Components of leaf-trait variation along environmental gradients

Ning Dong1,2; Iain Colin Prentice1,3,4; Ian J. Wright1; Bradley J. Evans2,5; Henrique Furstenau Togashi1; Stefan Caddy-Retalic,6,7,8; Francesca A. McInerney9; Ben Sparrow6,10; Emrys Leitch6,10; Andrew J. Lowe6,10

1Department of Biological Sciences, Macquarie University, North Ryde, NSW 2109, Australia

2Terrestrial Ecosystem Research Network, University of Sydney, NSW 2006, Australia

3AXA Chair of Biosphere and Climate Impacts, Imperial College London, Department of Life Sciences, Silwood Park Campus, Ascot SL5 7PY, UK

4Department of Earth System Science, Tsinghua University, Beijing 100084, China

5Department of Sciences, School of Physical Sciences, University of Sydney, NSW 2006, Australia

6School of Biological Sciences, University of Adelaide, North Terrace, Adelaide, SA 5005, Australia

7Botanic Gardens and State Herbarium of South Australia, Department for Environment and Water, Hackney Road, Adelaide, SA 5000, Australia

8School of Life and Environmental Sciences, University of Sydney, Sydney, New South Wales, Australia

9Department of Earth Sciences, School of Physical Sciences, University of Adelaide, Adelaide, SA 5005, Australia

10Terrestrial Ecosystem Research Network, University of Adelaide, Adelaide, SA 5005, Australia

Corresponding email: ning.dong@mq.edu.au ,telephone: +610298508137

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ORCID:

ND: 0000-0003-0793-8854, twitter: @biodning

ICP: 0000-0001-8338-9143, twitter: @LabPrentice

IJW: 0000-0001-8338-9143 twitter: @ianjwright1

BJE:

HFT:

SCR:

FAM: 0000-0002-2020-6650, twitter: @cescamcinerney

BW: [0000-0003-2566-1895](https://orcid.org/0000-0003-2566-1895) , twitter: @TERN\_Aus

EL: , twitter: @TERN\_Aus

AJL:

# Summary

* Leaf area (LA), mass per area (LMA), nitrogen per unit area (*N*area) and the leaf-internal to ambient CO2 ratio (χ) are fundamental traits for plant functional ecology and vegetation modelling. We aimed to assess how their variation, within and between species, tracks environmental gradients.
* Measurements were made on 705 species from116 sites within a broad north-south transect from tropical to temperate Australia. Trait responses to environment were quantified using multiple regression; within- and between-species responses were compared using analysis of covariance and trait-gradient analysis.
* Leaf area, the leaf economics spectrum (indexed by LMA and *N*area), and χ (from stable carbon isotope ratios) varied almost independently among species. Across sites, however, χ and LA increased with mean growing-season temperature (mGDD0) and decreased with vapour pressure deficit (mVPD0) and soil pH. LMA and *N*area showed the reverse pattern. Climate responses agreed with expectations based on optimality principles. Within-species variability contributed <10% to geographic variation in LA but >90% for χ, with LMA and *N*area intermediate.
* These findings support the hypothesis that acclimation within individuals, adaptation within species, and selection among species combine to create predictable relationships between traits and environment. However, the contribution of acclimation/adaptation versus species selection differs among traits.

**Keywords:**

Acclimation, adaptation, intraspecific variation, leaf area, plant functional traits, species selection, trait-gradient analysis, leaf economics spectrum.

**INTRODUCTION**

Leaf traits determine many aspects of plant function, including rates of photosynthesis and transpiration, investment costs for leaf construction, plant nutrient requirements and resilience to temperature extremes, amongst others. Trait values vary both within and among species, and along environmental gradients. Numerous empirical studies have addressed the nature of leaf-trait variation, and shown the existence of systematic relationships between leaf traits and site climate and/or, less commonly, soil properties (Wright *et al.,* 2005; Ordoñez *et al.*, 2009; Dwyer *et al.*, 2014; Maire *et al.*, 2015; Simpson *et al.*, 2016; Cornwell *et al.*, 2018). While these empirical descriptions are important for understanding plant ecology and evolution, they remain limited in their application to predictive vegetation modelling because (a) the choice of environmental variables as predictors generally lacks a clear basis in theory, and (b) there has been limited consideration, either in empirical studies or in model development, of the different mechanisms (phenotypic acclimation and genotypic adaptation within species, versus environmental selection among species) that underlie observed relationships between trait values and site properties. Here we take a different approach, in which statistical analysis is used (a) to test environmental dependencies of leaf-trait values on environment that are motivated by theoretical optimality considerations, and (b) to partition trait variation along environmental gradients into components due to acclimation/adaptation (within species) versus selection between species.

Four leaf traits in particular have been the subject of many empirical studies. Each has a clear functional significance. Importantly for this study, all can be determined on dry leaf material collected during wide-ranging field expeditions. Leaf dry mass per unit area (LMA), and nitrogen (N) content expressed either per unit area (*N*area) or mass (*N*mass) reflect different aspects of leaf carbon-capture strategies. The leaf economics spectrum (Wright *et al.*, 2004;Freschet *et al.*, 2010) embodies the trade-off between LMA and leaf lifespan, with low LMA implying low construction costs but also a high turnover rate of leaves. Many species growing on low-nutrient soils have high LMA, their longer leaf lifespans reducing nutrient uptake requirements (Aerts & Chapin 2000). *N*area generally increases with increasing LMA, while *N*mass declines, because increasing LMA requires an increasing investment of N in structure, defence and other non-photosynthetic functions but does not require a proportionate increase in photosynthetic proteins (Dong *et al.*, 2017a; Onoda *et al.*, 2017). High-LMA leaves with high *N*area are generally favoured in hot and dry climates (Wright *et al.*, 2005; Wieczynski *et al.*, 2019). Leaf area (LA) affects leaf temperature via its effect on boundary-layer conductance (Gates, 1968; Givnish & Vermeij, 1976; Dong *et al.*, 2017b). Larger leaves have a lower rate of heat exchange with their surroundings, allowing more effective transpirational cooling in hot, wet climates while potentially risking heat damage in dry climates (Gates, 1968) or risking frost damage in cold climates (Jordan & Smith, 1994). This trade-off leads to predictable biogeographic patterns in leaf area (Wright *et al.*, 2017). Finally, the ratio of leaf-internal partial pressure of CO2, *c*i, to the ambient partial pressure of CO2, *c*a – here termed *χ*, and commonly inferred from stable carbon isotope ratio (δ13C) measurements – is an integrative measure of the trade-off between carbon gain and water loss that is mediated by stomatal behaviour (Farquhar *et al.*, 1980; Katul *et al.*, 2010; Medlyn *et al.*, 2011). Small *χ* is typical in dry environments, allowing the conservation of water while also creating a demand for large photosynthetic capacity in order for leaves to function effectively at low *c*i (Prentice *et al.*, 2014; Wang *et al.*, 2017).

