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## RESEARCH ARTICLE

# Photosynthesis, water-use and nitrogen relate to both plant height and leaf structure in 60 species from the Mediterranean

Eric Garnier<sup>1</sup>  | Denis Vile<sup>1,2</sup>  | Sandrine Debain<sup>1,3</sup> | Lorraine Bottin<sup>1,4</sup>  |  
G rard Laurent<sup>1,5</sup> | Catherine Roumet<sup>1</sup> 

<sup>1</sup>CEFE, Univ Montpellier, CNRS, EPHE, IRD, Montpellier, France

<sup>2</sup>LEPSE, Univ Montpellier, INRAE, Institut Agro, Montpellier, France

<sup>3</sup>Songes de Sahuc, Mas Cougnot, Chemin du Recodier, Saint Roman de Codi res, France

<sup>4</sup>Universit  C te d'Azur, CNRS, ECOSEAS, Nice, France

<sup>5</sup>2 Place de l' toile, Pouzolles, France

## Correspondence

Eric Garnier

Email: [eric.garnier@cefe.cnrs.fr](mailto:eric.garnier@cefe.cnrs.fr)

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## Abstract

1. Within individuals and/or species of trees, the structure and gas exchange of sun-exposed leaves from the outer part of the canopy have been found to relate to sampling height. Across species, the gas exchange of such leaves has been shown to relate to their structure and biochemical composition, but not to canopy height. Why are leaf traits related to height within tree species but not across a broader range of species? And what are the components of leaf structure involved in leaf-level carbon, water and nitrogen economies?
2. Plant height, leaf mass *per* area (LMA) and its underlying components, gas exchange, leaf nitrogen and carbon isotopic discrimination were assessed for 60 species from different life and growth forms growing in the Mediterranean, spanning a wide range of height and LMA values.
3. Contrary to previous comparisons across species, our study shows that leaves of tall plants had a high LMA, a high leaf dry matter content (LDMC) and were slightly thicker; their stomatal conductance and photosynthetic rate were low, while their intrinsic water-use efficiency ( $\gamma$ WUE) was high. LMA was related to all gas exchange variables as well as to leaf nitrogen. These effects were mostly mediated through LDMC, with limited effects of leaf thickness. These conclusions were hardly modified when phylogeny was account for.
4. Across species of varying life and growth forms, leaf functioning relates to both plant height and leaf structure. These results provide a generalization of previous conclusions found in trees at the intra-individual and/or intraspecific levels. Inconsistencies among previous studies with respect to plant height are likely an issue of context-dependency, which should be explicitly taken into account for a better understanding of plant form and function.

## KEYWORDS

leaf carbon isotopic discrimination, leaf structure and function, leaf traits, photosynthesis, photosynthetic nitrogen-use efficiency, stomatal conductance, vegetative plant height, water-use efficiency

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## 1 | INTRODUCTION

In land plants, carbon dioxide uptake and water loss are closely linked via the stomatal pores on the leaf surface. On its way to the sites of carboxylation inside the chloroplasts, CO<sub>2</sub> moves from the atmosphere surrounding the leaf to the sub-stomatal internal cavities through stomata and from there to the sites of carboxylation inside the chloroplast stroma through the leaf mesophyll (cf. Parkhurst, 1994). Water vapour follows part of this path in the reverse direction, from the sites of water evaporation within leaves (which are currently not well known: Buckley et al., 2017) to the atmosphere through stomata. The controls on these key processes of plant functioning and the fine tuning of their coupling are a topic of intense debate (McDowell et al., 2011; Onoda et al., 2017; Reich, 2014; Sack et al., 2013; Xiong & Nadal, 2020 for reviews), which have been addressed from two largely independent perspectives. One accounts for the size of the plant, the other focuses on the underlying characteristics of the leaf.

In the first perspective, which aims at understanding the limits to tree height and tree growth, foresters have put an emphasis on constraints on water transport as trees grow taller (McDowell et al., 2011; Ryan & Yoder, 1997 for reviews). Hydraulic constraints increase with height because the length of the hydraulic path to lift water to the sites of evaporation in the leaves increases, as does the vertical water potential gradient (Fernández-de-Uña

