Search for top-down and bottom-up drivers of latitudinal trends in insect herbivory in oak trees in Europe

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Abstract

Aim

The strength of species interactions is traditionally expected to increase toward the Equator. However, recent studies have reported opposite or inconsistent latitudinal trends in the bottom-up (plant quality) and top-down (natural enemies) forces driving herbivory. In addition, these forces have rarely been studied together thus limiting previous attempts to understand the effect of large-scale climatic gradients on herbivory.

Location

Europe

Time period

2018-2019

Major taxa studied

Quercus robur

Methods

We simultaneously tested for latitudinal variation in plant-herbivore-natural enemy interactions. We further investigated the underlying climatic factors associated with variation in herbivory, leaf chemistry and attack rates in *Quercus robur* across its complete latitudinal range in Europe. We quantified insect leaf damage and the incidence of specialist herbivores as well as leaf chemistry and bird attack rates on dummy caterpillars on 261 oak trees.

Results

Climatic factors rather than latitude *per se* were the best predictors of the large-scale (geographical) variation in the incidence of gall-inducers and leaf-miners as well as in leaf nutritional content. However, leaf damage, plant chemical defences (leaf phenolics) and bird attack rates were not influenced by climatic factors or latitude. The incidence of leaf-miners increased with increasing concentrations of hydrolysable tannins, whereas the incidence of gall-inducers increased with increasing leaf soluble sugar concentration and decreased with increasing leaf C:N ratios and lignins. However, leaf traits and bird attack rates did not vary with leaf damage.

Main conclusions

These findings help to refine our understanding of the bottom-up and top-down mechanisms driving geographical variation in plant-herbivore interactions, and urge for further examination of the drivers of herbivory on trees.

Key words: leaf chemistry, plant defences, avian insectivory, climate, artificial prey

Introduction

Ecological theory predicts that the strength of species interactions increases toward the Equator due to warmer temperatures, longer growing seasons, and higher species abundance and diversity at lower latitudes (Janzen, 1970; Schemske *et al.*, 2009). Plant species at lower latitudes commonly experience higher rates of herbivory than plants growing further from the equator (Coley & Barone, 1996; Schemske *et al.*, 2009; Lim *et al.*, 2015; Moreira *et al.*, 2018) and thus tropical plant species may evolve higher levels of anti-herbivore defences (Johnson & Rasmann, 2011; Pearse & Hipp, 2012; Abdala-Roberts *et al.*, 2016; Hahn *et al.*, 2019). While early reviews reported patterns supporting these predictions (Coley & Aide, 1991; Coley & Barone, 1996; Dyer

& Coley, 2002), several studies in recent decades have found either no evidence for a latitudinal gradient in herbivory and plant defences (Moles & Westoby, 2003; Gaston *et al.*, 2004; Moles *et al.*, 2011) or increase in herbivory and defences with latitude (Gaston *et al.*, 2004; Stark *et al.*, 2008; Adams *et al.*, 2009; Martz *et al.*, 2009; Pennings *et al.*, 2009; Del-Val & Armesto, 2010; Woods *et al.*, 2012; Moreira *et al.*, 2018, 2020). Given these inconsistencies, it is of great importance to identify the mechanisms underlying the substantial variation in herbivory and plant defences across latitudes, as herbivory is an important ecological process that modulates primary productivity by altering the recruitment, mortality and growth of plants.

Latitudinal gradients can be used as 'natural laboratories' to study the relationship between climate and plant-herbivore interactions (De Frenne *et al.*, 2013; Kozlov *et al.*, 2015; Lim *et al.*, 2015; Moreira *et al.*, 2018). In the northern extratropical hemisphere, mean annual temperature drops by 0.73 °C and mean annual precipitation by 4.04 mm per degree of latitude northward (De Frenne *et al.*, 2013). Latitudinal variation in plant-herbivore interactions is therefore generally associated with large-scale variability in climatic conditions (Moreira *et al.*, 2018) and numerous studies demonstrate an effect of temperature and precipitation on plant traits (e.g. leaf N, phenolic compounds) (Chen *et al.*, 2013; Holopainen *et al.*, 2018; Gely *et al.*, 2019) and herbivory (Jamieson *et al.*, 2015; Gely *et al.*, 2019). However, many regions deviate from the global trend in temperature and precipitation toward higher latitudes due to their proximity to oceans or the presence of mountains (De Frenne *et al.*, 2013), which can markedly change the relationship between latitude and plant-herbivore-predator interactions (Roslin *et al.*, 2017; Loughnan & Williams, 2019; Moreira *et al.*, 2019).

Recent work identified several potential sources of variation in the reported directions and strengths of latitudinal gradients in herbivory and plant defences (Johnson & Rasmann, 2011; Anstett *et al.*, 2016). First, theory on latitudinal gradients in herbivory and plant defences assumes a plant-centred equilibrium in which plants at low latitudes have adapted to higher herbivory levels by evolving stronger defences. However, most studies have measured either herbivory patterns or plant defences, but not both (but see Anstett *et al.*, 2015; Moreira *et al.*, 2018), leading to an incomplete understanding of the relationship between latitudinal clines and plant-herbivore interactions. Second, little attention has been paid to latitudinal variation in tritrophic dynamics (Roslin *et al.*, 2017). Herbivore natural enemies, however, can drastically modify tritrophic interactions by suppressing herbivore populations or reducing herbivore feeding (Rosenheim, 1998; Maguire *et al.*, 2015). In the few published studies exploring latitudinal patterns in natural enemy activity, authors have found no variation in parasitism (Dyer & Coley, 2002; Moreira *et al.*, 2015), lower attack rates on artificial prey by ants (Roslin *et al.*, 2017), and higher (Zvereva *et al.*, 2019) or no variation (Roslin *et al.*, 2017) in attack rates on artificial prey by birds with increasing latitude. Third, while external feeders are directly exposed to enemies and adverse abiotic conditions, internal feeders (e.g., leaf-mining and gall-inducing insect herbivores) benefit from a buffered microhabitat and relative protection against enemies. It is therefore likely that latitudinal trends in tritrophic interactions would vary across herbivore feeding guilds. Thus, considering bottom-up and top-down forces simultaneously could be crucial for a comprehensive understanding of latitudinal clines in tritrophic interactions.

We aimed to test for latitudinal variation in plant-herbivore-natural enemy (*i.e.*, tritrophic) interactions, as well as the underlying climatic factors associated with variation in herbivory, bottom-up and top-down forces in the pedunculate oak (*Quercus robur*), a long-lived, common European tree. In particular, we asked the following questions: (1) Are there latitudinal clines in herbivory? (2) Is latitudinal variation in leaf chemical traits (bottom-up effects) and/or bird attack rates (top-down effects) on herbivorous insects associated with latitudinal variation in herbivory? (3) Are climatic correlates of latitude associated with clines in herbivory, leaf chemical traits and attack rates? We used data collected by professional scientists and schoolchildren across major parts of the geographical distribution range of *Q. robur*. We quantified insect leaf herbivory, leaf chemical traits (soluble sugars, nutrients and phenolics) and attack rates on dummy caterpillars placed on mature oak trees. Overall, our study attempted to refine our understanding of bottom-up and top-down mechanisms that may drive geographical variation in plant-herbivore interactions.

