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Research

A thinner jacket for frosty and windy climates? Global patterns in leaf cuticle thickness and its environmental associations

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Summary

- Plant cuticles protect the interior tissues from ambient hazards, including desiccation, UV light, physical wear, herbivores and pathogens. Consequently, cuticle properties are shaped by evolutionary selection.
- We compiled a global dataset of leaf cuticle thickness (CT) and accompanying leaf traits for 1212 species, mostly angiosperms, from 293 sites representing all vegetated continents. We developed and tested 11 hypotheses concerning ecological drivers of interspecific variation in CT.
- CT showed clear patterning according to latitude, biome, taxonomic family, site climate and other leaf traits. Species with thick leaves and/or high leaf mass per area tended to have thicker cuticles, as did evergreen relative to deciduous woody species, and species from sites that during the growing season were warmer, had fewer frost days and lower wind speeds, and occurred at lower latitudes. CT–environment relationships were notably stronger among nonwoody than woody species.
- Heavy investment in cuticle may be disadvantaged at sites with high winds and frequent frosts for 'economic' or biomechanical reasons, or because of reduced herbivore pressure. Alternatively, cuticles may become more heavily abraded under such conditions. Robust quantification of CT–trait–environment relationships provides new insights into the multiple roles of cuticles, with additional potential use in paleo-ecological reconstruction.

Introduction

The outermost layer of the leaf surface, like that of all aerial plant parts, is the cuticle. It covers the epidermis and differs in chemical composition from other plant components. Traditionally, the cuticle is described as consisting of two layers (Riederer & Muller, 2006). A waxy, cutin-rich *cuticular layer* with embedded polysaccharides, which interfaces with (and is distinct from) the outer cell wall of the epidermis. Outside that, the *cuticle proper* consists of a cutin-rich matrix with intracuticular wax deposits embedded within and extracuticular wax accumulations on its surface. Others dispute that the two-layer model (which dates back to the 18th century) can be consistently recognised across the great diversity of the plant kingdom, instead considering the cuticle far more generally: simply as the *lipidised region of the outer epidermal cell wall* (OECW) that, in turn, is covered with *epicuticular waxes* (Guzmán *et al.*, 2014; Fernández *et al.*, 2016). Key to this broader definition is the idea that the cuticle is not simply a layer on the epidermis but, rather, that the OECW and lipidised regions beyond that together form an integrated structure that protects the leaf. Recent studies showing that cuticle lipids can be covalently bound to epidermal cell wall materials, for example in *Pisum*, *Solanum* and *Hylotelephium* (Xin & Fry, 2021), are consistent with this broader definition.

Irrespective of the definition adopted, it is agreed that the cuticle performs numerous important functions, most notably minimising water loss outside the stomatal pathway, attenuating and/or reflecting visible and UV-B light (thus preventing damage to photosynthetic apparatus and DNA), forming a water-repellent, physical barrier to herbivores and pathogens and providing mechanical support to the leaf (Riederer & Muller, 2006; Onoda *et al.*, 2012; Yeats & Rose, 2013; Fernández *et al.*, 2016, 2017). These functions relate to a range of chemical and morphological properties of the cuticle. Variation in cuticle permeability to water seems driven more by chemistry (especially long-chain n-alkane content) and nanopore properties than by physical properties such as cuticle thickness (Riederer & Schreiber, 2001; Burghardt & Riederer, 2006; Kerstiens, 2006). That said, as leaves age and suffer physical damage (e.g. from wind-blown particles, and from repeated flexing in the wind), the development of microcracks in cuticles may lead to increased permeability (van Gardingen *et al.*, 1991; Jordan & Brodribb, 2007; Onoda *et al.*, 2012). Variation in optical properties is driven by cuticle thickness and surface features enhancing reflectivity (Cescatti & Niinemets, 2004), and also by chemistry (e.g. lipophilic phenolic molecules can absorb UV-B irradiance but transmit photosynthetically active wavelengths (Krauss *et al.*, 1997)). Onoda *et al.* (2012), studying 13 evergreen forest species, reported that the cuticle contributed up to 18% of resistance to leaf tearing. Such biomechanical properties are also driven by a combination of chemistry and morphology: the amount and nature of intracuticular waxes and polysaccharides affect cuticle stiffness, strength and extensibility (Khanal & Knoche, 2017), whereas cuticle thickness and tissue density both affect mechanical strength (Grubb, 1986). As cuticle thickness varies widely among species, while cuticle density is rather conservative (Schreiber & Schönherr, 1990; Onoda *et al.*, 2012), variation among species in leaf cuticle thickness presumably affects leaf mechanical properties. Furthermore, as the contribution of leaf tissue to leaf flexural rigidity increases with the distance to the central plane (Onoda *et al.*, 2015), the contribution of upper and lower cuticles (being the outermost elements) should be higher than expected on the basis of their thickness alone. Variation in leaf mechanical properties, in turn, is a key driver of interspecific variation in leaf lifespan (Wright & Westoby, 2002; Onoda *et al.*, 2011).

Interspecific variation in leaf cuticle thickness considered for fully mature leaves sampled from field plants (hereafter 'CT') was the focus of this study. In particular, we focussed on variation in CT_{sum}, the summed thickness of upper and lower cuticles, seeking (1) to establish whether there are robust latitudinal and taxonomic patterns in this trait at global scale; (2) to clarify the relationship of CT_{sum} to other leaf morphological traits (leaf thickness, leaf density, leaf dry mass per unit area (LMA) and leaf size); and (3) to assess the role of site climate and soil properties in driving variation in CT_{sum}. Based on common understanding of cuticle functions and leaf functional biology, we developed 11 testable hypotheses concerning putative developmental and environmental associations of CT_{sum} (Table 1). We expected that evergreen woody species would have higher CT_{sum} than deciduous woody species (Hypothesis 1); that leaves that were thicker, denser, had higher LMA or had larger laminas would have higher CT_{sum} (Hypotheses 2–5); that species from sites that had lower nutrient soils or were warmer, sunnier or drier would have higher CT_{sum} (Hypotheses 6–9); and that sites subject to stronger winds or more frequent frosts (i.e. more physically hazardous situations) would have higher CT_{sum} (Hypotheses 10 and 11). We focussed primarily on understanding interspecific variation in CT_{sum}, having found in preliminary analyses (also reported) that upper and lower cuticle thicknesses – also known for many species – were tightly and isometrically correlated. Also, CT–environment relationships were similar using CT_{sum}, CT_{upper} and CT_{lower}.

Plant scientists for more than a century have reported leaf anatomical measurements made on field-collected plants. To test our hypotheses, we gathered these data from the literature together with our own published and unpublished data, compiling a global, georeferenced dataset of CT and associated leaf traits covering 1212 species of plants growing in natural conditions from 293 sites. This was supplemented with climate and soil data from global gridded datasets and with data for leaf thickness, LMA, leaf density and leaf size (projected leaf area) from global trait datasets. The resulting dataset allowed us to test the hypotheses and to quantify taxonomic and biogeographical patterning in CT.

Materials and Methods

Compilation of leaf cuticle thickness data from field-based studies

Data on leaf cuticle thickness were collected from published studies (Clements, 1905; Cooper, 1922; Gäumann & Jaag, 1936; Helmers, 1943; Stover, 1944; Wylie, 1954; Lange & Schulze, 1966; Pyykkö, 1966, 1979; Stocker, 1970, 1971, 1972; Baig & Tranquillini, 1976; Riveros *et al.*, 1976; Krause & Kummerow, 1977; Johnson, 1980; Sobrado & Medina, 1980; Rao *et al.*, 1981; Malaisse & Colonval-Elenkov, 1982; Tanner & Kapos, 1982; DeLucia & Berlyn, 1984; Rao & Tan, 1984; Ridge *et al.*, 1984; Roth, 1984; Schulze, 1984; de Lillis & Valletta, 1985; Sugden, 1985; Körner *et al.*, 1986; Schouten, 1986; Christodoulakis & Mitrakos, 1987; Koike, 1988; Cavelier & Goldstein, 1989; Seshavatharam & Srivalli, 1989; Grace, 1990; Medina *et al.*, 1990; de Lillis, 1991; Choong *et al.*, 1992; Christodoulakis, 1992; Groom *et al.*, 1994; Turner *et al.*, 1995, 2000; Omino, 1996; Schreiber & Riederer, 1996; Arens, 1997; Hlwatika *et al.*, 1998; Santier & Chamel, 1998; Cunningham *et al.*, 1999; Gratani & Bombelli, 1999; Saha *et al.*, 1999; Edwards *et al.*, 2000; Burrows, 2001; Mediavilla *et al.*, 2001; Hlwatika & Bhat, 2002; Peeters, 2002; Velazquez-Rosas *et al.*, 2002; Boeger & Wisniewski, 2003; Gamage *et al.*, 2003; Semerdjieva *et al.*, 2003; Gras *et al.*, 2005; Sack & Frole, 2006) both as part of this study and in some cases as part of a previous data compilation (Niinemets, 1999). Additional data were contributed by coauthors (D. H. Duncan & M. Westoby, unpublished; G. J. Jordan, unpublished). Where reported, information on leaf lamina thickness and LMA were also extracted from all of these data sources.

Table 1 Testable hypotheses and justifications concerning leaf cuticle thickness, CT_{sum} and the degree of support found in this study.