et al., 2023; Koch et al., 2004; McDowell et al., 2011; Ryan et al., 2006). Within species or even individuals, these inter-related factors lead to a decrease in stomatal conductance and photosynthesis with the sampling height of sun-exposed leaves from the outer part of the canopy, and an increase in water-use efficiency (Fernández-de-Uña et al., 2023; McDowell et al., 2011), as assessed using leaf carbon isotopic discrimination ( $\Delta^{13}\text{C}$  hereafter: Farquhar et al., 1982; see Table 1 for abbreviations) in such leaves. The structure of these leaves also varies with sampling height, probably as a response to increased water stress higher up in the canopy: this is the case for leaf mass per area (LMA, the ratio between leaf mass and area) which increases with plant height (Fernández-de-Uña et al., 2023; Poorter et al., 2009 for reviews), while leaf porosity decreases (Oldham et al., 2010) resulting in denser leaves. Interestingly, leaf nitrogen, a key element involved in CO<sub>2</sub> gas exchange (cf. Evans, 1989; Field & Mooney, 1986), does not seem to vary systematically with height (McDowell et al., 2011).

Whether hydraulic constraints lead to comparable relationships between leaf traits and canopy height across species is far from clear. Although the relationship between  $\Delta^{13}\text{C}$  and height seems to hold for a wide range of tree species (McDowell et al., 2011), this was apparently not the case for stomatal conductance (Fernández-de-Uña et al., 2023), and unconvincing results

TABLE 1 Plant traits measured together with their abbreviations and units.

Trait	Abbreviation	Unit	Resource for definition
Vegetative plant height	VPH	cm	TOP69
Gas exchange			
Photosynthetic rate per unit leaf area	$A_{\text{area}}$	$\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$	TOP357
Photosynthetic rate per unit leaf mass	$A_{\text{mass}}$	$\text{nmol CO}_2 \text{ g}^{-1} \text{ s}^{-1}$	TOP359
Stomatal conductance to water vapour	$g_{\text{sw}}$	$\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$	TOP368
Intrinsic water-use efficiency	$i\text{WUE} (=A_{\text{area}}/g_{\text{sw}})$	$\mu\text{mol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$	<a href="#">ess-dive-community/essdive-leaf-gas-exchange</a>
Photosynthetic nitrogen-use efficiency	$\text{PNUE} (=A_{\text{area}}/\text{LNC}_{\text{area}})$	$\mu\text{mol CO}_2 \text{ mg}^{-1} \text{ N}$	The net CO <sub>2</sub> exchange per leaf area (TOP357) divided by the leaf nitrogen content per unit area (TOP481)
Morpho-anatomy			
Leaf mass per area	LMA	$\text{g m}^{-2}$	TOP53
Leaf dry matter content	LDMC	$\text{mg g}^{-1}$	TOP45
Leaf thickness	LT	$\mu\text{m}$	TOP21
Chemical composition			
Leaf nitrogen content per unit area	$\text{LNC}_{\text{area}}$	$\text{g m}^{-2}$	TOP481
Leaf nitrogen content per unit mass	$\text{LNC}_{\text{mass}}$	$\text{mg g}^{-1}$	TOP462
Leaf carbon isotope discrimination	$\Delta^{13}\text{C}$	‰	The relative deviation of the $^{13}\text{C}/^{12}\text{C}$ ratio of leaf biomass from the $^{13}\text{C}/^{12}\text{C}$ ratio of atmospheric CO <sub>2</sub>

Note: Definitions can be found in two terminological resources: the Thesaurus of Plant characteristics (TOP: Garnier et al., 2017: <https://www.top-thesaurus.org/>) and in the reporting format for leaf-level gas exchange data and metadata (Ely et al., 2021: <https://github.com/ess-dive-community/essdive-leaf-gas-exchange>). When not available in terminological resources, a full definition is given.

have been found for other traits (see [Tables S1](#) and [S2](#) for a literature overview). A global analysis conducted on more than 2200 species showed that plant height and LMA were orthogonal in the first two dimensions of a phenotypic space based on six traits (Díaz et al., 2016). The global analysis conducted by Price et al. (2014) led to similar results, with very weak or no relationship observed between plant height and either LMA, photosynthetic rate or leaf nitrogen (no analysis for stomatal conductance). However, in this latter study, when the analyses were conducted on data organized by biome instead of globally, some of the relationships became significant. This led the authors to hypothesize that the likelihood of observing size effects on leaf traits was greater at a local (community) than at a global scale. A further issue is that in these global compilations, trait data are averaged at the level of species, with values for the different traits not necessarily (not often, actually) measured on the same individuals and/or even in the same sites. This might further blur potential relationships which could be detected using trait data measured on individuals from the same populations.

