

# Complex trait–environment relationships underlie the structure of forest plant communities

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## Abstract

1. Traits differentially adapt plant species to particular conditions generating compositional shifts along environmental gradients. As a result, community-scale trait values show concomitant shifts, termed trait–environment relationships. Trait–environment relationships are often assessed by evaluating community-weighted mean (CWM) traits observed along environmental gradients. Regression-based approaches (CWMr) assume that local communities exhibit traits centred at a single optimum value and that traits do not covary meaningfully. Evidence suggests that the shape of trait–abundance relationships can vary widely along environmental gradients—reflecting complex interactions—and traits are usually interrelated. We used a model that accounts for these factors to explore trait–environment relationships in herbaceous forest plant communities in Wisconsin (USA).
2. We built a generalized linear mixed model (GLMM) to analyse how abundances of 185 species distributed among 189 forested sites vary in response to four functional traits (vegetative height—VH, leaf size—LS, leaf mass per area—LMA and leaf carbon content), six environmental variables describing overstorey, soil and climate conditions, and their interactions. The GLMM allowed us to assess the nature and relative strength of the resulting 24 trait–environment relationships. We also compared results between GLMM and CWMr to explore how conclusions differ between approaches.
3. The GLMM identified five significant trait–environment relationships that together explain ~40% of variation in species abundances across sites. Temperature appeared as a key environmental driver, with warmer and more seasonal sites favouring taller plants. Soil texture and temperature seasonality affected LS and LMA; seasonality effects on LS and LMA were nonlinear, declining at more seasonal sites. Although often assumed for CWMr, only some traits under certain conditions had centred optimum trait–abundance relationships. CWMr more liberally identified (13) trait–environment relationships as significant but failed to detect the temperature seasonality–LMA relationship identified by the GLMM.
4. *Synthesis.* Although GLMM represents a more methodologically complex approach than CWMr, it identified a reduced set of trait–environment relationships still capable of accounting for the responses of forest understorey herbs to environmental gradients. It also identified separate effects of mean and seasonal temperature on LMA that appear important in these forests, generating useful

insights and supporting broader application of GLMM approach to understand trait–environment relationships.

#### KEYWORDS

climate seasonality, community assembly, functional trait analysis, generalized linear mixed model, leaf traits, mean annual temperature, plant height, soil texture

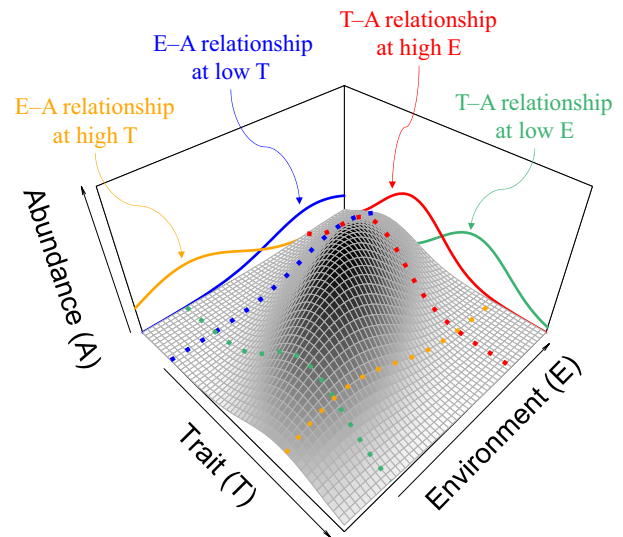
## 1 | INTRODUCTION

Understanding how plant species and communities respond to environmental gradients helps us predict future responses to global change (Kearney et al., 2010; Scheiter et al., 2013; Thuiller et al., 2008; van Bodegom et al., 2014). In general, we expect plant phenotypes to be distributed in ways that reflect how species are adapted to local environments providing a mechanistic link between environmental change and community responses (Keddy, 1992; Lavorel & Garnier, 2002; Shipley, 2010; Violle et al., 2007). This approach has led to considerable empirical research seeking to characterize how phenotypic traits relate to environmental gradients, that is, the so-called trait–environment relationships (Funk et al., 2017; Shipley et al., 2016). However, reported trait–environment relationships vary widely in strength and sign (see reviews in Funk et al., 2017; Garnier et al., 2016). This highlights the need to improve our understanding of these relationships, for example, to make useful predictions.

The analytical tools we use to represent how traits vary along environment gradients must be suitably structured and complete enough to adequately capture the complexity that underlies ecological relationships. Mechanistically, trait–environment relationships emerge as the consequence of an adaptive process wherein the relative fitnesses of species that vary in trait values change along environmental gradients (Laughlin & Messier, 2015; Shipley et al., 2016). Species or population-level fitnesses reflect multiple measures of local performance including rates of growth, survival and fecundity (Laughlin et al., 2020). These environment-dependent, trait-based fitness differences in turn translate into differences in species abundance (local population sizes), generating observable trait shifts along environmental gradients (Shipley et al., 2016). Traits and environments thus interact via their effects on fitness to influence relative species abundances across communities.

How trait–environment interactions affect species abundance can be viewed from two perspectives (Figure 1; see also ter Braak, 2019). The site-centric perspective focuses on how rules for local community assembly (Keddy, 1992), as represented by trait–abundance relationships (Loranger et al., 2018; Rolhauser & Pucheta, 2017), change along environmental gradients (Figure 1). The species-centric perspective instead focuses on how traits modulate species' abundances in response to environmental conditions, or environment–abundance relationships (Figure 1; Vesk, 2013). A corollary is that trait–environment relationships are inherently three-dimensional and cannot be readily reduced to, or inferred from, relationships viewed only along single trait or environmental

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



**FIGURE 1** Fixed-effect structure of our GLMM approach modelling variation in the abundance of species  $i$  at site  $j$  (equation for  $A_{ij}$  at the top).  $A_{ij}$  (white to black surface) is modelled as an exponential function (through a log link) of trait  $T$  and environmental variable  $E$ , both standardized to zero mean and unit variance. Parameters  $\beta_1$  and  $\beta_2$  are the linear and quadratic effects of  $T$  on  $A$ ;  $\beta_3$  and  $\beta_4$  are the linear and quadratic effects of  $E$  on  $A$ ; and  $\beta_5$  and  $\beta_6$  are the linear and quadratic effects of  $E$  on  $T$  (see Section 2.5). Combining the log link with negative quadratic terms (with  $\beta_2$  and  $\beta_4$  set to  $-1$  and  $-0.5$ , respectively, here), this model reproduces unimodal trait–abundance (green and red) and environment–abundance (orange and blue) relationships. In this example, the result is a directional and negative  $T$ – $E$  relationship ( $\beta_5$  and  $\beta_6$  set to  $-1$  and  $0$  respectively). Dotted lines are modelled relationships at different  $T$  or  $E$  levels while continuous lines are their counterparts projected onto 2D planes

axes. Rather, we must characterize how trait values interact with environmental conditions to affect abundance if we are to infer trait–environment relationships. A first step here is to acknowledge the different shapes these relationships can take.