Hypotheses for CT_{sum} being higher in given comparisons	Justifications	Degree of support observed in this study
1. In woody species vs nonwoody species and, among woody species, in evergreen vs deciduous species		Strongly supported in comparison of plant functional types
2. For thicker leaves	Cuticle thickness increases in parallel to leaf thickness during leaf development (Zhu *et al*., 2018) and in relation to stand age or plant height (England & Attiwill, 2006), in part because CT_{sum} directly contributes to leaf thickness	Strongly supported. However, observed scaling slope was shallower than isometric (thicker leaves have proportionally less cuticle)
3. For higher LMA leaves	CT_{sum} and LMA are known to be generally correlated (Roderick *et al*., 1999; Onoda *et al*., 2012; John *et al*., 2013; Soh *et al*., 2017), potentially via CT_{sum} correlations with both leaf thickness and density (remembering that LMA = leaf thickness × leaf density (Witkowski & Lamont, 1991))	Strongly supported, with trend driven more by variation in thickness than by variation in density
4. For higher density leaves	Cuticle material has higher density than other leaf components (Onoda *et al*., 2012); hence, higher CT_{sum} leaves will have higher overall tissue density	Supported, but with low explanatory power. However, leaves with proportionally thicker cuticles (higher $CT_{sum}\%$) had higher tissue density
5. For larger leaves	Larger leaves require greater structural reinforcement (Givnish, 1984; Niinemets *et al*., 2006). In part, this could be achieved via more reinforced (thicker) outer layers, that is thicker epidermis + cuticle (Grubb, 1986), in which case leaf size and CT_{sum} will be correlated	Not supported (all species), but supported for nonwoody species
6. At sites with lower soil nutrient concentrations	Species on low nutrient soils typically have long leaf lifespans (LL), an adaptation that reduces nutrient uptake requirements (Aerts & Chapin, 2000). Leaf mechanical strength is a key driver of LL variation (Wright & Westoby, 2002; Onoda *et al*., 2011). As CT_{sum} contributes directly to LMA and mechanical properties (Onoda *et al*., 2012), CT_{sum} will be higher on lower nutrient soils	Supported: CT_{sum} was weakly higher at lower cation exchange capacity (and at higher soil C : N or at lower pH)
7. At warmer sites	Globally, leaf dry mass per unit area (LMA) is higher at warmer sites (Wright *et al*., 2004), as is leaf thickness (Niinemets, 2001). Therefore CT_{sum} will also be higher at warmer sites, via secondary correlation with leaf thickness and LMA (as discussed previously)	Supported for all temperature metrics but still evident when variation in either LMA or thickness was controlled in partial correlation analyses
8. At higher irradiance sites	Thicker cuticles should provide better protection from damage by solar (especially UV-B) radiation to DNA, proteins (e.g. PSII), lipids and membranes (Jordan *et al*., 2005; Karabourniotis *et al*., 2021); therefore, CT_{sum} will be positively correlated with site irradiance, and CT_{upper} more strongly so (receiving more direct radiation)	Weakly supported, more so for CT_{upper} than CT_{sum}, and more so for PPFD than UV-B. Correlations notably stronger (and in expected directions) among nonwoody species
9. In arid vs mesic biomes, and at sites with lower rainfall or lower moisture index (ratio of precipitation to potential evapotranspiration)	Globally, species at drier sites tend to have thicker, higher LMA leaves (Niinemets, 2001; Wright *et al*., 2004), so CT_{sum} will also be higher at drier sites, via its contribution to leaf thickness and via secondary correlation with LMA	Partial support, in that thick cuticles were typical of species from the *Deserts and Xeric Shrublands* biome and, in multiple regressions, CT_{sum} was weakly and negatively correlated with site rainfall. By contrast, in the bivariate analyses, CT_{sum} was slightly higher at wetter sites
10. At windier sites	Expected on the basis that windier sites are in some sense more abrasive – there is higher likelihood of damage from airborne soil, ice and dust particles (Hadley & Smith, 1989; van Gardingen *et al*., 1991), and from physical contact with neighbouring leaves – and thus higher CT_{sum} (representing greater protection) would be advantageous in such situations. Higher wind sites also have higher evaporative demand due to stripping of the atmospheric boundary layer which, otherwise, slows water loss (Gates, 1968); this may also favour leaves with higher CT_{sum}	Not supported. Rather, CT_{sum} was clearly *lower* at sites with higher mean wind speed, both across all species and within PFTs (notably stronger in nonwoody PFT), and especially at sites experiencing frequent frost
11. At sites with more frequent frost	Structural reinforcement of leaves, including from the cuticle, helps protect against frost damage (Grace, 1990) and abrasive, icy rains. Thus, species from sites more prone to frost will have higher CT_{sum}	Not supported. Rather, CT_{sum} was *lower* at sites with more frost days in the growing season, both across all species and within PFTs (notably stronger in nonwoody PFT), and especially at sites experiencing higher average wind speeds

Only field-measured data were selected for inclusion; data from glasshouse, shade-house, common garden, manipulative experiments and any other clearly non-natural situations or treatments were excluded. Where data for multiple leaves or individual plants were reported for a species at a site, we included only the mean value in our dataset. Where data were reported separately for adult and juvenile plants, we included adult data only. Where data were reported separately for leaves sampled at high and low light, we used the mean value. Our final dataset consisted of species-mean trait values at each site, with species names standardised according to The Plant List (www.theplantlist.org; accessed on 18 January 2021), which also provided taxonomic information at higher levels.

The dataset represents more than a century of research on leaf anatomy, the oldest data source being the seminal work on Rocky Mountain (USA) species by Edith Schwartz Clements, a founding figure of ecology (Clements, 1905). Accordingly, there was substantial variation among studies in sample preparation, including histochemical staining procedures used for identifying the cuticle region of leaves, and in the optical resolution used for measuring leaf cuticle thickness via light microscopy (the rationale of excluding the small number of transmission electron microscopy (TEM)-based studies will be discussed later). Most studies reported anatomical data measured on freshly collected leaves; for three studies, the data were measured on rehydrated leaves sampled from herbarium specimens (Pyykkö, 1966, 1979; Johnson, 1980). Most studies reported thickness data for both upper and lower cuticles, others for upper cuticle only and others still for combined cuticle thickness only (i.e. the sum of upper and lower, '$\mathrm{CT_{sum}}$', μm). As already noted, we focussed primarily on understanding interspecific variation in this combined metric, '$\mathrm{CT_{sum}}$'. However, for completeness, analyses involving $\mathrm{CT_{upper}}$ and $\mathrm{CT_{lower}}$ are also reported.

As noted in the Introduction section, the relationship between the OECW and the cuticle is complicated, with increasing reason to consider these as an integrated structure or region of the leaf rather than as separate entities (Guzmán *et al.*, 2014; Fernández *et al.*, 2016; Xin & Fry, 2021). We suggest that this is especially the case when seeking a single inclusive definition that applies across a broad taxonomic sample of the world's plant taxa (e.g. as done here, for 171 plant families); that applies across the substantial variation in cuticle structure/organisation that this represents; and that applies across a dataset built from source studies employing a wide variety of methods, including the manner in which the OECW was treated. On that matter, rarely, depth of the OECW and cuticle were reported together as a single number (Malaisse & Colonval-Elenkov, 1982; Tanner & Kapos, 1982; Sugden, 1985; Cavelier & Goldstein, 1989; Medina *et al.*, 1990; Turner *et al.*, 2000). Mostly, these were studies with a focus on sclerophylly. This approach seemingly relates back to a phenomenon noted 50 yr ago (Grubb *et al.*, 1975) – in a study of tropical forest species from Japan and Papua New Guinea – in which the authors noted that the cuticle formed a clearly distinct outer layer (separate from the OECW) in only 44/100 species under study; in the other 56, lipid material, as judged by staining with Sudan III, was deposited through almost the whole wall thickness, usually becoming gradually less dense inward. Seemingly, this is the same phenomenon receiving more recent attention by Guzmán *et al.* (2014) and Fernández *et al.* (2016). Conversely, but also rarely, cuticle thickness in source studies was reported separately from the depth of the OECW (Pyykkö, 1966; Christodoulakis & Mitrakos, 1987; Christodoulakis, 1992; Burrows, 2001); thus, in these datasets, we were certain that CT represented the thickness of cuticle only. (The same was true for unpublished data contributed by author GJJ). However, no mention of the OECW was made in the great majority of source publications; furthermore, cuticle thickness was sometimes reported alongside data on epidermis depth and sometimes not. In either situation, we were unable to confidently ascertain whether or not the reported CT data were intended to include the OECW. We assume not, but even so, there is a possibility that histochemical staining for cuticle would simultaneously stain lipids embedded in the OECW, meaning that the depth of this structure could have been inadvertently included in CT values.

Consequently, the pragmatic approach adopted in this study was to consider all data reported for CT in source publications as equivalently indicative of the cuticle thickness of the study species. Our current, functional definition of CT is necessarily 'fuzzy' – by way of analogy with the use of this term in other areas of plant science to designate uncertainty (Pillar, 1999; Townsend, 2000) – considering the cuticle simply as *the protective outermost layer of the leaf, composed of cutin, cuticular waxes and embedded polysaccharides, varying in anatomical composition among taxa such that it may (or may not) include the OECW but, in common, protecting the leaf from desiccation, environmental hazards, herbivory, and so forth.*

Finally, we note that data from a small number of studies (for *c.* 30 species) using TEM were initially compiled but eventually excluded from the compilation as (1) $\mathrm{CT_{sum}}$ was dramatically and systematically lower in all cases, and (2) as these studies predominantly concerned high latitude and/or extreme high elevation sites such as the Ural Mountains (Kravkina, 2000) or the *Trans*-Altai Gobi desert (Kravkina & Miroslavov, 1980), meaning that the CT values therein disproportionately affected estimates of CT–latitude and CT–temperature relationships across our dataset (making reported relationships notably stronger).

Additional leaf trait data

Information on leaf size (one-sided projected surface area of leaves or leaflets, $\mathrm{cm^2}$) and LMA ($\mathrm{g\ m^{-2}}$) was rarely reported alongside information on cuticle thickness, so further data (considered as species means) were obtained from a global dataset for leaf size (Wright *et al.*, 2017), and (for both leaf size and LMA) from a gap-filled version of the TRY v5.0 database (Kattge *et al.*, 2020). Gap-filling of the TRY data product (not performed as part of this project) was achieved using Bayesian hierarchical probabilistic matrix factorisation (BHPMF) (Shan *et al.*, 2012; Fazayeli *et al.*, 2014; Schrodt *et al.*, 2015), and it was the gap-filled dataset that was used for providing missing data to this current analysis. BHPMF uses machine learning to estimate missing trait entries at the individual level in a sparse data matrix, in a process run iteratively at nested taxonomic levels. Model inputs are existing trait data (including their correlations) and taxonomic information. Neither environmental data nor cuticle thickness data were used in the gap-filling process, meaning that the resulting dataset was suitable for quantifying trait–environment associations, as per the goals of this study. To match CT data with that for leaf tissue density, leaf tissue density was estimated by dividing LMA by leaf thickness (Witkowski & Lamont, 1991). This gave rise to a small number of implausibly high and low values (e.g. < 0.1 g cm^{-3} or > 1 g cm^{-3}) that in approximately equal measure came from CT-source trait datasets and from the gap-filled LMA dataset (in other words, the extreme values were not simply due to species-level data coming from different sources). Hence, we discarded the lowest and highest 2.5 percentiles leaf density data, yielding a final data range of 0.17–0.99 cm^{-3}.