Three separate theory lineages concerning the optimal coordination of photosynthetic properties provide the background to this study. Cowan & Farquhar (1977) proposed that stomatal behaviour maximizes *A* – *Ε*/*λ*, where *A* and *E* are photosynthesis and transpiration, respectively, and *λ* represents the exchange rate between carbon and water (∂*E*/∂*A*). The “least-cost hypothesis” extends this logic, proposing that plants minimize the *combined* costs (per unit assimilation) of maintaining the required capacities for carboxylation and transpiration (Wright *et al.*, 2003). Stomatal behaviour then minimizes *aE*/*A* + *bV*cmax/*A* where *a* and *b* are the unit costs for transpiration and carboxylation capacity (*V*cmax), respectively (Prentice *et al.*, 2014). The least-cost hypothesis has been shown to generate realistic predictions of variation in both *N*area and *χ* in response to environmental variables, specifically site temperature during the growing season, vapour pressure deficit, and elevation (Dong *et al.*, 2017a; Wang *et al.*, 2017; Lavergne *et al.*, 2019). Finally, the “coordination hypothesis” states that the Rubisco- and electron transport-limited rates of photosynthesis are co-limiting under typical daytime conditions in the field (Chen *et al.*, 1993; Dong *et al.*, 2017a; Togashi *et al.*, 2018). This hypothesis has direct implications for the regulation of photosynthetic capacity and, therefore, the metabolic component of *N*area, which is approximately proportional to *V*cmax assessed at a reference temperature, by convention 25˚C (Dong *et al.*, 2017a; Togashi *et al.*, 2018; Bloomfield *et al.*, 2019).

These various, well understood principles suggest that there should, in consequence, be universal patterns in trait-environment relationships (Yang *et al.*, 2019). To test this proposition, and the degree to which trait responses to environment are similar or different among and within species, requires the analysis of extensive leaf-trait data on multiple environmental gradients. Here we analyse data gathered within a broad north-south transect across Australia that runs from temperate woodlands in the south, through extensive semi-arid and arid lands in the centre to tropical savannas in the north. Our dataset consisted of mean trait values per species and site, for 705 species in total, sampled across 116 sites. Many species occurred at multiple sites, providing information on within-species trait variation. Our study had three principal objectives:

1. To quantify the correlations among traits, taking all species together, irrespective of where they were growing. To this end we used principal components analysis (PCA).
2. To explore the relationships between species’ trait values and environmental variables, estimated from gridded climate and soil data sets: namely growth temperature, vapour pressure deficit (VPD), and soil pH. The independent effects of environmental predictors were quantified as partial slopes in a multiple regression analysis – primarily for analyses run across all species, and secondarily within those species that occurred at three or more sites.
3. To assess the relative contributions of within-species variation versus species turnover to the observed trait-environment relationships. This partitioning was achieved using trait-gradient analysis (Ackerly & Cornwell 2007), greatly expanding a previous study that focused on fewer sites and fewer traits (Dong *et al*. 2017).

**MATERIALS AND METHODS**

**Study sites and sampling**

We used leaf material previously collected by the Ecological Surveillance team of the Terrestrial Ecosystem Research Network. The 116 sites (each a 100 x 100 m plot) were chosen within a broad north-south transect across Australia (Fig S1). At each site all of the Australian native species’ matured leaves were sampled by the Ecological Surveillance team, following a standard protocol for sampling, processing and archiving (White *et al.*, 2012), genrally 5 individuals per species were sampled for enountered on each plot except for those whose abundance was low for sampling. From this resource we, sampled leaf material for all species which sufficient material were available, generally more than 5 leaves per specie. In total, 705 species were sampled across the 116 sites. The great majority of species sampled are woody and evergreen.

The selected sites span a wide range of environments (Fig. S1) from the wet-dry tropics of the Northern Territory to temperate coastal environments of South Australia.Vegetation types ranged from tropical grasslands, shrublands and savannas in the north, through deserts and xeric shrublands in the centre, to temperate shrublands and woodlands in the south (Caddy-Retalic *et al.,* 2017). Data from 27 of these sites were used in previous analyses by Dong *et al.* (2017a).

## Climate and soils data

In climates without a cold season, mean annual temperature is often a sufficient measure of thermal growing conditions. However, if any part of the year is too cold for plant growth, a better index is provided by the growing-season mean temperature (mGDD0, units ˚C day). This can be calculated as the ratio of accumulated annual growing degree days above 0˚C to the length of the period with temperatures above 0˚C (Harrison *et al.*, 2010). Vapour pressure deficit (VPD) is a measure of atmospheric aridity, and growing-season VPD can also be expressed as the mean value during the period with temperatures above 0˚C (mVPD0, units kPa). Stomata respond to VPD such that *χ* is low at high VPD (Franks & Farquhar, 1999; Prentice *et al.*, 2014), and low soil moisture can cause further reductions of both stomatal conductance and photosynthetic capacity (Zhou *et al.*, 2013; Stocker *et al.*, 2019). However, VPD and soil moisture are not independent, because VPD is partly determined by the regional evapotranspiration rate (Jarvis & McNaughton, 1986; Lhomme, 1997) – so dry soils, reduced vegetation cover, low *χ*, and high VPD tend to occur together.

Soil properties as well as climate influence plant traits. Soil pH in particular is often used as a proxy for several aspects of soil fertility, affecting the availability of major nutrients (Lucas & Davis, 1961). Increasing evidence suggests that soil pH, amongst soil properties, correlates most strongly with plant traits (Dwyer *et al.*, 2014; Maire *et al.*, 2015; Simpson *et al.*, 2016; Cornwell *et al.*, 2018).

Climatological data were derived from gridded climate datasets at 0.01° spatial resolution developed and archived by the TERN Ecosystem Modelling and Scaling Infrastructure facility (eMAST; <https://www.tern.org.au/Ecosystem-Modelling-and-Scaling-Infrastructure-e-MAST-pg17734.html>). Mean monthly values of temperature, precipitation, short-wave radiation and vapour pressure were extracted and averaged over 1970 to 2012. Soil pH (in H2O solution) and C:N ratio for each site were derived from the gridded soils datasets at 0.01° spatial resolution developed and archived by the TERN AusCover facility (Grundy *et al.,* 2015; <http://www.auscover.org.au/>). Variables used for the main analyses were mGDD0 (ranging from 13.5 to 27.6 ˚C), mVPD0 (0.48 to 2.28 kPa), and soil pH (4.7 to 7.3), also listed in Table S1. Mean canopy irradiance (*I*L) was calculated, according to equation (2) in Dong *et al.* (2017a), for a supplementary analysis of *N*area data.

**Foliage sampling and analysis**

LMA (dry leaf mass divided by leaf area) was measured on the archived leaf samples by scanning and weighing the leaves. This procedure assumes that leaf shrinkage has little effect on LMA, presumably a reasonable assumption (Baruch *et al.*, 2017) for the generally high-LMA evergreen species considered in this study, many from dry sites. LA was measured using ImageJ software. Subsamples (a mixture of material from at least two replicates) were analysed for C and N contents and bulk δ13C at the Stable Isotope Laboratory of the University of Adelaide, Australia, using a EuroVector elemental analyser coupled to a Nu Horizon isotope ratio mass spectrometer. Values of *χ* were derived from the δ13C data using equations given in Dong *et al.* (2017a).