Material and methods

The present study involved 30 professional scientists from 14 countries and 82 school teachers (with their pupils) from 10 countries, giving a total of 112 partners from 17 European countries and covering most of the native geographic range of the pedunculate oak **(Figure 1)**. Every partner received detailed instructions at the beginning of the project (Castagneyrol *et al.*, 2019). Here, we only provide a summary of these instructions. Only project partners who provided data that could be used in the present article were included.

Target species

The pedunculate oak is one of the dominant deciduous tree species in European forests and is of high ecological, economic and symbolic importance (Eaton *et al.*, 2016). Its distribution ranges from Central Spain (39°N) to southern Fennoscandia (62°N), thus this species experiences variable climatic conditions (Petit *et al.*, 2002). Pedunculate oak supports a large community of specialist and generalist herbivorous insects; especially suckers, chewers, skeletonizers, gall-inducers and leaf-miners (Southwood *et al.*, 2005; Moreira *et al.*, 2018), as well as xylophagous species (Marković & Stojanović, 2011). The wide distribution of pedunculate oak and the high diversity of associated herbivorous insects make it a suitable model species for research on the effect of climate on biotic interactions.

In total, the study included 261 mature oak trees surveyed by professional scientists (n = 115) and schoolchildren (n = 146) in 2018 (n = 148) and 2019 (n = 113) (**Figure 1**). However, not every partner measured or provided material allowing measurement of herbivory, bird attack rates and leaf chemistry simultaneously on every tree (Figure S1.1a, b and c, supplementary material).

Attack rates on dummy caterpillars

To control for latitudinal variation in environmental conditions, we matched the start of the experiment in each locality to the phenology of local oak trees. Six weeks after oak budburst, partners installed 20 dummy caterpillars per tree, *i.e.*, five caterpillars on each of four branches (facing north, south, east and west) with a minimum distance of 15 cm between caterpillars.

The project coordinators provided the same green plasticine (Staedler, Noris Club 8421, green[5]) to all partners to make the caterpillars. In order to standardize caterpillar size among partners, we made caterpillars from a 1 cm diameter ball of plasticine, and gently pressed/rolled this along a 12 cm long metallic wire until a 3 cm long caterpillar was obtained, with the wire in its center. Partners attached the caterpillars to branches by twisting the wire and left the caterpillars on trees for 15 days before recording predation marks. A second survey using the same procedure immediately followed the first one. In 2018, schoolchildren photographed every caterpillar with the suspected attack marks from any potential predatory taxon. In 2019, both schoolchildren and professional scientists sent caterpillars back to the project coordinators.

In order to be consistent and reduce bias due to multiple observers, photos and dummy caterpillars were screened by a single trained observer (first author, EVC). For each oak tree and survey period, we assessed attack rate as the proportion of dummy caterpillars with at least one attack mark. Although we asked partners to record attack rate marks left by different types of predators (in particular birds and arthropods), attacks by arthropod predators could not be verified on photos because of their low resolution. In addition, the relevance of marks left by arthropods on plasticine model prey has recently been questioned, in particular after mandibular marks were observed on lizards or frog models (Rößler *et al.*, 2018). For these reasons, we decided to discard arthropod attack rate from the study and focused on marks that were unambiguously attributed to birds, *i.e.*, conic holes or V-shaped beak marks. Attack marks left by reptiles or rodents were also disregarded, because only a few caterpillars were attacked by these potential predators. Most bird marks were directed towards the head or the body centre of the dummy caterpillars, which is typical to bird attacks and indicates prey recognition (Rößler *et al.*, 2018). We therefore refer to the proportion of dummy caterpillars with such marks as bird attack rate.

Between 2018 and 2019, 137 partners installed 12,760 dummy caterpillars on 319 oak trees. Despite clear instructions regarding caterpillar installation, removal and conditioning prior to shipping, the material sent by 22 school partners was of poor quality (with no particular geographic bias) such that only caterpillars returned by 115 partners (*i.e.*, 78.4%, collected on 254 oak trees) were screened for attack marks and included in subsequent analyses (**Table S1.1; Figure 1**).

Leaf herbivory

Professional scientists and schoolchildren were instructed to collect oak leaves after the second bird attack rate survey, *i.e.*, roughly 10 weeks after oak budburst, on the same branches where dummy caterpillars were installed. They haphazardly collected 30 leaves per branch, totalling 120 leaves from which they blindly drew 60 leaves. Professional scientists oven-dried leaves for a minimum of 48 h at 45°C immediately after collection, and leaves collected by schoolchildren were oven dried upon receipt by the project coordinators, to ensure optimal conservation prior to herbivory assessment.

We used three response variables to characterise leaf herbivory: leaf damage (the percentage of leaf area that was consumed or mined by insect herbivores), incidence of leaf-miners (the proportion of leaves with leaf-mines) and incidence of gall-inducers (the proportion of leaves with galls). For each leaf, we visually assessed leaf damage (attributed to ectophagous chewing and leaf-mining organisms) following eight levels of defoliation (0%, >0-5%, >5-10%, >10-15%, >15-25%, >25-50%, >50-75%, and >75%). We then averaged leaf damage at the tree level using the midpoint of each percentage class to obtain a mean value per tree. While this measurement also included the surface covered by leaf mines (*i.e.*, internally chewed by mining larva), it excluded both galls and punctures made by sap feeders. Leaf assessment was made by two trained observers who were blind to leaf origin to reduce unconscious bias. We expect that most of the leaf damage will be attributable to insects, as in our experience, mollusc herbivory (e.g. snails and slugs), although possible, is rare in adult oak trees. As of mites, they mostly cause discolouration that can easily be differentiated from insect herbivory. There are also few mammals consuming oak leaves, mostly rodents and ungulates, but usually they mostly consume seedlings and saplings.

Leaf chemical traits

We used leaves collected in 2018 to quantify several leaf chemical traits typically recognized as important determinants of plant quality for insect herbivores associated with oaks. Details of procedures used to analyse chemical leaf traits are reported in online Appendix S1.1.

We quantified leaf phenolics as oak defensive metabolites (Moreira *et al.*, 2018). We used only leaves collected by professional scientists in 2018. Unfortunately, we were not able to quantify other leaf defences that need to be estimated on fresh leaves (e.g. leaf toughness) because leaves were oven dried after collection to ensure optimal conservation. From each tree, we selected 10 mature, dried leaves with no evidence of insect damage and ground them to fine powder. We identified four groups of phenolic compounds: flavonoids, ellagitannins and gallic acid derivatives ("hydrolysable tannins" hereafter), proanthocyanidins ("condensed tannins" hereafter) and hydroxycinnamic acid precursors to lignins ("lignins" hereafter) (see Appendix S1.1 for further details).

We quantified C:N ratio, N:P ratio, cellulose and soluble sugars as proxies for leaf nutritional content to herbivores (Moreira *et al.*, 2019) as in many plant taxa these variables are correlated with herbivory (Mattson, 1980; Schoonhoven *et al.*, 2005; Smilanich *et al.*, 2016). We measured these traits on leaves collected by both professional scientists and schoolchildren. We ground the 50 oven dried leaves on which we scored herbivory to fine powder such that leaf nutritional traits reflected the content of leaves with different amounts of herbivore damage (see Appendix S1.1 for further details).

Statistical analysis

We were primarily interested in testing the effect of latitude on herbivory and in identifying the underlying ecological forces. We aimed to test whether the effect of latitude was driven by latitudinal gradients in climatic conditions, and by their direct and indirect consequences on leaf chemistry, herbivory and bird attack rate. We obtained temperature and precipitation data from the WorldClim database (Hijmans *et al.*, 2005) based on oak coordinates as retrieved on Google maps by project partners. Specifically, we extracted the mean temperature and precipitation from April to June, which roughly corresponded to the period when caterpillars were present on trees, irrespective of latitudinal cline in moth phenology. Yet, latitude was correlated with both temperature (Pearson's r = -0.85) and precipitation (r = -0.72). To avoid spurious estimates of model coefficients caused by collinearity among predictors, we tested the effects of latitude and climate separately.

Specifically, we built three types of Linear Mixed-effects Models (LMM): (i) geographic models analysing the effect of latitude on herbivory, leaf chemistry and bird attack rate, (ii) climatic models in which we substituted latitude with climatic data (temperature and precipitation) and (iii) abiotic and biotic models analysing the effects of leaf chemistry and bird attack rate together with temperature and precipitation or latitude (both linear and quadratic) on herbivory. As latitudinal gradients in plant-herbivore interactions can be non-linear (following Rodríguez-Castañeda 2013 and Kozlov *et al.*, 2015), we complemented our linear analyses with quadratic models. As leaf chemistry was only measured on a subset of trees (n = 64), we used a subsample of the dataset to quantify relationships between herbivory with its top-down and bottom-up drivers (Table S1.1).

In every LMM, we used Partner ID as a random factor to account for the fact that some partners surveyed multiple trees. For instance, the geographic models were of the form:

$$Y = \beta_0 + \beta_1 \times \text{Year} + \beta_2 \times \text{Partner} + \beta_3 \times \text{Latitude} + \beta_4 \times \text{Latitude}^2 + \gamma + \varepsilon + \sigma^2_{\text{Partner ID}}$$

where Y was the response variable, β_i model coefficient parameter estimates, Partner was the effect of partner type (the estimate for schoolchildren being compared with the estimate for professional scientists that was included in the intercept), Year was the effect of each year (2019 contrasted with 2018), Latitude (and their quadratic terms) the geographic conditions around sampled oak trees, $\sigma^2_{\text{Partner ID}}$ the random effect of Partner ID (assuming that $\gamma \in N(0, \sigma^2_{\text{Partner ID}})$ and ε the residuals (assuming $\varepsilon \in N(0, \sigma^2_e)$). When Y was bird attack rate, we added the survey (first vs. second) as a fixed effect and Tree ID as a random effect nested within Partner ID to account for repeated measurements on the same trees. When needed, we used arcsine square-root (bird attack rate) or ln (x + 1) transformation (leaf damage, soluble sugars, N:P ratio and leaf defences) of the response variable to satisfy model assumptions.

We ran geographic and climatic models on the complete dataset including 2018 and 2019 data collected by both professional scientists and schoolchildren. Note that because not every partner provided reliable data on both bird attack rates and herbivory, the sample sizes differed between models using bird attack rate or herbivory as response variables **(Figure 1, Figure S1.1a and b)**. We ran the geographic and climatic models on leaf phenolics as well as the biotic model on the 2018 data collected by scientific partners only, as we did not quantify leaf defences on leaves collected and sent by schoolchildren.

The tree-level response variables for each year and survey period (Y) were either leaf damage (% of leaf area removed and mined by herbivores), the incidence of leaf-miners or gall-inducers (proportions), mean bird attack rate (ratio of % attacked caterpillars on exposition period) or leaf chemistry (C:N ratio, N:P ratio, soluble sugar content [g L^{-1}], cellulose content (g), concentrations of condensed or hydrolysable tannins, flavonoids or lignins [mg g^{-1} d.m.]). We scaled and centred every continuous predictor prior to modelling to facilitate comparisons of their effect sizes, and made sure that none of the explanatory variables were strongly correlated using the variance inflation factor (VIF) (all VIFs < 5). We used LMM with a Gaussian error distribution, with the exceptions of geographic, climatic and process-based models with the incidence of leaf-miners or gall-inducers as response variables. In these cases, we used Generalized LMM with a binomial error distribution and logit-link.

We analysed the data within the information theory framework (Burnham & Anderson, 2002). We first built a set of geographic and climatic models as well as nested models for each response variable separately. Biotic models (models including also leaf chemistry and bird attack rates as explanatory variables) were run on the subset of samples where all data were measured simultaneously. We then applied a procedure of model selection based on AIC corrected for small sample size (AICc). In the first step, we ranked the models according to the difference in AICc between a given model and the model with the lowest AICc (ΔAICc). Models within 2 ΔAICc units of the best model (*i.e.*, the model with the lowest AICc) are generally considered as equally likely. We also computed AIC weight (w_i) that is the probability a given model to be the best model among the set of candidate models examined, as well as the relative variable importance (RVI) as the sum of w_i of every model including this variable. When several models competed with the best model (*i.e.*, when multiple models were such that their ΔAICc < 2), we applied a procedure of multimodel inference building a consensus model including the variables in the set of best models. We then averaged their effect size across all the models in the set of best models, using variable w_i as a weighting parameter (*i.e.*, model averaging). We considered that a given predictor had a statistically significant effect on the response variable when its confidence interval excluded zero.

In the results section, we report the degrees of freedom, log-likelihood, AICc, ΔAICc (delta), w_i and the variance explained by fixed and fixed plus random factors (R^2m and R^2c, respectively) (Nakagawa & Schielzeth, 2013) for every model, as well as averaged coefficient parameter estimates and RVI for all variables present in the set of competing best models. When appropriate, we plotted the relationship between raw data and explanatory variables together with the predictions of simplified models, holding undisplayed predictors constant. All analyses were run in the R language environment (Team, 2018) with packages *MuMIn* (Bartoń, 2018) and *lme4* (Bates *et al.*, 2018).

Results

Latitudinal and climatic gradients in herbivory, leaf chemistry and bird attack rates

Herbivores damaged on average (± se) 8.7 ± 0.4 % of leaf area (n = 182 trees, see **Table S1.1** for details). Model simplification identified the null model as the best model given the model set, indicating that none of the predictors had a consistent effect on leaf damage (**Figure 2j**, **k** and **l**, **Table S2.1**).

Insect galls were present on 7.1 ± 0.6 % of the inspected leaves (n = 182, **Table S1.1**). In the set of best models (**Table S2.1; Figure S1.3**), the incidence of gall-inducers increased linearly with increasing spring temperature (**Figure 2e**) and peaked at intermediate levels of spring precipitation (**Figure 2f**). It was on average higher in 2018 than in 2019 (**Figure S1.2**). Other predictors had no significant effects on the incidence of gall-inducers (**Figure 2d**).

Leaf-miners were present on 18.2 ± 1.3 % of the inspected leaves (**Table S1.1**). In the set of best models (**Table S2.1; Figure S1.3**), the incidence of leaf-miners peaked at intermediate mean spring temperatures (**Figure 2h**) and decreased linearly with increasing spring precipitation (**Figure 2i**). It was significantly higher in 2018 than in 2019 (**Figure S1.2**), and higher in leaves sampled by professional scientists than in those sampled by schoolchildren.

Some oak traits related to nutritional content, but not phenolic compounds, covaried with climate and latitude (**Table S1.1**). Specifically, leaf soluble sugar content (3.7 ± 0.2 $g \cdot L^{-1}$, n = 114, **Table**

S1.1) decreased with increasing precipitation (**Figure 3a**). Leaf C:N ratio (18.6 ± 0.2 , n = 114, **Table S1.1**) increased non-linearly with latitude (with concave up shape, **Figure 3b**) and was on average lower in leaves collected by professional scientists than those collected by schoolchildren. None of the predictors had a significant effect on N:P or cellulose content **(Table S1.1)**.

From a total of 10,000 exposed dummy caterpillars, 2,390 had bird beak marks (*i.e.*, 23.9%). Model selection identified the null model as the best model, with no other competing model within two units of ΔAICc of the best model.

Mechanisms underlying latitudinal and climatic variation in herbivory

Using a data subset limited to trees for which information on herbivory, leaf traits and bird predation rates was available, model selection identified the null model as the best model, indicating that none of the examined biotic and abiotic predictors had a significant effect on leaf damage (**Table S2.2**).

When leaf chemistry was included in the model, the incidence of gall-inducers increased with increasing soluble sugar concentration and decreased with increasing C:N ratio and lignin concentration (**Figure 4**), whereas the positive relationship between temperature and gall-inducers disappeared. When leaf traits were included in the models, the incidence of gall-inducers increased non-linearly with increasing latitude. The relative importance of leaf chemistry predictors (RVI = 0.65) was however higher than that of latitude (RVI = 0.05) or temperature (RVI = 0.30, **Figure S1.4**).

Leaf-miner incidence increased with increasing concentration of hydrolysable tannins. The relationship between temperature and leaf-miners remained significant, suggesting independent effects of leaf defences and temperature on leaf-miners. Other predictors had no significant effects on leaf-miners (**Figure 4**; **Table S2.2**).

Discussion

We found no evidence that either herbivory, oak chemical traits or bird attack rates varied with latitude linearly or non-linearly. Our work therefore supports the growing number of studies that have recently questioned the common view that biotic interactions are generally stronger at lower latitudes (Moles *et al.*, 2011; Mottl *et al.*, 2020). Several arguments may explain the absence of latitudinal patterns in herbivory or plant defences. On the one hand, Moles & Ollerton (2016) argued that the latitudinal herbivory-defence hypothesis should simply be dismissed because it is not convincingly supported despite decades of research. On the other hand, Anstett *et al.* (2016) called for a refinement of the concepts and methods in the field. We align with the latter perspective and henceforth discuss ecological sources of variation that may have obscured latitudinal patterns, and argue these could be real rather than methodological artefacts.

Herbivory responded to climate rather than to latitude, but in a guild-specific manner— Variation in the incidence of gall-inducers and leaf-miners, but not in leaf damage, was associated with variation in temperature and precipitation, rather than with latitude *per se* (Anstett *et al.*, 2018; Moreira *et al.*, 2018; Loughnan & Williams, 2019). The absence of a climatic or geographic effect on leaf damage is in line with previous reviews and meta-analyses that have shown herbivory does not vary consistently along climatic or geographic gradients (Moles *et al.*, 2011; Moles & Ollerton, 2016). Our analysis suggests that different herbivore species or guilds may be differently affected by abiotic conditions, such that grouping different types of herbivores may prevent the detection of patterns for each herbivore type (Abdala-Roberts *et al.*, 2015; Moreira *et al.*, 2015; Anstett *et al.*, 2016).

In line with this explanation, we found that the incidence of gall-inducers and leaf-miners was associated with broad scale climatic conditions. Specifically, the incidence of both gall-inducers and leaf-miners increased with increasing temperature, but the shape of this relationship was accelerating for gall-inducers and decelerating (*i.e.*, convex) for leaf-miners (**Figure 5**). Although we did not identify species of leaf-miners, this result is in line with that of Kozlov *et al.* (2013) who found that in northern Europe, the diversity of leaf miners on birch trees increased linearly toward lower latitudes and was most likely associated with the direct impact of temperature, especially during cold years. We also found that the incidence of gall-inducers peaked at intermediate precipitation (Blanche & Ludwig, 2001; Leckey *et al.*, 2014) whereas leaf-miners decreased significantly with precipitation. It has been hypothesized that endophagous feeding modes such as galling and mining have evolved partly as adaptation to abiotic factors such as UV radiation and desiccation (Fernandes & Price, 1992; Connor *et al.*, 1997; Danks, 2002). If so, gall-inducers and leaf-miners may be expected to be more common in the warmest and driest parts of the pedunculate oak range and at low latitudes where the light intensity is markedly higher (Fernandes & Price, 1992; Lara & Fernandesrs, 1996; Price *et al.*, 1998; Cuevas-Reyes *et al.*, 2004). However, even within the gall-inducer and leaf-miner groups, relationships to climate are highly variable among species and years (Blanche, 2000; Sinclair & Hughes, 2010; Kozlov *et al.*, 2013, 2016), thus suggesting that other factors are also important in the incidence of gall-inducers and leaf-miner herbivores.

Leaf chemical traits had an inconsistent response to latitude and climate—

We did not find detectable latitudinal and climatic gradients in plant chemical defences. This contradicts the Latitudinal Herbivory Defence Hypothesis which predicts that plant species at lower latitudes experience higher mean rates of herbivory than their temperate counterparts (Coley & Barone, 1996; Schemske *et al.*, 2009; Lim *et al.*, 2015) and, for this reason, should have evolved higher levels of anti-herbivore defences (Rasmann & Agrawal, 2011; Pearse & Hipp, 2012). However, the generality of this hypothesis is currently under debate (Moles & Ollerton, 2016). Several studies found no evidence for a latitudinal gradient in herbivory and plant defences

(Moles *et al.*, 2011) while others did (Salgado & Pennings, 2005; Woods *et al.*, 2012); there is also mixed evidence when comparing different herbivore species or plant defensive traits (Anstett *et al.*, 2015; Moreira *et al.*, 2015, 2018). A plausible explanation for the lack of latitudinal gradients in oak defences may be that there is no latitudinal gradient in leaf damage, hence there is no reason for latitudinal gradient for defences to exist either. It could also be that we sampled leaves at the middle of the growing season rather than at the end, and we did not measure constitutive and induced defences separately. This is an insightful point because oak leaves may have differentially accumulated phenolics in response to herbivory (*i.e.*, induced defences) or have experienced marked differences in light intensity toward the end of the growing season (Karolewski *et al.*, 2013). Furthermore, despite attempts to synchronize phenology across sites, seasonal changes in oak chemical defences (Salminen & Karonen, 2011) might have masked latitudinal patterns in defences. Therefore, further studies should include measurements at multiple time points during the growing season and distinguish between different types of defences, including physical vs. chemical defences (Wang *et al.*, 2018) as well as constitutive vs. induced defences (Anstett *et al.*, 2018) in order to address latitudinal gradients in plant defence more comprehensively.

Some leaf traits related to leaf nutrient content were associated with latitude or climatic conditions, but their overall response was inconsistent. Leaf C:N ratio and sugar content varied along latitudinal and climatic gradients, respectively. The leaf C:N ratios were lowest at intermediate latitudes. This outcome may be due to temperature-related plant physiological stoichiometry and biogeographical gradients in soil substrate age (limitation of soil N at higher latitudes) (Reich & Oleksyn, 2004). Leaf soluble sugar content decreased with increasing precipitation (Cao *et al.*, 2018). Soluble sugars, especially glucose and fructose, accumulate together with other osmolytes during drought (Nio *et al.*, 2011), resulting in high concentration in areas where precipitation is low.

Predation rate was not influenced by latitude or climatic conditions—

We found no latitudinal or climatic gradients in bird attack rates on dummy caterpillars (**Figure 5**). These results agree with the large-scale study performed by Roslin *et al.* (2017) who found an increase of the activity of predatory arthropods in several plant species toward the Equator, but no significant trend in avian predation. Several factors may explain the lack of response of avian predation to latitudinal or climatic gradients. First, some bird species are distributed through migration allowing them to breed at higher latitudes, resulting in a constant predation rate across climatic and geographical clines (Dufour *et al.*, 2020). In contrast, other predators with lower mobility such as arthropods (e.g. ants, ladybirds) are much more abundant at lower latitudes, resulting in a higher selection pressure toward the Equator (Roslin *et al.*, 2017). Second, bird communities are more influenced by forest habitat composition at lower latitudes, and more by food availability at higher latitudes (Charbonnier *et al.*, 2016) where the diet variability is lower

(Barnagaud *et al.*, 2019), suggesting a stronger effect of local habitat features (e.g. resource availability and habitat suitability) than climatic gradients. Third, we cannot exclude that the lack of latitudinal trend in bird attack rates resulted from methodological limitations due to the fact that we only exposed green dummy caterpillars in low hanging branches. Birds depend more on food accessibility than abundance *per se*, so that the exact location of dummy caterpillars regarding factors such as edge, light contrast and shrubby understory may have modified the perception and the accessibility to the prey (Zvereva *et al.*, 2019).

Mechanisms underlying latitudinal and climatic variation in herbivory—

We did not find any statistically significant relationship between leaf damage and leaf chemical traits. Although we cannot exclude that unmeasured traits (e.g. leaf toughness, inducible defences) may have correlated with herbivory, our results disqualify large-scale variation in bottom-up forces as important drivers of overall leaf damage at a continental scale. In contrast, the incidence of gall-inducers and leaf-miners was partially related to the variability in several leaf chemical traits (**Figure 5**). For instance, the incidence of gall-inducers increased with increasing leaf soluble sugars and N concentrations, which is consistent with gall-inducers being metabolic sinks (Huang *et al.*, 2014). However, the effect of precipitation on leaf-miners was likely indirectly mediated by climatic variation in defences, as such an effect became non-significant once hydrolysable tannins were included in the model. Similarly, the effects of temperature and precipitation on gall-inducers were indirectly mediated by climatic variation in defences, as such effects were also non-significant after soluble sugars, N concentrations and lignins were included in the models. These results agree with previous studies reporting indirect effects (via leaf defences) of climate on herbivory (Anstett *et al.*, 2018; Moreira *et al.*, 2018). For instance, Anstett *et al.* (2018) found indirect effects of climate on herbivory in 80 species of evening primroses, which were mediated by leaf chemicals (total phenolics and oenothein A). However, these conclusions need to be considered with caution because the dataset used to test the effect of bottom-up and top-down forces on herbivory along large-scale latitudinal and climatic gradients of the biotic and abiotic models only consisted of a subset of the complete dataset used in the geographic and climatic models.

We found no evidence that bird attack rate drove large-scale variability in herbivory. This result is in line with a recent study by Zverev *et al.* (2020) who found that birds are unlikely to shape the spatial patterns of insect herbivory in an Arctic ecosystem. More generally, associations between bird insectivory and insect herbivores can be positive (Mäntylä *et al.*, 2014; Gunnarsson *et al.*, 2018), negative (Maguire *et al.*, 2015; Kozlov *et al.*, 2017) or non-significant (Moreira *et al.*, 2019; Valdés-Correcher *et al.*, 2019), depending on the study and methods used. Arthropod predators (e.g. ants, ladybirds) play an important role in limiting herbivore populations and may respond to large-scale variation in climatic conditions at greater extent than vertebrate predators (Roslin *et al.*, 2017; Zvereva *et al.*, 2019). For example, a meta-analysis conducted by Rodríguez-Castañeda

(2013) found that ant predation on herbivores significantly increase at higher temperatures and precipitations, indicating that plants growing under warmer and wetter conditions exhibit lower levels of herbivory. Besides, birds are considered intraguild predators that not only eat insect herbivores but also arthropod predators (Gunnarsson, 2007) and intraguild predation may weaken herbivore suppression (Finke & Denno, 2005). Unfortunately, we were not able to quantify predation rates by such arthropods nor intraguild predation, which weakens our conclusions about the potential role of predators across climatic gradients.

Conclusion — By simultaneously investigating bottom-up and top-down forces driving herbivory along latitudinal and climatic clines in a widespread tree species in Europe, this study brings some new insights into the vivid debate about latitudinal variation in the direction and strength of biotic interactions (Schemske *et al.*, 2009; Moles *et al.*, 2013; Anstett *et al.*, 2016; Roslin *et al.*, 2017). We found no evidence that latitude or climate influenced insect herbivores feeding on oaks, but we found that climatic factors rather than latitude *per se* were the best predictors of the large-scale variation in the incidence of leaf-miner and gall-inducer herbivores as well as in variation in leaf nutritional content. In sharp contrast, we found no evidence that plant chemical defences and bird attack rates were influenced by latitude or climatic factors, which conflicts with the dominant view in ecology (Moles & Ollerton, 2016; Roslin *et al.*, 2017; Zvereva *et al.*, 2019). Because unravelling causes of latitudinal variation in the strength of biological interactions is one of the common approaches for the prediction of biotic interactions under global warming (Verheyen *et al.*, 2019), it is crucial that future studies simultaneously test for effects of latitude *per se* and climate on herbivory by different feeding guilds (Kozlov *et al.*, 2017), as well as investigate the complexity of biotic interactions in which plant-herbivores interactions are embedded.

Data accessibility

The datasets supporting this article are available via an open-access repository (https://doi.org/10.5061/dryad.18931zcw0).

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Biosketch

Elena Valdés-Correcher: Interested on plant-herbivore interactions. More specifically, she investigate the effect of different drivers of plant herbivore interactions including the effect of landscape composition, climate and tree genotype on plant-herbivore relationships.

Bastien Castagneyrol: Interested on citizen science and on the ecology of plant-herbivore interactions including predation, insect herbivory and leaf traits and how these relationships are influenced by factors that act at different scales.

Xoaquín Moreira: Interested on plant-herbivore interactions. More specifically, interested on the effect of latitude and elevation on biotic relationships.

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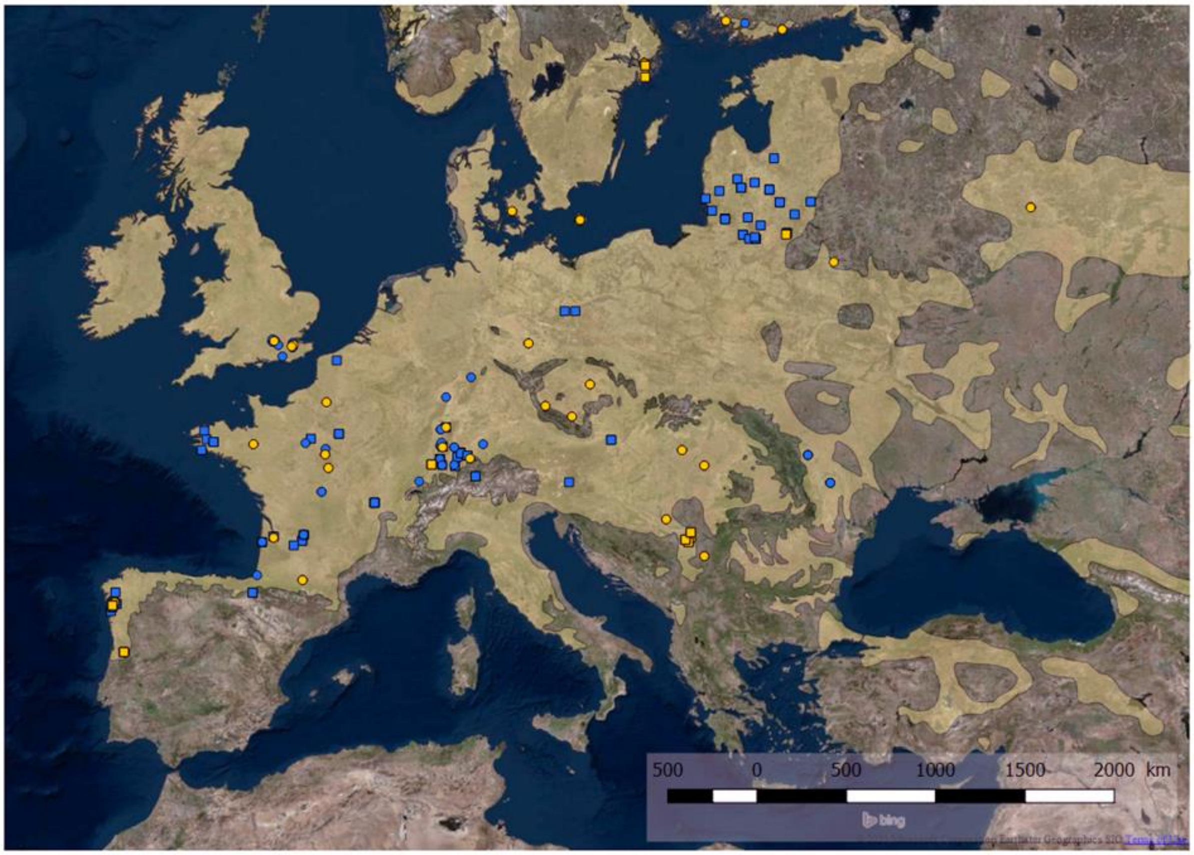

Figure 1. Distribution range of *Quercus robur* L. (shaded in yellow) and locations of trees sampled by professional scientists (orange symbols, 30 sites) and schoolchildren (blue symbols, 82 sites) in 2018 (circles, 57 sites) and 2019 (squares, 55 sites). Additional maps showing oak trees used for estimating leaf herbivory, attack rates on dummy caterpillars and trait analyses are provided in supplementary material (Figure S1.1).

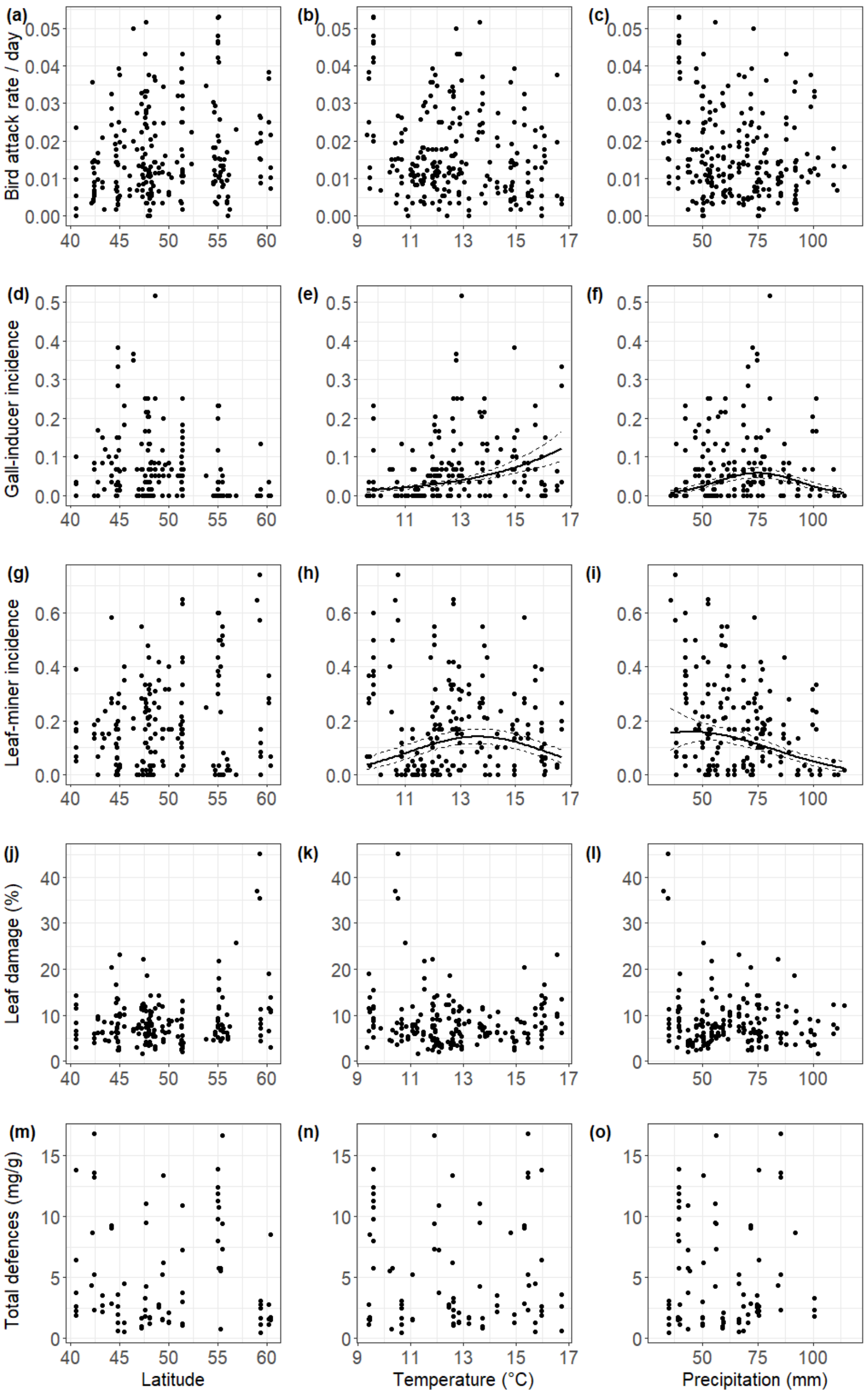

Figure 2. Effects of Latitude, mean spring temperature and mean spring precipitation on bird attack rates (a, b, c), gall-inducers incidence (d, e, f), leaf-miners incidence (g, h, i), leaf damage (j, k, l) and total phenolics (m, n, o). Dots represent raw data averaged at the tree level. Solid and dashed lines represent model predictions (and corresponding standard error) calculated after other significant variables (see Table S2.1) were set to their mean value. Only statistically significant relationships are shown. Regression line equations are as follows: **e**, $y = -3.32 + 0.44 \cdot x$; **f**, $y = -3.32 + 0.32 \cdot x - 0.51 \cdot x^2$; **h**, $y = -1.98 + 0.37 \cdot x - 0.36 \cdot x^2$; **i**, $y = -1.98 - 0.44 \cdot x$.

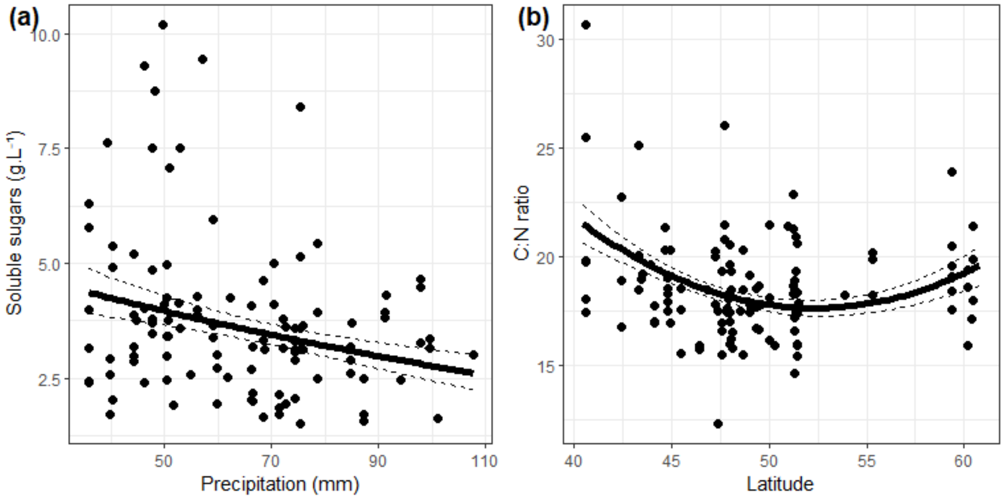

Figure 3. Effect of mean spring precipitation and latitude on soluble sugar (a) and C:N ratio (b) on leaves, respectively. Dots represent raw data averaged at tree level. Solid and dashed lines represent model predictions (and corresponding standard error) for temperature and latitude calculated after other significant variables (see Table S2.2) were set to their mean value. Only significant relationships are shown. Regression line equations are as follows: **a**, $y = 1.53 - 0.10 \cdot x$; **b**, $y = 17.9 - 0.86 \cdot x + 0.70 \cdot x^2$.

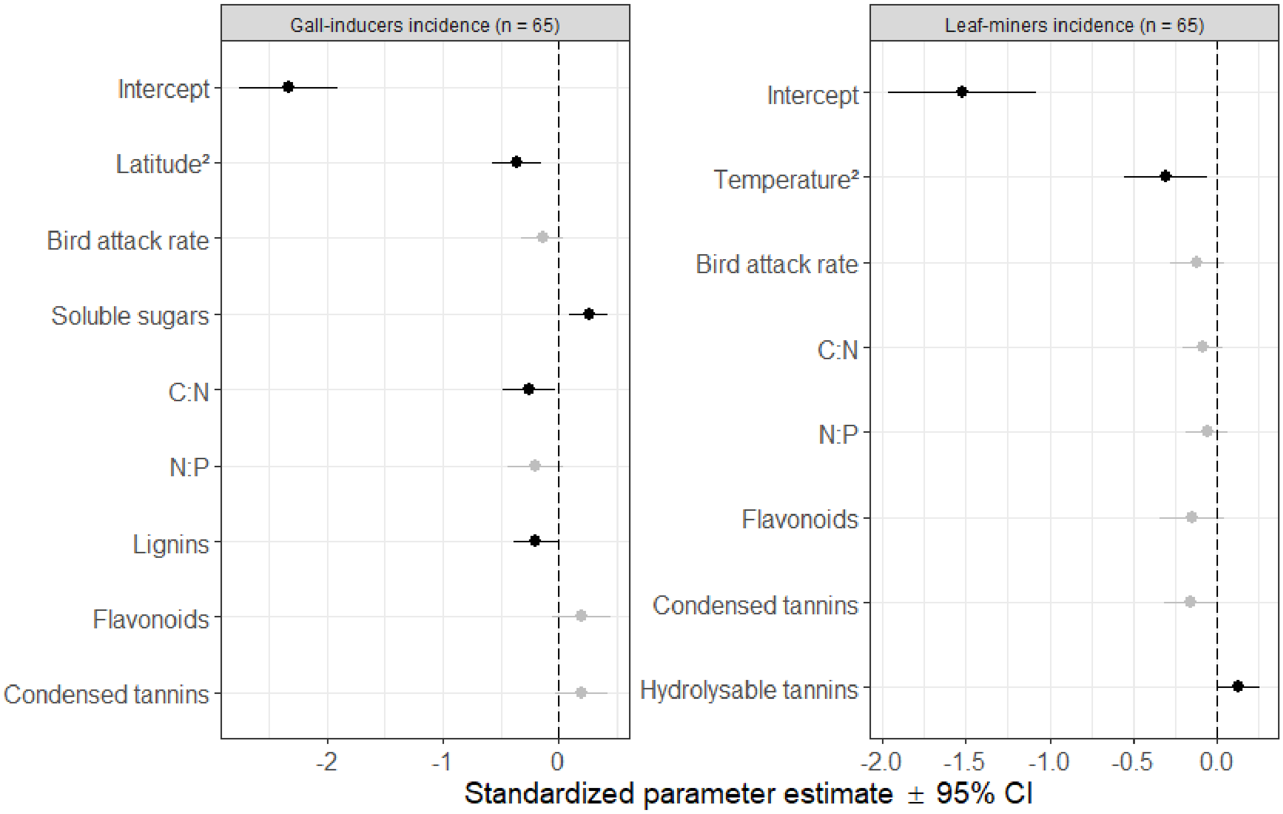

Figure 4. Effects of latitude, mean spring temperature, mean spring precipitation and leaf chemistry on gall-inducer (left) and leaf-miner (right) incidences. Circles and error bars represent standardized parameter estimates and corresponding 95% CI. The vertical dashed line centred on zero represents the null hypothesis. Black and grey circles represent significant and non-significant effect sizes, respectively.

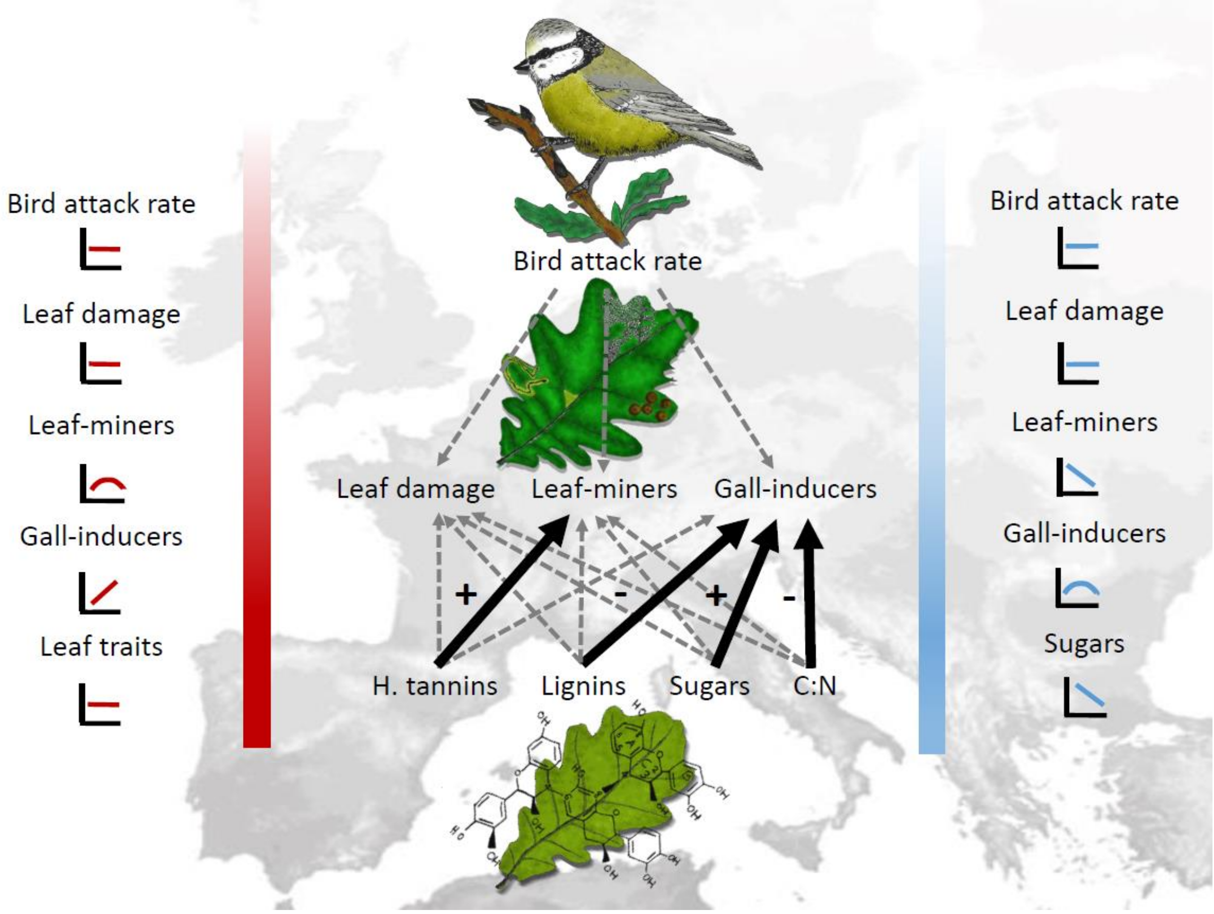

Figure 5. Summary illustrating plant-herbivore-predator relationships along a latitudinal gradient in Europe. The red and blue bands denote the variation in mean spring temperature and precipitation, respectively. The five figures on the left represent the correlation between the mean spring temperature and bird attack rate, leaf damage, the incidence of gall-inducers and leaf-miners and leaf traits. The five figures on the right correspond with the correlation between mean spring precipitation and bird attack rate, leaf damage, the incidence of leaf-miners and gall-inducers and the concentration of soluble sugar in leaves. Solid black arrows represent significant positive (+) or negative (-) relationships; dashed grey lines indicate non-significant relationships.

Figure S1.1. Location of the trees sampled for the assessment of herbivory (a), predation attack rate (b), leaf nutritional content (c) and leaf defences (d). An interactive version of these maps are also included in the supplementary material as Figures S1.1a, S1.1b, S1.1c and S1.1D.

Interactive version of the maps:

Figure S1.1a. Trees sampled for the assessment of herbivory.

Figure S1.1b. Trees sampled for the assessment of predation attack rate.

Figure S1.1c. Trees sampled for the assessment of leaf nutritional content.

Figure S1.1d. Trees sampled for the assessment of leaf defences.

Figure S1.2. Effects of partner type, year, mean spring temperature and mean spring precipitation on gall-inducers and leaf-miners incidences. Circles and error bars represent standardized parameter estimates and corresponding 95% CI. The vertical dashed line centered on zero represents the null hypothesis. Black and grey circles indicate significant and non-significant effect sizes, respectively.

Figure S1.3. Importance of every variable (RVI) included in the geographic and climatic models that considered the effect of longitude, latitude, temperature and precipitation on herbivory (gall-inducers and leaf-miners incidences; n = 182) and on leaf chemistry (soluble sugar and C:N ratio; n = 114).

Figure S1.4. Importance of every variable (RVI) included in the biotic model that considered the effect of leaf traits, bird attack rate, climatic variables on gall-inducers and leaf-miners incidence (n = 65).

Table S1.1. Summary of the different variables measured.

Table S2.1. Summary of model coefficient parameter estimates (i.e., effect sizes), degrees of freedom (df), log-likelihood, AICc, ΔAICc, AICc weight (w_i) and the variance explained by fixed (*R2m*) and fixed plus random factors (*R2c*) of the different climatic models. The gradient of colours from red to green corresponds to the effect size, from large negative (red) to large positive (green) effect sizes. The set of models competing with the best model within 2 units of ΔAICc is highlighted in bold font. *Partner type* is the effect of partner type (the estimate for schoolchildren being compared with the estimate for professional scientists that was included in the intercept) and *Year* is the effect of each year (2019 contrasted with 2018).

Table S2.2. Summary of model coefficient parameter estimates (i.e., effect sizes), degrees of freedom (df), log-likelihood, AICc, ΔAICc, AICc weight (w_i) and the variance explained by fixed (*R2m*) and fixed plus random factors (*R2c*) of the different climatic models. The gradient of colours from red to green corresponds to the effect size, from large negative (red) to large positive (green) effect sizes. The set of models competing with the best model within 2 units of ΔAICc is highlighted in bold font.