The full dataset for CT_{sum}, CT_{upper}, CT_{lower} and accompanying traits (Supporting Information Table S1) – the 'GlobCute database' – comprises 1531 rows of data, representing 1211 species from 171 families collected from 293 sites. CT_{upper} is reported for 1492 of the 1531 rows; CT_{lower} is reported for 1168 of the 1531 rows. Our final dataset for CT_{sum} consisted of 1207 rows of data, representing 983 species from 154 plant families and 275 sites, accompanied by data for leaf lamina thickness for 1137 of these data rows (936 species), LMA for 1040 rows/828 species, leaf density for 978 rows/750 species and leaf size for 1046 rows/834 species. The proportional contribution of leaf cuticle to lamina thickness ('CT_{sum}%') was calculated as a ratio, expressed as a percentage. The geometric mean percentage contribution of cuticle to leaf thickness ('CT_{sum}%') was 3.1%, and for 1092 of 1137 data rows, it was < 10%. The highest CT_{sum}% values were reported in species sampled from tropical monsoon forest in southern China (de Lillis, 1991).

There was insufficient replication of CT within species but across sites to explore within-species trends, or to explore the contribution of within-species traits variation to the overall trends across the dataset.

Plant functional types (PFTs)

Species were categorised into broad plant functional types (PFTs) (nonwoody, woody deciduous or woody evergreen species) based on information available from the TRY database (categorical lookup tables) and online species descriptions. 'Woody' species included trees, shrubs and subshrubs, lianas and four species of tree fern (all *Cyathea* genus). Nonwoody species included graminoids, forbs, vines and other ferns. A total of 85% of species in our datasets were woody: the CT_{sum} dataset contained 742 evergreen woody species, 92 deciduous woody species and 149 nonwoody species.

Soil and climate data

All studies included location, but in some cases, this was a geographic descriptor (e.g. nearest town) rather than a precise latitude and longitude. In such cases, a point location was estimated using Google Earth. Soil and climate data from global gridded datasets were attributed to each location based on latitude and longitude.

Soil information was extracted from the WISE 30 arc-second resolution global gridded dataset (Batjes, 2016), using data for 0–30 cm depth. Eight soil variables were used: three indicators of soil texture (sand, silt and clay % fractions), three indices of cation exchange capacity (CEC) (eCEC: 'effective' CEC of bulk soil; CECc: CEC of clay fraction; CECs: CEC of silt fraction), soil C : N ratio and soil pH. Variation in soil texture affects water holding capacity as well as providing an indication of relative nutrient availability. CEC more precisely indexes nutrient availability – specifically, the ability of a soil to supply three important plant nutrients: calcium, magnesium and potassium. Soil C : N ratio is a complementary index of soil fertility, especially relating to the amount and quality of organic matter (Heal *et al.*, 1997). Over much of its observed range, soil pH is a general index of fertility; cation availability and even solubility of organic matter tend to be higher at higher pH (Maire *et al.*, 2015), at least until one is considering strongly alkaline soils, in which availabilities may again decrease.

Based on site coordinates, a variety of potentially relevant climatic variables were extracted from global gridded datasets of long-term, monthly climate. Monthly data were linearly interpolated to daily values, then mean or summed growing-season values were calculated for all climatic variables. The growing season was defined as the continuous period with daily 24-h mean temperature > 5°C (Kikuzawa *et al.*, 2013); this ranged from 109 to 365 d yr^{-1} across sites. We used growing-season data on the basis that it is the most relevant period of the year for leaf function and therefore the best way to fairly compare trait–climate relationships across plant functional types differing in leaf phenology. It is the period when woody deciduous and nonwoody species are present or with-canopy, and also the period when the leaves of evergreen species are most functional: during the nongrowing season, evergreen species 'harden' their leaves, protecting them against extreme cold but rendering them essentially nonfunctional (Havranek & Tranquillini, 1995).

Site temperature was represented by average minimum, maximum and mean growing-season temperatures: Tmin_{gs}, Tmax_{gs} and Temp_{gs}, respectively (all in °C). Site aridity was described via summed precipitation $\text{Precip}_{\text{gs}}$ (mm) and via moisture index (MI_{gs}), MI_{gs} (units: mm/mm) being the ratio of $\text{Precip}_{\text{gs}}$ (mm) to Priestley–Taylor potential evapotranspiration also calculated across the growing season (mm). Top-of-canopy irradiance was summarised in terms of mean daily photosynthetically active photon flux density (PPFD_{gs}; mol photon m^{-2} d^{-1}) and mean daily ultraviolet B radiation (UVB_{gs}; J m^{-2}d^{-1}). Site windiness was characterised as mean daily wind speed (Wind_{gs}; m s^{-1}) and frost exposure as the average number of frost days per growing season (Frost_{gs}; days). Most climate variables were calculated using SPLASH v1.0 (Davis *et al.*, 2017), with input variables sourced from the CRU.CL 2.0 gridded climatology dataset (New *et al.*, 2002), which reports mean data for 1961–1990 considered at 30 arc-second resolution. UV-B data were extracted from the 15 arc-minute resolution glUV database (Beckmann *et al.*, 2014). Note that all of these descriptors consider site macroclimate. We note that, within ecosystems, irradiance, temperature, relative humidity and wind speed may vary with slope aspect, canopy exposure and surrounding vegetation; however, we did not consider microclimate effects in this study.

The 293 sites varied widely in environmental properties; for example, Temp_{gs} ranged from 7.5 to 28.6°C, Precip_{gs} from 67 to 4969 mm, PPFD_{gs} from 23.8 to 45.8 mol photon m^{-2} d^{-1}, Frost_{gs} from 0 to 134 d, Wind_{gs} from 1.0 to 7.7 ms^{-1}, soil pH from 4.4 to 8.2 and soil C : N from 8 to 27. The details of study site locations, climate, soils and source datasets are given in Table S2.

Biomes

Sample sites represented every vegetated continent (Fig. 1a) and 12 of 14 biomes (or 'ecoregions') from one commonly used classification (Olson *et al.*, 2001): *Tropical and Subtropical Moist Broadleaf Forests*; *Tropical and Subtropical Dry Broadleaf Forests*; *Temperate Broadleaf and Mixed Forests*; *Temperate Conifer Forests*; *Boreal Forests/Taiga*; *Tropical and Subtropical Grasslands, Savannas and Shrublands*; *Temperate Grasslands, Savannas and Shrublands*; *Montane Grasslands and Shrublands*; *Tundra; Mediterranean Forests, Woodlands and Scrub*; *Deserts and Xeric Shrublands*; and *Mangrove*. The two biomes from that scheme without data were *Tropical and Subtropical Coniferous Forests*, and *Flooded Grasslands and Savannas*.

Data analysis

As all leaf traits were right-skewed, they were $\log_{10}$-transformed before analyses in order to attain approximate normality in trait distributions and homogeneity of variance among residuals in regression models. Six of the climate and soil variables were also $\log_{10}$-transformed: eCEC, CECs, soil C : N, Precip_{gs}, MI_{gs}, Frost_{gs} and the other variables being approximately normally distributed. As Frost_{gs} had many zero values, a constant value of 1 was added before log-transformation.

First, general patterns in CT_{sum} distribution were described: in relation to biomes, broad taxonomic group (angiosperm, fern and gymnosperm), PFT, taxonomic family (for the 12 families with more than 20 records) and latitude. Group mean values were compared using ANOVA followed by Least Significant Difference *post hoc* tests. Relationships to latitude (all species; or with species grouped by PFT) were quantified with ordinary least squares (OLS) regressions – including a quadratic term, as this clearly improved the fit to data and the quadratic term was in all cases highly significant (all $P \leq 0.005$). Supplementary analyses of upper (CT_{upper}) and lower cuticle thickness (CT_{lower}) to latitude are also reported, as well as latitudinal trends in other leaf traits. Again, these were quantified with polynomial regressions including a quadratic term (and the quadratic term was highly significant in each case, $P < 0.001$). The relationship between CT_{upper} and CT_{lower} was quantified as its standardised major axis (SMA) slope (Warton *et al.*, 2006) as in that case, we were specifically interested in whether the relationship was isometric or allometric.

Second, using OLS regression, we quantified relationships between CT_{sum} (as dependent variable) and each of leaf lamina thickness, LMA, leaf density and leaf size (Hypotheses 2–5, Table 1), and also relationships between these traits and leaf cuticle fraction (i.e. CT_{sum} divided by leaf thickness, 'CT_{sum}%'), a previous analysis finding that thicker leaves have *proportionally* thinner cuticles than thinner leaves (Niinemets, 1999).

Third, regarding CT–soil/climate relationships (Hypotheses 6–8), we first quantified Pearson's correlations between CT_{sum} and a range of soil and climate variables. From these, we identified the strongest single correlate (highest correlation *r*) of CT_{sum} from each of four variable groups (soil, irradiance, temperature and moisture) for use in subsequent analyses, alongside frost days and wind speed (Hypotheses 10–11). For these six variables (CECc, Tmin_{gs}, Precip_{gs}, PPFD_{gs}, Wind_{gs} and Frost_{gs}), the relationships with CT_{sum} were further explored in relation to PFT.

Fourth, partial correlation analysis was used to quantify the effects of the six key environmental variables on CT_{sum} while simultaneously accounting for variation in other leaf traits.

Fifth, multiple linear regression was used for quantifying the joint explanatory power (for CT_{sum}) of the six key environmental variables and simultaneously the effect of each variable while variation in others was held constant. Having found that Wind_{gs} and Frost_{gs} were the two most important variables, we also explored regression models using just those variables, with and without an interaction term. To check that the apparent frost effect was not simply arising via secondary correlation with site temperature, we also ran regression models in which Frost_{gs} was replaced by Temp_{gs}, Tmax_{gs} or Tmin_{gs}.