**Statistical analyses**

The data analysed in this paper consist of species-mean trait values, for each species sampled at each site. For species sampled at more than one site, these data provide information about how trait variation within species varies with environment. Leaf-area data were square-root transformed to provide a measure of linear dimension. Natural log transformation was then applied to all leaf traits, except χ, to reduce skewness. Logit transformation was applied to χ. All data analysis and graphics were conducted in R (R 3.5.1 Core Team, 2018).

*Correlations between traits*

We used principal components analysis (PCA) to summarize the correlation structure of the trait data. No further standardization was applied to the (log- or logit-transformed) data. This approach results in each variable in the PCA carrying a weight proportional to its relative variability. So, for example, a variable that varies two-fold will carry five times less weight than one that varies ten-fold (Yang *et al.,* 2019). The PCA plot was carried out in R with the loadings and coordination was rescaled in range [-1,1] in Fig.1.

*Trait variation in relation to site environment*

Ordinary least-squares multiple linear regression (function *lm* in R) was used to relate trait values to environmental predictors. Partial residual plots were generated with the *visreg* package in R (Fig. 2). We also tried substituting generalized additive models, GAMs (package *gamm* in R) for linear regression models in order to detect potential departures from linearity.

Pairwise correlations among predictor variables were examined to identify potentially problematic levels of collinearity (Table S2). The correlations turned out to be moderate, supporting the use of multiple regression analyses (and partial residual plots) in this study. We also analysed the data in an analysis of covariance framework, including species identity as a factor and testing for heterogeneity among the within-species slopes using ANCOVA (package *rstatix* in R). Where deemed non-significant (species x covariate interactions being non-significant) a common fitted slope was calculated. This slope represents the most general within-species tendency of the trait in relation to that environmental variable. Within-species slopes were illustrated in Fig. 3**.** Trait-environment relationship within species were examined further by including species identity as a random effect in a mixed-effects multiple regression model, using the *lme4* package in R.

Dong *et al.* (2017a) showed that *N*area values can be independently predicted as a function of LMA and *V*cmax evaluated at 25˚C, which in turn – in the absence of field measurements – can be predicted from the coordination hypothesis as a function of χ, mGDD0 and *I*L (Smith *et al.*, 2019). Extending the analysis of Dong *et al.* (2017), we used multiple linear regression to quantify the relationship of *N*area to LMA, *χ*, mGDD0, *I*L, soil C:N ratio, soil pH, and “N-fixer” as a factor. This analysis allowed us to compare the fitted slopes of ln *N*area versus mGDD0 and *χ­­* with independent, theoretically derived slopes.

*Partitioning trait variation into different components*

Analysis of variance was used to quantify the contribution of variation within species to the total variation in each trait.In addition, trait-gradient analysis (Ackerly & Cornwell, 2007; R function *tga*) was used to partition the between-site variation of each trait into two components: one attributable to within-species variation, the other (by inference) to species turnover. (A similar approach was later proposed by Leps *et al.*, 2011.) In the Ackerly & Cornwell method, also used by Dong *et al.* (2017a), community-mean values are first calculated for each trait and site. For each species sampled at three or more sites, a regression slope is then obtained, relating all the trait values of species recorded at each site to the community-mean trait values. The median species-level slope for each trait indicates the fraction of geographic variation in community-mean trait values that is explained by variation within species. The difference between this median slope and unity indicates the remaining fraction of trait variation, which is inferred to be due to species turnover. (Note that within-species trait variability along gradients comprises phenotypic plasticity within individuals, and genotypic adaptation within species, in unknown proportions.) Kernel density estimation was used to visualize the probability density functions of trait-gradient slopes.

**RESULTS**

**Trait orthogonality versus correlation**

PCA (Fig. 1, Table 1) indicated the existence of three, nearly independent dimensions of trait variation corresponding to leaf area, the leaf economics spectrum (LMA and *Narea*), and χ. Note that LMA and *Narea* were closely positively correlated (Table S2), presumably because the structural component of *Narea* is large, and highly dependent on LMA (Dong *et al.*, 2017a): higher LMA implies thicker leaves with more developed mesophyll layers. Together, these three components accounted for 94.3 % of the total variation in the four leaf traits. Independent variation of *Narea* and LMA was shown on the fourth axis, accounting for only 5.7% of the total variation in leaf traits (Table 1).

**Interspecific trait variation in relation to climate and soil**

*Variation in χ*

Multiple regression analyses (Fig. 2, Table 2) indicated a significant positive relationship between logit χ (higher values implying a smaller difference between leaf-internal and ambient CO2) and temperature (mGDD0), and negative relationships with aridity (mVPD0) and soil pH (Table 2; Fig. 2a–c). The direction of each of these effects was as expected from the theoretical analysis by Wang *et al* (2017). The fitted slope for logit χ versus mGDD0 was 0.07 (95% CIs 0.05, 0.08; Table 2), which was statistically indistinguishable from the theoretical slope of 0.055 derived by Wang *et al.* (2017). The fitted slope for mVPD0 was –0.76 (95% CIs –0.92, –0.61), which is somewhat steeper than the theoretical slope of –0.5 derived by Wang *et al.* (2017). There was currently no available quantitative prediction for the slope of logit χ in response to soil pH, however the direction (with larger χ on acidic soils) was consistent with that found by Wang *et al.* (2017) and with independent experimental evidence (Yang *et al.*, 2015). The fitted model explained 34% of the total variance in logit χ (Table 2).

The alternative application of a GAM for logit *χ* yielded almost the same relationships as the linear model. Only the relationship to soil pH showed any noticeable departure from linearity, being fitted by a monotonic descending curve that was flatter in the central pH range and steeper at low and high pH values. GAMs for other traits showed no tendencies to non-linearity (Fig. S2).

*Variation in leaf area*

Temperature, aridity and soil pH together explained 25% variation in leaf area, with all three partial relationships significant (*p* < 0.001). Thus, on average, leaves were shown to be larger at hotter sites, less arid sites, and on more acid soils (Fig. 2g–i). However, the effect of soil pH was notably weaker than the climatic effects (Fig. 2i).

*Variation in Narea*

Leaf nitrogen (*N*area) showed a significant negative response to site temperature, but increased with aridity and soil pH (Fig. 2d­–f). The fitted model explained 25% of the variance of *N*area (Table 2).

In a supplementary analysis, we revisited the theoretical framework of Dong *et al.* (2017a) with our (now greatly expanded) data set. As in Dong *et al.* (2017a), we found significant (*p* < 0.001) relationships of *N*area to canopy irradiance, temperature, LMA and *χ*. The fitted slopes were consistent with expected values in those cases for which *a priori* predictions exist (Fig. S3, Table S3). We also found a significant tendency for low *Narea* at sites with a high soil C:N ratio. No significant partial relationship with soil pH remained. All predictors together explained 62.3% of the total variation in *Narea* in this analysis.