When species are best adapted to some particular intermediate environmental condition, environment–abundance relationships should be humped or unimodal (Austin, 1999; Curtis, 1959; ter Braak & Prentice, 1988; Whittaker, 1967). Similarly, we expect unimodal trait–abundance relationships when functional trade-offs lead to optimum trait values among coexisting species (Muscarella & Uriarte, 2016; Rolhauser et al., 2019; Rolhauser & Pucheta, 2017). However, environment–abundance relationships can also be bimodal

if one species is excluded from its optimum by strong competition, herbivory or some other negative interspecific interaction (Austin, 1999; Minchin, 1987; Mueller-Dombois & Ellenberg, 1974). Within-site environmental heterogeneity can also favour functional divergence of competitive, dominant species, generating a bimodal (or multimodal) trait–abundance relationship (Rolhauser et al., 2019; Rolhauser & Pucheta, 2017). Directional relationships may emerge when abundance maxima (in optimum relationships) or minima (in bimodal relationships) occur at the extremes or outside the range of observed trait and environmental values (Rolhauser et al., 2019; ter Braak & Prentice, 1988). Unlike trait–abundance and environment–abundance relationships, theoretical discussions on the shape of trait–environment relationships are rare. Both conceptual (Garnier et al., 2016; Laughlin & Messier, 2015; Shipley et al., 2016) and empirical studies (Moles et al., 2009, 2014; e.g. Niinemets, 2001; Wright et al., 2004, 2017) largely assume directional relationships. However, empirical work suggests that trait–environment relationships can be nonlinear (de Bello et al., 2013; Laughlin et al., 2011).

A simplification of the three-dimensional structure of trait–environment relationships is common, such as when studies regress single-trait values against environmental variables (Funk et al., 2017; Garnier et al., 2016). These trait–environment regressions may employ community-weighted mean (CWM) values of traits as response variables (Miller et al., 2019), use site-specific species trait averages (e.g. Dong et al., 2020) or use individual trait values (Laughlin et al., 2012). Trait–environment regressions have two conceptual limitations. First, because they do not explicitly model abundance, they cannot account for possible variations in trait–abundance and environment–abundance relationships. For example, CWM values represent the adaptive value of a trait only at sites showing centred optimum trait–abundance relationships (Muscarella & Uriarte, 2016) despite this being one of a number of potential relationships (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. Because traits are inherently interrelated within integrated phenotypes (Murren, 2012), this can be misleading. Evolutionary biologists have long recognized the importance of using multivariate approaches when trying to tease apart the adaptive significance of correlated traits (Lande & Arnold, 1983). Both complexities reduce the power, potentially generating false-negative conclusions (type II errors). Studies using simulated data show that CWM regressions (CWMr) can also lead to false-positive conclusions (type I errors) as they fail to account for differences in how species respond to environmental gradients (Miller et al., 2019; Peres-Neto et al., 2017). The fourth-corner method (Dray & Legendre, 2008) has better false-positive rates but does not provide information on the strength of trait–environment associations, limiting interpretability (Brown et al., 2014).

To better account for these complexities, we used a generalized linear mixed model (GLMM) approach (ter Braak, 2019; Veski, 2013; Warton et al., 2015) to evaluate trait–environment relationships across forest understorey communities in Wisconsin, USA. Our

model allows all three functional relationships (trait–abundance, environment–abundance and trait–environment) to be nonlinear (Figure 1; Figure S6). Also, the GLMM simultaneously estimates all effects as partial regression coefficients, estimating interactions after accounting for effects of other interactions present in the model. This allows us to rank trait–environment relationships according to their strength. In doing so, this approach provides an explanation of species–abundance variation across communities based on traits, environmental gradients and their interactions (Figure 1).

We used comprehensive data describing the abundance of 185 species across 189 forest stands, four plant traits, and six key environmental variables. The traits were maximum vegetative height (VH), leaf size (LS), leaf mass per area (LMA) and leaf carbon content (LCC). These traits are well-known to play central roles in plant growth and temperature regulation (Díaz et al., 2016; Lambers & Poorter, 1992; Westoby et al., 2002). The environmental variables were mean annual temperature (MAT), mean annual precipitation (MAP), temperature seasonality (i.e. the standard deviation of mean monthly temperatures, abbreviated TSD), soil sand (%Sand) and nitrogen (%N) contents and tree basal area (BA). MAT and MAP drive ecological dynamics at broad scales (Whittaker, 1970), while TSD may exert important effects at regional scales (Xu et al., 2013). %Sand usually limits soil water retention and availability, while %N reflects soil fertility. Basal area increases as forest stands mature and their canopy closes, reducing light availability for understorey plants (Canham et al., 1994). We expect increasingly benign conditions for plant growth (e.g. higher MAT, MAP and %N, and lower %Sand) to generally favour acquisitive species, that is, taller plants with larger and cheaper leaves in terms of both mass and carbon investments (Garnier et al., 2016; Moles et al., 2009, 2014; Niinemets, 2001; Wright et al., 2004, 2017). Indeed, a previous study of these same understorey species in Wisconsin confirms that taller plants with cheaper leaves dominate sites with higher MAT, MAP and %N while %Sand exerted the opposite effects (Amatangelo et al., 2014), although information on the strength of these associations was not available. Finally, we compare results between GLMM and CWMr to explore how conclusions differ between approaches. We expect the GLMM to identify fewer significant trait–environment relationships compared to CWMr given the general propensity of the latter to identify false-positive results (Miller et al., 2019; Peres-Neto et al., 2017).