In the second perspective, the focus is on the underlying structural and biochemical determinants of CO<sub>2</sub> uptake and transpiration at the level of the leaf itself (see e.g. Evans, 2021; Flexas et al., 2013; Onoda et al., 2017). At a macroscopic scale, LMA, which represents the leaf-level cost of light interception, is a key trait which relates to many aspects of leaf functioning. It varies several hundred-fold among the world's species (Díaz et al., 2016; Poorter et al., 2009), and is central to the so-called “leaf economics spectrum” (LES hereafter: Wright et al., 2004) which captures the pace of CO<sub>2</sub> return on investments of nutrients and dry mass in leaves. Across species, it correlates with several traits involved in leaf carbon, nitrogen and water economy ([Table S1](#)).

However, the effects of LMA on these processes result from the combined effects of its underlying components, leaf thickness and tissue density (cf. Garnier & Laurent, 1994; Poorter et al., 2009; Witkowski & Lamont, 1991), which are currently not well understood (cf. [Table S1](#)). Variations in LMA are indeed driven by the concomitant, independent variations of these two traits, which reflect different structural and chemical features (John et al., 2017; Poorter et al., 2009) whose effects on leaf functioning are independent, and sometimes antagonistic (Niinemets, 1999; Onoda et al., 2017). For example, in a global synthesis on trees, leaf density has been found to relate negatively to  $A_{\text{mass}}$ , while no relationship was found with leaf thickness, while the reverse was true when photosynthesis was expressed per unit leaf area (Niinemets, 1999). Strikingly, a recent synthesis involving a broader range of species (ferns, herbaceous and woody species) reached different conclusions for some of these trait combinations (Huang et al., 2022). Disentangling the relative impacts of leaf thickness and density on traits involved in the carbon, water and nitrogen economies would therefore further our understanding of the net effects of LMA on leaf functioning, which remains limited and/or inconsistent on many aspects (see Nadal et al., 2023; Wang et al., 2022 for recent attempts).

In the present study, we assessed how plant height, LMA and components thereof affect photosynthesis, leaf nitrogen and water use, using an original data set in which all traits were measured on individuals from the same populations of 60 species growing in the Mediterranean. First, we hypothesized that hydraulic constraints, which increase with plant height and have been identified at the intraspecific level essentially in trees, also operate across species from different life forms found in the same habitat type. This challenges several prominent conclusions derived from broad-scale studies, but would be in line with the hypothesis put forward by Price et al. (2014), stating that leaf traits are more likely to relate to plant height at a local than a global scale. In particular, our expectations are that stomatal conductance and photosynthesis should decrease with plant height, while LMA, leaf density and water-use efficiency should increase. Second, we sort out the relative impacts of LMA and its underlying components, leaf thickness and density, on leaf gas exchange and nitrogen content. Although a number of relationships involving LMA are well established across species and life forms, some of them are not, and the effects of leaf thickness and density need further explorations to clarify inconsistencies found among studies.

The species selected belong to different life forms (from herbaceous annuals to deciduous and evergreen trees), resulting in a 200-fold variation in plant height and an 8-fold variation in LMA. These substantial ranges spanned by both traits allowed us—for the first time to our knowledge—to test simultaneously how plant height and leaf structure affect carbon, nitrogen and water economies at the interspecific level, beyond distinctive groups of species.

## 2 | MATERIALS AND METHODS

### 2.1 | Study sites and plant species

The study was conducted at three sites located in the north-western part of the Mediterranean Basin: Cazarils and Les Agros in southern France and Garraf in north-eastern Spain ([Table 2](#)). In the three sites, the climate is Mediterranean with mild wet winters and warm dry summers. Mean annual temperature and rainfall are comparable in the two French sites, while temperature is about 3°C higher in Garraf and precipitation is almost half compared to that in the French sites. At the three sites, the bedrock is limestone and soils have comparable pH values ([Table 2](#)). Further details on site description and vegetation can be found in the references given in [Table 2](#). Formal permits were issued by the administrations of the Hérault Departmental Council and of the Garraf National Park to conduct fieldwork in Cazarils and Garraf, respectively. In Les Agros, a private property, the owner gave verbal permission to conduct the work.

Sixty species were selected for study, on the basis of their frequent occurrence in these different sites. Some species were measured in more than one site, making a total of 70 ‘populations’ (i.e. species × site combinations): 49 in Cazarils, 10 in Les Agros and 11 in Garraf ([Table S3](#)). There were 8 therophytes, 21 hemicryptophytes and 2 geophytes

TABLE 2 Main characteristics of the three study sites: Cazarils, Les Agros and Garraf.