*Variation in LMA*

LMA showed a significant (*p* < 0.001) negative relationship to temperature and positive relationships to aridity and soil pH (i.e., thicker leaves on more alkaline soils and drier sites; Table 2, Fig. 2j–l). Although each separate effect was significant, the fitted model explained only 9% of variance in LMA – much less than the fitted models for other three traits.

**Trait-environment relationships within species**

We tested whether the sets of within-species slopes for each trait-environment relationship were heterogeneous (Fig. 3). Leaf area showed significant heterogeneity among within-species slopes with respect to all three environmental variables. LMA and leaf nitrogen, however, showed significant heterogeneity only in their responses to soil pH. The within-species responses of *χ* to environmental predictors, and the responses of LMA and leaf nitrogen to climatic predictors, did not show significant differences among species, noting that common slopes (Table S4) all fall within the 95% CIs of the all-species slopes in Table 2.

Further analyses of a mixed-effects model including species identity as a random effect were reported in Fig. S4 and Table S5. Logit χ showed generally similar environmental relationships to the simple regression model (Table 2). LA and *Narea* showed still-significant relationships, but with reduced slopes. LMA showed a significant (*p* < 0.01), negative partial relationship with temperature only.

**Components of trait variation**

Across the whole dataset, variation between species explained substantially different fractions of total variance for different traits: 22.1% for *χ*, 55.6% for leaf nitrogen, 69.6% for LMA and 86.5% for leaf area. Trait gradient analysis (Fig. 4) revealed comparable differences in the fraction of variation between sites that could be attributed to within- versus between-species variation. These differences were visually apparent in Fig. 4a–d, where within-species trait shifts aligned with the overall gradient (slope ≈ 1) were in principle due to plasticity (acclimation) and/or genotypic adaptation, while slopes ≪ 1 indicate relative constancy of trait values within species – so that community-mean values represented shifts in species composition. Most slope values lied in the range from zero to unity. Slope values less than zero, which signified trends opposite to the community mean, and values greater than unity, which indicated “over-reaction”, occur but are uncommon.

Fig. 4e summarized the distributions of trait-gradient slopes among species for the four traits. *χ* showed near-perfect acclimation or adaptation, with a median within-species slope of 0.91 (Fig. 4a). The median within-species slope for LA was 0.08, indicating limited acclimation or adaptation and (instead) a dominant control of community-level trait variation by species turnover. However, individual species varied rather widely in their responses for this trait, with negative slopes in a few cases (Fig. 4b). The median slope for LMA was 0.34 (Fig. 4d) while *N*area was intermediate between LMA and χ with a median slope of 0.53. Thus, the ranking of traits in terms of the contributions of within-species variability to environmental differences between sites was consistent with their ranking in terms of the contributions of within-species variability to total trait variance, with the greatest acclimation or adaptation shown by *χ*, the least by leaf area, and LMA and *N*area in between.

**DISCUSSION**

Our analyses have demonstrated that leaf area, the leaf economics spectrum (indexed by leaf mass and nitrogen per unit area), and *χ* represent *largely independent dimensions* of interspecific trait variation – highlighting the great diversity of trait combinations that exist in nature (Wright *et al.*, 2004; Maire *et al.*, 2012; Díaz *et al.*, 2015). On the other hand, all four traits showed significant relationships to site environment, with LA and χ showing one pattern (increasing with temperature, declining with aridity and soil pH), and LMA and *N*area showing the reverse pattern. Thus, despite the diversity of species overall, the growth environment apparently selects in favour of particular combinations of traits (Yang *et al.,* 2019). These environmental effects are discussed one-by-one below.

**Temperature effects**

High values of *χ* in warm environments can be explained by the least-cost hypothesis (Wright *et al.*, 2003; Prentice *et al.*, 2014). This hypothesis predicts a shift towards decreased investment in water transport capacity (and relatively increased investment in carboxylation) as (a) photorespiration increases, and (b) the viscosity of water declines.

The prevalence of larger leaves in warmer environments has been explained (Wright *et al.*, 2017) in part by the lack of frost danger. In colder climates, night-time conditions cause greater cooling of large leaves (as shown experimentally by Lusk *et al*., 2019). This implies a constraint to maximum leaf area in cold climates. In warmer environments, however, large leaves allow effective transpirational cooling (Wright *et al.*, 2017).

*N*area has previously been shown to decline with increasing temperature (Dong et al, 2017a), and the same is observed in the current study. This negative tendency is an expected consequence of the coordination hypothesis. It can be explained by the increased catalytic capacity of Rubisco at higher temperature, which means that a smaller quantity of Rubisco is needed to achieve a given rate of photosynthesis (Scafaro *et al.*, 2017; Togashi *et al.*, 2018).

The observed negative relationship of LMA to temperature may be linked to the well-known positive relationship between LMA and leaf longevity. Long-lived leaves required robust construction in the form of high LMA (Wright *et al.*, 2004; Poorter *et al.*, 2009). Moreover, for any given LMA, there is an optimal leaf longevity that maximizes the return on carbon invested in leaf construction (Kikuzawa, 1991; Kikuzawa *et al.*, 2013). This optimum declines with increasing growth temperature in evergreen plants (Kikuzawa *et al.*, 2013).

**Aridity effects**

The observed decrease in *χ* towards arid environments reflects a shift towards investment in photosynthetic capacity as the drying power of the air increases, in order to economise on photosynthetic water requirements (Wright *et al* 2003). We showed a steeper slope (–0.76, 95% CIs –0.92, –0.6) among our sites than the global theoretical slope (–0.5) proposed by Wang *et al* (2017). The reason for the discrepancy may be that low soil moisture can cause further reductions of stomatal conductance to prevent hydraulic conductivity loss (Martínez‐Vilalta *et al.*, 2014), and even, under very dry soil conditions, reductions in photosynthetic capacity (Zhou *et al.*, 2013; Reich *et al.*, 2018). These additional effects have not yet been quantified in the least-cost framework, but their direction is clear: low soil moisture increases the cost of water transport, resulting in a further shift of optimal investment towards increased photosynthetic capacity and reduced stomatal conductance.

Small leaves are characteristic of dry environments everywhere, so the negative relationship between leaf area and aridity is no surprise (Wright *et al*., 2017). The accepted explanation is that restricted transpiration in dry climates precludes the survival of large leaves, which would be forced to reach excessively high daytime temperatures. A small surface area allows leaves to avoid such overheating by remaining close to ambient air temperature (Gates, 1968; Dong *et al.*, 2017b).

Species inhabiting arid and semi-arid climates commonly have leaves with high LMA and *Narea*, consistent with high photosynthetic capacity (compensating for low χ) and small leaf area (Givnish & Vermeij, 1976). Higher LMA in arid environments may also help leaves to avoid transient overheating when wind speeds fall (Leigh *et al.*, 2012).

**Soil** **fertility effects**

A growing number of studies has shown apparently substantial effects of soil pH on photosynthetic traits (Maire *et al.*, 2015; Wang *et al.*, 2017; Cornwell *et al.*, 2018). However, the mechanisms involved are not fully understood. Soil pH has often been considered as an indicator of soil fertility (including cation availability).