Results

Biogeographic, taxonomic and functional-type variation in leaf cuticle thickness

Summed cuticle thickness, CT_{sum}, ranged widely from 0.60 to 87.5 µm, representing 145-fold variation. Still, 98% of data (i.e. 1^{st} to 99^{th} percentile) fell between 1.5 and 47.4 µm, representing 32-fold variation, and 95% of data (i.e. from the 2.5^{th} to 97.5^{th} percentile) fell between 2.0 and 37.0 µm, representing 18-fold variation. Comparing biomes, on average, species from *Deserts and Xeric Shrublands* and from *Mediterranean Forest, Woodland and Scrub* had higher CT_{sum}, while species from *Boreal Forests/Taiga*, *Tundra* and *Tropical and Subtropical Dry Broadleaf Forests* had lower CT_{sum} (Fig. 1b). That said, the range seen within any given biome was notably wide, commonly 10-fold or more, and wide variation was observed among co-occurring species also. As an extreme example, an *c.* 30-fold range in CT_{sum} was observed among perennial species from a single site in eastern Australia (Fig. S1).

CT_{sum} did not differ significantly between angiosperms, gymnosperms and ferns (Fig. 2a); however, sample sizes for ferns and gymnosperms were just 8 and 15 species, respectively (compared with 960 species of angiosperms or 1155 species-site combinations). At the species level, these sample sizes equate to *c.* 0.3% of the *c.* 300 000 known angiosperm species, 1.4% of the *c.* 1100

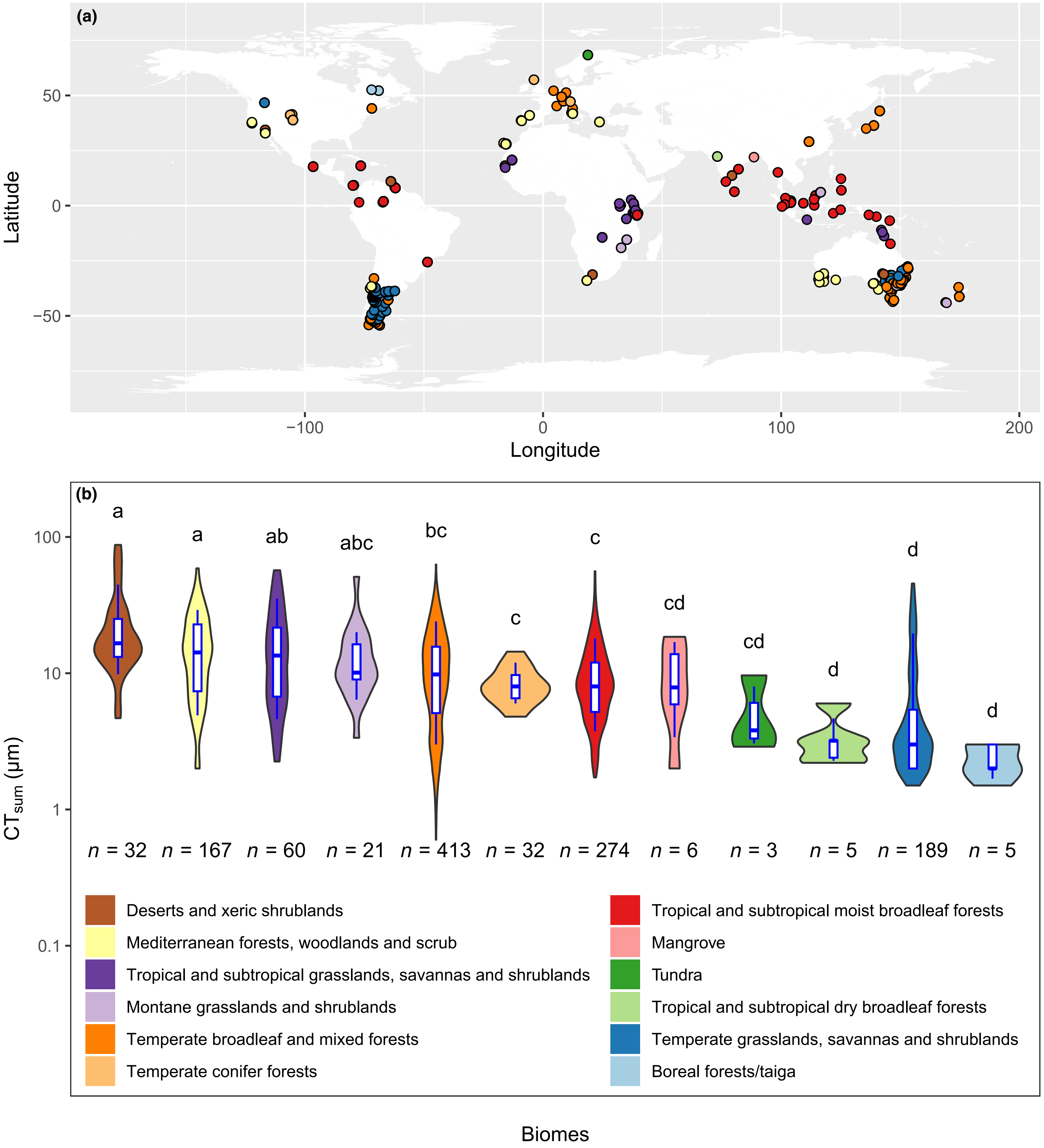

Fig. 1 Locations and biogeographic coverage of leaf cuticle dataset. (a) Locations of the 293 sites represented in the dataset. (b) Variation in leaf cuticle thickness (CT_{sum}) by biome, ordered by decreasing median CT_{sum}. Violin plots (ordered by decreasing median CT_{sum}) indicate the shape and density of the data distribution for each group; within those, the boxplots show interquartile ranges, and the whiskers show 0.1 and 0.9 quantiles. Sample size per group (i.e. number of species) is also indicated. ANOVA with LSD *post hoc* comparisons was used to make comparisons among biomes; different letters indicate significant differences at the level of $P < 0.05$.

known gymnosperm species and 0.08% of the *c.* 10 000 known fern species (i.e. ferns were most notably under-represented). Within angiosperms, there was distinct taxonomic patterning in CT_{sum} (Fig. 2c): Among the 12 families having more than 20 entries in our dataset, Proteaceae and Myrtaceae possessed the thickest leaf cuticles, on average, while Rosaceae and Asteraceae

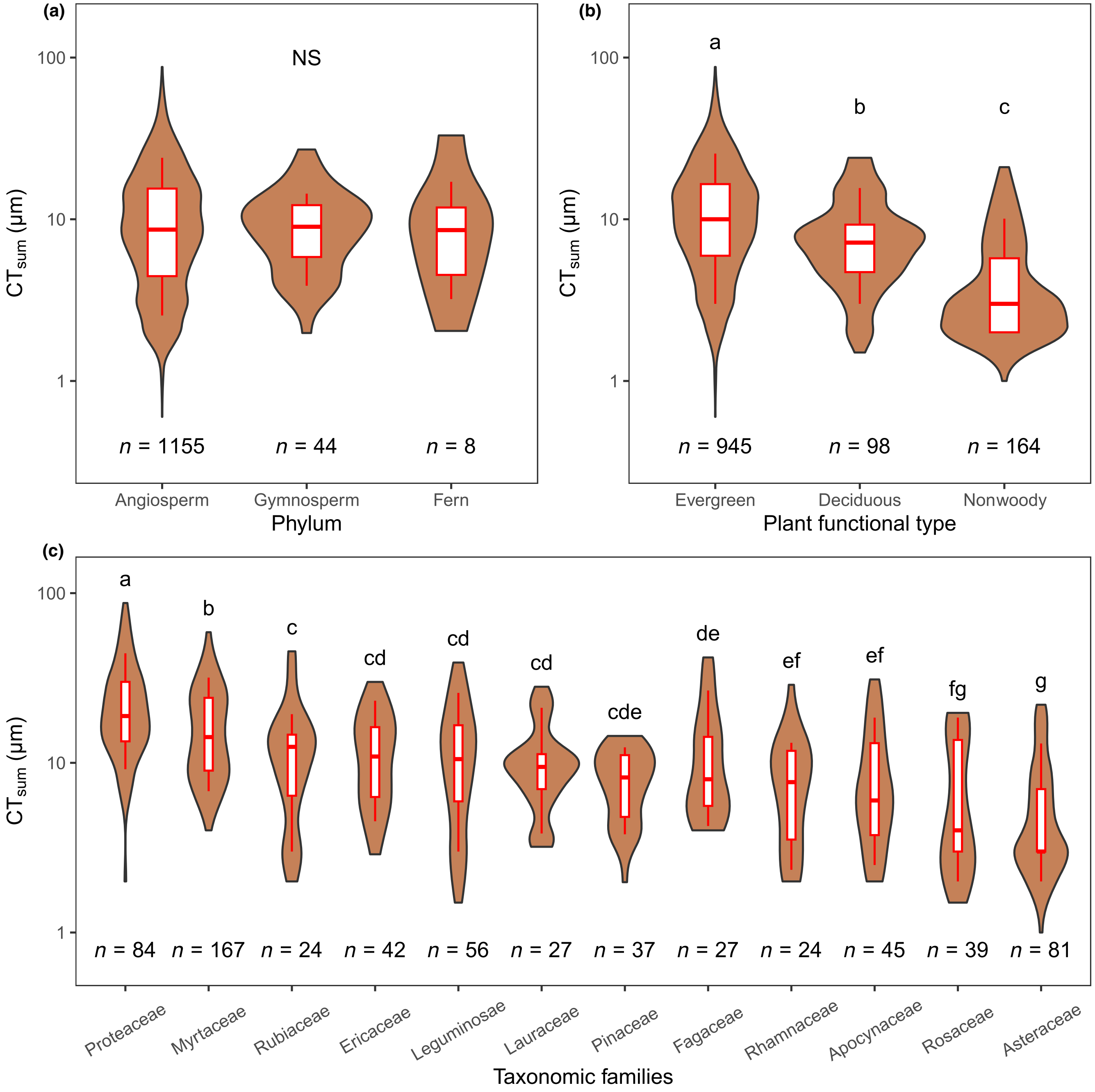

Fig. 2 Patterning of leaf cuticle thickness in relation to (a) broad taxonomic group, (b) plant functional types (woody evergreen, woody deciduous and nonwoody) and (c) taxonomic family. In panel C, only families with more than 20 species in our dataset are illustrated. Violin plots (ordered by decreasing median CT_{sum}) indicate the shape and density of the data distribution for each group; within those, the boxplots show interquartile ranges, and the whiskers show 0.1 and 0.9 quantiles. Sample size per group (i.e. number of species) is also indicated. ANOVA was used to make comparisons of CT_{sum} among taxonomic families. Different letters indicate significant differences at the level of $P < 0.05$. NS, non-significant.

had the thinnest. The 10 highest CT_{sum} records came from Myrtaceae (three species), Malvaceae (one species) and Proteaceae (six species, including the three highest values: *Leucadendron nervosum* E.Phillips & Hutch., *Protea longifolia* Salisb. and *Hakea dactyloides* (Gaertn.) Cav. Taxonomic patterning was less clear among low CT_{sum} values, the lowest 10 being contributed by species in eight families (Asteraceae, Brassicaceae, Calyceraceae, Dilleniaceae, Euphorbiaceae, Goodeniaceae, Lamiaceae and Schoepfiaceae)). The full dataset is provided as Table S1.

