Accounting for underlying complexities identifies simple hierarchy of trait–environment relationships in Wisconsin forest understory communities

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Abstract

Adaptive relationships between traits and the environment are often inferred from observational data by regressing community-weighted mean (CWM) traits on environmental gradients. However, trait–environment relationships are better understood as the outcome of trait–abundance and environment–abundance relationships, and the interaction between traits and the environment. Accounting for this functional structure and for interrelationships among traits should improve our ability to accurately describe general trait–environment relationships. Using forest understory communities in Wisconsin, we applied a generalized mixed model (GLMM) incorporating this structure. We identified a simple hierarchy of trait–environment relationships dominated by a strong positive effect of mean temperature on plant height. Compared to the traditional CWM approach, the GLMM was more conservative in identifying significant trait–environment relationships, and also detected important relationships that CWM regressions overlooked. This work highlights the need to consider the complexity underlying trait–environment relationships in future analyses.

Keywords Community assembly, environmental gradient, generalized linear mixed model, leaf size, leaf mass per area, mean annual temperature, plant height, selection, soil sand content, temperature seasonality.

Introduction

Understanding how environmental gradients drive plant community composition and species distributions is essential for predicting future responses to global change (Thuiller *et al.* 2008; Kearney *et al.* 2010; Scheiter *et al.* 2013; van Bodegom *et al.* 2014). Theory proposes that the distribution of phenotypic plant traits (e.g. leaf size and potential plant height) across co-occurring species should be adaptively associated with local environments, thus providing a mechanistic link between environmental change and community responses (Keddy 1992; Lavorel & Garnier 2002; Violle *et al.* 2007; Shipley 2010). A considerable amount of empirical research has therefore been devoted to characterizing how phenotypic traits relate to environmental gradients, i.e. the so-called trait–environment relationships (Funk *et al.* 2017). However, reported trait–environment relationships vary widely in strength and sign (see reviews in Garnier *et al.* 2016; Funk *et al.* 2017), highlighting the need to improve our understanding of these relationships if we are to make useful predictions. To this end, we should use analytical tools that better represent and capture the complexities that shape trait–environment relationships.

Mechanistically, trait–environment relationships arise because individual fitness or performance depend on the interaction between functional traits and environmental conditions (Laughlin & Messier 2015; Shipley *et al.* 2016; Laughlin *et al.* 2018). In general, we say that traits and environments are related if the relative performance (or adaptive value) of trait values change along an environmental gradient. This is a two-way interaction and can therefore be viewed from two perspectives (ter Braak 2019). From a site-centric perspective, the trait–performance relationship among coexisting individuals would change when environmental conditions change, while a species-centric perspective would show that environment–performance relationships change among species that differ in their trait values (Fig. 1). The corollary is that trait–environment relationships are conceptually three-dimensional and cannot be understood on the information conveyed by trait and environmental axes alone. Rather, detecting trait–environment relationships depends on properly characterizing how both trait values and environmental conditions affect performance. For this, we must acknowledge the different shapes that all these relationships may take.

69 Species' performances typically show unimodal responses to *environmental* variables
70 reflecting intermediate optima along the gradient (Curtis 1959; Whittaker 1967; Ter Braak &
71 Prentice 1988; Austin 1999). Similarly, we expect unimodal performance responses to *traits*
72 when functional trade-offs lead to optimum trait values within sites (Muscarella & Uriarte 2016;
73 Rolhauser & Pucheta 2017; Rolhauser *et al.* 2019). Environment–performance relationships can
74 also be bimodal because of intense negative interspecific interactions (Mueller-Dombois &
75 Ellenberg 1974; Minchin 1987; Austin 1999), nonlinear trait–environment relationships
76 (Laughlin & Joshi 2015), or unmeasured environmental factors (Austin *et al.* 1984). Similarly,
77 trait–performance relationships can be bimodal (or multimodal) when within-site
78 environmental heterogeneity favors the functional divergence of competitive, dominant species
79 (Rolhauser & Pucheta 2017; Rolhauser *et al.* 2019). Rectilinear or directional relationships
80 emerge as particular cases when performance maxima (in optimum relationships) or minima (in
81 bimodal relationships) occur at the extremes or outside the range of observed trait and
82 environmental values (Rolhauser *et al.* 2019). Unlike trait–performance and environment–
83 performance relationships, theoretical discussions on the shape of trait–environment
84 relationships are rare. Both conceptual (Laughlin & Messier 2015; Garnier *et al.* 2016; Shipley *et*
85 *al.* 2016) and empirical studies (e.g. Niinemets 2001; Wright *et al.* 2004, 2017; Moles *et al.* 2009,
86 2014) largely assume rectilinear relationships. Nonetheless, some empirical work exists to show
87 that trait–environment relationships can be nonlinear (Laughlin *et al.* 2011; Bello *et al.* 2013).
88 Studies of trait–environment relationships often resort to various simplifications of the
89 three-dimensional problem to infer trait–environment relationships. Mechanistic studies that
90 directly assess how individual performance responds to conditions remain rare and typically
91 assume rectilinear trait–performance and environment–performance relationships (e.g.
92 Laughlin *et al.* 2018). Other studies make even stronger assumptions, e.g., by directly regressing
93 trait values against environmental variables (see reviews in Garnier *et al.* 2016; Funk *et al.* 2017).
94 These often involve regressing community-weighted means (CWM) on environmental
95 variables, termed CWM regression (CWMr) (Miller *et al.* 2019), but we can also find regressions
96 of site-specific species trait averages (e.g. Dong *et al.* 2020) and individual trait values (Laughlin
97 *et al.* 2012). These trait–environment regression approaches assume that the abundance or

presence of a species at a given site indicates their performance. They also have two other important limitations. First, because they do not explicitly model abundance, they cannot assess plausible differences in trait–abundance and environment–abundance relationships. For example, CWMs represent the adaptive value of a trait at a given site only when the most abundant phenotype happens to be close to the trait mean (Muscarella & Uriarte 2016), although this is a special case (Rolhauser & Pucheta 2017). Second, they tend to evaluate traits one at a time using separate regressions, implicitly assuming independence between traits and their responses to environments. However, traits are inherently interrelated and evolutionary biologists have long recognized the importance of using multivariate regressions to tease apart the adaptive significance of correlated traits (Lande & Arnold 1983).