Name	Cazarils	Les Agros	Garraf
Site location	Bas Languedoc, France	Provence, France	Catalonia, Spain
Latitude (decimal degrees)	43.764	43.625	41.304
Longitude (decimal degrees)	3.703	6.586	1.892
Altitude range (m a.s.l.)	240–310	405–465	250–350
Average soil pH (water)	7.1	8.0	7.2–8.2
Mean annual temperature (°C)	13.5	13.6	16.1
Mean annual precipitation (mm)	1075	1041	588
References for detailed description of site and vegetation	Le Floc'h et al. (1998) and Navas et al. (2010)	Garnier, Laurent, et al. (2001)	Lloret and Vilá (2003) and Garnier et al. (2019)

Note: Temperature and precipitation data over the 1981–2010 period were taken from the meteorological stations of Saint-Martin-de-Londres for Cazarils (3.5 km north-east of the field site), Bargemon for Les Agros (1.5 km south-west of the field site) and of the Barcelona airport for Garraf (20 km east of the field site).

(sensu Raunkiaer, 1934), making a total of 31 herbaceous species, and 6 chamaephytes, 11 deciduous phanerophytes, 12 evergreen phanerophytes (sensu Raunkiaer, 1934), making a total of 29 woody species.

## 2.2 | Trait selection and measurements

Twelve traits were assessed on individuals from the 70 populations (Table 1) during the spring peak of growth (between May and July) in 1998, 1999 and 2001 (Table S3).

*Vegetative plant height* (plant height hereafter) was recorded on 10–30 healthy individuals of each population, as the upper 20% of the foliage of each adult plant, taking only vegetative structures into account. It was determined with a measuring tape for individuals up to 150 cm, and with a telescopic stick for higher individuals, climbing up the trees when necessary.

*Leaf traits* were assessed on intact, full-grown leaves taken from plants in full light situations (i.e. not under tree cover) or from the outer canopy for tall woody species. Gas exchange and leaf mass per area of the samples present in the chamber of the measurement device (see below) were determined on 365 leaf samples ('gas exchange samples' hereafter). As these samples were generally too small for chemical analyses and were not rehydrated to complete saturation—required to determine leaf dry matter content (LDMC, the ratio of dry mass to water-saturated fresh mass: cf. Garnier, Shipley, et al., 2001 and see below)—all other traits were assessed on a second set of leaves collected from individuals of the same populations ('screening samples' hereafter) growing under comparable conditions. Methods, which mostly follow Pérez-Harguindeguy et al. (2013), are briefly summarized below. Leaf traits can be grouped into three categories:

### 2.2.1 | Gas exchange

Traits were measured or derived from gas exchange measurements (Table 1), conducted using a LI-6400 portable photosynthesis system (LI-COR Biosciences Inc., Lincoln, Nebraska, USA). They were

determined on an average of 5 well-lit leaves *per* population (range: 3–7, generally taken from different individuals), sampled at the top of the plant for small to medium stature species, and as close as possible to the upper part of the canopy for tall woody species. After measurements, leaves were severed from the plant and the actual leaf sample that was present in the chamber was collected and placed in a cool box until further processing in the laboratory (see below). To avoid midday depression of photosynthesis, most measurements were made between 9:30 AM and 1:00 PM. Light intensity was held constant at  $1500 \mu\text{mol photons m}^{-2}\text{s}^{-1}$  to ensure light-saturated photosynthesis. Across all measurements ( $n=365$ ), the average values ( $\pm$  standard error) of  $\text{CO}_2$  partial pressure of incoming air, leaf temperature and vapour pressure deficit based on leaf temperature were  $358 \pm 11 \mu\text{mol mol}^{-1}$ ,  $27.1 \pm 2.6^\circ\text{C}$  and  $1.93 \pm 0.58 \text{ kPa}$ , respectively. Net photosynthetic ( $A_{\text{area}}$ ), transpiration rates *per* unit leaf area and stomatal conductance to water vapour ( $g_{\text{sw}}$  hereafter) were recorded after reaching steady-state.  $A_{\text{mass}}$  was calculated as the ratio between  $A_{\text{area}}$  and the LMA of the gas exchange samples (see below). To account for differences in air vapour pressure deficit among measurements, we calculated intrinsic water-use efficiency ( $\Psi_{\text{WUE}}$ ) as  $A_{\text{area}}/g_{\text{sw}}$  (Osmond et al., 1980).