## 2 | MATERIALS AND METHODS

### 2.1 | Study area

Wisconsin covers a large area (169,639 km<sup>2</sup>) of fairly uniform elevation (177–595 m). Nonetheless, there are strong north–south and east–west gradients in MAT and temperature seasonality (Figure S1). A marked climatic and floristic ‘tension zone’ running NW to SE bisects the state separating forest types and the ranges of many species (Curtis, 1959; Figure S1). Climatic changes have shifted these

gradients between the 1950s and 2000s with forest species shifting their distributions to various degrees in response (Ash et al., 2017). Prairies and savannas originally dominated the unglaciated region in southwestern Wisconsin until European settlement and fire suppression led to increasing forest cover (Figures S2 and S3; Wisconsin DNR, 2015). Upland forests in southern Wisconsin are now mostly dominated by oaks, maples and basswood (*Quercus*, *Acer* and *Tilia*) persisting as fragments within a matrix of agricultural and developed lands (Figure S4). Beech forests exist only in eastern Wisconsin under the moderating effect of Lake Michigan. Upland forests in northern Wisconsin were originally dominated by sugar and red maple (*Acer saccharum* and *rubra*), hemlock (*Tsuga*), northern red oak (*Quercus rubra*) and white and red pine (*Pinus strobus* and *resinosa*) with jack pine (*P. banksiana*) and small oaks dominating pine barrens communities on sandy sites. Northern forests are more continuous but now tend to be dominated by *Populus*, *Betula*, and other early successional species except in scattered mature stands (disproportionally represented among our study sites).

## 2.2 | Vegetation sampling

Surveys of forested sites through much of Wisconsin (Figure S1) occurred between 2000 and 2012 (Waller et al., 2012; see Appendix S1 for details). Only sites with intact forest canopy, no nearby edges and few signs of understorey disturbance were surveyed. All vascular plants were tallied within each of many replicate (20–403) 1-m<sup>2</sup> quadrats to estimate species abundances (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 access to locally collected trait data. We then selected sites for analysis with high trait coverage, that is, those for which the cumulative relative abundance of species with known trait values was higher than 0.8. We discarded a few sites missing full environmental data, yielding 189 sites.

## 2.3 | Environmental variables

Descriptions of the four climatic variables, 10 soil variables and one overstorey variable (tree BA) appear in Appendix S1. Climate variables for each site derive from 10-year averages for the period 1995–2004. Replicate soil samples from each site were combined and analysed for cations and physical properties. We estimated tree BA at each site using tree surveys. We used pairwise Pearson correlations and principal components analyses to select relatively independent subsets of climatic and soil variables and so avoid collinearity among predictors (see details in Appendix S1). This generated six predictors: MAT, MAP, temperature seasonality (or monthly temperature standard deviation, TSD), soil nitrogen content (%N), soil sand content (%Sand) and tree BA. Correlation coefficients between all retained predictors were below 0.5 (Table S2). Before analysis, environmental variables were transformed as needed to reduce

the weight of extreme values, then standardized to zero mean and unit variance (Appendix S1).

## 2.4 | Plant traits

Amatangelo et al. (2014) characterized functional trait variation in the common herbaceous species in these communities using standard methods (see Appendix S1 for trait descriptions and methods). Briefly, at least 12 individuals ( $\geq 4$  plants from each of  $\geq 3$  sites) were collected in Wisconsin between 2008 and 2014 and processed following standardized protocols (Pérez-Harguindeguy et al., 2013). From 10 traits available, we selected four for analysis which both represented leading ecological dimensions and showed low pairwise Pearson correlations ( $< 0.3$ ; Table S3). These are: VH, leaf size (LS, calculated as the product of length and width), LMA and LCC. Traits were transformed as needed to reduce the weight of extreme values and standardized (Appendix S1).

## 2.5 | Statistical methods

### 2.5.1 | GLMM

We sought to infer the significance, strength and shape of trait–environment relationships while modelling meaningful site and species responses to, respectively, trait and environmental axes (Figure 1). We used a negative binomial GLMM to model the abundance of species  $i$  at site  $j$  ( $A_{ij}$ ) as a function of the selected traits and environmental variables using 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 our analysis for one trait and one environmental variable, both standardized. Given the log link function, the fixed effects in the GLMM are (cf. Figure 1):

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

where  $\beta_0$  is the y-intercept of the fitted surface. Since trait  $T$  is centred at zero,  $\beta_1$  and  $\beta_2$  respectively estimate the mean slope (or 'gradient') and the mean curvature of the trait–abundance relationship for the particular case when  $E_j$  is set to its average value: zero (see e.g. Aiken et al., 1991).  $\beta_1$  thus measures the direction (positive or negative) and strength of trait  $T_i$ 's effect on species abundance. Negative values of  $\beta_2$  indicate 'n' shaped (optimum or unimodal) relationships, while positive values indicate 'u' shaped (bimodal) relationships. Similarly,  $\beta_3$  and  $\beta_4$  reflect the mean slope and curvature of the environment–abundance relationship (ter Braak, 2019). Due to the combination of quadratic effects ( $\beta_2$  and  $\beta_4$ ) and the log link associated with the negative binomial distribution, Equation 1a can reproduce bell-shaped trait–abundance and environment–abundance relationships (Figure 1; Figure S6a).

While doing this, we assess trait–environment relationships by estimating interactions between  $T$  and  $E$ . These parameters 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  $\beta_5$  and  $\beta_6$ , as explained below.

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

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

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

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

In this quadratic function,  $\beta_5$  and  $\beta_6$  are respectively the mean slope and mean curvature of the trait–environment relationship (Figure S6).  $\beta_5$  thus measures the overall direction and strength of the trait–environment interaction. Furthermore, since  $T_i E_j^2 = (T_i E_j) E_j$ ,  $\beta_6$  estimates how the strength of the trait–environment interaction in a given site depends on the environmental value at that site (see Equation 1a), a quadratic relationship. Concordantly, the properties of the fitted surface (e.g. the position of optimum trait values) change along the environmental gradient depending on  $\beta_5$  and  $\beta_6$  (Figure S6).

The full GLMM included four traits (VH, LMA, LS and LCC) and six environmental variables (MAT, MAP, TSD, %Sand, %N and BA), yielding 24 trait–environment relationships to be estimated. 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 full model in R code notation is shown in Appendix S1. In this formulation, we estimated random-effect slopes for each trait,  $T$ , for each site (denoted  $b_{(T)j}$  here). We similarly estimated random-effect slopes for each environmental variable,  $E$ , for each species ( $c_{(E)i}$ ) (ter Braak, 2019). We accounted for search effort by including the log number of quadrats sampled at each site, used here as an offset (Kéry, 2010, pp. 188–189). We fitted the full, multi-trait GLMM using the R-package `GLMMTMB` (Brooks et al., 2017).

Importantly, standardization of traits and environmental variables in the GLMM results in the estimation of standardized partial regression coefficients ( $\beta_1$  to  $\beta_6$  in Equation 1). Therefore, the strength and curvature of trait–environment relationships (controlled by  $\beta_5$  and  $\beta_6$ ) can be readily compared across the 24 trait–environment combinations (Schielzeth, 2010). Standardization also allows for the interpretation of linear effects in the presence of polynomials (Lande & Arnold, 1983; Schielzeth, 2010).