Our analysis shows a clear trend towards higher *χ* on more acid soils. This is consistent with nutrient acquisition costs increasing with declining pH (Högberg *et al.,* 2006; Janssens *et al.*, 2010; Ye *et al.*, 2018), and the prediction from least-cost theory that species should operate at higher *χ* when (all else equal) the unit-costs for nutrient acquisition are higher (Wright *et al* 2003). The observed negative influence of soil pH on leaf area requires further investigation and, assuming that higher pH connotes higher nutrient availability, is opposite to the nutrient-effect predicted by Givinish & Vermeij (1976) in their seminal paper on optimal leaf area.

**Implications for vegetation modelling**

Vegetation models are moving towards a basis in continuous trait variation, instead of discrete plant functional types (PFTs) (e.g. Prentice & Cowling, 2013; Fyllas *et al.*, 2014; van Bodegom *et al.*, 2014; Scheiter *et al.*, 2013; Ali *et al.*, 2015; Fisher *et al*., 2015; Meng *et al.*, 2015). We have described relationships of four key leaf traits (LA, LMA, χ and Νarea) to environmental predictors (describing growing-season temperature, atmospheric dryness and soil fertility) that apply irrespective of PFT. We have also shown that in cases where optimality hypotheses make quantitative predictions, the magnitudes of these effects are close to those predicted. In other cases, quantitative predictions do not yet exist, but the basic functional relationships are known and they result in qualitative predictions consistent in direction with the empirical relationships. Therefore, this research overall supports a “plant-centred” approach to the further development of vegetation models, in which key traits are predicted with values representing optimal values for plants as a function of different aspects of their growth environment.

**Implications for overall understanding of trait variability**

Our analysis has also revealed sharp contrasts among traits in the relative contribution to community-mean trait gradients from acclimation/adaptation within species *versus* species turnover. This finding implies that the processes by which plant communities achieve optimal function differ among traits. For example, Cornwell *et al* (2018) showed that about 60% of global variation in leaf δ13C was explained by site climate and soils at species level. This is the strongest environmental dependency that has been demonstrated so far for any plant trait. We have shown here that over 90% of the community-mean variation in χ is expressed within species. For this trait, it can probably be assumed that species can track changes in their growth environment principally via physiological acclimation.

The opposite holds for leaf area. The trait gradient analysis indicated that, although species do show some LA variation in the same direction as the community mean, the median slope of this variation was less than 10% of the community-mean slope. Thus, the ability of species to track environmental changes by adjustment of leaf area seems to be limited; the relationship between community-mean leaf area and environmental gradients is primarily a consequence of species turnover. Full adjustment to environmental change will then require changes in the competitive balance of co-existing species, and possibly the replacement of some or all species (depending on the magnitude of environmental change) by others. For the transient response of communities to a changing environment this is an important difference – because the rates of change of acclimation and migration are all different. This distinction, to our knowledge, has been rarely considered in dynamic vegetation models (Sakschewski et al, 2015, Walker et al. 2017).

The rather flat median slope for LA in the trait gradient analysis is a surprise because it has often been considered as a highly plastic trait –for example being usually larger in the shade, and in well-watered versus droughted plants (Jensen & Zwieniecki, 2013). However, our results suggest that such within-species variability is dwarfed by very large differences among species that are driven by climatic gradients (Wright *et al.*, 2017).

LMA and *N*area show intermediate levels within-species trait variation. Geographic patterns of LMA are generally weaker than those for other traits (Wright *et al.*, 2004). We showed that less than 10% of variation in LMA can be explained by the climate and soil variables that we considered. Nonetheless, there is some coordinated variability in LMA within species, amounting to about a third of the variability in the community-mean LMA. *N*area includes both morphological (proportional to LMA) and physiological (proportional to photosynthetic capacity) components and, as such, not surprisingly shows a level of acclimation or adaptation intermediate between that of LMA and *Narea*.

Our results provided empirical information on trait-environment relationships by demonstrating general, quantitative linkages of four key leaf traits to growth conditions in the form of temperature, atmospheric aridity and soil pH. Theoretical predictions of the partial relationships between each trait and climate variables were largely supported in so far as they are available. However, our results also indicated the existence of consistent quantitative relationship with soil pH that have not yet been analysed in an optimality framework, and the need to incorporate stomatal responses to soil moisture in addition to VPD in the least-cost framework.

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**Author contributions**

ND, ICP and BJE conceived the study, ND, HFT, SCR, BS, EL and FAM undertook data collection, ND performed the data analyses, ND and ICP wrote the first draft, and ND, ICP, IJW, BJE, HFT, SCR, BS, FAM and AJL contributed to sequent manuscripts.

**Data Availability Statement**

Data will be available in TRY.

**Supporting information**

**Figure S1.** Site locations and climate distributions.

**Figure S2.** Partial residual plots of for key plant traits as a function of mean growing-season temperature (mGDD0, ˚C), mean growing-season vapour pressure deficit (mVPD0, kPa) and soil pH, where soil pH was used gam function to treat it nonlinear variable in the regression model.

**Figure S3.** Partial residual regression plots for ln *Narea* and its predictors.

**Figure S4.** Partial residual plots for a mixed-effects regression analysis with species identity as a random effect.

**Table S1** List of environment variables and plant traits.

**Table S2.** Cross-correlations of all traits and environmental variables.

**Table S3** Linear regression coefficients for ln *N*area, with or without soil variables included.

**Table S4** The heterogeneity table for the regression models of leaf traits as a function of mean growing-season temperature (mGDD0, ˚C), mean growing-season vapour pressure deficit (mVPD0, kPa) and soil pH in Figure 2.

**Table S5.** Coefficients for the mixed-effects regression of leaf traits as a function of mean growing-season temperature (mGDD0, ˚C), mean growing-season vapour pressure deficit (mVPD0, kPa) and soil pH (Fig. S4) with species identity as a random effect.

# References

**Ackerly DD, Cornwell WK. 2007.** A trait-based approach to community assembly: partitioning of species trait values into within- and among-community components. *Ecology Letters* **10**(2): 135-145.

**Aerts R, Chapin FS 1999.** The Mineral Nutrition of Wild Plants Revisited: A Re-evaluation of Processes and Patterns. In: Fitter AH, Raffaelli DG eds. *Advances in ecological research*: Academic Press, New York,1-67.

**Ali AA, Xu C, Rogers A, McDowell NG, Medlyn BE, Fisher RA, Wullschleger SD, Reich PB, Vrugt JA, Bauerle WL. 2015.** Global‐scale environmental control of plant photosynthetic capacity. *Ecological Applications* **25**(8): 2349-2365.

**Baruch Z, Christmas MJ, Breed MF, Guerin GR, Caddy‐Retalic S, McDonald J, Jardine DI, Leitch E, Gellie N, Hill K. 2017.** Leaf trait associations with environmental variation in the wide‐ranging shrub Dodonaea viscosa subsp. angustissima (Sapindaceae). *Austral Ecology* **42**(5): 553-561.

**Bloomfield KJ, Prentice IC, Cernusak LA, Eamus D, Medlyn BE, Rumman R, Wright IJ, Boer MM, Cale P, Cleverly J, et al. 2019.** The validity of optimal leaf traits modelled on environmental conditions. *New Phytologist* **221**(3): 1409-1423.

**Brown AM, Warton DI, Andrew NR, Binns M, Cassis G, Gibb H. 2014.** The fourth‐corner solution–using predictive models to understand how species traits interact with the environment. *Methods in Ecology and Evolution* **5**(4): 344-352.

**Caddy-Retalic S, Andersen AN, Aspinwall MJ, Breed MF, Byrne M, Christmas MJ, Dong N, Evans BJ, Fordham DA, Guerin GR, et al. 2017.** Bioclimatic transect networks: Powerful observatories of ecological change. *Ecology and Evolution* **7**(13): 4607-4619.

**Chen J-L, Reynolds JF, Harley PC, Tenhunen JD. 1993.** Coordination theory of leaf nitrogen distribution in a canopy. *Oecologia* **93**(1): 63-69.

**Cornwell WK, Wright IJ, Turner J, Maire V, Barbour MM, Cernusak LA, Dawson T, Ellsworth D, Farquhar GD, Griffiths H. 2018.** Climate and soils together regulate photosynthetic carbon isotope discrimination within C3 plants worldwide. *Global Ecology and Biogeography* **27**(9): 1056-1067.

**Cowan I, Farquhar G 1977**. Stomatal function in relation to leaf metabolism and environment. *Symposia of the Society for Experimental Biology* **31:**471-505.

**Díaz S, Kattge J, Cornelissen JHC, Wright IJ, Lavorel S, Dray S, Reu B, Kleyer M, Wirth C, Prentice IC, et al. 2015.** The global spectrum of plant form and function. *Nature* **529**: 167.

**Dong N, Prentice IC, Evans BJ, Caddy-Retalic S, Lowe AJ, Wright IJ. 2017a.** Leaf nitrogen from first principles: field evidence for adaptive variation with climate. *Biogeosciences* **14**(2): 481-495.

**Dong N, Prentice IC, Harrison SP, Song QH, Zhang YP. 2017b.** Biophysical homoeostasis of leaf temperature: A neglected process for vegetation and land-surface modelling. *Global Ecology and Biogeography* **26**(9): 998-1007.

**Dwyer JM, Hobbs RJ, Mayfield MM. 2014.** Specific leaf area responses to environmental gradients through space and time. *Ecology* **95**(2): 399-410.

**Farquhar GD, von Caemmerer Sv, Berry JA. 1980.** A biochemical model of photosynthetic CO2 assimilation in leaves of C 3 species. *Planta* **149**(1): 78-90.

**Fisher R, Muszala S, Verteinstein M, Lawrence P, Xu C, McDowell N, Knox R, Koven C, Holm J, Rogers B. 2015.** Taking off the training wheels: the properties of a dynamic vegetation model without climate envelopes, CLM4. 5 (ED). *Geoscientific Model Development* **8**(11): 3593-3619.

**Franks PJ, Farquhar GD. 1999.** A relationship between humidity response, growth form and photosynthetic operating point in C3 plants. *Plant, Cell & Environment* **22**(11): 1337-1349.

**Freschet GT, Cornelissen JHC, Van Logtestijn RSP, Aerts R. 2010.** Evidence of the ‘plant economics spectrum’ in a subarctic flora. *Journal of Ecology* **98**(2): 362-373.

**Fyllas N, Gloor E, Mercado L, Sitch S, Quesada C, Domingues TF, Galbraith D, Torre-Lezama A, Vilanova E, Ramírez-Angulo H. 2014.** Analysing Amazonian forest productivity using a new individual and trait-based model (TFS v. 1). *Geoscientific Model Development* **7**(4): 1251-1269.

**Gates DM. 1968.** Transpiration and leaf temperature. *Annual Review of Plant Physiology* **19**(1): 211-238.

**Givnish TJ, Vermeij GJ. 1976.** Sizes and Shapes of Liane Leaves. *The American naturalist* **110**(975): 743-778.

**Grundy MJ, Rossel RAV, Searle RD, Wilson PL, Chen C, Gregory LJ. 2015.** Soil and Landscape Grid of Australia. *Soil Research* **53**(8): 835-844.

**Harrison SP, Prentice IC, Barboni D, Kohfeld KE, Ni J, Sutra JP. 2010.** Ecophysiological and bioclimatic foundations for a global plant functional classification. *Journal of Vegetation Science* **21**(2): 300-317.

**HÖGBERG P, FAN H, QUIST M, BINKLEY D, TAMM CO. 2006.** Tree growth and soil acidification in response to 30 years of experimental nitrogen loading on boreal forest. *Global Change Biology* **12**(3): 489-499.

**Jordan DN, Smith WK. 1994.** Energy balance analysis of nighttime leaf temperatures and frost formation in a subalpine environment. *Agricultural and Forest Meteorology* **71**(3): 359-372.

**Janssens I, Dieleman W, Luyssaert S, Subke J-A, Reichstein M, Ceulemans R, Ciais P, Dolman AJ, Grace J, Matteucci G. 2010.** Reduction of forest soil respiration in response to nitrogen deposition. *Nature Geoscience* **3**(5): 315.

**Jarvis PG, McNaughton KG 1986.** Stomatal Control of Transpiration: Scaling Up from Leaf to Region. In: MacFadyen A, Ford ED eds. *Advances in ecological research*: Academic Press, **New York,**1-49.

**Jensen KH, Zwieniecki MA. 2013.** Physical limits to leaf size in tall trees. *Physical review letters* **110**(1): 018104.

**Katul G, Manzoni S, Palmroth S, Oren R. 2010.** A stomatal optimization theory to describe the effects of atmospheric CO2 on leaf photosynthesis and transpiration. *Annals of Botany* **105**(3): 431-442.

**Kikuzawa K. 1991.** A cost-benefit analysis of leaf habit and leaf longevity of trees and their geographical pattern. *The American naturalist* **138**(5): 1250-1263.

**Kikuzawa K, Onoda Y, Wright IJ, Reich PB. 2013.** Mechanisms underlying global temperature-related patterns in leaf longevity. *Global Ecology and Biogeography* **22**(8): 982-993.

**Lavergne A, Voelker S, Csank A, Graven H, de Boer HJ, Daux V, Robertson I, Dorado-Liñán I, Martínez-Sancho E, Battipaglia G, et al. 2020.** Historical changes in the stomatal limitation of photosynthesis: empirical support for an optimality principle. *New Phytologist* **225**(6): 2484-2497.

**Leigh A, Sevanto S, Ball MC, Close JD, Ellsworth DS, Knight CA, Nicotra AB, Vogel S. 2012.** Do thick leaves avoid thermal damage in critically low wind speeds? *New Phytologist* **194**(2): 477-487.

**Lepš J, de Bello F, Šmilauer P, Doležal J. 2011.** Community trait response to environment: disentangling species turnover vs intraspecific trait variability effects. *Ecography* **34**(5): 856-863.

**Lhomme JP. 1997.** An examination of the Priestley‐Taylor equation using a convective boundary layer model. *Water Resources Research* **33**(11): 2571-2578.

**Lucas RE, Davis JF. 1961.** RELATIONSHIPS BETWEEN PH VALUES OF ORGANIC SOILS AND AVAILABILITIES OF 12 PLANT NUTRIENTS. *Soil Science* **92**(3): 177-182.

**Lusk CH, Clearwater MJ, Laughlin DC, Harrison SP, Prentice IC, Nordenstahl M, Smith B. 2018.** Frost and leaf-size gradients in forests: global patterns and experimental evidence. *New Phytologist* **219**(2): 565-573.

**Maire V, Martre P, Kattge J, Gastal F, Esser G, Fontaine S, Soussana J-F. 2012.** The coordination of leaf photosynthesis links C and N fluxes in C3 plant species. *PLOS ONE* **7**(6): e38345.

**Maire V, Wright IJ, Prentice IC, Batjes NH, Bhaskar R, Bodegom PM, Cornwell WK, Ellsworth D, Niinemets Ü, Ordonez A, et al. 2015.** Global effects of soil and climate on leaf photosynthetic traits and rates. *Global Ecology and Biogeography* **24**(6): 706-717.

**Martínez‐Vilalta J, Poyatos R, Aguadé D, Retana J, Mencuccini M. 2014.** A new look at water transport regulation in plants. *New Phytologist* **204**(1): 105-115.

**Medlyn BE, Duursma RA, Eamus D, Ellsworth DS, Prentice IC, Barton CV, Crous KY, De Angelis P, Freeman M, Wingate L. 2011.** Reconciling the optimal and empirical approaches to modelling stomatal conductance. *Global Change Biology* **17**(6): 2134-2144.

**Meng TT, Wang H, Harrison SP, Prentice IC, Ni J, Wang G. 2015.** Responses of leaf traits to climatic gradients: adaptive variation versus compositional shifts. *Biogeosciences* **12**(18): 5339-5352.

**Niinemets Ü, Díaz-Espejo A, Flexas J, Galmés J, Warren CR. 2009.** Role of mesophyll diffusion conductance in constraining potential photosynthetic productivity in the field. *Journal of Experimental Botany* **60**(8): 2249-2270.

**Onoda Y, Wright IJ, Evans JR, Hikosaka K, Kitajima K, Niinemets Ü, Poorter H, Tosens T, Westoby M. 2017.** Physiological and structural tradeoffs underlying the leaf economics spectrum. *New Phytologist* **214**(4): 1447-1463.

**Ordoñez JC, Van Bodegom PM, Witte JPM, Wright IJ, Reich PB, Aerts R. 2009.** A global study of relationships between leaf traits, climate and soil measures of nutrient fertility. *Global Ecology and Biogeography* **18**(2): 137-149.

**Poorter H, Niinemets Ü, Poorter L, Wright IJ, Villar R. 2009.** Causes and consequences of variation in leaf mass per area (LMA): a meta‐analysis. *New Phytologist* **182**(3): 565-588.

**Prentice IC, Cowling SA 2013.** Dynamic global vegetation models. In Levin **SA** ed. *Encyclopedia of biodiversity*(2th edition). Amsterdam:elsevier,670-689.

**Prentice IC, Dong N, Gleason SM, Maire V, Wright IJ, Penuelas J. 2014.** Balancing the costs of carbon gain and water transport: testing a new theoretical framework for plant functional ecology. *Ecology Letters* **17**(1): 82-91.

**Reich PB, Sendall KM, Stefanski A, Rich RL, Hobbie SE, Montgomery RA. 2018.** Effects of climate warming on photosynthesis in boreal tree species depend on soil moisture. *Nature* **562**(7726): 263-267.

**Sakschewski B, von Bloh W, Boit A, Rammig A, Kattge J, Poorter L, Peñuelas J, Thonicke K. 2015.** Leaf and stem economics spectra drive diversity of functional plant traits in a dynamic global vegetation model. *Global Change Biology* **21**(7): 2711-2725.

**Scafaro AP, Xiang S, Long BM, Bahar NH, Weerasinghe LK, Creek D, Evans JR, Reich PB, Atkin OK. 2017.** Strong thermal acclimation of photosynthesis in tropical and temperate wet‐forest tree species: the importance of altered Rubisco content. *Global Change Biology* **23**(7): 2783-2800.

**Scheiter S, Langan L, Higgins SI. 2013.** Next‐generation dynamic global vegetation models: learning from community ecology. *New Phytologist* **198**(3): 957-969.

**Smith NG, Keenan TF, Prentice IC, Wang H, Wright IJ, Niinemets Ü, Crous KY, Domingues TF, Guerrieri R, Yoko Ishida F, et al. 2019.** Global photosynthetic capacity is optimized to the environment. *Ecology Letters* **22**(3): 506-517.

**Simpson AH, Richardson SJ, Laughlin DC. 2016.** Soil–climate interactions explain variation in foliar, stem, root and reproductive traits across temperate forests. *Global Ecology and Biogeography* **25**(8): 964-978.

**Stocker BD, Zscheischler J, Keenan TF, Prentice IC, Seneviratne SI, Peñuelas J. 2019.** Drought impacts on terrestrial primary production underestimated by satellite monitoring. *Nature Geoscience* **12**(4): 264-270.

**Togashi HF, Atkin OK, Bloomfield KJ, Bradford M, Cao K, Dong N, Evans BJ, Fan Z, Harrison SP, Hua Z, et al. 2018.** Functional trait variation related to gap dynamics in tropical moist forests: A vegetation modelling perspective. *Perspectives in Plant Ecology, Evolution and Systematics* **35**: 52-64.

**van Bodegom PM, Douma JC, Verheijen LM. 2014.** A fully traits-based approach to modeling global vegetation distribution. *Proceedings of the National Academy of Sciences* **111**(38): 13733-13738.

**Wang H, Prentice IC, Keenan TF, Davis TW, Wright IJ, Cornwell WK, Evans BJ, Peng C. 2017.** Towards a universal model for carbon dioxide uptake by plants. *Nature Plants* **3**(9): 734-741.

**Walker AP, McCormack ML, Messier J, Myers-Smith IH, Wullschleger SD. 2017.** Trait covariance: the functional warp of plant diversity? *New Phytologist* **216**(4): 976-980.

**White A, Sparrow B, Leitch E, Foulkes J, Flitton R, Lowe AJ, Caddy-Retalic S. 2012.** Ausplots Rangelands Survey Protocols Manual. *The University of Adelaide Press*.

**Wieczynski DJ, Boyle B, Buzzard V, Duran SM, Henderson AN, Hulshof CM, Kerkhoff AJ, McCarthy MC, Michaletz ST, Swenson NG, et al. 2019.** Climate shapes and shifts functional biodiversity in forests worldwide. *Proceedings of the National Academy of Sciences* **116**(2): 587-592.

**Wright I, Reich P, Westoby M. 2003.** Least-cost input mixtures of water and nitrogen for photosynthesis. *The American naturalist* **161**(1): 98-111.

**Wright IJ, Dong N, Maire V, Prentice IC, Westoby M, Díaz S, Gallagher RV, Jacobs BF, Kooyman R, Law EA, et al. 2017.** Global climatic drivers of leaf size. *Science* **357**(6354): 917-921.

**Wright IJ, Reich PB, Cornelissen JHC, Falster DS, Groom PK, Hikosaka K, Lee W, Lusk CH, Niinemets Ü, Oleksyn J, et al. 2005.** Modulation of leaf economic traits and trait relationships by climate. *Global Ecology and Biogeography* **14**(5): 411-421.

**Wright IJ, Reich PB, Westoby M, Ackerly DD, Baruch Z, Bongers F, Cavender-Bares J, Chapin T, Cornelissen JHC, Diemer M, et al. 2004.** The worldwide leaf economics spectrum. *Nature* **428**(6985): 821-827.

**Yang Y, Wang H, Harrison SP, Prentice IC, Wright IJ, Peng C, Lin G. 2019.** Quantifying leaf-trait covariation and its controls across climates and biomes. *New Phytologist* **221**(1): 155-168.

**Ye C, Chen D, Hall SJ, Pan S, Yan X, Bai T, Guo H, Zhang Y, Bai Y, Hu S. 2018.** Reconciling multiple impacts of nitrogen enrichment on soil carbon: plant, microbial and geochemical controls. *Ecology Letters* **21**(8): 1162-1173.

**Zhou S, Duursma RA, Medlyn BE, Kelly JWG, Prentice IC. 2013.** How should we model plant responses to drought? An analysis of stomatal and non-stomatal responses to water stress. *Agricultural and Forest Meteorology* **182-183**: 204-214.

**Table 1.** **Principal components analysis of leaf traits.** Trait loadings have been rescaled to the range [–1, 1] for each component, following the method of Wieczynski *et al.* (2019). Rescaled values with magnitude >0.5 are shown in **bold**.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  |  | PC1 | PC2 | PC3 | PC4 |
| trait | logit χ | 0.43 | **1** | **1** | 0.26 |
| ln *Narea* | **1** | **–1** | –0.74 | **1** |
| ln LMA | **0.82** | **–0.92** | –0.25 | **–1** |
| ln√LA | **–1** | –0.04 | **–1** | 0.07 |
| eigenvalue  percentage explained  cumulative percentage | | 0.81 | 0.52 | 0.17 | 0.09 |
| 51.1 | 32.5 | 10.7 | 5.7 |
| 51.1 | 83.6 | 94.3 | 100 |

**Table 2.** Coefficients for the regression of leaf traits as a function of mean growing-season temperature (mGDD0, ˚C), mean growing-season vapour pressure deficit (mVPD0, kPa) and soil pH (Fig. 2). Abbreviations: ln LMA, log-transformed leaf mass per unit area; ln *Narea*, log-transformed leaf nitrogen per unit area;ln LA, log-transformed leaf area; logit χ, logit-transformed ratio of leaf-internalto ambient CO2; ln mVPD0, log-transformed mVPD0.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| trait | environmental variable | slope | 95% confidence intervals | | *R*2 | *p*-value |
| logit *χ* | mGDD0 | 0.07 | | (0.05, 0.08) | 34% | <0.001 |
| ln mVPD0 | –0.76 | | (–0.92, –0.61) | <0.001 |
| pHsoil | –0.14 | | (–0.19, –0.08) | <0.001 |
| ln *N*area  (g m–2) | mGDD0 | –0.10 | | (–0.12, –0.07) |  | <0.001 |
| ln mVPD0 | 0.99 | | (0.77, 1.2) | 25% | <0.001 |
| pHsoil | 0.10 | | (0.03, 0.17) |  | <0.001 |
| ln LA  (cm2) | mGDD0 | 0.33 | | (0.27, 0.39) |  | <0.001 |
| mVPD0 | –1.66 | | (–2.11, –1.20) | 25% | <0.001 |
| pHsoil | –0.46 | | (–0.65, –0.27) |  | <0.01 |
| ln LMA  (g m–2) | mGDD0 | –0.04 | | (–0.07, –0.02) |  | <0.001 |
| ln mVPD0 | 0.30 | | (0.08, 0.5) | 9% | <0.001 |
| pHsoil | 0.12 | | (0.05, 0.19) |  | <0.001 |

**Figure legends**

**Figure 1** Principal components analysis of key leaf traits in panels (a) to (c). Abbreviations: ln LMA, log-transformed leaf mass per unit area (g m–2); ln *Narea*, log-transformed leaf nitrogen per unit area (g m–2); ln√LA, log-transformed square root of leaf area (mm); logit χ, logit-transformed ratio of leaf-internalto ambient CO2 (dimensionless). Trait loadings (given in Table 1) have been rescaled to the range [–1, 1] for each component, following Wieczynski *et al.* (2019).

**Figure 2** In panels (a) to (l), partial residual plots for each key leaf traits are as a function of mean growing-season temperature (mGDD0, ˚C), mean growing-season vapour pressure deficit (mVPD0, kPa) and soil pH. Abbreviations: ln LMA, log-transformed leaf mass per unit area (g m–2); ln *Narea*, log-transformed leaf nitrogen per unit area (g m–2); ln LA, log-transformed leaf area (mm2); logit χ, logit-transformed ratio of leaf-internalto ambient CO2 (dimensionless). Back lines are the partial regression lines, the grey shading areas are the 95% confident bands.

**Figure 3** In panels (a) to (l), partial residual plots for each key leaf trait are as a function of mean growing-season temperature (mGDD0, ˚C), mean growing-season vapour pressure deficit (mVPD0, kPa) and soil pH. Abbreviations: ln LMA, log-transformed leaf mass per unit area (g m–2); ln *Narea*, log-transformed leaf nitrogen per unit area (g m–2); ln LA, log-transformed leaf area (mm2); logit χ, logit-transformed ratio of leaf-internalto ambient CO2 (dimensionless). Thin coloured lines illustrate individual within-species regression lines for species with at least three occurrences, showing individual species trends in different colours.

**Figure 4** Trait gradient analyses of key leaf traits. In panels (a) to (d), species’ trait values (grey dots) are plotted against the community-mean trait value for all 705 species and 116 study sites. Black dashed lines represent the overall regression line, which has a slope of unity by definition. Thin coloured lines illustrate individual within-species regression lines for species with at least three occurrences. The general tendency from each set of regressions is summarized in panel (e) with the median slope indicated by a vertical dashed line. Abbreviations: ln LMA, log-transformed leaf mass per unit area (g m–2); ln *Narea*, log-transformed leaf nitrogen per unit area (g m–2); ln LA, log-transformed leaf area (mm2); logit χ, logit-transformed ratio of leaf-internalto ambient CO2 (dimensionless).