CT_{sum} differed between PFTs, decreasing in the order: woody evergreen > woody deciduous > nonwoody (Fig. 2b). CT_{sum} was on average 2.6-fold higher in woody than in nonwoody species (geometric means 9.4 vs 3.6 µm), and 1.4-fold higher in evergreen than in deciduous woody species

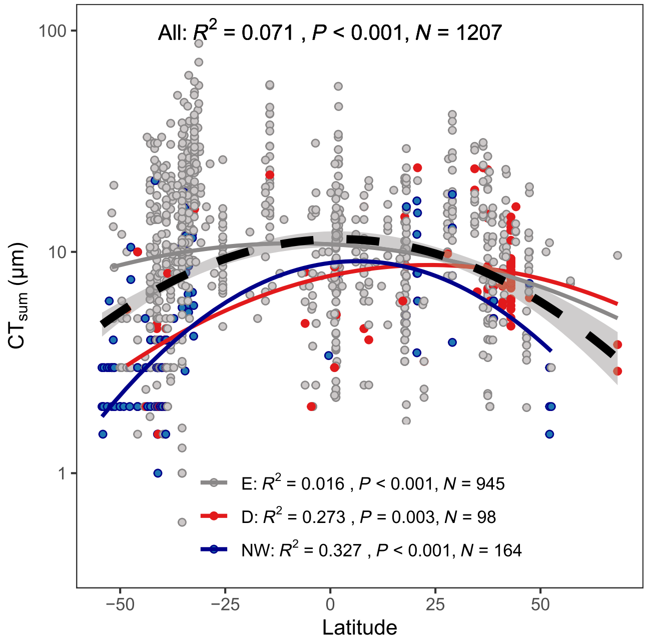

Fig. 3 Latitudinal trends in leaf cuticle thickness, $\mathrm{CT_{sum}}$. Quadratic functions were fitted to the data, largely for illustration purposes but also because these provided a better fit than linear functions, and the quadratic terms were all highly significant (all $P \leq 0.005$). All species fit: black dashed line (95% CI indicated with grey shading). Evergreen woody species: grey points and line; deciduous woody species: red points and line; nonwoody species: blue points and line. Negative latitudes indicate sites in the southern hemisphere, and positive latitudes indicate northern sites.

(geometric means 9.7 vs 6.9 µm). Both trends were in agreement with Hypothesis 1.

$\mathrm{CT_{sum}}$ showed an approximately parabolic relationship with latitude, being higher towards the equator and lower towards the poles (Fig. 3; quadratic fit: $R^2 = 0.071$, quadratic term, $P < 0.001$). Considering the three PFTs separately, a parabolic relationship with latitude was clearly evident in nonwoody species ($R^2 = 0.327$) and deciduous woody species ($R^2 = 0.273$) but less so among woody evergreens ($R^2 = 0.016$; still, the relationship was significant, $P < 0.001$). With $\mathrm{CT_{upper}}$ and $\mathrm{CT_{lower}}$ being tightly correlated ($R^2 = 0.68$; Fig. S2) and with an SMA slope of 1.006 (95% CIs 0.97, 1.04) indicating isometric scaling, it was no surprise to observe similar latitudinal trends in $\mathrm{CT_{upper}}$ and $\mathrm{CT_{lower}}$ as for $\mathrm{CT_{sum}}$ (indeed, with slightly higher R^2 values; Fig. S3A,B). In the 1168 rows of the dataset with both upper and lower CT data, $\mathrm{CT_{upper}}$ was on average 1.3-fold higher than $\mathrm{CT_{lower}}$ (geometric means = 4.5 vs 3.5 µm; paired t-test, $P < 0.0001$; Fig. S2).

Relationships of $\mathrm{CT_{sum}}$ to other leaf traits

Generally, species with thicker leaves had thicker cuticles ($R^2 = 0.\ 236$; Fig. 4a; in support of Hypothesis 2). As the log–log scaling slope was somewhat flatter than 1 (0.83; 95% CIs = 0.75, 0.91), by definition, the slope of $\log_{10}(\mathrm{CT_{sum}}\%)$ vs $\log_{10}$(leaf thickness) was significantly negative (−0.17; 95% CIs = −0.30, −0.13; Fig. 4b); that is, thicker leaves had thicker cuticles (as might be expected) but the *proportion* of leaf thickness contributed by cuticle was slightly lower in thicker leaves, on average.

In agreement with Hypothesis 3, species with higher LMA tended to have thicker cuticles ($R^2 = 0.228$; Fig. 4c) and, more weakly, a higher $\mathrm{CT_{sum}}\%$ ($R^2 = 0.018$; Fig. 4d). Cuticle thickness and leaf density were only weakly associated ($R^2 = 0.013$, Fig. 4e; Hypothesis 4). However, species with higher $\mathrm{CT_{sum}}\%$ had higher density leaf tissue overall ($R^2 = 0.100$; Fig. 4f). Finally, neither $\mathrm{CT_{sum}}$ nor $\mathrm{CT_{sum}}\%$ was significantly associated with leaf size (Fig. 4g,h), in disagreement with Hypothesis 5.

Generally, the same trait relationships were observed within PFTs (Table S3), with two of the most consistent relationships being between $\mathrm{CT_{sum}}$ and LMA, and between $\mathrm{CT_{sum}}\%$ and leaf density. By contrast, within-PFT relationships involving leaf size differed notably from the nonsignificant all-species results, in that (1) among nonwoody species, leaf size and $\mathrm{CT_{sum}}$ were clearly correlated (positively; $R^2 = 0.186$, $P < 0.001$); (2) among woody species, the leaf size–$\mathrm{CT_{sum}}$ relationship was weakly positive in deciduous species and weakly negative among evergreens (both P *c.* 0.05); and (3) leaf size and $\mathrm{CT_{sum}}\%$ were positively correlated in deciduous and nonwoody species (most strongly in deciduous), but unrelated in evergreen species; that is, in nonwoody and in deciduous species, there was some evidence supporting Hypothesis 5, that bigger leaves receive proportionally more physical support from their cuticles; but not in evergreens.

Bivariate relationships between $\mathrm{CT_{sum}}$ and environmental variables

We identified the single strongest correlates with $\mathrm{CT_{sum}}$ within each of the four chief groups of abiotic variables (soil fertility; and growing-season temperature, irradiance and moisture; Table S4). On average, $\mathrm{CT_{sum}}$ was higher at sites with lower CECc ($R^2 = 0.030$; Fig. 5a supporting Hypothesis 6), higher $\mathrm{Tmin_{gs}}$ ($R^2 = 0.052$; Fig. 5b; supporting Hypothesis 7), and higher $\mathrm{Precip_{gs}}$ ($R^2 = 0.024$; Fig. 5d; in opposition to Hypothesis 9). Hypothesis 8 (that $\mathrm{CT_{sum}}$ would be thicker at higher irradiance) found no support (Fig. 5c). In terms of relationship sign, almost identical results emerged when considering $\mathrm{CT_{upper}}$ and $\mathrm{CT_{lower}}$ rather than $\mathrm{CT_{sum}}$ (Table S4). In terms of relationship strength (R^2), associations between $\mathrm{CT_{sum}}$ and temperature variables were stronger than for either $\mathrm{CT_{upper}}$ or $\mathrm{CT_{lower}}$; whereas for several soil, irradiance and rainfall variables, the associations were strongest for $\mathrm{CT_{upper}}$.