We assessed the significance of model terms based on parametric Wald chi-square tests of Type II (which follow the principle of marginality) implemented by the ANOVA function in the R-package `CAR` (Fox & Weisberg, 2019). Wald tests are commonly used because

they are straightforward and easy to implement, although they can be relatively liberal or anti-conservative, especially for smaller sample sizes (e.g. Luke, 2017). To account for this, even though our sample size is reasonably large (34,965 species–site combinations), we used a lower significance level (aka alpha) of 0.01.

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). We implemented  $R^2$  calculations using the `r.squaredG-LMM` function in the R-package `MuMIn` (Barton, 2019). We visualized uncertainty around estimates of  $\varphi_j$  (for a given  $T$ – $E$  combination) by plotting overall site effects calculated as the sum of the fixed ( $\varphi_j$ ) and the random slopes associated with the corresponding trait ( $b_{(T)j}$ ) (ter Braak, 2019).

## 2.5.2 | Comparing GLMM and CWMr approaches

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

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

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 the six standardized environmental variables ( $E_j$ ) through four, separate (one for each trait) multiple regression models. In each model, we included both linear and quadratic terms for  $E_j$ , generally denoted  $\gamma_1$  and  $\gamma_2$  respectively. We fitted CWMr models using the least squares method implemented in the `lm` function in R.

First, we compared the multi-trait GLMM and CWMr approaches in terms of the statistical significance of homologous model parameters for each trait–environment combination, that is,  $\beta_5$  versus  $\gamma_1$  and  $\beta_6$  versus  $\gamma_2$ . We divided test statistics ( $z$  and  $t$  in the case of GLMM and CWMr respectively) based on whether they led to the rejection of the null hypothesis or not ( $p < 0.01$ ) and compared these between approaches (see Appendix S1). We preferred test statistics because they are directly linked with statistical hypothesis testing; the associated  $p$ -values are virtually identical to those calculated via Type II chi-square tests, at least for trait–environment interactions. This allowed us to identify different clear-cut outcomes into which each trait–environment relationship can be classified (Table S4; Laughlin et al., 2018). We interpreted these outcomes based on the assumption that the GLMM would provide a more realistic description of trait–environment relationships compared to CWMr, given its ability to accommodate the ecological complexities discussed above as well as the statistical advantages shown by others (Miller et al., 2019; Peres-Neto et al., 2017; ter Braak, 2019). However, we note that this comparison is not conclusive with respect to the true existence of trait–environment relationships since both approaches are correlative; that is, adaptive processes are inferred from species' spatial distributions and not from direct fitness estimations (Laughlin et al., 2020; Laughlin & Messier, 2015).

When both CWMr and GLMM show nonsignificant effects, they agree that the trait has little adaptive value along that environmental gradient (Outcome #1). A nonsignificant trait–environment relationship with CWMr that is significant with GLMM suggests that the trait does have adaptive value along the gradient, but that CWMr lacked the sensitivity to detect it (Outcome #2). If, conversely, the CWMr trait–environment interaction is significant but the GLMM term is not, the CWMr result may possibly be spurious or indirect as found by others who noted highly inflated false-positive rates (Miller et al., 2019; Peres-Neto et al., 2017) (Outcome #3). Therefore, we interpreted Outcomes #2 and #3 as *possible* false negatives and false positives respectively. Finally, if both methods identify a significant relationship with the same sign, we can conclude the trait has adaptive value along that gradient (Outcome #4). However, significant relationships for both approaches might also emerge with opposite signs (Outcome #5), indicating a clear disagreement between methods that may be interpreted as possible spurious results from the CWMr, similarly to Outcome #3 (Table S4).

Second, we compared both full GLMM and CWMr approaches with an intermediate approach. Here, we intend to differentiate between the effect of modelling abundance responses to trait and environmental variation and modelling trait covariation in driving the discrepancies between full GLMM and CWMr. The intermediate approach consists of single-trait GLMMs that follow the structure in Equation 1a. That is, we fitted four separate GLMMs each one featuring a single trait, all six environmental variables and their interactions with the trait, and all quadratic terms for main predictors. Single-trait GLMMs are then completely analogous to the CWMr described above in that they estimate trait–environment relationships for one trait in the absence of other traits. Therefore, comparing single-trait GLMMs and CWMr may give us a sense of the impact of modelling trait–abundance and environment–abundance relationships on the inference of trait–environment relationships. Comparing single-trait and multi-trait GLMMs would then explore the role of trait covariation in determining trait–environment relationships. We used Pearson correlations ( $n = 24$ ) to measure the overall agreement between approaches in terms of test statistics.

## 3 | RESULTS

### 3.1 | Multi-trait GLMM

#### 3.1.1 | Overview

The full, multi-trait model used 113 parameters (69 fixed effects, 43 random effects and the overdispersion parameter) to explain abundance across 34,965 species–site combinations. The marginal  $R^2$  of this model was 0.385 with a conditional  $R^2$  of 0.977. We focus on trait–environment relationships, but full model results appear in Appendix S2. All significant quadratic main effects were negative (Table S5), indicating unimodal trait–abundance and environment–abundance relationships. Importantly, removing either all quadratic or all interaction terms from the full model resulted in large AIC increments (Table S6), supporting their retention in the final model.

#### 3.1.2 | Trait–environment relationships

The multi-trait GLMM identified five trait–environment relationships as significant, defined as those combinations of a trait and an environmental variable where either or both interaction parameters ( $\beta_5$ ,  $\beta_6$ ) were significant ( $p < 0.01$ ; Table S5). Three of these relationships were largely directional (only  $\beta_5$  was significant; blue lines in Figure 2). The interaction between MAT and vegetation height (VH) was the strongest ( $\beta_5 = 1.031$ ;  $SE = 0.146$ ). All remaining significant linear interactions ( $\beta_5$ ) had slopes below 0.33 (Table S5; note unitless standardized coefficients). Warmer sites (higher MAT) favoured taller plants (Figure 2, top line second plot, and Figure 3a1,a2). Height also increased linearly with temperature seasonality (TSD:  $\beta_5 = 0.328$ ,  $SE = 0.082$ ; Figure 2, top line third plot, and Figure 3b1 and b2). In addition, LS declined linearly as soil sand content increased ( $\beta_5 = -0.266$ ,  $SE = 0.075$ ; Figure 2, row 2 column 4, and Figure 3c1,c2).