### 2.2.2 | Morpho-anatomy

LMA was first determined on the gas exchange samples. Immediately after return to the laboratory, the projected area (one side of the leaves) of these samples was determined with an area meter (Delta-T Devices, Cambridge, UK, model MK2). Samples were then oven-dried at  $60^\circ\text{C}$  for at least 2 days, and their dry mass measured. LMA was then calculated as leaf dry mass/leaf area.

LMA and underlying components were then assessed on 10 'screening samples' *per* population, mostly taken from different individuals. Immediately after being severed from the plant, leaves were put into a test tube with the proximal end sunken in deionized water and put into a cool box; traits were measured after complete leaf rehydration ( $>6 \text{ h}$  in test tubes under cool conditions: Garnier, Shipley,

et al., 2001). LMA determination followed the same procedure as that used for gas exchange samples. The relationship between the two sets of LMA values, obtained, respectively, for partially and fully rehydrated leaves, was very tight with a slope and an intercept not significantly different from 1 and 0, respectively ( $LMA_{\text{screening}} = 1.05 [\pm 0.05] * LMA_{\text{gas exchange}} - 1.93 [\pm 4.75]$ ,  $r^2 = 0.85$ ,  $p < 0.001$ ,  $n = 70$ ; not shown). We thus considered that the characteristics of two sets of leaves (gas exchange and screening) were similar and that the trait values obtained on the two batches could be combined safely.

Leaf thickness (LT hereafter) was measured using linear variable displacement transducers (IFELEC T.N.C., Paris, France, GDL case, models L2 and L5), calibrated with metal strips of known thickness. Depending on the size of the individual leaves, thickness was measured on 5–10 points per leaf (blade), avoiding the mid-vein, and an average value *per* leaf was calculated. LDMC was used as an estimate of leaf density (Castro-Díez et al., 2000; Garnier & Laurent, 1994; Nadal et al., 2023; Niinemets, 1999), based on the assumption that leaf water-saturated fresh mass is a good estimate of leaf volume (see Poorter et al., 2009; Vile et al., 2005 for discussions). It was assessed as the ratio between the dry mass of oven-dried leaves and the fresh mass of leaves following rehydration (Garnier, Shipley, et al., 2001).

### 2.2.3 | Chemical composition

Screening samples were pooled to obtain three to four bulked samples for nitrogen and stable carbon isotope analyses. These samples were ground individually, and their  $LNC_{\text{mass}}$  determined with an elemental analyser (Carlo Erba Instruments, Milano, Italy, model EA 1108). Area-based nitrogen content ( $LNC_{\text{area}}$ ) was calculated as ( $LNC_{\text{mass}} \times LMA$ ). Leaf carbon isotopic composition ( $L\delta^{13}C$  values relative to PDB standard) was determined on separate batches from the same samples, using a CHN elemental analyser coupled online to an isotope mass spectrometer (Europa Scientific Ltd., Crewe, UK, ANCA-MS system).  $L\delta^{13}C$  values are missing for *Genista scorpius* in Cazarils, *Bituminaria bituminosa* in Les Agros, and those for *Hyparrhenia hirta* were not included in the analyses, as it is the only  $C_4$  species in the data set ( $L\delta^{13}C = -12.66 \text{‰} \pm 0.18$ ,  $n = 4$ ).

### 2.3 | Replication statement

Scale of inference	Scale at which the factor of interest is applied	Number of replicates at the appropriate scale
Species	Species	60 species (70 populations) in 3 sites

### 2.4 | Data transformation and statistical analyses

For all traits, individual measurements were averaged for each species-site combination.

$L\delta^{13}C$  values were used to calculate discrimination against  $^{13}C$  during photosynthetic  $CO_2$  assimilation ( $L\Delta^{13}C$ ) as (cf. Farquhar et al., 1989):

$$L\Delta^{13}C = \frac{a\delta^{13}C - L\delta^{13}C}{1 + L\delta^{13}C},$$

where  $a\delta^{13}C$  is the carbon isotopic ratio of the air, fixed at  $-8\text{‰}$  (Farquhar et al., 1989).  $L\Delta^{13}C$ , which relate to  $L\delta^{13}C$  with a Pearson correlation coefficient of  $-1$ , is used as an estimate of time-integrated values of  $iWUE$  (Farquhar et al., 1982; Farquhar & Richards, 1984; discussed in Cernusak, 2020; Seibt et al., 2008). Plant height and leaf traits were approximately log-normally distributed (Shapiro–Wilk test,  $p > 0.05$ ) and were thus log transformed prior to analyses to fulfil normality requirements and minimize the heterogeneity of residuals.