On average, species at sites experiencing higher growing-season wind speeds had thinner cuticles ($R^2 = 0.125$; Fig. 5e), as did species at sites experiencing more frost days ($R^2 = 0.085$; Fig. 5f). These trends were in the opposite direction to those predicted (Hypotheses 10 and 11). Again, similar results were found for $\mathrm{CT_{upper}}$ and $\mathrm{CT_{lower}}$ as for $\mathrm{CT_{sum}}$ (Table S4). Fitting locally weighted (LOESS) lines to the plots indicated notable nonlinearity in the $\mathrm{CT_{sum}}$–$\mathrm{Frost_{gs}}$ relationship (~ no response until 10 frost days, thereafter decreasing; Fig. 5f) and in the $\mathrm{CT_{sum}}$–$\mathrm{Temp_{gs}}$ relationship ($\mathrm{CT_{sum}}$ increasing up to $\mathrm{Tmin_{gs}}$

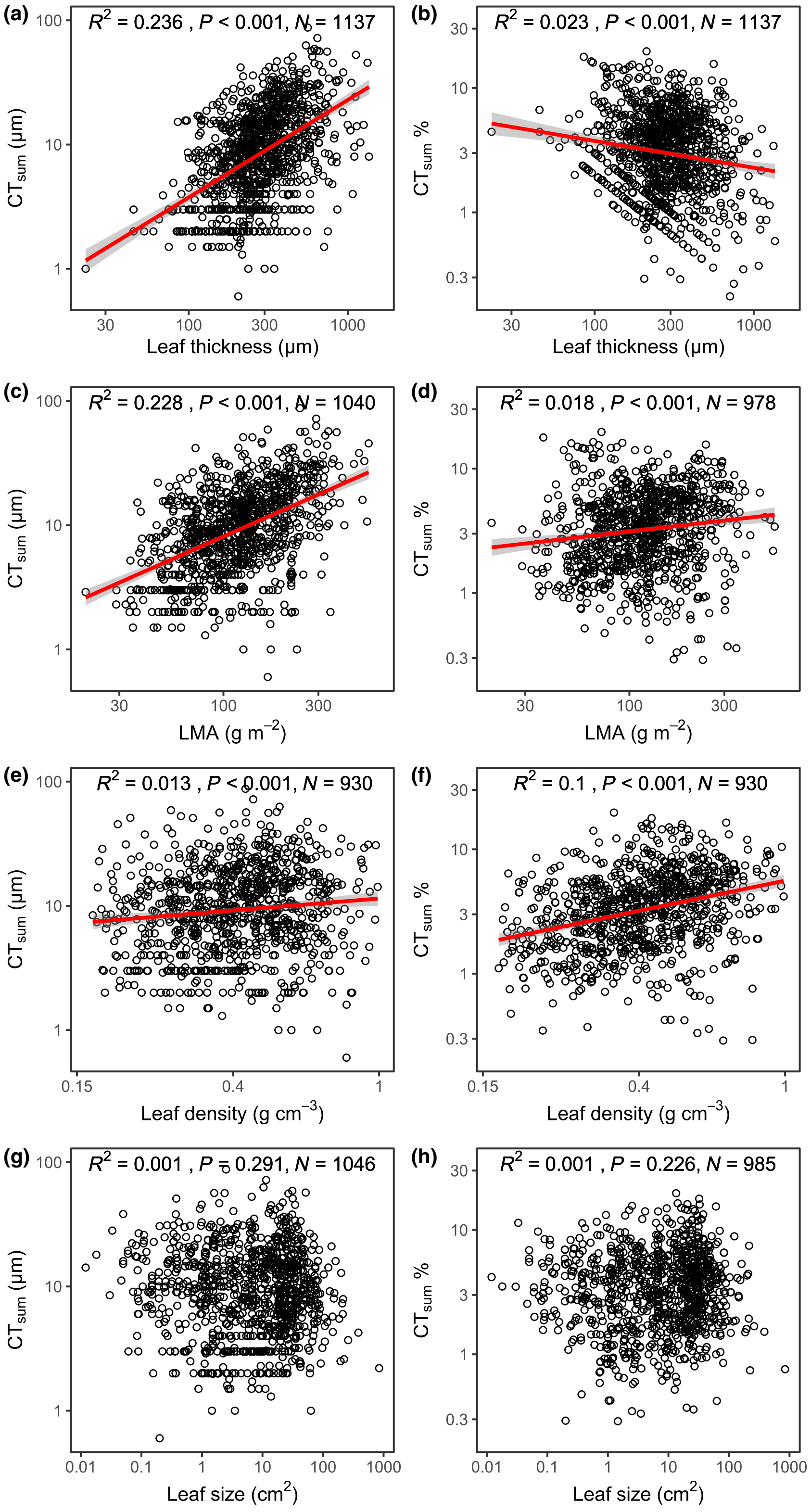

Fig. 4 Relationships between leaf cuticle properties (thickness and proportional contribution to leaf thickness, $\mathrm{CT_{sum}}$%) and other leaf traits. (a, b) leaf thickness, (c, d) leaf mass per area, LMA, (e, f) leaf density, (g, h) leaf size. Fitted lines (95% CIs shown in grey) illustrate statistically significant ordinary least squares regression relationships.

c. 12, thereafter flat; Fig. 5c). These trends were almost mirror images of each other.

Relationships between $\mathrm{CT_{sum}}$ and the six key environmental variables were generally stronger (higher R^2) in nonwoody species than in the two other PFTs (Table S4), most especially for $\mathrm{CT_{sum}}$ vs $\mathrm{Frost_{gs}}$ (R^2 = 0.336), $\mathrm{Wind_{gs}}$ (R^2 = 0.244), all temperature metrics (R^2 = 0.283–0.313) and $\mathrm{PPFD_{gs}}$ (R^2 = 0.045, $P < 0.001$). Notably, for nonwoody species, $\mathrm{PPFD_{gs}}$ and $\mathrm{UVB_{gs}}$ explained *c.* 20–25% variation in $\mathrm{CT_{upper}}$, which being far more variation explained than for either $\mathrm{CT_{sum}}$ or $\mathrm{CT_{lower}}$ (Table S4),

Table 2 Multiple regressions (Type III sums of squares) of CT_{sum} on environmental variables.

(a)	SS	DF	*F*	*P*	Estimate	SE	VIF
(Intercept)	16.395	1	151.522	< 0.001	2.153	0.175	
$Wind_{gs}$	13.332	1	123.215	< 0.001	−0.092	0.008	1.585
$Frost_{gs}$	5.864	1	54.196	< 0.001	−0.207	0.028	3.999
$Tmin_{gs}$	3.021	1	27.924	< 0.001	−0.018	0.003	4.523
$Precip_{gs}$	0.657	1	6.071	0.014	−0.089	0.036	1.833
$PPFD_{gs}$	0.444	1	4.102	0.043	−0.005	0.002	1.137
CECc	0.226	1	2.086	0.149	−0.001	0.001	1.888
Residuals	129.841	1200					
$R^2 = 0.174$							

(b)	SS	DF	*F*	*P*	Estimate	SE
(Intercept)	20.876	1	204.402	< 0.001	0.767	0.054
$Wind_{gs}$	3.177	1	31.104	< 0.001	0.097	0.017
$Frost_{gs}$	5.538	1	54.222	< 0.001	0.280	0.038
$Wind_{gs} \times Frost_{gs}$	11.402	1	111.642	< 0.001	−0.114	0.011
Residuals	122.867	1203				
$R^2 = 0.218$						

(a) Analysis of CT_{sum} in relation to wind speed, frost days and the four variables showing the strongest individual correlations from the temperature, irradiance, moisture and soil variable groups. (b) CT_{sum} as a function of frost days, wind speed and the frost × wind interaction. CECc, cation exchange capacity of the soil clay fraction, $Frost_{gs}$, number of growing-season frost days, $PPFD_{gs}$, growing-season annual photosynthetic photon flux density, $Precip_{gs}$, growing-season mean annual precipitation, $Tmin_{gs}$, growing-season minimum annual temperature, $Wind_{gs}$, growing-season mean annual wind speed. Note that $Precip_{gs}$ was $\log_{10}$-transformed for analyses; $Frost_{gs}$ was $\log_{10} + 1$-transformed.

suggesting that protection from UV-B may be an important role of the leaf cuticle in nonwoody species.

Multivariate relationships between CT_{sum} and environmental variables

In a multiple regression considering CT_{sum} as a function of all six environmental variables from Fig. 5 (Table 2a), $Wind_{gs}$ again had the strongest explanatory power, *c.* 2.5-fold stronger as judged by the *F*-value than the next most influential variable, $Frost_{gs}$ (i.e. $F = 123.2$ vs 54.2) which, itself, had ~ twofold more explanatory power than the third most influential variable, $Tmin_{gs}$ ($F = 27.9$). The *F*-values of other variables were considerably lower. Illustrated as partial residual plots (Fig. S4), the previous nonlinearity in bivariate $Frost_{gs}$ and $Tmin_{gs}$ relationships with CT_{sum} (Fig. 5) was no longer apparent. Furthermore, the weakly positive CT_{sum}–$Tmin_{gs}$ relationship (Fig. 5b) was now weakly negative (Fig. S4B). Relationships among the environmental variables (Table S5) help understand this effect, variation in both $Frost_{gs}$ and $Wind_{gs}$ being significantly correlated with that in $Tmin_{gs}$ (*r c.* −0.8 and −0.5, respectively).

Considering just $Frost_{gs}$ and $Wind_{gs}$ (positively correlated, $r = 0.35$, $P < 0.001$; Table S5), an additive regression model explained 14.5% variation in CT_{sum}, with both variables showing highly significant and negative partial effects (Table S6). In the related model now including a $Frost_{gs} \times Wind_{gs}$ interaction term, the explanatory power increased to 21.8%, with the interaction term strongly influential (high *F*-value) and significantly negative (Table 2b). Plotting that regression equation (Fig. 6) helps understand its implications: the negative effect of wind speed on CT_{sum} was stronger at sites with more frequent frost, and the negative effect of frost was strongest at windier sites. Species with the thinnest cuticles were mostly observed at sites with both strong winds and frequent frosts. These sites were mostly high-latitude, high-elevation sites in Patagonia, South America (Pyykkö, 1966). By contrast, at the lowest-wind sites frost had little effect on CT_{sum} and, at the lowest-frost sites (many in tropical biomes; Fig. S5), wind had little effect on CT_{sum}. In equivalent regression models replacing $Frost_{gs}$ by $Temp_{gs}$, $Tmin_{gs}$ or $Tmax_{gs}$, the explanatory power was notably lower in every case (15.2, 16.9 and 13.4%, respectively; Table S7); that is, there was no support for the alternative explanation for the frost effect (and its interaction with wind), which is that it simply reflected temperature effects more broadly.

Taken together, multiple lines of evidence suggested that high $Frost_{gs}$ and $Wind_{gs}$ were the strongest environmental correlates of low CT_{sum}. This conclusion was not altered by simultaneously considering variation in other leaf traits (thickness, LMA or density) for bivariate relationships between CT_{sum} and each of the six key environmental variables (Table S8).