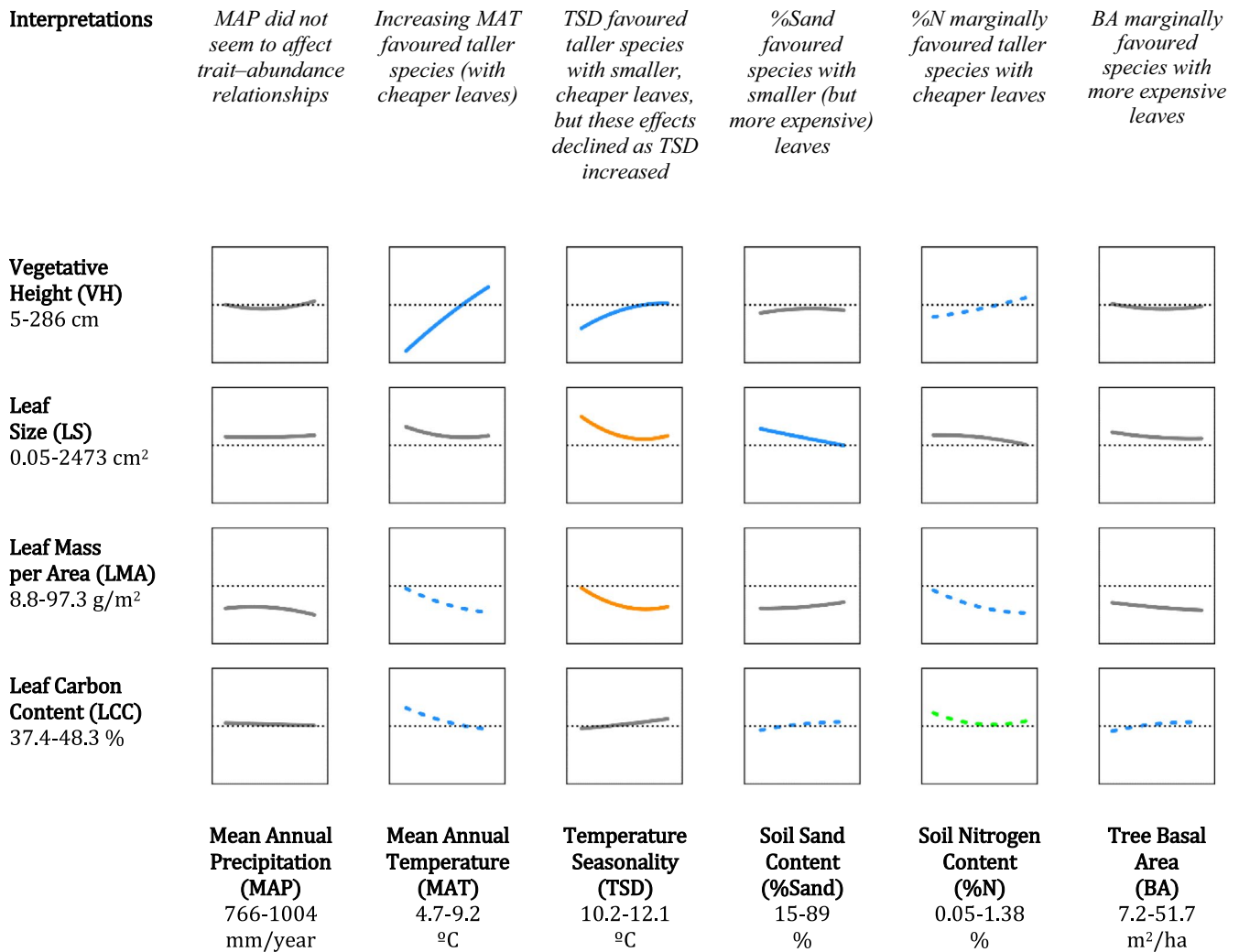
Sites with more seasonal temperatures (TSD) supported species with smaller leaves (LS,  $\beta_5 = -0.242$ ,  $SE = 0.082$ ; Figure 3b3 and b4) and lower leaf mass per area (LMA,  $\beta_5 = -0.239$ ,  $SE = 0.082$ ; Figure 3b5 and b6). In both cases, these decreases were nonlinear ( $\beta_6 \neq 0$ , orange lines, Figure 2, column 3; Table S5) with decelerating effects of seasonality on LS and LMA as seasonality increased.

Other linear trait–environment interactions were marginally significant ( $0.01 < p < 0.05$ , dashed lines in Figure 2). These included increases in plant height with soil N (row 1, column 5), declines in LMA with increasing temperatures and increasing soil N (row 3, columns 2 and 5), declines in LCC with increasing temperatures (row 4, column 2) and increases in LCC as soil sand (row 4, column 4) and BA (row 4, column 6) increased. LCC also showed a marginally significant nonlinear response to soil N (green dashed line, row 4, column 5). Surprisingly, annual precipitation (MAP) did not appear to affect any of these traits (column 1).

Significant trait–environment interactions mean that the shapes of trait–abundance relationships change along environmental gradients (Figure 3, column 1). We did detect roughly centred optimum trait–abundance relationships (the form assumed by the CWMr approach) for some trait–environment combinations, sometimes for restricted regions along environmental gradients. These centred optimum patterns appeared most notably for VH around the mean MAT (Figure 3a1) and at mid-to-high TSD (Figure 3b1). These relationships, however, become directional away from these environmental regions. We found largely directional or flat trait–abundance relationships for the remaining three significant trait–environment relationships (Figure 3b3, b5 and c1).

### 3.2 | Comparing GLMM and CWMr approaches

In contrast to the five significant trait–environment relationships (of 24) identified using the multi-trait GLMM, the CWMr approach identified 13 regressions where either or both parameters ( $\gamma_1$  and  $\gamma_2$ ) showed  $p < 0.01$  (Table S7). Nine of these were only directional (only  $\gamma_1 \neq 0$ ; e.g. LMA–MAT), two were curved with a directional component (both  $\gamma_1 \neq 0$ ,  $\gamma_2 \neq 0$ ; e.g. LCC–%N) and two were curved but lacked directionality (only  $\gamma_2 \neq 0$ ; VH–BA; Table S7).