Differences in trait values between life forms were tested using one-way analyses of variances (ANOVAs) followed by *post hoc* Tukey tests when significant differences were found (the two geophytes *Aristolochia rotunda* and *Convolvulus arvensis* were pooled with hemicryptophytes in all analyses). The degree of bivariate association between traits was assessed both at the 'population' (some species were measured in more than one site) and at the species level, with and without accounting for phylogenetic distances between species. These distances were obtained based on the botanical nomenclature of the World Plants (WP) database (<https://www.worldplants.de>) using the phylo.maker function from U.PhyloMaker package (Jin & Qian, 2023). Population-level trait relationships were assessed both with ordinary least-square regression and standardized major axis (SMA) regression (note that *Clematis vitalba*, *Hedera helix*, *Smilax aspera* and *Vitis vinifera* which are lianas, were excluded from the relationships between plant height and leaf traits). Average trait value for each species was estimated from a mixed-effect model with site as random factor using lme4 and emmeans R packages. Species-level trait relationships were then assessed in generalized least squares (GLS) models that took phylogenetic correlation structure into account using the gls function from nlme package with Pagel's correlation structure calculated using corPagel from ape package (Paradis & Schliep, 2019). In order to assess a possible effect of growth form, species-level trait relationships were tested separately for herbaceous and woody species using the same SMA and GLS procedures.

### 2.5 | Network analysis

A correlation network using the 12 traits was constructed based on significant Pearson's  $r$  correlations with  $r^2 > 0.25$ , as correlations with lower  $r$  are unlikely to have a mechanistic basis (see Poorter et al., 2014), after taking the phylogenetic signal into account. Phylogenetic distance correction was performed on correlations between species marginal means using species phylogenetic tree in the corphylo function of ape package (Paradis & Schliep, 2019). The degree and strength of connectivity as well as betweenness of each trait

were determined using the corresponding functions in iGraph package and were used as metrics of trait centrality in the network. Modularity Q scores were calculated using the different algorithms of the iGraph package (maximum variation [-1; 1]), and the identification of groups of traits was also performed using the clique percolation community detection algorithm of the CliquePercolation package (Lange, 2021).

