

# Intraspecific herbivory variability, rather than species' turnover, drives latitudinal patterns in community-wide herbivory

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## Abstract

Attempts over the past 30 years to explain geographic variation in the strength of herbivore pressure gave rise to the latitudinal herbivory hypothesis. However, this long-standing hypothesis has rarely been tested using community-level data. In this study, we selected 43 grassland sites along a 1,500-km latitudinal gradient (c. 27°N to 39°N) on the Qinghai-Tibetan Plateau. We calculated community-wide herbivory at each site by summing herbivory across plant species weighted by the biomass of each species; we then investigated how abiotic and biotic latitudinal correlates drove community-wide herbivory via their effects on intraspecific herbivory variability and species' turnover. We found that community-wide herbivory decreased with latitude, mirroring intraspecific herbivory variability. Furthermore, intraspecific herbivory variability was driven by climatic factors, but not edaphic or plant community factors. Overall, our study highlights the importance of considering both intraspecific herbivory variability and species' turnover for predicting how climate change will alter community-wide herbivory.

## Running head: Latitudinal gradient in community-wide herbivory

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## AUTHORSHIP

ML conceived and designed the study. XL, ML, KH, YX, XW, LZ and PZ collected the data. ML, XL and ZL analysed the data. ML and XL wrote the first draft. All authors provided critical feedback on manuscript draft and approved the final manuscript.

## DATA AVAILABILITY STATEMENT

The data used in this paper can be accessed via Data available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.7h44j0zwv>.

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The authors declare no competing financial interests.

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## Abstract

Attempts over the past 30 years to explain geographic variation in the strength of herbivore pressure gave rise to the latitudinal herbivory hypothesis. However, this long-standing hypothesis has rarely been tested using community-level data. In this study, we selected 43 grassland sites along a 1,500-km latitudinal gradient ( $c. 27^{\circ}\text{N}$  to  $39^{\circ}\text{N}$ ) on the Qinghai-Tibetan Plateau. We calculated community-wide herbivory at each site by summing herbivory across plant species weighted by the biomass of each species; we then investigated how abiotic and biotic latitudinal correlates drove community-wide herbivory via their effects on intraspecific herbivory variability and species' turnover. We found that community-wide herbivory decreased with latitude, mirroring intraspecific herbivory variability. Furthermore, intraspecific herbivory variability was driven by climatic factors, but not edaphic or plant community factors. Overall, our study highlights the importance of considering both intraspecific herbivory variability and species' turnover for predicting how climate change will alter community-wide herbivory.

**Keywords:** alpine meadow, community composition, community weighted mean, plant defense, plant-herbivore interactions, latitudinal herbivory hypothesis

## Introduction

Increases in species' abundances and diversity towards the equator are well documented (Dobzhansky, 1950; Willig *et al.*, 2003), and may be associated with a heightened intensity of biotic interactions at lower latitudes (*i.e.*, the "biotic interactions hypothesis"; Mittelbach *et al.*, 2007; Schemske *et al.*, 2009). Evidence for stronger biotic interactions in the tropics has been found for many mutualisms and antagonisms (Schemske *et al.*, 2009; Zvereva & Kozlov, 2021); however, evidence for latitudinal gradients in herbivory, one of the most ecologically significant biotic interactions, remains mixed (Anstett *et al.*, 2016; Zhang *et al.*, 2016).

Herbivory is particularly important because it involves a large proportion of the biodiversity and energy flows in terrestrial ecosystems (Becerra, 2007; Agrawal *et al.*, 2012). The classical latitudinal herbivory hypothesis (LHH) suggests that plant losses to herbivory decrease with latitude (Coley & Aide, 1991; Johnson & Rasmann, 2011). Although this hypothesis has been popular in the literature, its generality has been called into question in the last ten years because latitudinal trends in herbivory tend to be highly variable in both strength and direction (Moles *et al.*, 2011; Moles & Ollerton, 2016).

Some biases among LHH studies may partly explain the inconsistency in patterns reported thus far. The LHH was initially formulated as a community-level hypothesis, in which herbivory was expected to be greater in tropical versus temperate communities (Coley & Aide, 1991; Coley & Barone, 1996). However, many recent studies have focused on herbivory gradients within specific clades or even single species (*i.e.*, species found along a latitudinal gradient), rather than community-wide herbivory (see review in Anstett *et al.*, 2016). Such non-random, species-level measurements of herbivory are likely to bias conclusions regarding both absolute levels of herbivory and spatial patterns in community-wide herbivory (Zvereva & Kozlov, 2019). Furthermore, plant species may be unevenly distributed among communities (varying in abundance), and thus the results of LHH studies may be affected by species-specific factors, as well as limited by species' ranges (Anstett *et al.*, 2016). Therefore, direct measurements of community-wide herbivory are better suited to studies of the LHH, as originally proposed, and essential for understanding the consequences of herbivory for biodiversity and ecosystem functioning (*e.g.*, nutrient cycling, productivity and trophic transfer rates) (Anstett *et al.*, 2016; Zvereva *et al.*, 2020).

Abiotic and biotic correlates of latitude can potentially affect community-wide herbivory through both intraspecific and interspecific pathways. At the intraspecific level, abiotic and biotic factors can drive variability in herbivory by directly influencing the abundance of insect herbivores (Maron *et al.*, 2014) and/or by indirectly altering plant susceptibility to herbivore damage (Moreira *et al.*, 2018; Loughnan & Williams, 2019). For example, plant populations growing under more benign climates may suffer greater herbivore damage, as longer growing seasons increase the number of insect generations and warmer winters result in lower insect mortality (Sakata *et al.*, 2017). In addition, plants growing in high resource environments may also invest less in defense traits (Hahn & Maron, 2016) and are therefore more susceptible to herbivore damage.

Apart from causing intraspecific variability in herbivory, latitudinal abiotic and biotic correlates are also predicted to alter the composition and diversity of natural plant assemblages and to modify the abundance of individual plant species (*i.e.*, community evenness) (Yang *et al.*, 2011; Ma *et al.*, 2017), ultimately affecting community-wide herbivory. These "species turnover effects" result from species' differences in susceptibility to herbivory and also levels of adaptation to abiotic environmental conditions (Agrawal, 2007). For example, the resource availability hypothesis posits that lower-resource environments can select plant species with slow growth rates, but enhanced herbivore resistance (Coley *et al.*, 1985; Hahn & Maron, 2016). Individual species differences lead to habitat-driven turnover in plant community composition, potentially resulting in divergent patterns of community-wide herbivory over space. Overall, abiotic and biotic latitudinal correlates can affect community-wide herbivory through both intraspecific and/or interspecific pathways. However, the relative importance of these two pathways remains largely uninvestigated.

To test for latitudinal patterns in community-wide herbivory and to evaluate the relative roles of intraspecific herbivory variability versus species' turnover effects, we conducted a survey of both population- and community-wide herbivory along a 1,500-km latitudinal gradient in the grasslands of the Eastern Qinghai-Tibetan Plateau. Grasslands are the dominant ecosystem on the Qinghai-Tibetan Plateau, a diverse region with highly variable abiotic conditions (Yao, 2019); grassland communities in this region vary dramatically

in composition, productivity and species richness, and plant defense traits have been shown to vary along environmental gradients within the region (Xiao *et al.*, 2021). In this study, we asked: 1) Does community-wide herbivory decrease with latitude and, if so, what is the relative importance of intraspecific herbivory variability versus species' turnover in driving this latitudinal pattern? 2) What abiotic (*i.e.*, climatic and edaphic factors) and biotic (*i.e.*, plant community factors) factors drive latitudinal variation in population- and community-wide herbivory, and how are these drivers related to intraspecific herbivory variability and/or species' turnover effects?

## Materials and methods

### Study region and study sites

In July 2021, at the peak of the growing season, we surveyed 43 field sites spanning a latitudinal gradient of 26.95–38.60°N and a longitudinal gradient of 96.40–103.41°E (Figure 1). Our survey covered the full latitudinal range for grasslands occurring in the Eastern Qinghai-Tibetan Plateau. At the survey sites, the mean annual temperature and mean annual precipitation ranged from -3.0 to 9.3°C and from 250 to 1,350 mm, respectively. This represents a significant climatic gradient, capable of driving variation in resident plant communities (Zhang *et al.*, 1988).

Sampling sites were situated far from any cities and can be considered representative areas for natural vegetation. The sites were chosen based on the following criteria: (i) at least 500 m away from major roads; (ii) under pristine or unmanaged conditions without visible signs of domestic animal grazing, grass/wood collection, human-engineered restoration plantings, and/or human infrastructure. We recorded geographical information (*i.e.*, longitude, latitude and altitude) for each sampling site, before establishing four 0.5 × 0.5 m quadrats at the corners of a 50 × 50 m square. The quadrats were used to survey plant community composition, the aboveground biomass and herbivory of each species present, and the associated soil properties. Plant specimens were sorted to species level and identified using the “Flora of China” (Wu *et al.*, 2013). Samples from each species were clipped from the quadrats for herbivory assessments and weighed (to determine the aboveground biomass) after drying at 65°C for 72 h to a constant weight.

### Community-wide herbivory measurements

We recorded foliar herbivory for each species following the methods outlined in Chen *et al.* (2019). For each plant species in a quadrat, we randomly sampled 25 leaves from at least five individuals. For those species with less than 25 leaves, we sampled all leaves available. To quantify herbivory severity, we predefined six damage categories according to how much of the leaf was consumed: 0%, <5%, 5–10%, 10–20%, 20–50% and >50% (Scherber *et al.*, 2010; Ness *et al.*, 2011). For needlelike leaves (*e.g.*, from *Kobresia myosuroides*), all incidences of leaf wounding were placed into the <5% category as field observations revealed that insect predators only ever consumed the tip of the leaves.

We calculated the overall herbivory percentage (ranging from 0% to 100%) for each plant species by first multiplying the number of leaves in each damage class by the median removal value for that class (*i.e.*, 0%, 2.5%, 7.5%, 15.0%, 35.0% and 75.0%). Obtained values were then summed across all damage classes and the sum divided by the total number of leaves (including undamaged ones) in a sample.

We calculated community-wide herbivory (hereafter, CWM herbivory) as the summed species' (herbivory) means weighted by their abundance (Chen *et al.*, 2019) using the following formula:

$$CWM \text{ herbivory} = \frac{\sum_{i=1}^S a_i h_i}{\sum_{i=1}^S a_i}$$

where  $S$  is the total number of plant species in a quadrat,  $a_i$  is the aboveground biomass of plant species  $i$ , and  $h_i$  is the herbivory on plant species  $i$ .

Using the method proposed by Lepš *et al.* (2011) to decompose effects into their direct and indirect components (*i.e.*, “intraspecific variability” and “turnover”, respectively), we subtracted species' turnover

effects from the total CWM herbivory to obtain the intraspecific variability in herbivory among sampling sites. Turnover effects represent the expected CWM herbivory (based on constituent host plant species) independent of the actual herbivory measured in a given plot (Chen et al., 2019).

### Plant defense trait measurements

We investigated whether abiotic and biotic factors influenced intraspecific variation in herbivory by altering the susceptibility of individual plants to herbivore damage. We also quantified plant chemical defenses in three common species (*Polygonum macrophyllum*, *Saussurea pulchra* and *Taraxacum mongolicum*). Among all species identified in the study survey, these three species had the broadest latitudinal distributions (Figure S1).

We measured the total flavonoid, saponin and tannin contents; these compounds are widely used by plants as toxins (*i.e.*, flavonoids and saponins, Treutter, 2005; Faizal & Geelen, 2013) and digestibility reducers (*i.e.*, tannins, Feeny, 1968). They are also protective against abiotic stresses; for example, flavonoids play a role in drought resistance and UV radiation stress tolerance (Treutter, 2005). The quantification of these defense chemicals is described in *Supplementary Methods*.

### Climatic data availability

To characterize the climatic conditions at each site, we extracted eight variables from the BIOCLIM database (Hijmans *et al.*, 2005; available online): BIO1 (mean annual temperature, °C; AT), BIO4 (temperature seasonality, expressed as the standard deviation of temperature across months; ST), BIO5 (maximum temperature of the warmest month, °C; MAXT), BIO6 (minimum temperature of the coldest month, °C; MINT), BIO12 (mean annual precipitation, mm; AP), BIO13 (precipitation of the wettest month, mm; WP), BIO14 (precipitation of the driest month, mm; DP) and BIO15 (precipitation seasonality, expressed as the standard deviation of precipitation across months; SP). These variables include annual climatic means, climatic extremes and seasonality aspects.

### Soil physicochemical property measurements

To characterize the soil properties at each site, we collected six upper mineral soil cores (0–10 cm) from random locations within each quadrat and mixed them thoroughly to produce a composite sample. The soil samples were then stored at 4 for later analysis. Samples were first sieved through a 2-mm screen to remove stones and visible plant roots, before air-drying at 25. Soil pH and electrical conductivity (mc/cm; EC) were determined using a 1:5 (w/v) soil-to-water solution. Soil total carbon (mg/g; Soil C) and total nitrogen (mg/g; Soil N) were measured on ground soil samples using an elemental analyzer (Elementar vario MACRO cube, Germany). Soil water content (SWC, %) was determined gravimetrically by quantifying the weight lost after oven-drying 100 g of fresh soil at 105 for 48 h.

### Statistical analyses

To summarize the climatic variables (AP, AT, DP, MAXT, MINT, SP, ST and WP) and soil properties (EC, pH, Soil C, Soil N and SWC), we employed the “rda” function from the R package “vegan” (v. 2.5.7; Oksanen *et al.*, 2020) to conduct two principal component analyses (PCAs): one for climate and one for edaphic factors. For the climate PCA, PC1 was negatively correlated with AP, AT, DP, MINT and WP and positively correlated with MAXT, SP and ST; in the soil PCA, PC1 was negatively correlated with pH and positively correlated with EC, Soil C, Soil N and SWC. We then calculated Spearman rank-order correlations among the climatic, geographic, plant community, plant defense and soil variables. Implementing the “diversity” function for each plot, we also calculated plant species richness (Richness), the Shannon diversity index (Shannon) and Pielou’s evenness index (Pielou). To summarize plant community composition, a detrended correspondence analysis (DCA) was used (implemented using the “decorana” function), given the potential for arch effects in plant community composition data (Borcard *et al.*, 2011).

We evaluated how altitude, latitude and longitude affected CWM herbivory, intraspecific variability and

turnover using a series of linear mixed-effect models; these were implemented using the “lmer” function, with “site” as a random effect, and the corresponding t-values, P-values and marginal  $R^2$  values were calculated. Various climatic, geographical, plant community and soil factors were also used as independent variables in linear mixed-effect models to test their effects on CWM herbivory, intraspecific variability and turnover. We then compared these models with a null model (*i.e.*, an intercept-only model) using Akaike’s information criterion corrected for small sample sizes ( $AIC_c$ ); comparisons were performed using the  $AIC_c$  function in the “AICcmodavg” package (v. 2.3.1; Mazerolle, 2020). The log-likelihood (LL) was calculated for each model using the “logLik” function and  $AIC_c$ -based parameters, including the change in  $AIC_c$  relative to the top-ranked model ( $\Delta AIC_c$ ),  $AIC_c$  weight ( $w AIC_c$ ) and the marginal  $R^2$  (Burnham *et al.*, 2011). Linear mixed-effect models (with “site” as a random effect) were also used to test the effects of “better” predictors (*i.e.*, predictors better than the null model as determined using  $AIC_c$ ) on CWM herbivory, intraspecific variability and turnover.

We then built a piecewise structural equation model (piecewiseSEM) to test how the climatic, plant community and soil variables affected CWM herbivory through intraspecific variability and turnover effects. In the SEM, standardized path coefficients (scaled by their mean and standard deviation) were calculated, as well as the corresponding significance (P-values) for each path; the overall fit of both full and final models was determined using Fisher’s C statistic and  $AIC_c$  using the “piecewiseSEM” package (v. 1.3.2; Lefcheck 2016).

For population-level herbivory, we tested how altitude, latitude and longitude affected herbivory in *Polygonum macrophyllum*, *Saussurea pulchra* and *Taraxacum mongolicum* using linear mixed-effect models with “site” as a random effect. Linear mixed-effect models were also used to predict population-level herbivory using various climatic, geographic, plant community, plant defense and soil factors;  $AIC_c$ ,  $\Delta AIC_c$ ,  $w AIC_c$  and the marginal  $R^2$  served as indicators of model goodness of fit. All statistical analyses were performed in R v4.1.1 (R Development Core Team, 2021).

## Results

### Community-wide herbivory

Consistent with our hypothesis, we found that community-wide (CWM) herbivory decreased as latitude increased from N 26°59’ to N 38deg34’ (Slope = -0.203,  $R^2 = 0.079$ ,  $P = 0.012$ ) (Figure 2); this latitudinal pattern remained even after accounting for the effects of longitude and altitude (Slope = -0.155,  $P = 0.069$ ) (Table S1). Model selection based on  $AIC_c$  suggested that both latitude ( $AIC_c = 222.557$ ,  $w AIC_c = 0.525$ ) and altitude ( $AIC_c = 224.140$ ,  $w AIC_c = 0.238$ ) better predicted community-wide herbivory than did the null (intercept-only) model ( $AIC_c = 224.391$ ,  $w AIC_c = 0.210$ ) (Table 1). Among the climatic factors, temperature seasonality decreased CWM-herbivory (Slope = -0.005,  $R^2 = 0.107$ ,  $P = 0.003$ ), while precipitation in the wettest month (Slope = 0.023,  $R^2 = 0.101$ ,  $P = 0.004$ ) and mean annual precipitation (Slope = 0.004,  $R^2 = 0.098$ ,  $P = 0.004$ ) increased CWM-herbivory (Table 1; Figure S2). As for the edaphic factors, soil nitrogen (Slope = 0.185,  $R^2 = 0.078$ ,  $P = 0.003$ ) and soil water content (Slope = 0.037,  $R^2 = 0.076$ ,  $P = 0.005$ ) increased CWM-herbivory, while soil pH showed the opposite effect (Slope = -0.639,  $R^2 = 0.093$ ,  $P = 0.003$ ) (Table 1; Figure S2). Community-wide herbivory was not associated with any plant community factors in the linear mixed-effect models (Table 1).

Intraspecific variability and species’ turnover represent additive components of community-wide herbivory; intraspecific variability increased with latitude (Slope = -0.131,  $R^2 = 0.054$ ,  $P = 0.018$ ), while turnover was not related to altitude, latitude or longitude (Figure 2). Among the predicted factors, turnover was not well explained by any climatic, plant community or soil factors (Table S2). Meanwhile, soil electrical conductivity increased intraspecific variability (Slope = 0.007,  $R^2 = 0.053$ ,  $P = 0.013$ ), but temperature seasonality (Slope = -0.003,  $R^2 = 0.051$ ,  $P = 0.022$ ) and soil pH (Slope = -0.405,  $R^2 = 0.059$ ,  $P = 0.010$ ) decreased intraspecific variability (Table S3; Figure S3).

### Structural equation modeling

Given the high collinearity among the climatic and edaphic factors (Figure S4), we extracted the first PCA axis for both sets of variables: PC1 explained 94.36% and 68.99% of the variance for the climatic and edaphic variables, respectively (Table S4, S5). The final piecewise SEM (standardized path coefficients provided in Table S6; Figure S5), which adequately fit the data (Fisher's  $C = 10.302$ ,  $d.f. = 8$ ,  $P = 0.244$ ;  $AIC_c = 54.302$ ), explained 63.7% of the variance in species' turnover ( $R^2 = 0.637$ ) and 23.1% of the variance in intraspecific variability in herbivory ( $R^2 = 0.231$ ) (Figure 3). In the final piecewise SEM, intraspecific variability decreased with the climatic PC1 (standardized path coefficient  $\beta = -0.265$ ,  $P = 0.016$ ), while it was not affected by the plant community composition DC1 ( $\beta = 0.128$ ,  $P = 0.222$ ) or soil PC1 ( $\beta = 0.023$ ,  $P = 0.808$ ); this suggests that climatic effects on intraspecific herbivory variability drove changes in community-wide herbivory along the study latitudinal gradient. The plant community composition DC1 ( $\beta = -0.251$ ,  $P = 0.222$ ), but not the climatic PC1 ( $\beta = -0.091$ ,  $P = 0.487$ ) or soil PC1 ( $\beta = 0.098$ ,  $P = 0.291$ ), decreased species' turnover effects.

### Population-level herbivory

Herbivory on three common plant species, *Saussurea pulchra* (Slope = -0.946,  $R^2 = 0.352$ ,  $P = 0.012$ ), *Taraxacum mongolicum* (Slope = -0.583,  $R^2 = 0.220$ ,  $P = 0.009$ ) and *Polygonum macrophyllum* (Slope = -1.273,  $R^2 = 0.151$ ,  $P = 0.041$ ), decreased consistently with latitude (Figure 4). Precipitation of the wettest month (Slope = 0.214,  $R^2 = 0.438$ ,  $P = 0.005$ ), mean annual precipitation (Slope = 0.030,  $R^2 = 0.414$ ,  $P = 0.006$ ) and soil N (Slope = 1.191,  $R^2 = 0.309$ ,  $P = 0.001$ ) increased, but temperature seasonality decreased (Slope = -0.035,  $R^2 = 0.418$ ,  $P = 0.003$ ), herbivory on *S. pulchra* (Table S7; Figure S6). Mean annual precipitation (Slope = 0.016,  $R^2 = 0.269$ ,  $P = 0.004$ ), precipitation of the wettest month (Slope = 0.099,  $R^2 = 0.254$ ,  $P = 0.004$ ) and electrical conductivity (Slope = 0.039,  $R^2 = 0.200$ ,  $P = 0.014$ ) increased, but temperature seasonality (Slope = -0.017,  $R^2 = 0.238$ ,  $P = 0.006$ ) and soil pH (Slope = -2.932,  $R^2 = 0.317$ ,  $P = 0.001$ ) decreased, herbivory on *T. mongolicum* (Table S8; Figure S7). Mean annual precipitation (Slope = 0.056,  $R^2 = 0.240$ ,  $P = 0.010$ ), minimum temperature of the coldest month (Slope = 1.071,  $R^2 = 0.187$ ,  $P = 0.031$ ) and tannin content (Slope = 0.418,  $R^2 = 0.193$ ,  $P = 0.003$ ) increased, but temperature seasonality decreased (Slope = -0.037,  $R^2 = 0.183$ ,  $P = 0.032$ ), herbivory on *P. macrophyllum* (Table S9; Figure S8). Plant community factors did not explain significant amounts of variation in herbivory for any of the three common species (Table S7, S8, S9). In addition, the biomass of each species in each site did not significantly vary with latitude.

### Discussion

This study is unique in that it systematically assessed a latitudinal gradient in community-wide herbivory and further explored the mechanistic underpinnings of this gradient. Our assessment of community-wide herbivory provides strong evidence for the latitudinal herbivory hypothesis. Moreover, our results suggest that the observed latitudinal variation in community-wide herbivory was primarily driven by decreasing intraspecific values of leaf herbivory along the latitudinal gradient (*i.e.*, intraspecific herbivory variability), rather than by shifts in plant community composition (*i.e.*, species' turnover effects). Meanwhile, we found that climate also played a major role in driving intraspecific herbivory variability, ultimately affecting latitudinal variation in community-wide herbivory. Together, these findings highlight the importance of community-wide herbivory in macroecological research on plant-herbivore interactions.

### Intraspecific herbivory variability, rather than species' turnover effects, determined latitudinal variation in community-wide herbivory

The evidence found here for greater herbivory at lower latitudes is in agreement with the LHH, and concurs with studies of other systems including salt marsh plants (Pennings & Silliman, 2005; Pennings *et al.*, 2009) and terrestrial plants (Anstett *et al.*, 2014; Baskett & Schemske, 2018). However, to the best of our knowledge, our study is the first to show a decrease in community-wide herbivory with latitude. Several studies have conducted multispecies analyses by sampling diverse sets of representative species in two or more locations (*e.g.*, Pennings *et al.*, 2009; Zvereva *et al.*, 2020); however, this is no substitute for the direct evaluation of community-wide herbivory in most instances (Zvereva *et al.*, 2020). When considering single species, a decline in herbivory at higher latitudes might also result from a reduction in

abundance; abundance is a well-studied species-level feature positively affecting herbivory (the so-called “resource concentration effect”; Underwood *et al.* , 2014; Kim & Underwood, 2015). Therefore, earlier studies, which did not account for the abundance of individual plant species, likely yielded biased estimates of community-wide herbivory and, consequently, of the overall effects of background herbivory on ecosystem-level processes (Anstett *et al.* , 2016; Zvereva *et al.* , 2020; Moreira *et al.* , 2021).

Latitudinal variation in community-wide herbivory is expected to result from two additive components, intraspecific herbivory variability and species’ turnover effects. In this study, we found that the decline in community-wide herbivory with latitude mirrored similar declines in both intraspecific herbivory variability and observed herbivory levels for three common plant species. This indicates that latitudinal variation in community-wide herbivory was mainly driven by decreases in herbivory on individual plant species, rather than by shifts in community composition. This relatively weak role for species’ turnover effects is inconsistent with a recent study of boreal forests (taiga) (Zvereva *et al.* , 2020). Zvereva *et al.* (2020) found that latitudinal shifts in community composition (the proportion of deciduous plants increased with latitude) counterbalanced a simultaneous decrease in herbivory on deciduous species; the net result was a lack of latitudinal variation in community-wide herbivory (Zvereva *et al.* , 2020).

This inconsistency among studies may be attributed to the higher plant species richness in grasslands (*c.* 20–30 plant species in a  $0.5 \times 0.5 \text{ m}^2$  quadrat) and correspondingly low abundance of each plant species (*e.g.* , 96.3% of 54 plant species had percent cover  $< 5\%$ ; Ma *et al.* , 2017); for species showing significant latitudinal gradients in herbivory, there were no corresponding latitudinal shifts in abundance. For example, the three most broadly-distributed plant species (*P. macrophyllum* , *S. pulchra* and *T. mongolicum* ) showed latitudinal gradients in herbivory, but the biomass of each species did not vary with latitude. The relatively high herbivory levels observed here could affect plant fitness, and thus competitive ability (Agrawal *et al.* , 2012); as a result, a compensatory increase in abundance or biomass may have been lacking. Hence, we conclude that the abundance of each plant species in a community should be taken into account when studying latitudinal variation in community-wide herbivory and detangling the relative importance of intraspecific herbivory variability versus species’ turnover.

### **Climate played a primary role in driving intraspecific herbivory variability**

Structural equation modeling revealed stronger effects of climate versus other environmental factors on both intraspecific herbivory variability and community-wide herbivory. Similarly, of all factors considered, temperature seasonality was the most often included factor in the best models; seasonality was negatively associated with community-wide herbivory, intraspecific herbivory variability and herbivory of the three focal plant species. These findings are consistent with previous studies of individual species which found that large-scale latitudinal variation in herbivore pressure was predominantly explained by climatic variables (*e.g.* , Moreira *et al.* , 2015; Moreira *et al.* , 2018; Loughnan & Williams, 2019). Furthermore, while climatic factors were strongly associated with herbivory in our three focal plant species, plant defense traits were only weakly linked to herbivory in these species. These findings suggest that (indirect) climatic effects were unlikely to be mediated by plant chemical defense traits. Instead, previous studies have found that climatic conditions strongly restrict the abundance and distribution of insect herbivores on the Qinghai-Tibetan Plateau (Xi *et al.* , 2013; Li *et al.* , 2019; Du *et al.* , 2020). Therefore, we speculate that climate may directly determine herbivore pressure on the Qinghai-Tibetan Plateau, primarily by altering the distribution and identity of insect herbivores affecting plant communities.

Apart from climatic factors, the effect of soil properties on herbivore pressure should not be ignored. In this study, although there was no overall association between the tested edaphic factors and intraspecific herbivory variability, soil nitrogen and water content were both positively associated with community-wide herbivory. Herbivore pressure has often been posited to be related to resource availability, both because high-resource environments sustain more herbivores (particularly generalists) (Weiblen *et al.* , 2006; Louthan *et al.* , 2015) and because plants growing in high-resource environments typically have higher tissue quality than plants growing in low-resource environments (Coley & Aide, 1991; Price, 1991). In addition, we found that soil pH was negatively correlated with community-wide herbivory and intraspecific herbivory variability.

These negative associations may be mediated by the soil biota. For example, a recent study demonstrated that higher soil pH and nutrient availability (*e.g.*, of phosphorus and potassium) can increase microbial abundance and diversity; this can further suppress aboveground insect herbivory via enhancing plant tolerance (by increasing plant total biomass) and resistance (by decreasing concentrations of sugars and amino acids) (Jiang *et al.*, 2020). Therefore, future research on the soil biota may enhance our understanding of the factors driving latitudinal variation in herbivory.

## Conclusion

Our study has emphasized the importance of taking a community-wide approach when quantifying latitudinal variation in herbivore pressure, revealing how community-wide herbivory varies latitude. We also provide new empirical evidence that climate plays a primary role in driving variation in community-wide herbivory, via effects on intraspecific herbivory variability. More broadly, as plant and insect species may undergo range shifts to higher latitudes in response to global warming (Buse *et al.* 2013; Cavanaugh *et al.* 2014), the higher latitudes of the Qinghai-Tibetan Plateau are expected to experience greater herbivore pressure. Therefore, field data from latitudinal surveys, together with evidence from experimental temperature treatments, are needed to evaluate the indirect impacts of climate change on plant communities via changing herbivore pressure.

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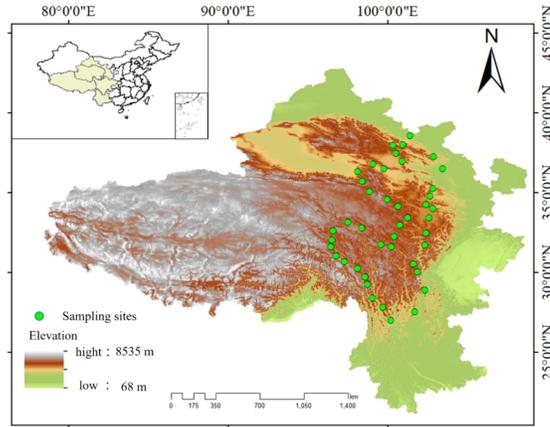
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**Table 1.** Linear mixed-effect model results for community-wide (CWM) herbivory as a function of multiple climatic, edaphic, geographic and plant community factors. The relative support for all variables is provided, as well as for the intercept-only (null) model. For each model, the maximum log-likelihood (LL), estimated number of model parameters ( $k$ ), Akaike’s information criterion corrected for small samples ( $AIC_c$ ), change in  $AIC_c$  relative to the top-ranked model ( $\Delta AIC_c$ ),  $AIC_c$  weight ( $w AIC_c$  = model probability) and marginal  $R^2$  are provided as measures of the model’s goodness-of-fit.

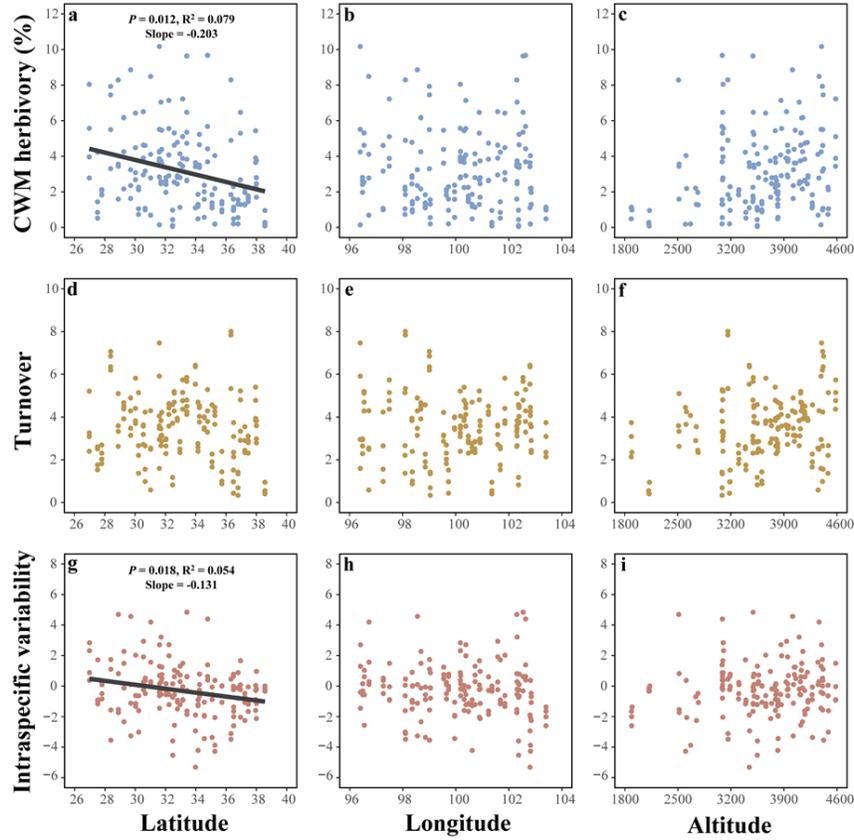
Model	LL	k	$AIC_c$
Phase 1: geographic factors	Phase 1: geographic factors	Phase 1: geographic factors	Phase
<i>Latitude</i>	-107.156	1	222.55
<i>Altitude</i>	-107.948	1	224.14
~1 (null)	-109.123	0	224.39
<i>Longitude</i>	-110.110	1	228.46
Phase 2: climatic factors	Phase 2: climatic factors	Phase 2: climatic factors	Phase
<i>Temperature seasonality</i>	-105.884	1	220.01
<i>Precipitation of the wettest month</i>	-106.129	1	220.50
<i>Mean annual precipitation</i>	-106.281	1	220.80
<i>Maximum temperature of warmest month</i>	-107.832	1	223.90
~1 (null)	-109.123	0	224.39
<i>Precipitation of the driest month</i>	-108.295	1	224.83
<i>Precipitation seasonality</i>	-108.944	1	226.13
<i>Minimum temperature of coldest month</i>	-109.760	1	227.76
<i>Mean annual temperature</i>	-110.340	1	228.92
Phase 3: edaphic factors	Phase 3: edaphic factors	Phase 3: edaphic factors	Phase
<i>Soil N</i>	-106.558	1	221.36
<i>pH</i>	-106.658	1	221.56
<i>SWC</i>	-106.733	1	221.71
<i>EC</i>	-107.321	1	222.88
<i>Soil C</i>	-107.625	1	223.49
~1 (null)	-109.123	0	224.39
Phase 4: plant community factors	Phase 4: plant community factors	Phase 4: plant community factors	Phase

Model	LL	k	AIC <sub>c</sub>
~1 (null)	-109.123	0	224.39
<i>Richness</i>	-109.200	1	226.64
<i>Shannon's index</i>	-109.865	1	227.97
<i>Plot biomass</i>	-110.470	1	229.18
<i>Pielou's evenness</i>	-110.740	1	229.72

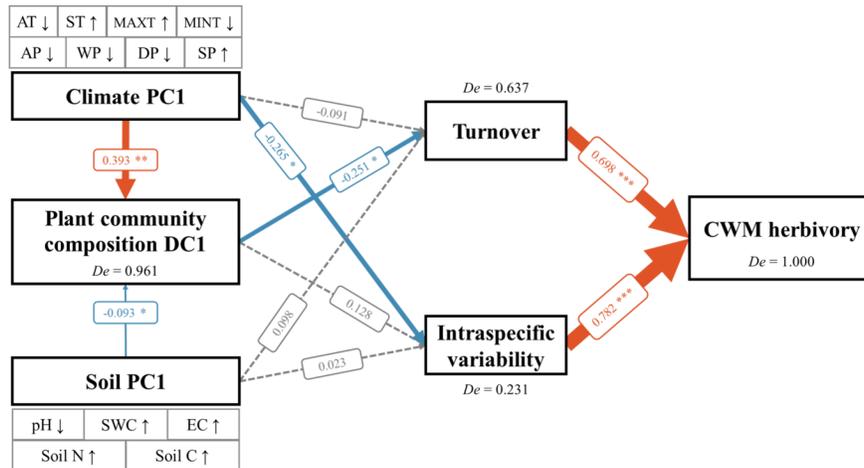
**Figure 1.** The distribution of sample sites across the Qinghai-Tibetan Plateau.



**Figure 2.** Relationships between community-wide (CWM) herbivory, species' turnover and intraspecific variability in herbivory on the Qinghai-Tibetan Plateau and latitude, longitude and altitude.



**Figure 3.** Illustration of the final piecewise structural equation model: Fisher’s  $C = 10.302$  ( $d.f. = 8$ ,  $P = 0.244$ ) and  $AIC_c = 54.302$ . Numbers on arrows are standardized path coefficients (scaled by their mean and standard deviation), while asterisks indicate statistical significance ( $***P < 0.001$ ;  $**P < 0.01$ ;  $*P < 0.05$ ). Solid red arrows indicate positive relationships, solid blue arrows negative relationships, and gray arrows insufficient statistical evidence for a path coefficient ( $P > 0.05$ ). Arrow width is indicative of the strength of the causal relationship, and the marginal  $R^2$  is shown.



**Figure 4.** Relationships between herbivory and latitude, longitude and altitude for three common plant species (*Saussurea pulchra*, *Taraxacum mongolicum* and *Polygonum macrophyllum*) in Qinghai-Tibetan Plateau plant communities.