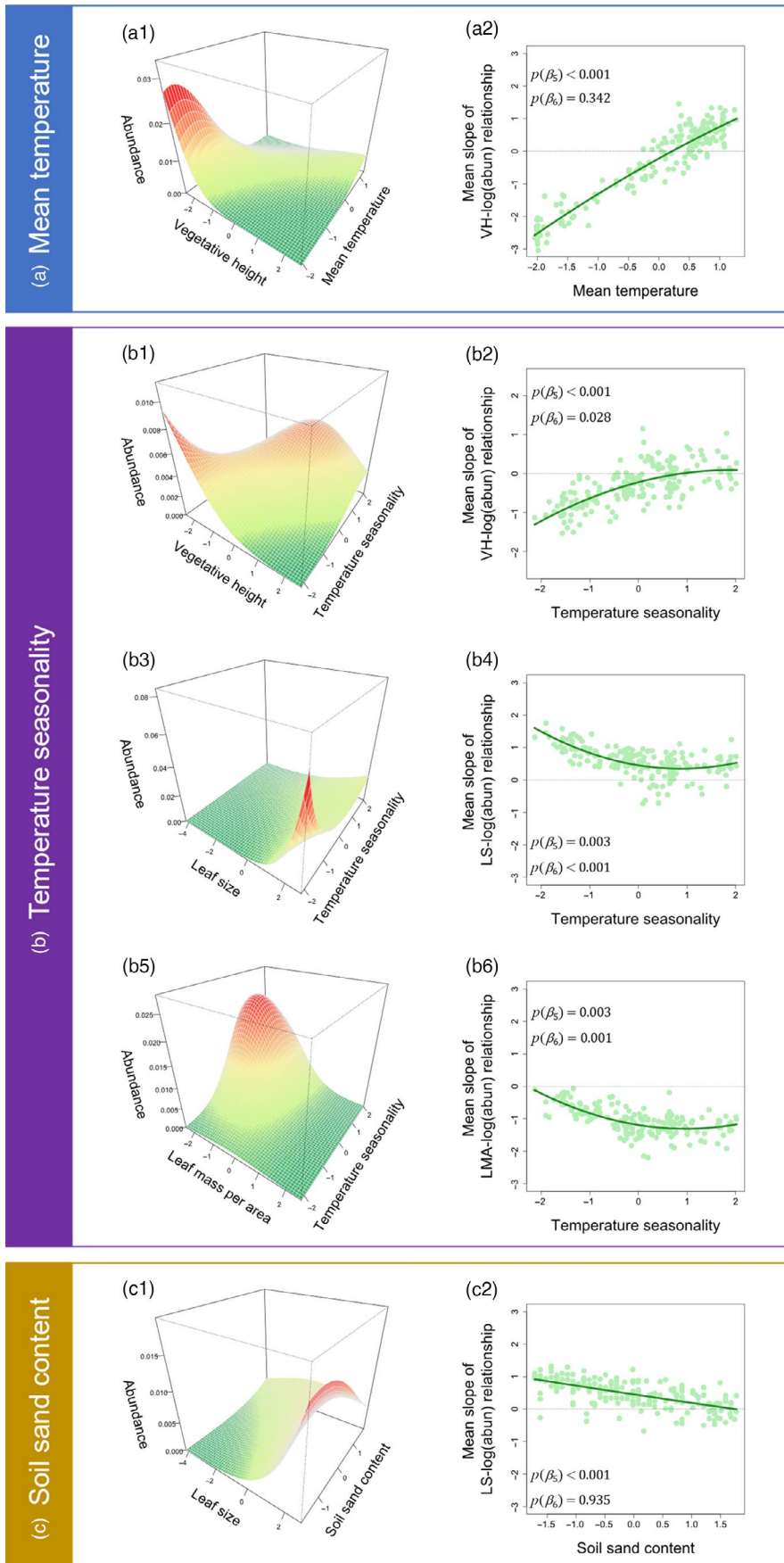


**FIGURE 2** Trait–environment relationships estimated via the multi-trait GLMM (Equation 2). Relationships between traits (y-axes) and environmental variables (x-axes) are plotted based on estimates of their linear ( $\beta_5$ ) and quadratic ( $\beta_6$ ) coefficients (Table S5). Traits and environmental variables are shown together with their observed ranges below. Blue lines reflect relationships where only linear effects are significant ( $\beta_5 \neq 0, \beta_6 \sim 0$ ). Green lines show relationships with only nonlinear effects significant ( $\beta_5 \sim 0, \beta_6 \neq 0$ ). Orange lines reflect situations where both effects are significant ( $\beta_5$  and  $\beta_6 \neq 0$ ). Dashed lines show relationships of lower significance ( $0.01 < p < 0.05$ ) while grey lines show those lacking any significance. Dotted horizontal lines show  $y = 0$  for reference. ‘Interpretations’ above describe overall patterns (with marginally significant patterns parenthesized). See Figure 3 for further details regarding the five significant relationships [Correction added on 1 October 2021, after first online publication: Figure 2 has been replaced with missing lines included.]

CWMr and multi-trait GLMM approaches agreed that 12 linear effects (including all four interactions with BA, Figure 4a) and 19 quadratic effects lacked significance (Figure 4b), representing Outcome #1. In addition, four linear effects of environmental conditions on plant traits (VH–MAT, VH–TSD, LS–TSD and LS–%Sand) and one quadratic term (LS–TSD<sup>2</sup>) emerged as significant in both approaches (Outcome #4). Reassuringly, we found no cases where the two approaches yielded opposite conclusions (Outcome #5). However, inferences often differed conspicuously between GLMM and CWMr. One linear and one quadratic effect emerged as significant in the GLMM but not in the CWMr approach (Outcome #2), both involving the relationship between LMA and TSD. More worrying, seven linear and three quadratic terms judged significant by the CWMr approach lacked significance under the GLMM

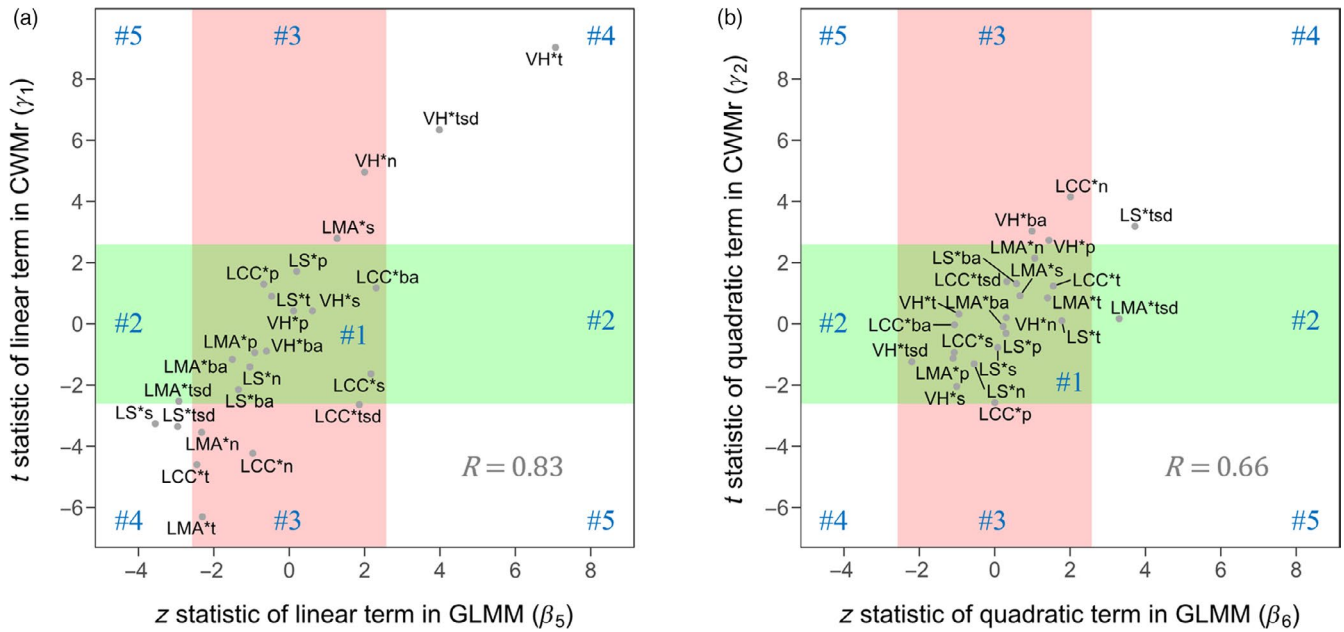
(Outcome #3). These included the following relationships: VH with %N, MAP and BA (the latter two being nonlinear); LMA with MAT, sand and %N; and LCC with MAT, TSD and %N (the latter being nonlinear; Figure 4; Table S7).

All trait–environment relationships classified as Outcome #3 (significant in CWMr and not significant in the multi-trait GLMM) were also not significant in the intermediate, single-trait GLMMs (Table S8). As a result, test statistics were generally more similar between multi- and single-trait GLMMs than between the former and CWMr (Table S9 and associated text). This suggests that accounting for trait–abundance and environment–abundance relationships contributed more to discrepancies between multi-trait GLMM and CWMr approaches than accounting for trait covariation. Nonetheless, single-trait GLMMs, as CWMr did, failed to detect LMA–TSD interactions



**FIGURE 3** Details of significant interactions ( $p < 0.01$ ) from the multi-trait GLMM visualized for (a) mean annual temperature, (b) temperature seasonality and (c) soil sand content. Surfaces in the first column show species abundance fitted as a function of trait values and environmental conditions (holding other variables constant at their mean value). Plots in the second column show average slopes ( $\varphi_j$  in Equation 2) of the given trait-abundance relationship (green line).  $\varphi_j$  depends on  $\beta_5$  and  $\beta_6$  which estimate the linear and quadratic components of the trait-environment relationships respectively. Each green dot represents the overall effect of site  $j$ , that is, the sum of the fixed ( $\varphi_j$ ) and the random slope associated with a trait ( $b_{(T)j}$ ). Dotted horizontal lines show  $y = 0$  for reference. Surfaces are coloured to enhance the perception of relief





**FIGURE 4** Scatterplots relating test statistics from community-weighted mean regressions (CWMr) to statistics for homologous terms from the multi-trait GLMM. Plot (a) compares linear terms ( $\gamma_1$  and  $\beta_5$ ) while plot (b) compares quadratic terms ( $\gamma_2$  and  $\beta_6$ ). Each data point corresponds to one of the 24 trait–environment relationships. Pearson’s correlations ( $R$ ) show that estimated linear terms agree better between the two approaches than the quadratic terms. Green and red rectangles show ranges of test statistic values failing to reject the null hypothesis (no significant effect;  $\alpha = 0.01$ ) under the CWMr and GLMM models respectively. The five Outcome codes shown in blue refer to those outlined in Materials and Methods and Table S4. Briefly, CWMr and GLMM may agree that trait–environment relationships are either significant (Outcome #4 for equal signs, Outcome #5 for different signs) or nonsignificant (outcome #1). Approaches may disagree when trait–environment relationships are significant in the GLMM but not in CWMr (Outcome #2) or vice versa (Outcome #3). Environmental variable and trait codes as in Figure 2, except that p = mean annual precipitation, t = mean annual temperature, s = soil sand content, n = soil nitrogen content

(Table S8), indicating that accounting for trait covariation is also important, at least for this trait–environment relationship.

## 4 | DISCUSSION

From the 24 trait–environment relationships, only five emerged as significant in the multi-trait GLMM, including one not supported by the CWMr approach. These, however, were sufficient to explain nearly 40% of the total variation in abundance among 185 plant species distributed over 189 sites. Recent GLMM applications achieved similar explanatory power in analysing presence–absence data (Löbel et al., 2018; Pollock et al., 2018). Our work confirms that trait–environment mixed-effects models provide powerful tools to explain not only distributions of species but also their abundances across large spatial scales. As we expected, the CWMr approach identified many relationships as significant that lacked significance in the GLMM. Conclusions derived from our multi-trait GLMM also contrast with those obtained from the fourth-corner method applied by Amatangelo et al. (2014) as discussed below.

### 4.1 | Prominent trait–environment relationships

The interaction between MAT and VH emerged as the strongest trait–environment relationship in the multi-trait GLMM in terms of

the size and significance of its slope ( $\beta_5$ ). It was also the only relationship where the slope of the trait–abundance relationship ( $\varphi_j$ ) switched signs along the environmental gradient (Figure 3), reflecting clear shifts in the rank order of species abundances. The positive height–temperature relationship matches global patterns (Moles et al., 2009, 2014). It may be explained by the fact that warmer climates favour taller plants as they compete for light (Falster & Westoby, 2003; Givnish, 1982) and/or that shorter plants are less prone to freeze-embolism in cold climates (Moles et al., 2009).

Temperature seasonality tended to increase both VH and plant abundance, confirming how many understorey species thrive in highly seasonal environments. This may reflect how spring and fall periods of higher light in these deciduous forests and warmer forest floor conditions favour understorey plant growth. If the growth of many species is favoured at more seasonal sites, these sites could generate the more competitive, crowded conditions that favour taller plant species (Falster & Westoby, 2003; Givnish, 1982).

Both LS and leaf mass per area (LMA, the inverse of specific leaf area) declined as temperature seasonality (TSD) increased. Low-LMA leaves are ‘cheaper’ in terms of dry-mass investment and therefore tend to be fast growing, although short lived (Poorter et al., 2009). Faster-growing species with low-LMA leaves might thus be favoured at sites with short spring windows of full light, for example, shorter seasons (Kikuzawa et al., 2013; Mason & Donovan, 2015). Given that leaves often start to generate carbohydrates at about half their

final size (Turgeon, 1989), shorter seasons might also favour smaller leaves for their ability to start exporting carbohydrates sooner than larger leaves.

Leaf size declined at sites with sandier soils in agreement with global patterns where water-limited environments tend to favour plants with smaller leaves (Wright et al., 2017). The standardized coefficient ( $\beta_s$ ) for the LS-%Sand interaction was only a quarter the size of that for the VH-MAT interaction. Thus, soil properties may play a smaller role in forest plant community assembly than climate factors at our scale of observation.

Among environmental factors, only temperature seasonality (TSD) interacted nonlinearly with plant traits with effects of TSD on LS and LMA declining as seasonality increased. Nonlinear patterns were also reported in other studies (de Bello et al., 2013; Laughlin et al., 2011), suggesting that trait-environment relationships may not be constant enough along some environmental gradients to expect consistent patterns to emerge. Such nonlinear effects could help to explain some of the inconsistencies found in the literature in terms of the strength and sign of these relationships (Funk et al., 2017; Garnier et al., 2016). The scale of observation and extent of sampling, in particular, could affect outcomes if studies differ in which portions of environmental gradients they sample (see Pollock et al., 2018 for further discussion on the role of scale).

## 4.2 | Missing trait-environment relationships

Many regional to global-scale studies find that levels of precipitation can strongly affect plant traits (Moles et al., 2009, 2014; Niinemets, 2001; Wright et al., 2017). In contrast, we found no trait-precipitation interactions. This may reflect the fact that these Wisconsin sites span only 5% of the 0 to 4,500 mm/year range spanned at the global scale. In contrast, soil sand varied from 15% to 89% among sites, allowing it to affect LS (and LCC somewhat). Thus, sand content may better reflect water availability for plants among our sites.

Basal area, our surrogate for understorey light, only affected LCC weakly, perhaps reflecting how widely understorey light levels vary within sites (Canham et al., 1994). In contrast, BA emerged as the single best predictor of species occurrences among sites (Beck et al., 2020). The weak linear effects we found for soil N (favouring taller plant species with cheaper leaves) could also reflect high local variability in soil conditions or perhaps that soil N mainly affects plants through interactions with other environmental variables. Such environment-environment interactions were omitted in our already complex GLMM.

Relationships between temperature and leaf characteristics have attracted the attention of many ecologists (e.g. the 'leaf economics spectrum', Wright et al., 2004). The negative LMA-MAT relationship we found was marginally significant, consistent with other regional-scale studies finding weakly negative or nonsignificant relationships (Balazs et al., 2020; Dong et al., 2020; Laughlin

et al., 2012, 2018; Mason & Donovan, 2015; Rosbakh et al., 2015). Such weak effects are surprising given the strong negative effects found under controlled growth conditions (Poorter et al., 2009). LMA responded more to temperature seasonality in the multi-trait 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. However, in our dataset, the MAT-TSD correlation was mild ( $-0.37$ ; Table S2), suggesting that the weak MAT effect on LMA was not an artefact of seasonality soaking up variation in the model. Instead, our results suggest that variation in LMA along our regional gradient is more linked with how favourable, warm temperatures are distributed within years than with the total amount of cumulated temperature per year.

## 4.3 | Contrast with previous work in Wisconsin understorey vegetation

Using the fourth-corner method and a less restrictive alpha of 0.05, Amatangelo et al. (2014) found plants to increase in height and decrease in LMA at sites with higher MAT, MAP and %N while %Sand exerted the opposite effects on these traits, as predicted. If we had adopted this alpha, we would have obtained the same results only for MAT and %N, with the effects of MAP and %Sand remaining nonsignificant (Figure 2). Our multi-trait GLMM therefore narrowed the list of relevant trait-environment relationships and highlighted that temperature effects on trait variation dominated over those due to differences in precipitation.

## 4.4 | GLMM versus CWMr approaches

The large number of significant but potentially spurious relationships identified here using the CWMr approach supports conclusions based on simulated data that CWMr often leads to false-positive conclusions (Miller et al., 2019; Peres-Neto et al., 2017). Many terms in the CWMr identified as significant (including LMA with MAT, %Sand and %N and VH with %N) lacked significance in the more nuanced and complete GLMM, reflecting Outcome #3. These relationships have been commonly reported in the literature, suggesting some reports may reflect spurious false positives. In the case of LMA, a widely studied trait, the lack of significance in the multi-trait GLMM cannot be ascribed to collinearity with competing traits as all inter-trait correlations were small ( $<0.18$ , Table S3). As suggested by comparisons with single-trait GLMMs, it is more likely to reflect how important it is to account for trait-abundance and environment-abundance relationships.

Accounting for trait covariation in the multi-trait GLMM appeared to be important for the LMA-TSD relationship, which was not significant in both CWMr (Outcome #2) and single-trait GLMMs. This result suggests that CWMr approaches are not only prone to

false positives but also to false negatives. False negatives related to predictor covariation might account for why such TSD effects are under-reported in the literature, particularly in cases where MAT and TSD are highly correlated. All these inferential differences are particularly striking given that compared approaches used exactly the same data.

## 5 | CONCLUSIONS

Modelling quadratic effects of traits and environmental gradients incorporates more realistic shapes for functional relationships, which showed to be important for the explanation of understory species abundance in Wisconsin. Including trait–environment combinations together in one model allowed us to account for non-independent effects on species abundance arising from collinearity. This complexity appeared to be important in detecting and characterizing the effect of temperature seasonality on LMA. Also, the nonlinear trait–environment interactions we introduced in our GLMM emerged as important for characterizing effects of temperature seasonality on both LMA and LS. Paradoxically, the more complex GLMM identified a simpler hierarchy of trait–environment relationships relative to CWMr, with temperature effects strongly driving variation in plant height while soil texture (and temperature seasonality) affected leaf traits more modestly. This hierarchy may help prioritize future work aimed at determining just how traits affect fitness in Wisconsin understory plant species. These might focus, for example, on temperature rather than precipitation effects. Future work based on simulated data could also pin down whether modelling these underlying complexities always improves the precision of our inferences regarding how traits interact with environmental conditions to affect plant performance.

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### CONFLICT OF INTEREST

The authors declare no conflict of interest.

### AUTHORS' CONTRIBUTIONS

A.G.R. and C.M.T. conceived the ideas and designed the study, with input from D.M.W., who also provided the data; A.G.R. analysed the data and led the writing. All authors contributed critically to the drafts and gave final approval for publication.

### PEER REVIEW

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### DATA AVAILABILITY STATEMENT

Data available from the Dryad Digital Repository <https://doi.org/10.5061/dryad.4qrfj6qb0> (Rolhauser et al., 2021).

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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