Discussion

The leaf cuticle plays multiple roles, for example protecting leaves from desiccation (Schreiber & Riederer, 1996), UV damage (Jordan *et al.*, 2005) and herbivory (Peeters, 2002), while also contributing to biomechanical integrity (Onoda *et al.*, 2012). Thus, it is perhaps unsurprising that in a dataset with a near-global span, CT_{sum} was patterned in relation to a number of environmental variables, other leaf traits and also latitude and/or plant taxonomic grouping. A challenge is to make sense of these patterns, noting the limitations of using inherently correlative data to

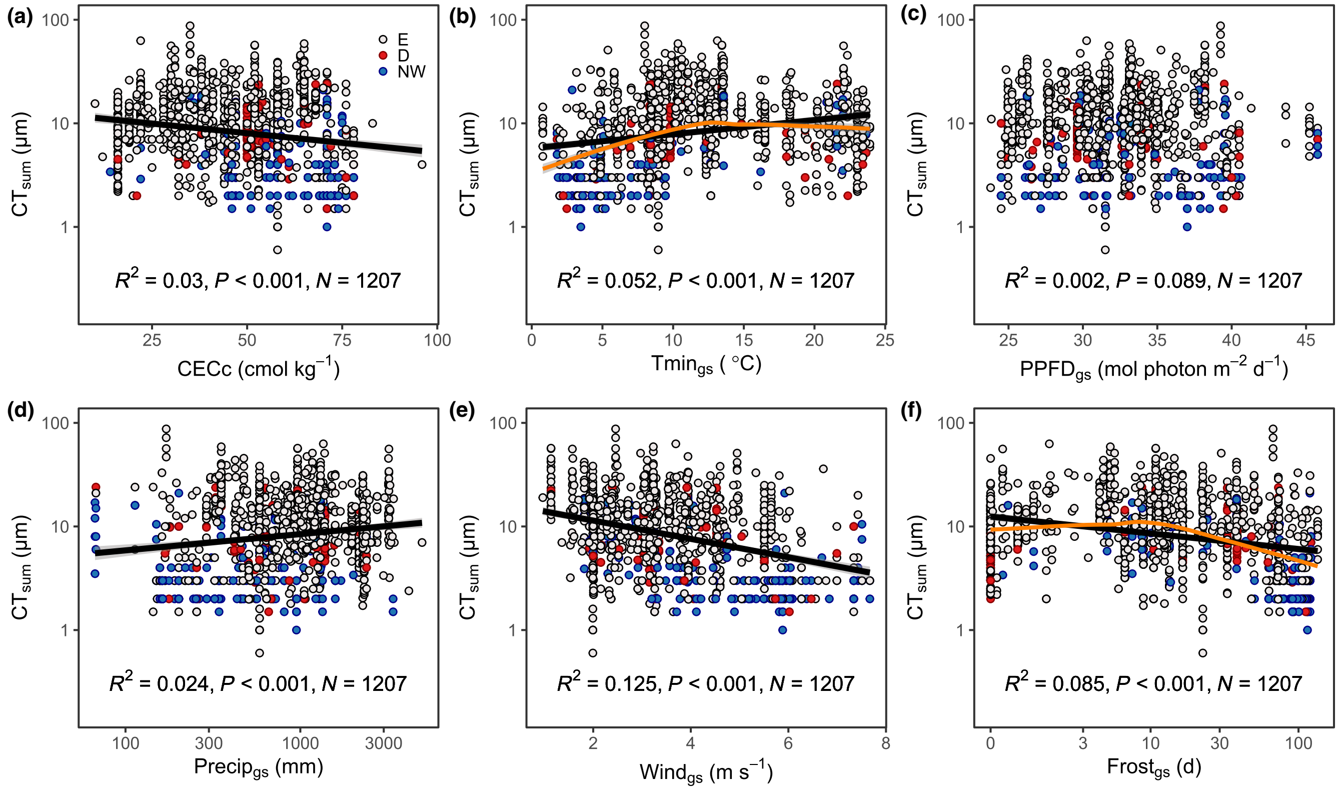

Fig. 5 Relationships of leaf cuticle thickness (CT$_{sum}$) to six key environmental variables. (a) Cation exchange capacity of the soil clay fraction (CECc), (b) growing-season minimum temperature (Tmin$_{gs}$), (c) growing-season photosynthetic photon flux density (PPFD$_{gs}$), (d) growing-season precipitation (Precip$_{gs}$), (e) growing-season wind speed (Wind$_{gs}$), (f) growing-season frost days (Frost$_{gs}$). The four variables, CECc, Tmin$_{gs}$, PPFD$_{gs}$ and Precip$_{gs}$, showed the strongest bivariate correlations from those considered in the soil, temperature, light and rainfall groups, respectively. All species fit: black line (linear) and orange line (LOESS). Evergreen woody species: grey points; deciduous woody species: red points; nonwoody species: blue points.

assess the relative likelihood of competing and/or complementary causal explanations, and remembering the variation in cuticle definition adopted by authors responsible for source datasets (see the Materials and Methods section).

In this study, we tested 11 hypotheses concerning the patterning of CT$_{sum}$ with other leaf traits and with site climate and/or soil properties (Table 1). Hypotheses 2–5 concerned CT$_{sum}$ in relation to other leaf traits. As expected, species with thicker and/or higher LMA leaves tended to have higher CT$_{sum}$. Species with proportionally thicker cuticles (higher CT$_{sum}$%) had higher leaf density, also as predicted, which in part may be due to cuticle material being denser than other leaf construction materials (Onoda *et al.*, 2012). We were less certain in hypothesising that leaf size and CT$_{sum}$ would be positively correlated and, indeed, the results were mixed. Across all species, the traits were unrelated. However, among nonwoody species, larger leaved species indeed tended to have thicker cuticles, consistent with the idea that the cuticle plays a role in the structural integrity of herbaceous leaves. This resonates with the concept of a spectrum of leaf types running from 'kites' to 'chocolate boxes' (D. Hanke, quoted by (Grubb, 1986)), *kites* being species with structural integrity predominantly achieved via heavily sclerified vasculature, *chocolate boxes* achieving strength predominantly via thickening of outer tissue layers. While Grubb emphasised the role of the epidermis in the 'chocolate box' type, arguably the concept should be extended to cuticles, the cuticle and epidermis together forming the outer protective layer of leaves. Another potential interpretation for leaf size and CT$_{sum}$ being correlated in nonwoody species is that the two traits respond to similar environmental drivers in this PFT more so than in other PFTs, and thus their leaf size–CT$_{sum}$ correlation arises via secondary correlation with climate. Indeed, nonwoody species showed the most marked latitudinal trend in CT$_{sum}$, being thicker in equatorial regions and thinner towards the poles (Fig. 3), echoing the pattern reported previously for leaf size – larger leaves towards the equator (Wright *et al.*, 2017) – also observed here (Fig. S3F).

Thinner cuticles at windier and more frost-prone sites

Overall, the two strongest environmental correlates of leaf cuticle thickness were wind speed (Wind$_{gs}$) and number of frost days per growing season (Frost$_{gs}$). On average, species at windier or more frost-prone sites had thinner cuticles. Multiple lines of evidence indicated this, and several complementary analyses indicated that these trends were not simply secondary correlations flowing via relationships between CT$_{sum}$ and LMA or other leaf traits (Table S8), or between CT$_{sum}$ and other environmental variables (Table S7). What might be the cause or causes of such patterns?

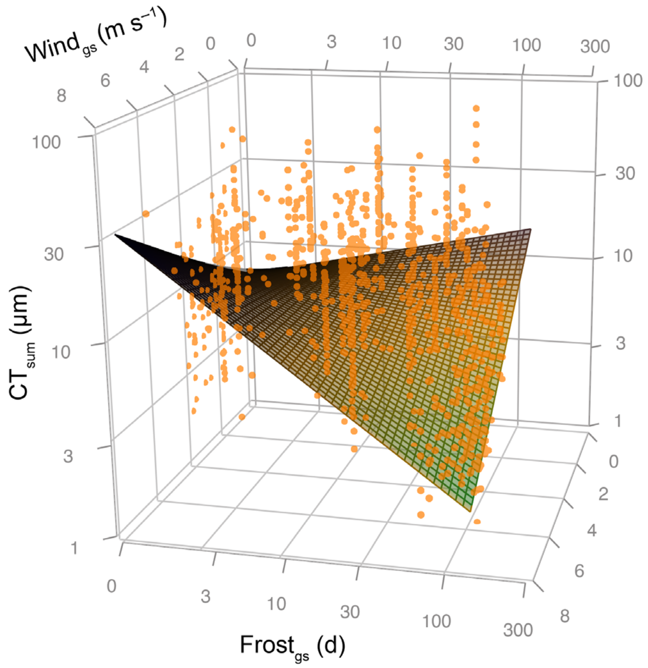

Fig. 6 Modelled mean trend in CT$_{sum}$ as a function of Wind$_{gs}$ (growing-season wind speed, ms^{-1}), frost (d per growing season) and their interaction, plotted across the range of observed data. Frost frequency ranged from 0 to 134 d per growing season. Equation plotted (Table 2b): $\log_{10}(CT_{sum}) = 0.767 + 0.280 \times \log_{10}(Frost_{gs} + 1) + 0.097 \times Wind_{gs} - 0.114 \times Wind_{gs} \times \log_{10}(Frost_{gs} + 1)$.

Under Hypothesis 11, we incorrectly predicted higher CT$_{sum}$ at more frost-prone sites on the basis that structural reinforcement of leaves, and especially the cuticle, might help protect against frost damage and abrasive, icy rains. This prediction and interpretation were not supported; instead, we found generally *lower* CT$_{sum}$ at more frost-prone sites. Similarly, we incorrectly predicted higher CT$_{sum}$ at windier sites (Hypothesis 10) on the basis that greater physical reinforcement limits damage from frequent flexing of the leaf lamina, thrashing of leaves against other leaves and the abrasive nature of wind-borne soil and ice particles (van Gardingen *et al.*, 1991). Instead, we found generally *lower* CT$_{sum}$ at higher wind sites, and the thinnest cuticles at sites that are both windy and heavily frost-prone.

These observations are consistent with cuticles being more abraded at higher wind speeds, as has been reported for conifers growing at high-elevation and high-latitude timberlines (Hadley & Smith, 1989; Grace, 1990; van Gardingen *et al.*, 1991), and with the combination of high winds and frequent frost days being an especially abrasive combination. One possible mechanism favouring thinner cuticles in abrasive environments is that the repair of damage to the waterproof outer wax layer (Koch *et al.*, 2004) could be slower in leaves with thicker cuticles due to the longer pathway for transport of polar compounds in such leaves. Rates of repair vary markedly among species (Koch *et al.*, 2009), but as yet little is known about the impact of cuticle thickness on repair. It is an open question whether cuticle abrasion is likely to be a general explanation for thinner cuticles at windy and frost-prone sites. Another possibility is that leaves facing predictably frequent frosts are in some sense disposable (~ inevitably lost), meaning that less investment in physical protection – that is, thinner cuticles – would be favoured or, equivalently, heavy investment in thick cuticle would be disadvantaged. Similarly, perhaps leaves facing predictably strong winds are also somewhat disposable – indeed, loss of some leaves might protect the remaining canopy from wind load. If so then, again, less investment in physical protection would be favoured. Perhaps, this type of reasoning helps understand why CT$_{sum}$ is typically lower in deciduous species than in evergreens, and lower again in nonwoody species; that is, high investment in cuticle is disadvantaged in leaves likely to have shorter lifespans, with less opportunity (a shorter time frame) to pay back investments in physical defences.

In relation to the negative CT$_{sum}$–wind relationship, another possible explanation is that it may be advantageous for leaves at windier sites to be less stiff; the ability to flex and even curl into a cone under high winds reduces drag and therefore reduces leaf loss (Vogel, 2009). Arguably, having a thinner cuticle aids this behaviour and, conversely, very thick cuticles would be damaged by frequent flexing or coning; thus, their ability to minimise cuticular water loss would be affected.

Herbivory is an additional or alternative consideration that may be important for understanding the observed relationships between CT$_{sum}$ and each of Wind$_{gs}$, Frost$_{gs}$, site temperature and latitude. As already noted, leaf cuticles are important in defence against insect herbivory (Müller, 2006; Gorb & Gorb, 2017). It is commonly held that in warm, frost-free tropical regions (Fig. S5), biotic interactions such as herbivory are more intense – meaning that plants suffer greater levels of herbivory or need to be better defended against herbivory, or perhaps both contentions are true (Coley & Barone, 1996). Actually, it is unclear whether or not there is *generally* higher herbivory at lower latitudes (Moles *et al.*, 2011a), broadscale analyses showing support in evergreen but not deciduous species (Lim *et al.*, 2015) and support in the northern but not the southern hemisphere (Zhang *et al.*, 2016). Global data compilations have also cast doubt on whether lower latitude species tend to be better defended than those at higher latitudes (Onoda *et al.*, 2011; Moles *et al.*, 2011b). Still, if one accepts that, at least among some plant groups, herbivore pressure is greater at lower latitudes, then one might expect to see a general tendency for thicker leaf cuticles in such regions, as observed here. Extending this idea, if herbivore pressure is generally less in sites that are windier or frost-prone, one might expect generally thinner leaf cuticles in such places, as observed here.

Thicker cuticles at warmer sites: role of biomechanics?

Across all species, CT$_{sum}$ was positively correlated with all of mean, minimum and maximum growing-season temperatures, albeit rather weakly, and this was confounded with the wind and frost effects. However, within nonwoody species, the relationships were very clear (R^2 ranging from 0.28 to 0.31; Table S4). If there were a causal reason for generally thicker leaf cuticles at warmer sites, what might that cause be?

Cuticles become more permeable to water at higher temperatures, in general showing a phase change between 35°C and 40°C after which permeability increases sharply (Burghardt & Riederer, 2006; Schuster, 2016; Duursma *et al*., 2019). Could higher CT_{sum} help counteract this increased tendency for water loss? Possibly, although cuticle thickness is generally thought to play only a minor role in cuticle permeability, whereas chemistry and the presence/absence of microcracks are thought generally to be more decisive (van Gardingen *et al*., 1991; Riederer & Schreiber, 2001; Burghardt & Riederer, 2006). However, biomechanical properties of cuticles also change with temperature: At higher temperatures, the polymer chains become more mobile and the cuticle becomes viscoelastic (having both fluid and solid properties), decreasing in both viscosity and stiffness (Heredia-Guerrero *et al*., 2018). Conceivably, being thicker helps to maintain the structural integrity of cuticles at higher temperatures in the face of these changes, an effect that would only confer benefit in warm sites.

Considering most trait–environment relationships (and also latitudinal trends) for individual PFTs, explanatory power clearly increased in the order evergreen woody < deciduous woody < nonwoody. One possible interpretation of this pattern is that the ecological 'tuning' of cuticle thickness variation is in some sense more important in species with generally more fragile leaves (i.e. herbaceous and deciduous species) and, conversely, somehow less decisive in evergreen species for which it is economically feasible to have additional modes of costly protection, such as heavily sclerified vasculature (*cf.* discussion of kite vs chocolate box leaf structure is described previously).

Conclusions and potential future directions

As summarised in Table 1, in this study, we demonstrated broadscale patterning of leaf cuticle thickness with other leaf traits (LMA, leaf thickness), biome, taxonomic group, plant functional type, latitude and numerous environmental properties – most especially wind speed and frost frequency. For nonwoody species, site temperature was also identified as a key correlate of CT_{sum}. For other soil and climate properties, even when observed trends were consistent with the hypothesised directionality (e.g. thicker cuticles on lower fertility soils; thicker upper cuticles at high irradiance sites; Table 1), the explanatory power tended to be low (Table S4). This is not to say that these factors are never important but, rather, that they have low explanatory power at a global scale.

Considering all species, the two strongest environmental variables, $Frost_{gs}$ and $Wind_{gs}$, explained 8.5–12.5% variation in CT_{sum} individually, or 21.8% when in combination and including their interaction (Fig. 6). In part, this rather low general explanatory power of environmental variables reflects the fact that much of the observed total range in leaf cuticle thickness can be seen at any given latitude or at any given site temperature, precipitation, wind speed, soil fertility or irradiance. Cuticle thickness is not predominantly shaped by identifiable single factors, and perhaps, this is because it is so important for so many different aspects of leaf biology (see the Introduction section). Furthermore, CT_{sum} is convincingly correlated with (and indeed contributes to) traits such as LMA and leaf thickness, which themselves are strongly shaped by site climate and soil nutrients (Givnish, 1987; Niinemets, 2001; Poorter *et al*., 2009), complicating matters. That said, there is clear scope for taking advantage of these trait–trait relationships. For example, the positive correlation between LMA and cuticle thickness has been used for estimating the LMA of fossil species and, from that information, shifts in ecosystem properties across the end-Triassic mass extinction event (Soh *et al*., 2017). The link between CT_{sum}, LMA and leaf mechanical properties may have potential for estimating leaf lifespan (McElwain *et al*., 2024), another trait with high utility for paleo-ecological reconstruction. In principle, the CT_{sum}–climate relationships reported here may usefully contribute to such efforts.

A study such as this can only identify broadscale patterns. It can only hint at mechanisms, and caution must be used in interpreting the results. Presumably, there was considerable 'noise' in the dataset resulting from different methods in source studies related to species selection, choice of material for study (e.g. leaf age, canopy position), sample preparation and choice of stains, optical resolution of microscopes, the precise definition of 'cuticle' (Fernández *et al*., 2016) and so forth. However, we have no reason to believe that these differences would have led to systematic biases when it comes to identifying global patterns in leaf cuticle thickness and its environmental associations. Despite this uncertainty, broadscale data compilation studies such as this one play an important role in plant science, providing a valuable context for more detailed investigations. We suggest that future studies could usefully concentrate on questions such as:

- How much does cuticle abrasion contribute to the observed trends in CT_{sum} with frost and wind?
- What role do economic considerations play? That is, can broadscale trends be understood in relation to the carbon costs and benefits of cuticles, and the risks of leaf loss in different environments? Should CT_{sum} be considered part of the leaf economic spectrum?
- To what extent does protection against herbivory (and the costs thereof) help understand the latitudinal and other trends observed here?
- To what extent do the observed broadscale patterns reflect contributions from within-species trait variation along environmental gradients vs changes in species composition?
- Why are CT_{sum}–environment properties so much stronger in nonwoody species than in other PFTs?
- To what extent can effects on CT_{sum} of short-term experimental manipulations (e.g. of wind speed, temperature or soil moisture) help understand broadscale patterns in relation to site climate and soil properties?

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Competing interests

None declared.

Author contributions

The leaf anatomy database was compiled by TIL and IJW, incorporating an earlier leaf anatomy data compilation from ÜN and also gap-filled leaf trait data from the TRY database, v5. ND helped to extract environmental data. IJW and XL analysed the dataset and drafted the manuscript. XL produced all the figures. All authors (XL, GEB, ND, GJJ, JK, TIL, ÜN, YO, HP, LS, RV, MW, IJW) contributed substantially to the interpretation of the results and to successive manuscript versions.

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Data availability

The leaf trait database is published as part of this article, in Tables S1 and S2.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Cross sections of photosynthetic organs of a set of co-occurring species from eastern Australia (Burrows, 2001).

Fig. S2 Relationship between upper and lower leaf cuticle thickness.

Fig. S3 Latitudinal patterns in leaf cuticle thickness and other leaf traits.

Fig. S4 Partial residual relationships between CT_{sum} and each of the six key environmental variables.

Fig. S5 Patterning of growing-season frost days, wind speed, minimum temperature and growing-season length by biome.

Table S1 Leaf cuticle thickness dataset with citation information for source papers.

Table S2 Dataset of site locations and environmental variables.

Table S3 Relationships between CT_{sum}, $CT_{sum}\%$ and other leaf traits, comparing all-species trends to within-plant functional type trends.

Table S4 Correlations of leaf cuticle (summed, upper and lower) thickness with the full set of 17 soil and climate variables.

Table S5 Correlation matrix of 17 soil and climate variables used to describe the environment at the 293 study sites.

Table S6 Multiple regression of CT_{sum} as a function of growing-season frost days and growing-season wind speed.

Table S7 Multiple regressions reporting the explanatory power of growing-season wind speed and temperature for CT_{sum}.

Table S8 Comparison of CT_{sum}–environment relationships before and after controlling for variation in other leaf traits.