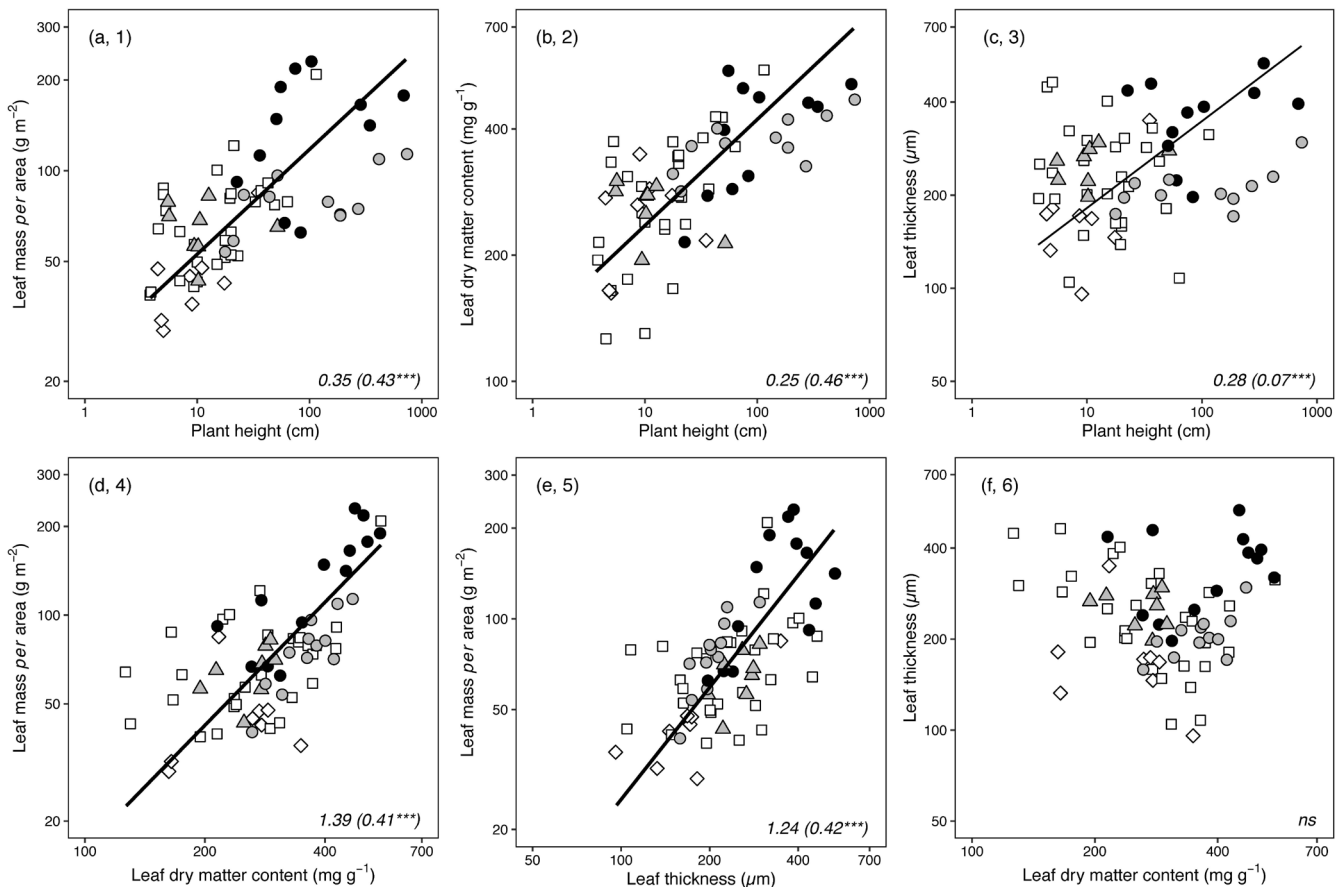
### 3 | RESULTS

Across species, trait values varied ~200-fold for plant height, ~8-fold for leaf mass per area, ~5-fold for leaf thickness and ~4.5-fold for LDMC. Figures 1–4 show SMA slopes between these four traits on the one hand, and leaf gas exchange, water use and nitrogen on the other hand, across the 60 species of the data set. Figure S3, which compares these SMA slopes with those obtained using the GLS method, shows that in most cases, accounting for phylogeny does not modify the significance of the observed relationships.

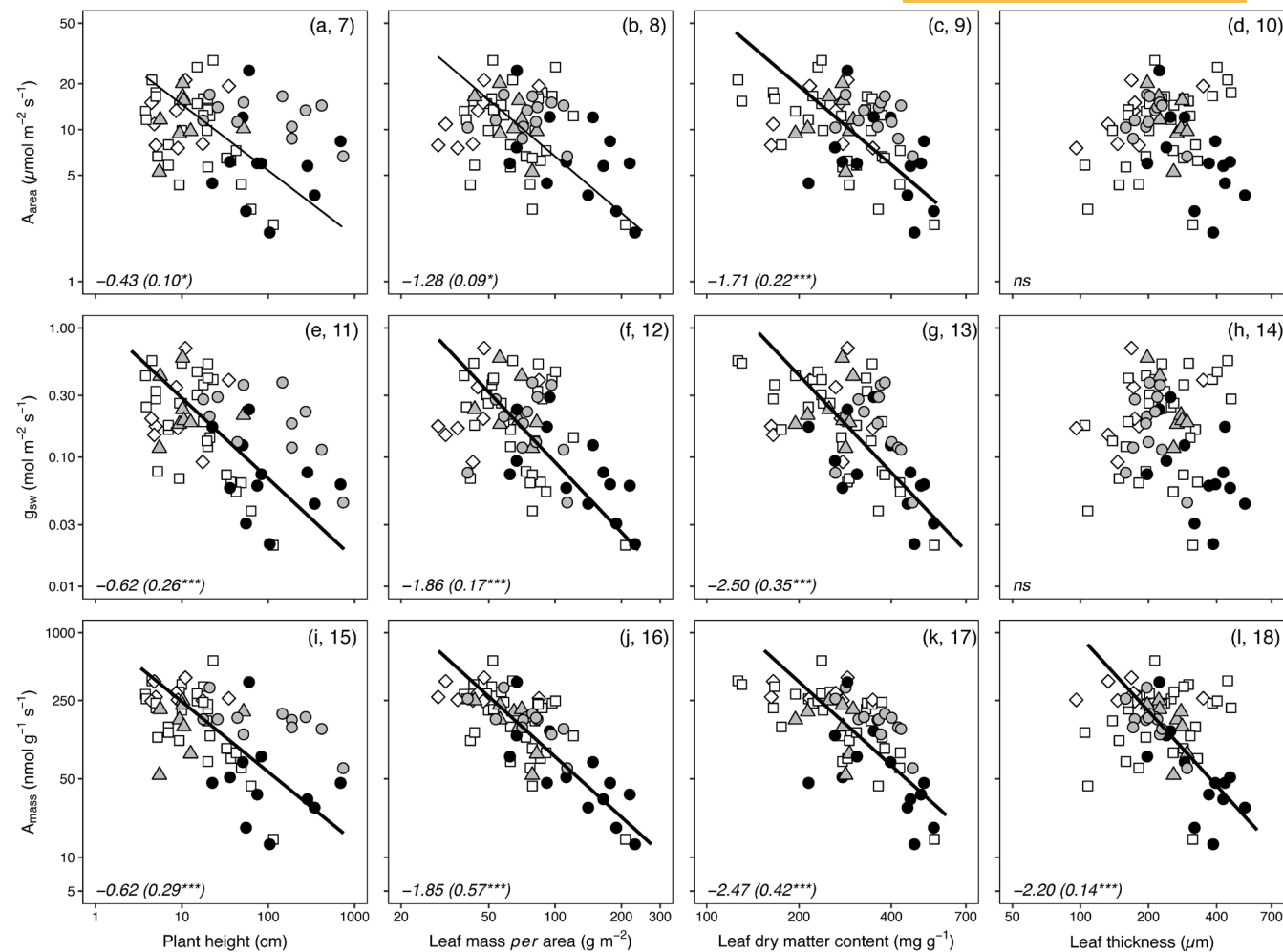
As a support for the discussion, the distributions of values for the 12 traits and how they differ among life forms are shown in Figure S2, and pairwise OLS regressions between all traits are shown in Figure S1.

#### 3.1 | Plant height, leaf mass per area and its components

Vegetative plant height varied from 3.8 cm for the herbaceous *Convolvulus arvensis* to 738 cm for the woody deciduous *Quercus pubescens* and was positively related to LMA, LDMC and LT (Figure 1a–c). The  $r^2$  of the relationships with LMA and LDMC were much higher than that with LT. LMA varied between  $29.5 \text{ g m}^{-2}$  for the annual dicot *Kickxia spuria* and  $230.4 \text{ g m}^{-2}$  for the evergreen palm *Chamaerops humilis*, while LDMC varied between  $126 \text{ mg g}^{-1}$  for the perennial dicot *Catananche caerulea* and  $553 \text{ mg g}^{-1}$  for the perennial grass *Ampelodesmos mauritanicus*; leaf thickness varied between



**FIGURE 1** Relationships between vegetative plant height (plant height) and (a) leaf mass per area, (b) leaf dry matter content, (c) leaf thickness; relationships between leaf mass per area and (d) leaf dry matter content and (e) leaf thickness, and between (f) leaf dry matter content and leaf thickness, for 60 species (70 populations) from three sites in the Mediterranean Basin. Symbols are for different life forms sensu Raunkiaer (1934;  $\diamond$ : Therophytes;  $\square$ : Hemicryptophytes;  $\blacktriangle$ : Chamaephytes;  $\bullet$ : Deciduous phanerophytes;  $\bullet$ : Evergreen phanerophytes). Solid lines denote significant relationships (the thicker the line, the highest the  $p$  value). SMA slopes and coefficients of determination ( $r^2$ , between brackets) are given for each relationship; significance levels: \*\*\*  $p < 0.001$ ; ns  $p > 0.05$ . All variables are log transformed. The relationships shown on the panels of Figures 1–4 are numbered from 1 to 38 (next to the letter identifying the panel). These numbers are used in the various comparisons between the slopes of the relationships (see text and Figures S3 and S4).



**FIGURE 2** Relationships between vegetative plant height (plant height: a, e, i), leaf mass per area (b, f, j), leaf dry matter content and (c, g, k), leaf thickness (d, h, l), and net photosynthetic rate per unit leaf area ( $A_{\text{area}}$ : a–d), stomatal conductance to water vapour ( $g_{\text{sw}}$ : e–h), and net photosynthetic rate per unit leaf mass ( $A_{\text{mass}}$ : i–l), for 60 species (70 populations) from three sites in the Mediterranean Basin. