and plant defense are in fact highly variable in strength and direction (Moles and Ollerton 2016). Studies have found either no evidence of a latitudinal gradient in herbivory and plant defences (Moles and Westoby 2003, Moles et al. 2011), greater levels of herbivory (Anstett et al. 2015) and defences (Moreira et al. 2014) at higher latitudes, or mixed evidence when comparing different herbivore species or plant defensive traits (Penning et al. 2009, Anstett et al. 2014, 2015, Moreira et al. 2015a, Abdala-Roberts et al. 2016a). Given these mixed findings, a better understanding of the underlying ecological factors driving herbivory and plant traits is needed in order to advance macro-ecological research on plant–herbivore interactions (Anstett et al. 2016).

There are some problematic assumptions in the literature of latitudinal variation in herbivory and plant defences, and these may explain the inconsistency in patterns reported thus far. First, most studies assume that higher levels of plant secondary metabolites or physical defences predominantly drive lower levels of herbivory. However, other traits relating to plant tolerance, such as re-growth capacity or overcompensation in reproduction (Carmona et al. 2011, Lehdal and Ågren 2015), as well as plant traits associated with nutritional quality (Mattson 1980, Agrawal 2007), may also strongly influence herbivory. For example, to date very few studies have examined latitudinal clines in plant nutritional quality (Lovelock et al. 2007), and thus the role of plant nutrients in shaping herbivory levels across latitudes remains unclear. Second, recent studies have demonstrated that abiotic correlates of latitude may concurrently influence levels of herbivory and plant allocation to defences (Pearse and Hipp 2012, Anstett et al. 2015, Abdala-Roberts et al. 2016a). For example, Abdala-Roberts et al. (2016a) recently found that climatic conditions influenced latitudinal variation in plant defences independently of herbivory in the perennial herb *Ruellia nudiflora* (see also Pearse and Hipp 2012, Moreira et al. 2014). Likewise, soil characteristics may also be important but remain largely ignored in latitudinal studies. For example, plant species growing in nutrient-poor soils invest more in chemical defences relative to species growing in nutrient-rich soils (Coley et al. 1985, Coley and Barone 1996), and such effects may operate concurrently with herbivory along latitudinal gradients (Johnson and Rasmann 2011, Abdala-Roberts et al. 2016a). Further, the effect of abiotic factors may not vary latitudinally or may show latitudinal trends opposite to those of herbivory which may lead to unexpected latitudinal trends in plant defence investment (Abdala-Roberts et al. 2016a, Stevens et al. 2016). Therefore, addressing the simultaneous effects of multiple abiotic drivers is necessary for understanding how abiotic forcing influences plant traits, herbivores, or both along latitudinal gradients.

In the present study, we tested concomitantly for latitudinal variation in herbivory, plant chemical defences (multiple groups of phenolic compounds) and nutritional traits (phosphorus and nitrogen) in leaves of the English

oak *Quercus robur* (Fagaceae), a long-lived tree common in western Europe. In addition, we investigated the underlying climatic and edaphic factors associated with variation in herbivory and plant traits. To this end, we sampled 38 populations of *Q. robur* distributed along an 18° latitudinal gradient extending from northern Spain to central Sweden, covering almost the entire latitudinal range of this species, as well as most of the climatic gradient experienced by this species throughout its distribution range (Fig. 1). We note that the latitudinal gradient sampled is restricted to a temperate zone and expected trends may vary when comparing tropical vs temperate zones relative to work conducted within each zone (Anstett et al. 2016). However, in both cases we expect many of the same underlying factors to influence plant traits and herbivory and thus both types of studies contribute to our understanding of the driving forces shaping latitudinal variation in plant–herbivore interactions along environmental clines. In addition, while much of the work on latitudinal variation in herbivory and plant defences has focused on comparisons among species (Rasmann and Agrawal 2011, Marquis et al. 2012, Moreira et al. 2014), our study contributes to an equally rich body of research addressing environmental clines in plant intra-specific variation (Woods et al. 2012, Abdala-Roberts et al. 2016a). Research

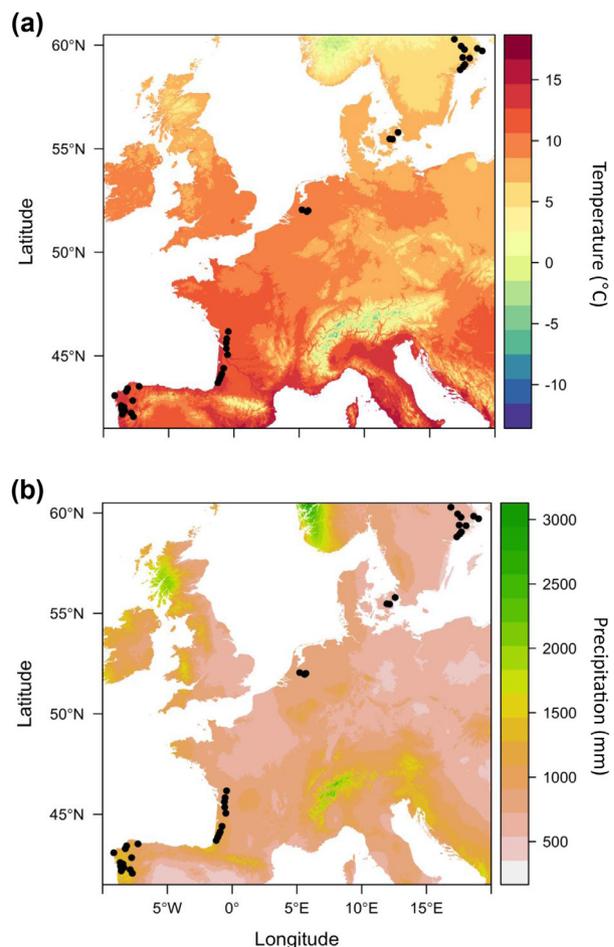


Figure 1. Maps for (a) mean annual temperature in °C and (b) annual precipitation in mm including a spatial layout of *Quercus robur* populations sampled from northern Spain to southern Sweden. Circles represent the location of each population (n = 38).

on intra-specific variation in ecological clines is of central importance for understanding selection on plant traits along biotic and abiotic gradients (Pratt and Mooney 2013) and may inform on among-species macro-ecological patterns (Anstett et al. 2015, Hahn and Maron 2016).

Specifically, we sought to answer the following questions: 1) are there latitudinal clines in herbivory, leaf chemical defences and leaf nutrient concentration? 2) Is latitudinal variation in leaf defences and/or nutrients associated with concomitant latitudinal variation in herbivory? 3) Are abiotic correlates of latitude (climate and soil conditions) associated with latitudinal variation in herbivory, plant chemical defences and nutrients? *Quercus robur* is an ideal system to address these questions because it is a widely distributed tree species in the Northern Hemisphere, particularly in western Europe, and grows in a wide range of climatic and soil conditions (Jones 1959). Overall, this study provides an assessment of latitudinal variation in plant defences and herbivory as well as the biotic and abiotic factors underlying such variation for a single widespread tree species in temperate regions.

Material and methods

Natural history

The English oak *Q. robur* is a long-lived, deciduous tree native to most of Europe (Schwarz 1964), and is distributed from northern Portugal (40°N) to southern Scandinavia (62°N) (Petit et al. 2002). This species tolerates an extremely wide range of climatic conditions and is frequently associated with nutrient-rich and moist soils in valleys or near rivers (Jones 1959). Leaf burst in this species usually occurs during April in southern Europe and in May for northern Europe. There may be up to four bursts during the season such that herbivores feeding in late season are exposed to a mix of young and old leaves. Leaf senescence and leaf drop typically start in September for northern Europe and in October for southern Europe. Leaves are approximately 8–12 cm long with four to seven pairs of lobes, and have almost no petiole. In its native range, *Q. robur* supports a large community of specialist (and some generalist) insect herbivores, mainly leaf chewers, miners and galls (Southwood et al. 2005, Tack et al. 2010, Tack and Roslin 2011, Castagneyrol et al. 2012, Giffard et al. 2012, Moreira et al. 2017a).

Field sampling and leaf herbivory measurements

At the end of the growing season (from late August to early October), and before leaf senescence, we sampled a total of 38 *Q. robur* populations distributed across Spain, France, the Netherlands, Denmark, and Sweden spanning 18° in latitude (from 42°N to 60°N; Fig. 1). The sampled transect covers most of the latitudinal and climatic gradients experienced by this tree over its range (Fig. 1). From north to south, plants and herbivores experience over a three-fold increase in precipitation (506 to 1563 mm yr⁻¹) and an increase of 9.4°C in mean annual temperature (from 5.1 to 14.5°C) (Fig. 1). We sampled all populations at the end of the

corresponding growing season in each site, i.e. late August to early September in northern populations and from late September to early October in southern populations. This procedure effectively minimized phenological differences in herbivory and plant defensive traits among populations.

Each study population included at least 10 mature, reproductive oak trees. We randomly selected five individuals per population, and for each tree we visually estimated leaf herbivory separately for two randomly chosen branches from the lowest part of the tree. Previous visual inspections of trees at each site indicated that the distribution of herbivore damage was relatively consistent throughout the canopy, so we therefore sampled only low-hanging branches due to easier access. Following Tack et al. (2010), we calculated the proportion of leaves damaged by herbivores by randomly choosing 25 leaves per branch and counting the number of leaves attacked by herbivores. To standardize our method and avoid biases in our estimations due to different observers scoring herbivory differently, leaf samples were sent to and scored by two authors (XM and AJMT) with extensive experience with both oak and the damage types by its local herbivores. Measurements of the proportion of damaged leaves may not necessarily match estimates of the amount of leaf area consumed. We therefore also conducted supplementary measurements of percent leaf area removed for a subset of 16 out of the 38 populations based on stored samples (i.e. five leaves from five trees selected in five populations in Spain and France and three populations in the Netherlands and Denmark). We found a significant positive relationship between the frequency of damage and the percentage of leaf area removed (Supplementary material Appendix 1 Fig. A1), indicating that the frequency of attacked leaves was a good proxy of percent leaf area loss to herbivory during the study. Personal observations showed that leaves measured at our study sites were mostly damaged by insect chewers, miners and galls, whereas damage by other invertebrates (e.g. snails and slugs) and vertebrates was rare. It is important to note that our methods may underestimate damage inflicted by mammalian herbivores (e.g. deer), which might have removed entire leaves. Such effects might have been particularly important at higher latitudes considering that herbivory by vertebrates tends to increase toward the poles (Peco et al. 2014). In addition, our method did not consider leaf abscission (Blundell and Peart 2000), which may result in an underestimation of damage levels. However, there was no a priori reason to expect damage-induced abscission levels to vary with latitude and introduce a bias in the results. Because there might be some loss to mammals and other invertebrates (e.g. molluscs), hereafter we broadly refer to our measurement of frequency of herbivore damage as ‘leaf herbivory’.

For each surveyed branch per tree, we also collected two fully expanded (mature) leaves with little or no herbivore damage for chemical analyses. By sampling undamaged leaves, we aimed at reducing variation in defence levels caused by site-specific induction (Abdala-Roberts et al. 2016a). However, this approach does not fully eliminate induction as induced responses may be systemic. Therefore, the measured levels of chemical defences likely represented a combination of constitutive defences, plus an unknown level of systemic induction of defences (Abdala-Roberts et al.

2016a). After collection, we oven-dried the leaf material for 48 h at 40°C, ground the leaves with liquid nitrogen, and stored the samples for subsequent quantification of phenolic compounds and nutrients (Moreira et al. 2012, 2014). Finally, we note that having conducted our sampling at the end of the growing season has two advantages: it provides an assessment of cumulative leaf herbivory occurring over the entire growing season (considering that *Q. robur* leaf longevity spans most of the growing season), and it is the time of year when leaf herbivory and chemistry are more stable (Salminen et al. 2004).

Quantification of phenolic compounds

We chose phenolic compounds as defensive traits because they are widely recognized as herbivore feeding deterrents common to many plant taxa (Salminen and Karonen 2011, Mithöfer and Boland 2012, Moreira et al. 2014, Anstett et al. 2015), and specifically, confer resistance against leaf herbivores feeding on *Q. robur* (Feeny 1970, Roslin and Salminen 2008, Abdala-Roberts et al. 2016b). We extracted phenolic compounds using 20 mg of dry plant tissue with 1 ml of 70% methanol in an ultrasonic bath for 15 min, followed by centrifugation (Moreira et al. 2014). We diluted these methanolic extracts (1:5 vol:vol) with the extraction solvent and transferred them to chromatographic vials to perform phenolic profiling according to Moreira et al. (2015b) with some modifications. Briefly, we used ultrahigh-pressure liquid chromatography-quadrupole-time-of-flight mass spectrometry (UHPLC-QTOF-MS) to detect, identify and quantify phenolic compounds. The separation was carried out on a 50 × 2.1 mm Acquity UPLC BEH C18 column (Waters, Milford, CT, USA) thermostated at 25°C. Solvents were water + 0.05% vol. formic acid (A), and acetonitrile + 0.05% vol. formic acid (B). The gradient program was performed at a flow rate of 0.4 ml min⁻¹ under the following conditions: 5–30% B for 6 min, 30–100% B for 2 min, holding at 100% B for 2 min followed by re-equilibration at 5% B for 2 min with an injection volume of 2 µl. The QTOF-MS was operated in MS^E negative mode over an m/z range of 85–1200 Da with the following parameters: capillary voltage at –2.5 kV, cone voltage –25 V, source temperature 120°C, desolvation gas temperature 350°C, desolvation gas flow 800 l h⁻¹. The instrument was internally calibrated by infusing a solution of leucine-enkephaline at 400 ng ml⁻¹ at a flow rate of 15 µl min⁻¹ through the Lock SprayTM probe. We identified phenolic compounds on the basis of their molecular formula (as determined from exact mass measurements), fragment ions, and comparison with available databases such as the Dictionary of Natural Products (Chapman and Hall, CRC Informa, London; ver. 20.2) or ReSpect for Phytochemicals (Sawada et al. 2012). We quantified flavonoids as rutin equivalents, condensed tannins as catechin equivalents, hydrolysable tannins as gallic acid equivalents, and lignins as ferulic acid equivalents. We achieved the quantification of these phenolic compounds by external calibration using calibration curves at 0.2, 0.8, 2, 5 and 20 µg ml⁻¹. We calculated total phenolics as the sum of flavonoids, lignins, condensed tannins and hydrolysable tannins, and expressed phenolic compound concentrations in mg g⁻¹ tissue on a dry weight basis.

Quantification of nutrients

We chose phosphorus and nitrogen as proxies for leaf nutrient status because these macro-nutrients are typically found in low concentrations in plant tissues across many plant taxa and can therefore be limiting with respect to herbivore nutritional requirements (Mattson 1980). Accordingly, these macro-nutrients are frequently strongly correlated with levels of leaf herbivory (Mattson 1980, Huberty and Denno 2006), and this has been shown for several oak species (Forkner and Hunter 2000, Jones et al. 2008). To quantify nitrogen and phosphorus concentration in leaves, we digested approximately 0.1 g of ground dried leaf material in a mixture of selenous sulphuric acid and hydrogen peroxide (Moreira et al. 2012). We then used a colorimetric analysis of diluted aliquots of the digestion to quantify nitrogen (indophenol blue method) and phosphorus (molybdenum blue method) concentration using a Biorad 650 microplate reader (Bio-Rad Laboratories, Philadelphia, PA, USA) at 650 nm and 700 nm, respectively (Walinga et al. 1995). We expressed nitrogen and phosphorus concentration in mg g⁻¹ tissue on a dry weight basis.

Geographic and climatic and soil variables

We determined the geographic coordinates of each *Q. robur* population using a Global Positioning System device (Garmin, KS, USA). To characterize the climatic and soil conditions present at each population, we used a subset of the bioclimatic variables of the WorldClim database (<www.worldclim.org/>) at the 30-second resolution, and soil variables from the SoilGrids database (<www.soilgrids1km.isric.org>) at 1 km resolution. Specifically, we used BIO1 (annual mean temperature, °C), BIO4 (temperature seasonality, expressed as the standard deviation of temperature among months × 100), BIO5 (maximum temperature of the warmest month, °C), BIO6 (minimum temperature of the coldest month, °C), BIO12 (annual precipitation, mm), BIO13 (precipitation of the wettest month, mm), BIO14 (precipitation of the driest month, mm), and BIO15 (precipitation seasonality, expressed as standard deviation of precipitation across months) as climatic variables. For the soil variables (1 km grid resolution), we used bulk density (kg m⁻³), cation exchange capacity (cmolc kg⁻¹), coarse fragments (cm³ cm⁻³), organic carbon stock (tonnes ha⁻¹), organic carbon content (parts per thousand), soil pH, percentage of clay, percentage of silt, and percentage of sand in the topsoil (2.5 cm) and subsoil (150 cm). The procedures used to calculate these climatic and soil variables are fully described in Hijmans et al. (2005) and Hengl et al. (2014), respectively.

Statistical analyses

Latitudinal variation in leaf herbivory and plant traits

To assess whether there were latitudinal gradients in leaf herbivory (logit-transformed), leaf chemical defences, or nutrients, we performed simple linear regressions between latitude and each variable using population means which were obtained by averaging values across plants within each population (PROC REG in SAS 9.4). We also ran

generalized linear mixed models testing the effect of latitude (fixed factor) on herbivory and each plant trait using data at the individual tree level (PROC MIXED in SAS 9.4). Each of these models included population as a random factor. Results were very similar to those found using population means (Supplementary material Appendix 1 Table A1), and we therefore only report results for population-level analyses in the main manuscript.

Correlates of latitudinal variation in herbivory and plant traits

We used path analysis to investigate the associations between climatic factors, soil factors, leaf traits and herbivory, as well as the indirect effects of climate and soil factors on leaf herbivory (PROC CALIS and the RAM statement in SAS 9.4). Instead of total phenolics, we used condensed tannins as defensive traits because this group of phenolics was the only predictor of leaf herbivory based upon results from a multiple regression where leaf herbivory was predicted by flavonoids, lignins, condensed tannins, and hydrolysable tannins using PROC REG in SAS 9.4 (Supplementary material Appendix 1 Table A2). The rationale for including condensed tannins rather than total phenolics is that using the latter might obscure the detection of effects of one or a few key groups of phenolic compounds driving herbivory (i.e. condensed tannins in this case).

For the path analysis, we used the information from all climatic variables without inflating Type I error due to multiple tests for each variable by summarizing climatic variables in two independent principal component analyses (PCA) (PROC FACTOR, rotation=varimax in SAS 9.4). We obtained one PCA for temperature variables and another for precipitation variables (Moreira et al. 2014, 2015a). Temperature variables were summarized by the first principal component that explained 87% of the variance in the four temperature variables across populations ('PC temperature' hereafter), and was positively related to mean annual temperature, maximum temperature of the warmest month and minimum temperature of the coldest month, and negatively related to temperature seasonality. Precipitation variables were summarized by the first principal component that explained 62% of the variance in the four precipitation variables across populations ('PC precipitation' hereafter), and was positively related to annual precipitation, precipitation of the wettest month, and precipitation seasonality. The standardized *z*-scores of the first PC in each case were used in the path analysis. Latitude was significantly negatively related to both PC temperature ($R^2=0.64$, $p < 0.001$) and PC precipitation ($R^2=0.71$, $p < 0.001$). Similarly, two axes explained 67% of the variance in the eighteen soil variables across populations. PC1 ('PC1 soil' hereafter) was positively related to soil pH, percentage of clay, and percentage of silt in the topsoil and subsoil, and negatively related to percentage of sand in the topsoil and subsoil. PC2 ('PC2 soil' hereafter) was positively related to coarse fragments in the topsoil and subsoil and negatively related to bulk density in the topsoil and subsoil, and thus characterizes the level of soil porosity. Latitude was significantly positively related to PC2 soil ($R^2=0.45$, $p < 0.001$), but not to PC1 soil ($R^2=0.01$, $p=0.670$). We used the standardized *z*-scores of the first two soil PC's in the path analysis. However, a preliminary path

model indicated that PC1 soil was not significantly associated with leaf herbivory or any plant trait, so we removed this PC in the final path model reported in the results.

Data deposition

Data available from the Dryad Digital Repository: <<http://dx.doi.org/10.5061/dryad.6r813>> (Moreira et al. 2017b).

Results

Latitudinal variation in leaf herbivory and plant traits

Univariate regressions revealed a significant negative relationship between leaf herbivory and latitude (Fig. 2a). In sharp contrast, we found a positive relationship between the concentration of total phenolics and latitude (Fig. 2b), and this trend was consistent for most groups of phenolic compounds (Fig. 2c, e, marginal effect for hydrolysable tannins, Fig. 2f). The exception was for lignins, which were negatively associated with latitude (Fig. 2d). In addition, we found positive associations between latitude and leaf nitrogen and phosphorus concentration (Fig. 2g, h).

Correlates of latitudinal variation in leaf herbivory and plant traits

The path analysis indicated that the PCA axes of temperature, precipitation and soil variables were significantly associated with leaf condensed tannins (Fig. 3). Specifically, we found that *Q. robur* populations from colder and drier regions and growing on soils with high porosity (higher coarse fragments and lower bulk density, 'PC2 soil') exhibited higher concentrations of leaf condensed tannins (Fig. 3).

The path analysis also indicated that temperature (but not precipitation or soil variables) had a significant influence on leaf phosphorus concentration (Fig. 3), and that populations from colder regions had a higher concentration of phosphorus in leaves (Fig. 3). In addition, we found that temperature and precipitation (but not soil variables) significantly influenced leaf nitrogen concentration (Fig. 3), where populations from warmer and drier regions had higher levels of leaf nitrogen (Fig. 3).

Path analysis indicated a significant (negative) effect of condensed tannins (but not of phosphorus or nitrogen) on leaf herbivory, and further revealed significant indirect effects of PC2 (soil porosity) and precipitation (but not temperature) on leaf herbivory mediated by leaf condensed tannins (Fig. 3). There was no evidence of direct effects of climatic and soil factors on leaf herbivory after accounting for plant traits (Fig. 3).

Discussion

This study is unique in that it jointly considers latitudinal variation in leaf chemical defences, nutrient content, and herbivory, and further investigates the abiotic factors underlying such variation. We found strong but divergent latitudinal gradients in leaf herbivory and leaf chemical defences and nutritional traits in *Q. robur*. Oak populations

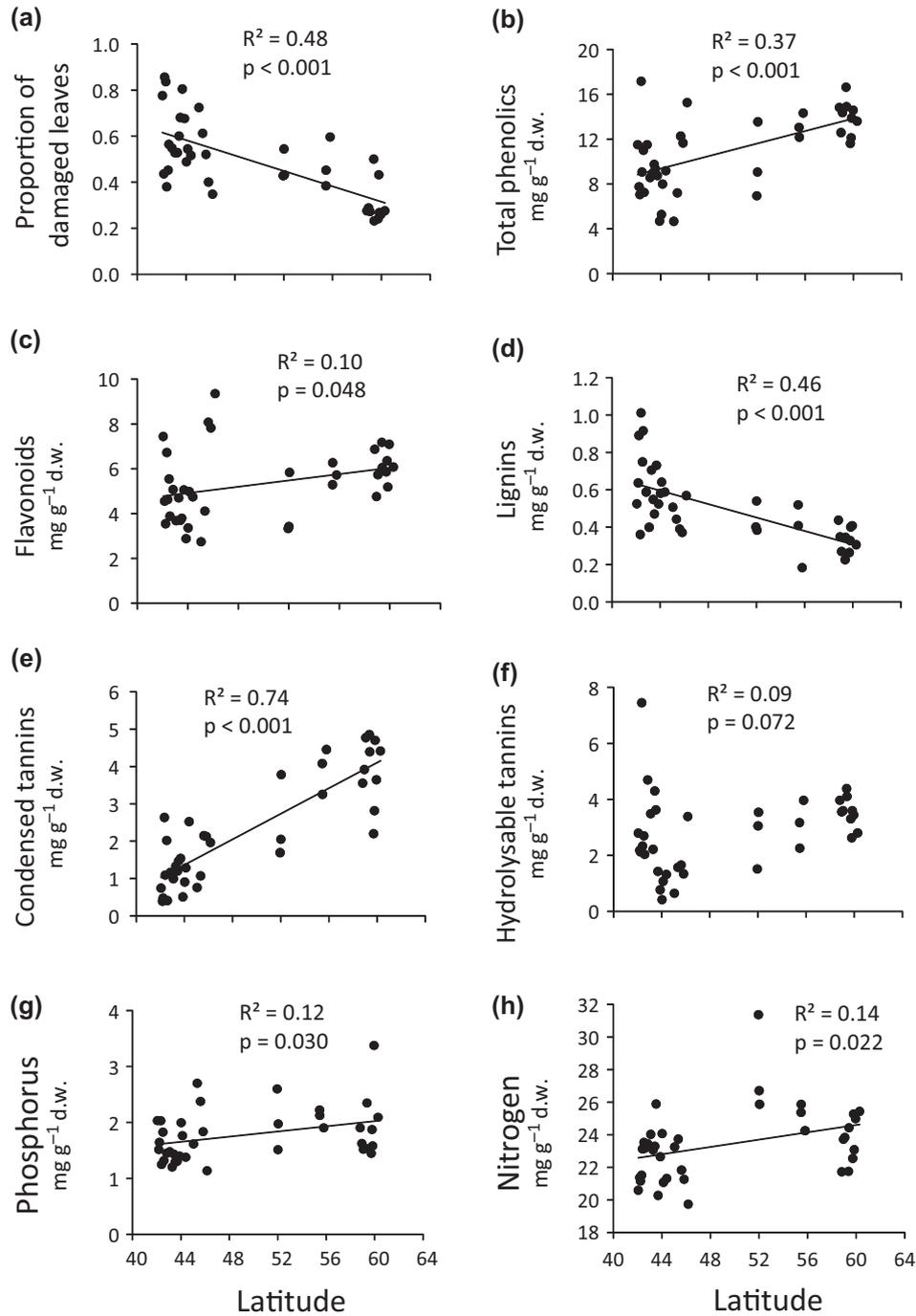


Figure 2. Latitudinal variation in (a) leaf herbivory (measured as proportion of herbivore-damaged leaves), (b) the concentration of total phenolics, (c) flavonoids, (d) lignins, (e) condensed tannins, (f) hydrolysable tannins, (g) phosphorus, and (h) nitrogen in leaves of *Quercus robur* individuals sampled from 38 populations distributed from northern Spain to southern Sweden. R-squared coefficients (R^2) and p-values are shown. Circles represent population means ($n = 5$ trees per population).

from lower latitudes exhibited higher levels of leaf herbivory, but had lower concentrations of defence compounds, phosphorus and nitrogen in leaves. Notably, path analysis indicated that the concentration of leaf condensed tannins (a key group of phenolic compounds) was the only plant trait associated with leaf herbivory in *Q. robur*, and the fact that this relationship was negative suggests that the latitudinal cline in leaf herbivory was driven by an inverse gradient in leaf tannin investment. Furthermore, although there

were no detectable direct effects of climatic and soil factors on herbivory, these abiotic factors (e.g. temperature and soil porosity) influenced leaf condensed tannin content and, in doing so, indirectly influenced herbivory. Together, these findings highlight the linkages between biotic and abiotic factors concurrently shaping geographic variation in plant defences and herbivory, and thus improve our understanding of the underlying processes driving latitudinal variation in plant defence and herbivory.

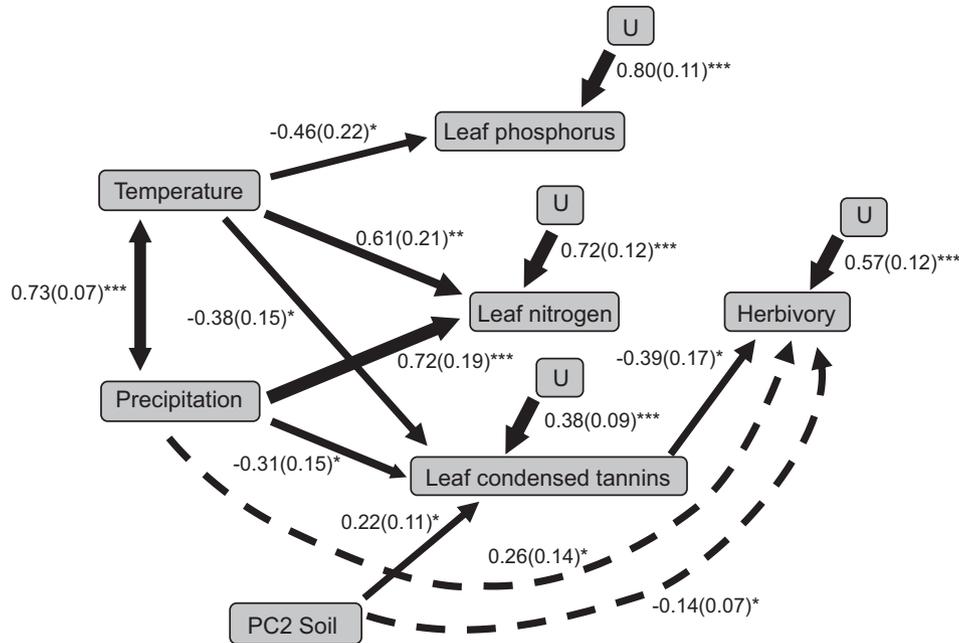


Figure 3. Path analysis model testing for the effects of climatic factors (temperature, precipitation) and soil factors (soil porosity) on *Quercus robur* leaf traits (condensed tannins, nitrogen and phosphorus), as well as effects of climate, soil, and leaf traits on leaf herbivory on this oak species. Plants of *Quercus robur* were sampled from 38 populations distributed along a latitudinal gradient from Spain to Sweden. Values next to each arrow represent path coefficients (i.e. standardized regression coefficients) and standard errors are provided in parenthesis in each case. Continuous arrows indicate direct effects whereas broken arrows indicate indirect effects, in this case of precipitation and PC2 soil on herbivory (mediated by leaf condensed tannins). Arrow thickness corresponds to the magnitude of the path coefficient. U=unexplained variance. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Only significant path coefficients are shown.

The assertion that plant species at lower latitudes experience higher rates of herbivory and that this should result in selection for greater investment in defence by plants has a long historical basis (Schemske et al. 2009, Johnson and Rasman 2011). In agreement with this prediction, we found that levels of herbivory on leaves of *Q. robur* markedly increased towards lower latitudes. Nonetheless, contrary to predictions, investment in leaf chemical defences showed an inverse trend where the concentration of most groups of phenolic compounds (except lignins) increased towards higher latitudes. Path analysis indicated a strong negative relationship between the herbivory and the concentration of condensed tannins, suggesting that these defensive compounds drive leaf damage. This confirms previous work showing that condensed tannins are abundant secondary metabolites in oak leaves (representing about 16% of dry weight; Barbehenn and Peter 2011), and confer resistance against leaf herbivores in several oak species (Feeny 1970, Forkner et al. 2004). Similar to our findings, Anstett et al. (2015) also found that latitudinal variation in the concentration of plant phenolic compounds was negatively associated with and presumably drove a latitudinal gradient in herbivory in leaves and fruits of the forb *Oenothera biennis*. To date, however, few studies have investigated the underlying factors driving latitudinal associations between plant defence and herbivory (but see Anstett et al. 2015, Abdala-Roberts et al. 2016a). Although our results indicate an association between latitudinal variation in phenolics and herbivory, it remains to be determined whether greater damage at lower latitudes is associated exclusively with lower plant defences or if it is also due to greater herbivore

abundance or species richness. Further work aimed at sampling the herbivore community is necessary to determine whether herbivore species richness and abundance are correlated with leaf damage and increase towards lower latitudes independently of plant chemical defences (Salazar and Marquis 2012). In addition, it is important to note that we assessed leaf herbivory at the end of the growing season and that the growing season is substantially longer in the southernmost populations (Spain) than in the northernmost populations (Sweden), such that leaves with the highest levels of damage might have been also the ones that were exposed to herbivores for a larger amount of time. Nonetheless, this does not take away that southern populations experience higher levels of leaf herbivory overall.

Recent work has shown that abiotic factors such as climatic and soil conditions are strongly associated with geographic variation in plant defences (Pearse and Hipp 2012, Moreira et al. 2014, 2015a). Similarly, our study showed that the concentration of condensed tannins in leaves of *Q. robur* was strongly influenced by both climatic factors and soil variables. Oak populations from colder and drier regions or growing on more porous soils (with lower water and nutrient retention), i.e. conditions frequently associated with low plant resource availability, exhibited higher investment in leaf condensed tannins. These findings agree with the resource availability hypothesis (Coley et al. 1985, Endara and Coley 2011), which holds that plants growing under resource-limited conditions exhibit slow growth, higher tissue construction costs, and thus higher investment in defences as tissues consumed by herbivores are costly to replace (Coley et al. 1985, Fine et al. 2004). Other recent

studies have similarly reported that resource availability and abiotic stress are important factors shaping latitudinal gradients in plant defences. For example, constitutive defences in pine species (*Pinus* spp.) increased at higher latitudes and altitudes with decreasing temperature (Moreira et al. 2014). In addition, precipitation was negatively associated with leaf phenolics across populations of the herb *Ruellia nudiflora*, suggesting that plants growing in sites with lower water availability (and presumably also lower resource availability) are more highly defended (Abdala-Roberts et al. 2016a). The fact that climate and soil conditions had strong effects on plant investment in phenolics and such compounds were associated with herbivory argues that the latitudinal association between plant defences and herbivory observed for *Q. robur* was driven to some extent by abiotic factors. Accordingly, we found that precipitation and soil porosity indirectly influenced herbivory, likely through their direct effects on the concentration of condensed tannins in leaves (Fig. 3), suggesting that abiotic factors shape investment in plant defences and that this, in turn, affects patterns of herbivory. Although additional work involving common gardens and manipulative field experiments (e.g. reciprocal transplants) is needed to test this hypothesis (Pennings et al. 2009), our study clearly demonstrates independent contributions of multiple abiotic factors to latitudinal trends in plant defence and herbivory.

Our results also revealed a latitudinal trend in the concentration of leaf nutrients where nitrogen and phosphorus both increased towards higher latitudes, as well as an influence of climate on such traits. Nonetheless, neither of these nutrients were significantly associated with leaf herbivory as indicated by the path analysis, suggesting that they did not underlie the latitudinal gradient in leaf herbivory. These findings are unresponsive of recent work arguing that nutrient concentrations in leaf tissues are strong predictors of herbivory, and in some cases may be more important than secondary compounds (Carmona et al. 2011). So, despite there being latitudinal variation in leaf nutrients, we speculate that throughout the sampled transect leaf nutrient levels were above a limiting threshold for leaf herbivores on *Q. robur*, beyond which increasing nutrient concentrations do not influence damage as herbivores have their nutrient requirements met. This could be the case in deciduous tree species (particularly in the case of deciduous oaks) which exhibit high rates of resource acquisition and high leaf nutrient concentrations (Reich et al. 1998).

An important aspect regarding the interpretation of our results is the extent to which the observed trends are products of plant local adaptation or phenotypic plasticity. Latitudinal studies often assume that natural selection imposed by herbivores has resulted in genetic differentiation among populations and that observed clines in plant traits reflect a process of adaptive evolution (particularly at broad spatial scales; Schemske et al. 2009, Woods et al. 2012). However, geographic variation in plant traits does not necessarily reflect population genetic differentiation, but may also be the result of phenotypic plasticity in response to environmental variation. Unfortunately, the nature of our data does not allow us to distinguish between these two possibilities. Although there are difficulties in

relating these results to manipulative experiments using seedlings or juvenile plants due to ontogenetic variation in long-lived species, reciprocal transplant experiments are ideal for separating genetic and environmental sources of trait variation, and latitudinal studies are slowly moving towards employing this type of experimental approach (Pennings et al. 2009). In this sense, work addressing intra-specific clines in species traits is key to determine how plant traits evolve along environmental gradients (Woods et al. 2012, Pratt and Mooney 2013). Further, a good handle on the factors driving intra-specific clines may inform and help us understand macro-ecological (i.e. among-species) patterns (Abdala-Roberts et al. 2016a, Anstett et al. 2016, Hahn and Maron 2016). Having said this, it is also important to note that there are some benefits from in situ measurements of plant traits versus common garden experiments. For example, the plant chemotypes being estimated in the field represent the phenotypes that herbivores encounter in the natural environment, regardless of the relative contribution of environment and genotype in shaping this phenotypic variation.

Research on latitudinal variation in plant defence and herbivory has resurged over the last decade (Schemske et al. 2009, Johnson and Rasmann 2011, Anstett et al. 2016), but the processes underlying such variation remain elusive. In this sense, our study delivers a broad-scale assessment of latitudinal variation in phenolic compounds and herbivory in a widespread tree species and sheds insight into the biotic and abiotic factors behind such variation. Research on latitudinal gradients is now starting to combine observational data with common garden experiments and reciprocal transplants (Salgado and Pennings 2005, Pennings et al. 2009, Anstett et al. 2015), and measurements of damage by multiple herbivore guilds are more common (Pennings et al. 2009, Anstett et al. 2014, Moreira et al. 2015a). Recent work also recognizes the importance of conducting a more comprehensive characterization of plant defences, spanning from inspection of trends for individual key compounds to assessing variation in defence syndromes (Pellissier et al. 2016), as well as differentiating between constitutive and induced defences (Moreira et al. 2014), and between tolerance and resistance (Więski and Pennings 2014). Addressing these aspects will contribute to develop more rigorous evaluations and a better understanding of latitudinal variation in plant–herbivore interactions.

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Supplementary material (Appendix ECOG-03326 at <www.ecography.org/appendix/ecog-03326>). Appendix 1.