Here, evaluated trait–environment relationships across forest understory communities in Wisconsin using a generalized linear mixed model (GLMM) (ter Braak 2019) that incorporated the underlying complexity discussed above. We modeled nonlinearities in all three functional relationships: trait–abundance, environment–abundance, and trait–environment (Figs. 1, S3). We also evaluated multiple traits and trait–environment relationships simultaneously. We used a large dataset (Waller *et al.* 2012) that included: abundances of 185 species distributed among 189 sites; ten functional traits characterizing tissues (e.g. leaf mass per area, or LMA), organs (e.g. leaf size, or LS), and whole plants (e.g. maximum vegetative height, or VH); and fourteen environmental variables reflecting local environmental abiotic (e.g. mean annual temperature and soil sand content) and biotic (tree basal area) conditions. Functional traits like VH, LS, and LMA have been widely studied as controls of plant performance and tend to vary along abiotic and biotic gradients (see review in Pérez-Harguindeguy *et al.* 2013). To demonstrate the usefulness of modelling key underlying ecological complexities using a single GLMM, we compared the resulting trait–environment relationships to those found using the traditional CWMr. We show that our approach allowed us to achieve two disparate goals: explaining a large proportion of the variability in species abundance across sites while improving the accuracy and reliability of inferring which trait–environment relationships matter.

Material and methods

Study area

Wisconsin covers 169,639 km^2 yet its elevation ranges only between 177-595 m. Its highly seasonal north temperate climate shows conspicuous north-south gradients in summer and winter temperatures and east-west gradients in seasonality and rainfall that reflect the moderating maritime influence of Lake Michigan to the East. The vegetation is dominated by deciduous forests that can be divided into two floristic provinces: the prairie-forest province to the southwest and the northern hardwoods province to the north, separated by a marked "tension zone" running from the SE to the NW edges of the state (Curtis 1959; see Fig. S1 in Supporting Information).

Vegetation sampling

Data were collected through contemporary resurveys of forests stands (sites) originally surveyed by J.T. Curtis and his students in the 1950s (see Fig. S1 in Supporting information). Only sites that remained less disturbed (i.e. with an intact forest canopy, no nearby edges, and few signs of understory disturbance) were resurveyed. Resurveys in the 2000s employed Curtis-era and similar methods covering similar areas (Waller et al. 2012; see Appendix S1 for details). Briefly, all vascular plants were tallied in each of many replicate (20-403) 1-m^2 quadrats to estimate their abundance (frequency) at each site. In total, 536 species were found at 293 sites. We restricted our attention to the commonest 185 species, for which we had registered trait values. We then selected sites to ensure high trait coverage (80+% of cumulative species occurrences). We also discarded a few sites missing full environmental data, yielding a total of 189 sites.

Environmental variables

Descriptions of the four climatic variables, ten soil variables and one biological variable (tree basal area) appear in Appendix S1. Climatic variables for each site were derived from 10-year

154 averages for the period 1995-2004. Soil and basal area data derive from samples and surveys at
155 each site. To limit collinearity among predictors, we first performed pairwise correlations and
156 principal components analyses to select more independent subsets of variables (all correlations
157 <0.5) to represent major climatic and soil gradients (Appendix S1). We selected six predictors for
158 analysis: mean annual temperature (MAT), mean annual precipitation (MAP), temperature
159 seasonality (i.e. the standard deviation of mean monthly temperatures, abbreviated TSD), soil
160 nitrogen content (%N), soil sand content (%Sand) and tree basal area (BA). Before analysis,
161 environmental variables were transformed as needed to reduce the weight of extreme values,
162 then standardized to zero mean and unit variance (Appendix S1).
163

164 ## *Plant traits*

165 In a similar fashion, we initially analyzed data for ten functional traits in the most common 185
166 herbaceous species (see Appendix S1 for descriptions of traits and data collection methods).
167 Briefly, 12+ individuals (at least four plants from each of three sites) were collected in Wisconsin
168 and processed between 2008 and 2014 following standardized protocols (Pérez-Harguindeguy
169 et al. 2013) as first described by Amatangelo *et al.* (2014). We then selected four traits for analysis
170 with low pair-wise correlations (<0.3; Table S3): vegetative height (VH), leaf size (LS, calculated
171 as the product of length and width), leaf mass per area (LMA), and leaf carbon content (LCC).
172 Traits were transformed as needed to reduce the weight of extreme values and standardized
173 (Appendix S1).
174

175 ## *Statistical methods*

176 ### <u>*GLMM*</u>

177 The aim of the approach below is at inferring the shape and significance of trait–environment
178 relationships while modeling meaningful site and species responses to, respectively, trait and
179 environmental axes (Fig. 1). We used a negative binomial generalized linear mixed model
180 (GLMM) to model the abundance of species i at site j (A_{ij}) as a function of selected traits and

environmental variables via the natural logarithm link function. A_{ij} is a count variable calculated as the frequency of quadrats in which species i was present at site j. Negative binomial models directly estimate an overdispersion parameter from the data (Agresti 2015). Before describing the full multivariate model, we outline an analysis for one trait and one environmental variable, both standardized. Given the log link function, the fixed effects in the GLMM are (cfr. Fig. 1):

$$\ln(A_{ij}) = \beta_0 + \beta_1 T_i + \beta_2 T_i^2 + \beta_3 E_j + \beta_4 E_i^2 + \beta_5 T_i E_j + \beta_6 T_i E_j^2 \quad (1a)$$

where β_0 is y-intercept of the fitted surface. Since trait T is centered at zero, β_1 and β_2 respectively estimate the mean slope (or "gradient") and the mean curvature of the trait–abundance relationship (see e.g. Aiken et al. 1991) for the particular case when E_j is set to its average value (zero). That is, β_1 measures the direction (positive or negative) and the strength of trait T_i's effect on species abundance. Negative and positive values of β_2 indicate a n- and u-shaped relationships, respectively. Similarly, β_3 and β_4 reflect the mean slope and curvature of the environment–abundance relationship (ter Braak 2019). Due to the combination of quadratic effects (β_2 and β_4) and the log link associated with the negative binomial distribution, equation 1a can reproduce realistic, bell-shaped trait–abundance and environment–abundance relationships (Figs. 1, S3a). While doing this, we can assess trait–environment relationships by estimating interactions between T and E, i.e. parameters that quantify how different environmental conditions modulate the mean effect of the trait on the response variable (Laughlin et al. 2018). In our model, these environmentally mediated trait effects are estimated by β_5 and β_6.

Rearranging Eqn. 1a to gather terms for the mean (linear) effect of trait T_i on A_{ij} yields:

$$\ln(A_{ij}) = \beta_0 + T_i(\beta_1 + \beta_5 E_j + \beta_6 E_j^2) + \beta_2 T_i^2 + \beta_3 E_j + \beta_4 E_j^2 \quad (1b)$$

Then, the environment-dependent mean slope of trait–abundance relationships (denoted φ_j) is:

$$\varphi_j = \beta_1 + \beta_5 E_j + \beta_6 E_j^2 \quad (2)$$

In this quadratic function, β_5 and β_6 are respectively the mean slope and mean curvature of the trait–environment relationship (Fig. S3). Indeed, since $T_i E_j^2 = (T_i E_j) E_j$, β_6 estimates how much the strength of the trait–environment interaction depends on the values of the environment (see equation 1a), i.e. a quadratic relationship. Importantly, the properties of the fitted surface (e.g. the position of optimum trait values) change along the environmental gradient depending on β_5 and β_6 (Fig. S3).

The full GLMM included four traits (VH, LMA, LS, LCC) and six environmental variables (MAT, MAP, TSD, %Sand, %N and BA). Following equation 1a, the fixed effects included linear and quadratic terms for all traits and environmental variables as well as all trait–environment interactions. We formulated random effects after ter Braak (2019) including random intercepts and slopes for all traits and environmental variables (i.e. MLM3 sensu ter Braak 2019). The model in R code notation, is thus:

```
glmmTMB(abundance~(MAP + MAT + TSD + %Sand + %N + BA + I(MAP^2)
+ I(MAT^2) + I(TSD^2) + I(%Sand^2) + I(%N^2) + I(BA^2)) * (VH + LS + LMA + LCC)
+ I(VH^2) + I(LS^2 + I(LMA^2) + I(LCC^2))
+ (1 + VH + LS + LMA + LCC | site)
+ (1 + MAP + MAT + TSD + %Sand + %N + BA | sp)
+ offset(log(quadrats))
```

Note that the random slopes with respect to a given trait, T, are estimated for each site (denoted $b_{(T)j}$ here). Random slopes with respect to environmental variables E are also estimated for each species ($c_{(E)i}$) (ter Braak 2019). We included the log number of quadrats sampled per site to measure search-effort, used in our model as an offset (Kéry 2010, pp188-189). We fitted the GLMM using the R-package glmmTMB (Brooks et al. 2017). We calculated marginal and conditional R^2 (the proportion of the total variance explained by fixed effects and by both fixed and random effects, respectively) following the delta method (Nakagawa et al. 2017)

implemented by the r.squaredGLMM function in the R-package MuMIn (Barton 2019). We visualized uncertainty around estimates of φ_j (for a given *T-E* combination) by plotting overall site effects calculated as the sum of the fixed (φ_j) and the random slopes associated with the corresponding trait ($b_{(T)j}$) (ter Braak 2019).

Comparing GLMM and CWMr approaches

We calculated community-weighted means (CWM) as usual:

$$CWM_{(T)j} = \frac{\sum_{i=1}^{n} A_{ij} T_{ij}}{\sum_{i=1}^{n} A_{ij}} \quad (3)$$

where A_{ij} and T_{ij} are the abundance and the standardized trait value for species *i* in site *j*. We then related CWM values to standardized environmental variables (E_j) through univariate regressions (separate for each trait). We included both linear and quadratic terms for E_j, leading to the following general form

$$CWM_{(T)j} = \gamma_0 + \gamma_1 E_j + \gamma_2 E_j^2 \quad (4)$$

We fitted CWMr models using the least squares method implemented by the lm function in R.

We evaluated the agreement between GLMM and CWMr approaches by comparing the statistical significance of homologous model parameters for each trait–environment combination, i.e. β_5 vs γ_1 and β_6 vs γ_2 (cf. equations 2 and 4). We divided test statistics (*z* and *t* in the case of GLMM and CWMr, respectively) into those that led to the rejection of the null hypothesis or not and compared these between approaches (see Appendix S1 for details). There are several possible outcomes for each trait–environment relationship tested (Table S4; Laughlin et al. 2018). If both approaches yield non-significant effects, then they agree that the trait has no adaptive value along that environmental gradient (Outcome #1, or O#1). A trait–environment relationship found to be not significant with the CWMr model but significant with GLMM indicates that the trait has likely adaptive value along the gradient, but the CWMr method lacked sensitivity to detect it, possibly due to confounding abiotic or biotic factors (O#2). If

conversely the trait–environment interaction is significant by CWMr but not by GLMM, the CWMr result may be spurious or indirect reflecting the highly inflated type I error rates found in CWMr models (Peres-Neto et al. 2017; Miller et al. 2019) (O#3). Finally, if both methods identify a significant relationship with the same sign, we can safely conclude that the trait has adaptive value along that environmental gradient (O#4). It is also possible that the relationship is significant via both approaches, but with opposite signs (O#5, Table S4).

Results

GLMM

Overview

The full model included 113 parameters (overdispersion, 43 random effects, and 69 fixed effects) to explain abundance across 34,965 species-site combinations. The marginal R^2 of this model was 0.385 and the conditional R^2 was 0.977. We focus our discussion on trait–environment relationships, but full model results appear in Appendix S2. Quadratic terms associated with main trait and environment effects on abundance were negative, indicating optimum relationships (Table S5). Removing either all quadratic or all interaction terms from the full model increased AIC>70 units (Table S6), confirming the importance of including these complexities when analyzing abundance.

Trait–environment relationships

The model identified five significant trait–environment relationships, defined as those combinations of a trait and an environmental variable where either of both interaction parameters (β_5 and β_6) showed $p<0.01$ (Table S5). Three relationships were linear, where with only β_5, not β_6, was significant (shown as blue lines in Fig. 2). Among these, the interaction between mean annual temperature (MAT) and vegetation height (VH) was the strongest, with β_5=1.031 (SE=0.146), whereas the remaining significant linear interactions all were below 0.34 (Table S5). Warmer sites (higher MAT) favored taller plants (Fig. 2, top line, second plot and

294 Fig. 3, a1 and a2). VH also increased linearly with temperature seasonality (TSD) (β_5=0.328,
295 SE=0.082): more seasonal sites (higher TSD) favored taller plants (Fig. 2, top line, third plot and
296 Fig. 3, b1 and b2). In addition, leaf size (LS) declined linearly as soil sand content increased (β_5=-
297 0.266, SE=0.075; Fig. 2, row 2, column 4 and Fig. 3, c1 and c2).

298 Temperature seasonality (TSD) was negatively associated with both LS (β_5=-0.242,
299 SE=0.082) and leaf mass per area (LMA) (β_5=-0.239, SE=0.082), and in both cases these decreases
300 were nonlinear ($\beta_6 \neq 0$) (orange lines, Fig. 2, column 3; Table S5). More seasonal sites (higher TSD)
301 favored plants whose leaves were smaller and had lower LMA (Fig. 3, b4, b5, b7 and b8). In
302 these cases, nonlinearity meant that TSD negative effects on LS and LMA were maximal at low
303 seasonality and tended to disappear as TSD increased.

304 Other linear trait–environment interactions were marginally significant ($0.05<p<0.01$, Fig.
305 2). These included the increase in plant height with soil N (row 1, column 5), the declines in
306 LMA and leaf carbon content (LCC) with MAT (rows 3 and 4, col 2), and the increase in LCC
307 with soil sand (row 4, column 4). Basal area (BA) also slightly increased LCC (row 4, column 6).
308 Surprisingly, annual precipitation (MAP) did not appear to affect any of the evaluated traits
309 (column 1).

310

311 *Comparing GLMM and CWMr approaches*

312 The CWMr approach identified 17 significant trait–environment relationships (out of 24), i.e.
313 single regressions where either of both parameters (γ_1 and γ_2) showed $p<0.01$ (Table S7). Twelve
314 were exclusively rectilinear or directional (only $\gamma_1 \neq 0$; e.g. LMA–MAT), four were curved with a
315 directional component (both $\gamma_1 \neq 0$, $\gamma_2 \neq 0$; e.g. LMA–%N), and one was curved with no significant
316 directionality (only $\gamma_2 \neq 0$; LCC–MAP) (Table S7).

317 Test statistics for linear trait–environment parameters (β_5 and γ_1) were quantitatively
318 similar between the GLMM and CWMr approaches (R=0.61; t=3.572, df=22, p=0.002; Fig. 4a).
319 Test statistics for the quadratic terms (β_6 and γ_2) were less similar (R=0.41; t=2.095, df=22,
320 p=0.048; Fig. 4b). Both approaches agreed that six linear terms (including all four interactions
321 with BA; Outcome #1, Fig. 4a) and 16 quadratic statistics lacked significance (also O#1, Fig. 4b).

In addition, three linear effects of environmental conditions on plant traits (LS–TSD, LS–%Sand, and VH–MAT) and one quadratic term (LS–TSD^2) emerged as significant in both models (O#4). Reassuringly, we found no cases where the two models yielded opposite conclusions (O#5). However, inferences differed conspicuously between approaches in several instances. Two linear and one quadratic term emerged as significant in the GLMM but not in CWMr model (O#2). As a result, among the five significant trait–environment relationships in the GLMM (second column in Fig. 3), two were largely missed by the CWMr approach and both involved TSD (third column in Fig. 3). Perhaps more worrying were many apparent Type I errors wherein 13 linear and four quadratic terms that emerged as significant from the CWMr model lacked significance using the GLMM approach (O#3).

Discussion

Trait–environment relationships provide a useful tool to understand and predict community responses to environmental change, assuming their accuracy is not compromised by using an inappropriate modelling approach. Unlike community-weighted mean regressions (CWMr), generalized linear mixed models (GLMM) are compatible with the ecological notions that trait–environment relationships are inherently three-dimensional and not independent from one another. Applied to Wisconsin understory vegetation, we found that CWMr inferences granted significance to far more relationships than the more functionally informed GLMM. Only five relationships emerged as statistically significant (of 24 evaluated) in the GLMM, and two of them were largely missed by the CWMr approach. Nonetheless, this handful of trait–environment relationships contributed to explain nearly 40% of the variation in abundance of 185 plant species distributed among 189 sites.

Prominent trait–environment relationships

The interaction between mean annual temperature (MAT) and vegetative height (VH) emerged as the strongest in the GLMM, both in terms of its standardized coefficient (β) and statistical significance. It was also the only one where the mean slope of the trait–abundance relationship

(φ_j) clearly switched signs over the length of the environmental gradient (Fig. 3), indicating a clear change in the rank order of species abundances. As such, the VH–MAT relationship would be the only one considered as "strong" by others (Laughlin et al. 2018). This relationship predicts increases in plant height as MAT increases, consistent with global patterns (Moles et al. 2009, 2014). This may be explained by the fact that warmer climates promote taller plants as they compete for light (Givnish 1982; Falster and Westoby 2003) and/or that shorter plants are less prone to freeze-embolism in colder climates (Moles et al. 2009).

The fact that leaf size (LS) declined at sites with sandier soils agrees with global patterns showing that water-limited environments tend to favor plants with smaller leaves (Wright et al. 2017). The standardized coefficient (β_5) for the LS–%Sand interaction was only a quarter the size of that for the VH–MAT interaction. This may reflect the subordinate role of soil properties on community assembly at the scale of our observations.

Both leaf size (LS) and leaf mass per area (LMA, the inverse of specific leaf area) declined with the increase of temperature seasonality (TSD). Temperature seasonality also strongly increased plant abundance, suggesting that many understory species thrive in more seasonal environments. This could reflect how Spring and Fall periods of higher light in these deciduous forests and warmer forest floor conditions favor understory plant growth. Consequently, species in more seasonal sites would also face more competitive conditions. This could explain why these sites support taller plant species as height is favored under crowded conditions (Givnish 1982; Falster and Westoby 2003). Because low-LMA leaves tend to be short-lived (Poorter et al. 2009), they could be favored at sites with short Spring windows of full light or shorter seasons (Kikuzawa et al. 2013; Mason and Donovan 2015). Given that leaves often start to generate carbohydrates at about half their final size (Turgeon 1989), shorter seasons might also favor smaller leaves for their ability to start exporting carbohydrates sooner than larger leaves.

Among the environmental factors, only temperature seasonality (TSD) showed significant nonlinear relationships with plant traits. Specifically, TSD effects on LS and LMA diminished as seasonality increased. Others that have noted these nonlinearities have

speculated on their theoretical consequences (Laughlin and Joshi 2015). However, we are not aware of theory to explain the mechanisms behind nonlinear trait–environment relationships.

Missing trait–environment relationships

Despite many published global-scale studies about how precipitation affects plant traits (Niinemets 2001; Moles et al. 2009, 2014; Wright et al. 2017), we found no trait–precipitation interactions in this study. This may reflect the fact that these Wisconsin sites span only 5% of the 0 to 4500 mm/year range spanned at the global scale. In contrast, soil sand varied from 15% to 89% among our sites and we found it to affect leaf size (and leaf carbon content, marginally). This may imply that sand content better reflects water availability for plants at this scale. Basal area, our surrogate for understory light, only affected LCC weakly, probably reflecting how notoriously variable understory light levels are within sites (Canham et al. 1994). The weak linear effects we found for soil N (favoring taller plant species with flimsier leaves) could also reflect local variability (e.g., in soil conditions) or perhaps that soil N mainly affects plants through interactions with other environmental variables. Such interactions were not included in our GLMM.

Relationships between MAT and LMA have also attracted the attention of ecologists. The negative LMA–MAT relationship we found was marginally significant, consistent with other regional-scale studies finding weakly negative or non-significant relationships (Laughlin et al. 2012, 2018; Mason and Donovan 2015; Rosbakh et al. 2015; Balazs et al. 2020; Dong et al. 2020). Such weak field effects are surprising given the strong negative effects found under controlled growth conditions (Poorter et al. 2009). We found, however, that LMA actually responded more to temperature seasonality in the GLMM. In larger, especially global, datasets, mean temperature and seasonality are so closely correlated (see e.g., Kikuzawa et al. 2013) that we cannot decouple their separate effects. In our dataset, on the contrary, the MAT–TSD correlation was mild (-0.37; Table S2) suggesting that the weak MAT effect was not an artifact of seasonality soaking up species abundance variation. Building on efforts linking leaf longevity to

MAT and seasonality (Kikuzawa et al. 2013) it seems that much remains to be understood regarding the individual and interactive effects of these variables on LMA.

GLMM vs. CWMr approaches

Community-weighted mean regressions are already known to have inflated type I errors, which manifest as falsely identifying particular trait–environment associations as significant even in simulations involving no actual association (Peres-Neto et al. 2017; Miller et al. 2019). In contrast, GLMM approaches show good balance between type I error control and power (Miller et al. 2019), particularly when random slopes for both traits and environmental variables are included in the model (MLM3 sensu ter Braak 2019). Our results support these conclusions and highlight limitations with CWMr approaches. Many of the terms in the CWMr that emerged as significant (including commonly reported trait–environment relationships like LMA with MAP, MAT and %N, LS with MAP and MAT, and VH with MAT and %N) lacked significance in the GLMM (Outcome #3). Given how frequently these relationships are described in the literature, we have concerns that some reports may reflect spurious false positives from CWMr approaches. Conversely, two trait–environment relationships featuring TSD were highly significant in the GLMM (with VH and LMA) but lacked significance under the CWMr approach (Outcome #2). Importantly, these hidden relationships emerged in a GLMM able to control for effects of other trait–environment relationships. This suggests that CWMr approaches may also generate false negatives, possibly accounting for why this TSD relationships are under-reported in the literature, particularly in cases were MAT and TSD are highly correlated. All these inferential differences are particularly striking given that both approaches are based on exactly the same data.

Strengths, limitations, and future directions

Using a single model enabled us to directly rank trait–environment relationships underlying species distributions. This cannot be done with separated regressions using single traits as response variables because they fail to account for unmodeled factors, such as other traits. A paradigmatic case is that of LMA, which showed extremely significant linear correlations with three environmental variables (sand and nitrogen content and MAT; see Fig. 4a) that were deemed nonsignificant in the GLMM. The lack of significant LMA interactions in the GLMM cannot be ascribed to collinearity with a single competing trait since all correlations with LMA were below 0.18 (Table S3). Rather, it might have to do with the combination of the many effects included in the model. Modelling several trait–environment combinations simultaneously accounts for any possible overlapping effect on species abundance arising from collinearity, while quadratic effects allow for flexibility in functional relationships. In particular, we introduced nonlinear trait–environment into the GLMM approach which showed to be important to characterize temperature seasonality effects. Paradoxically, despite its complexity, the GLMM points out to a much simpler hierarchy of relationships compared to the unstructured plethora suggested by the CWMr approach. Here, temperature (mean and seasonality) appeared to drive plant height variation, while soil texture (in combination with temperature seasonality) played a subordinate role affecting leaf traits.

We interpreted φ_j as reflecting how strongly trait values are "favored", "selected" or "filtered" along particular environmental gradients (Shipley 2010; Rolhauser and Pucheta 2017; Loranger et al. 2018). We implicitly assumed that trait–environment effects on plant abundance are not being strikingly confounded by other processes like dispersal and demographic stochasticity. Yet we acknowledge that stochasticity may play important roles in community dynamics (Hubbell 2001; Alonso et al. 2006) and could indeed help explain some of the ~60% of abundance variation unaccounted for by fixed effects in our GLMM. Dispersal limitation now limits the presence or abundance of some species given extensive habitat fragmentation particularly in southern Wisconsin forests (Rogers et al. 2009). However, the traits we used here are more related to local plant performance (see Pérez-Harguindeguy et al. 2013) than to

dispersal among forest patches; for instance, differences in plant height likely affect dispersal distances, but primarily at fine scales (e.g. Gómez 2007).

Approaches to trait–environment relationships based on the actual distribution of plants, rather than on individual or population-level performance (such as demographic rates), have been criticized due to their phenomenological nature (Laughlin and Messier 2015; Laughlin et al. 2018). Here, we argue that many of the problems associated with phenomenological approaches arise when we collapse or aggregate the abundance dimension, as in CWMr. It might thus be useful to evaluate agreement between GLMMs that use abundance vs. individual or population-level performance as response variables. Ultimately, the mismatch between these approaches will depend on the link between performance and abundance, which still remains as a foundation stone in community ecology with loose empirical support (McGill et al. 2006; Adler et al. 2013; Shipley et al. 2016). We also hope that our field-based results stimulate much needed theoretical developments on nonlinear trait–environment relationships, particularly considering temperature seasonality which may be a key environmental driver of community assembly along regional gradients.

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Supporting Information

Appendix S1 – Supplementary Material and Methods

Appendix S2 – Supplementary Results

Figure legends

Figure 1. Fixed-effect structure of our GLMM approach, were we measure species *i* performance in terms of its abundance in site *j* (A_{ij}). Conceptually, A_{ij} (white to black surface) is modeled as an exponential function (through a log-link) of a trait (T) and an environmental variable (E), as shown by the equation at the top. Parameters β_1 and β_2 are respectively linear and quadratic effects of T on A, β_3 and β_4 are linear and quadratic effects of E on A, and β_5 and β_6 are linear and quadratic effects of E on T (see details in Statistical methods). Combining the log link with negative quadratic terms (β_2 and β_4 set to -1 and -0.5, respectively) this model reproduces unimodal trait–abundance (orange and blue) and environment–abundance (green and red) relationships. In this example, the result is a rectilinear negative T-E relationship (β_5 and β_6 set to -1 and 0, respectively). Dotted lines are modeled relationships at different T or E levels while continuous lines are their counterparts projected onto 2-D planes.

Figure 2. Trait–environment relationships as estimated by the GLMM (Equation 2). Relationships between traits (y-axes) and environmental variables (x-axes) are plotted based on independent estimates of their the linear (β_5) and quadratic (β_6) coefficients (Table S5, ignoring statistical significance). The observed range of traits and environmental variables are shown below axes labels. Orange lines show significant nonlinear relationships (i.e. $\beta_6 \neq 0$), while blue lines show relationships where only linear effects are significant. Dotted lines show marginal

significance ($0.10<p<0.05$) while grey lines show nonsignificant ones. "Interpretations" describe overall patterns (with marginally significant patterns parenthesized).

Figure 3. Details of significant interactions ($p<0.01$) from the GLMM visualized for (a) temperature seasonality, (b) mean annual temperature, and (c) soil sand content. Surfaces in the first column show species abundance fitted as a function of trait values and environmental condition (holding other variables constant at their mean value). Plots in the second column show average slopes (B in Equation 2) of the given trait–abundance relationship (green line). B depends on β_5 and β_6 which respectively estimate the linear and quadratic components of the trait–environment relationships. Each green dot represents the overall effect of site j, i.e. the sum of the fixed (φ_j) and the random slope associated with the corresponding trait ($b_{(T)j}$). Plots in the third column show traditional community-weighted mean regressions (CWMr's) between observed CWM trait values (grey dots) and observed environmental variables (Equation 3; dark grey line fitted by Equation 4). Significance levels are shown for γ_1 and γ_2 (the linear and quadratic terms) and overall R^2. The dotted horizontal lines indicate y=0. (Surfaces are colored to enhance the perception of relief.) [Note: figure in next page]

Figure 4. Scatterplots relating test statistics from univariate CWM regressions to statistics for homologous terms from the GLMM. Plot (a) compares linear terms (γ_1 and β_5) while plot (b) compares quadratic terms (γ_2 and β_6). Each data point corresponds to one of the 24 trait–environment relationships. The purple line is fitted to all 24 points with corresponding Pearson's correlations (R) shown. Green and red rectangles show the range of test statistic values failing to reject the null hypothesis under the CWM and GLMM models, respectively. Codes shown in blue refer to the five outcomes presented in Material and Methods and Table S4. Environmental variable and trait codes as in Fig. 2, except for p = mean annual precipitation, t = mean annual temperature, s = soil sand content, n = soil nitrogen content.

Figure 1

$$A_{ij} = exp\left(\beta_0 + \beta_1 T_i + \beta_2 T_i^2 + \beta_3 E_j + \beta_4 E_i^2 + \beta_5 T_i E_j + \beta_6 T_i E_i^2\right)$$

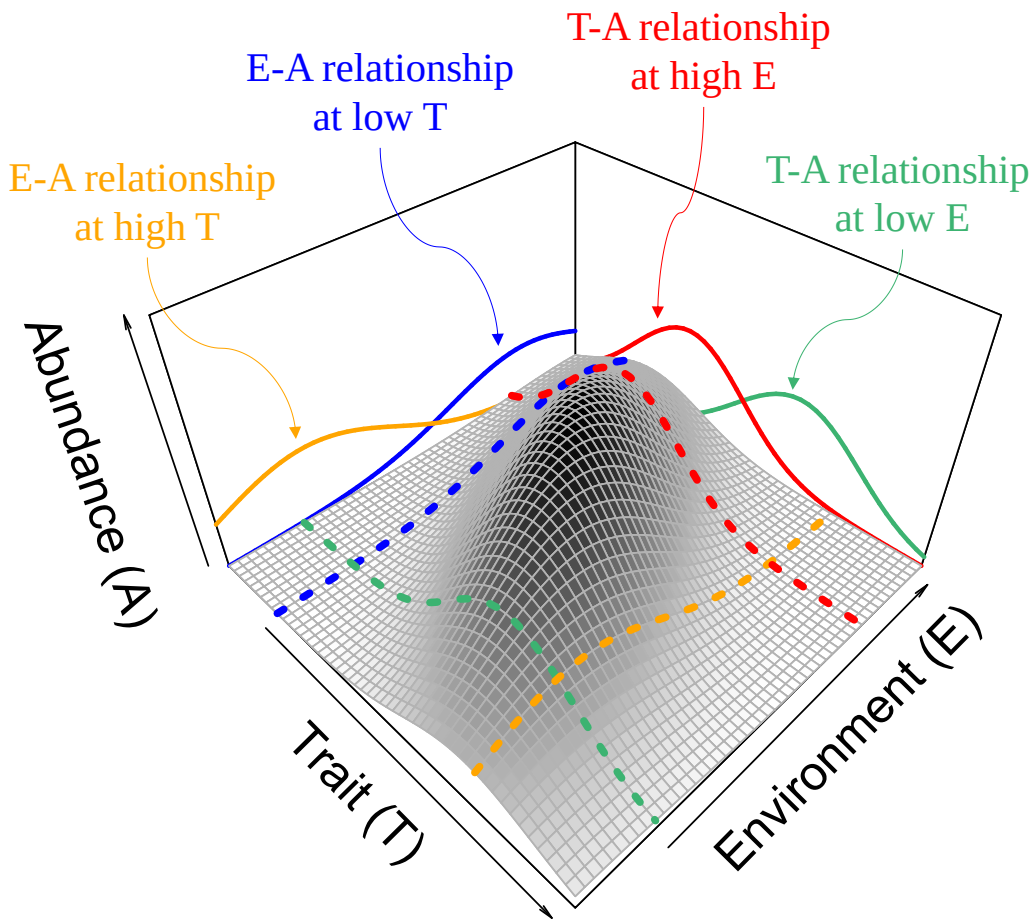


Figure 2

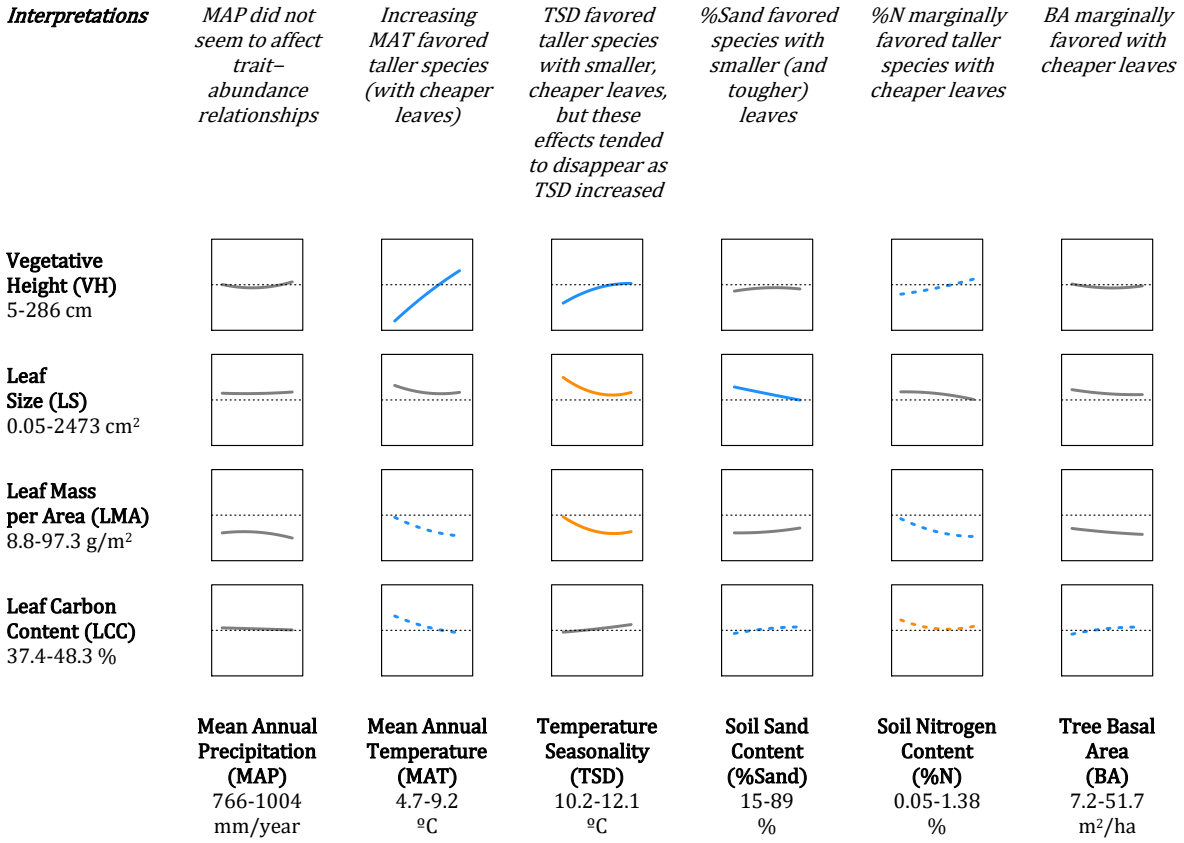

Interpretations
MAP did not seem to affect trait–abundance relationships
Increasing MAT favored taller species (with cheaper leaves)
TSD favored taller species with smaller, cheaper leaves, but these effects tended to disappear as TSD increased
%Sand favored species with smaller (and tougher) leaves
%N marginally favored taller species with cheaper leaves
BA marginally favored with cheaper leaves
Vegetative Height (VH) 5-286 cm
Leaf Size (LS) 0.05-2473 cm²
Leaf Mass per Area (LMA) 8.8-97.3 g/m²
Leaf Carbon Content (LCC) 37.4-48.3 %
Mean Annual Precipitation (MAP) 766-1004 mm/year
Mean Annual Temperature (MAT) 4.7-9.2 ºC
Temperature Seasonality (TSD) 10.2-12.1 ºC
Soil Sand Content (%Sand) 15-89 %
Soil Nitrogen Content (%N) 0.05-1.38 %
Tree Basal Area (BA) 7.2-51.7 m²/ha

Figure 3

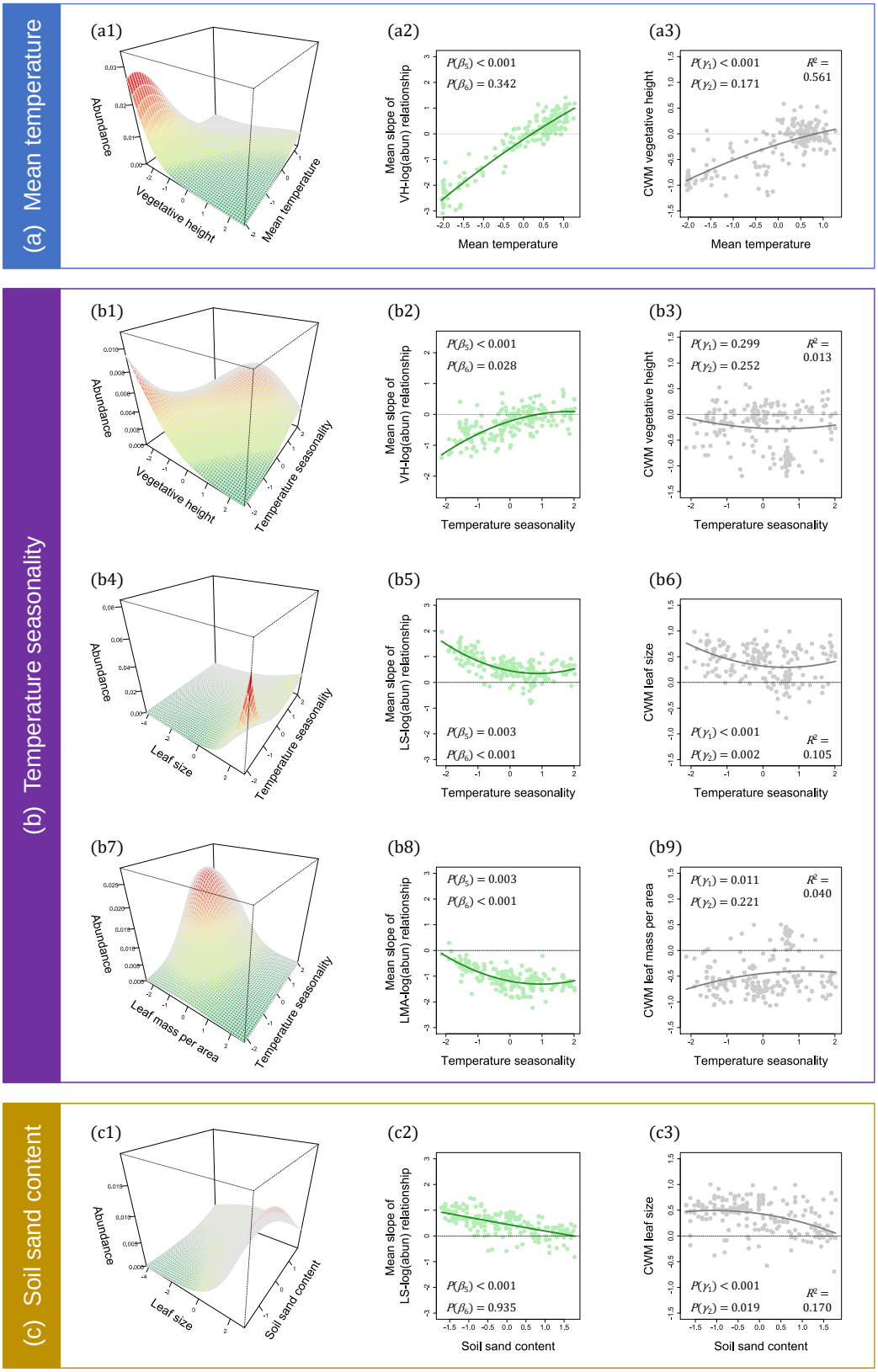
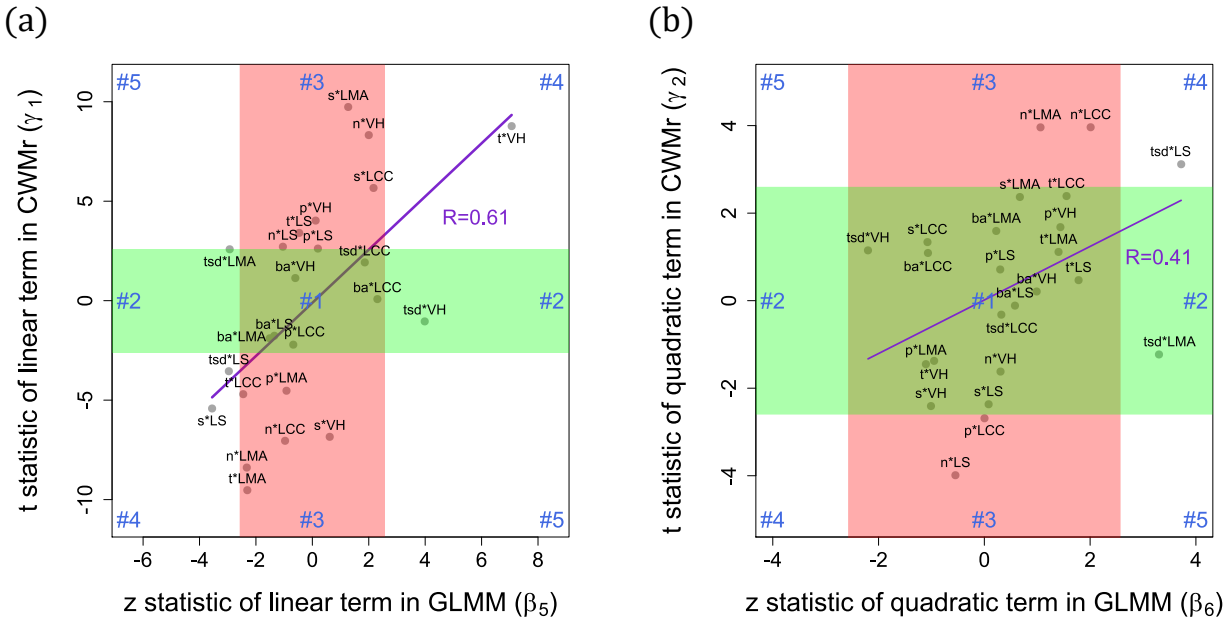

(a) Mean temperature
(a1)
Abundance
Vegetative height
Mean temperature
(a2)
Mean slope of VH-log(abun) relationship
$P(\beta_5) < 0.001$
$P(\beta_6) = 0.342$
Mean temperature
(a3)
CWM vegetative height
$P(\gamma_1) < 0.001$
$P(\gamma_2) = 0.171$
$R^2 = 0.561$
Mean temperature
(b) Temperature seasonality
(b1)
Abundance
Vegetative height
Temperature seasonality
(b2)
Mean slope of VH-log(abun) relationship
$P(\beta_5) < 0.001$
$P(\beta_6) = 0.028$
Temperature seasonality
(b3)
CWM vegetative height
$P(\gamma_1) = 0.299$
$P(\gamma_2) = 0.252$
$R^2 = 0.013$
Temperature seasonality
(b4)
Abundance
Leaf size
Temperature seasonality
(b5)
Mean slope of LS-log(abun) relationship
$P(\beta_5) = 0.003$
$P(\beta_6) < 0.001$
Temperature seasonality
(b6)
CWM leaf size
$P(\gamma_1) < 0.001$
$P(\gamma_2) = 0.002$
$R^2 = 0.105$
Temperature seasonality
(b7)
Abundance
Leaf mass per area
Temperature seasonality
(b8)
Mean slope of LMA-log(abun) relationship
$P(\beta_5) = 0.003$
$P(\beta_6) < 0.001$
Temperature seasonality
(b9)
CWM leaf mass per area
$P(\gamma_1) = 0.011$
$P(\gamma_2) = 0.221$
$R^2 = 0.040$
Temperature seasonality
(c) Soil sand content
(c1)
Abundance
Leaf size
Soil sand content
(c2)
Mean slope of LS-log(abun) relationship
$P(\beta_5) < 0.001$
$P(\beta_6) = 0.935$
Soil sand content
(c3)
CWM leaf size
$P(\gamma_1) < 0.001$
$P(\gamma_2) = 0.019$
$R^2 = 0.170$
Soil sand content

Figure 4


(a)
(b)
#1
#2
#3
#4
#5
R=0.61
R=0.41
t statistic of linear term in CWMr (γ_1)
z statistic of linear term in GLMM (β_5)
t statistic of quadratic term in CWMr (γ_2)
z statistic of quadratic term in GLMM (β_6)
s*LMA
n*VH
t*VH
s*LCC
p*VH
t*LS
n*LS
p*LS
tsd*LCC
tsd*LMA
ba*VH
ba*LCC
tsd*VH
ba*LS
ba*LMA
p*LCC
tsd*LS
t*LCC
p*LMA
s*LS
n*LCC
s*VH
n*LMA
t*LMA
n*LMA
n*LCC
tsd*LS
s*LMA
t*LCC
ba*LMA
p*VH
tsd*VH
s*LCC
t*LMA
ba*LCC
p*LS
t*LS
ba*VH
ba*LS
tsd*LCC
tsd*LMA
p*LMA
n*VH
t*VH
s*VH
s*LS
p*LCC
n*LS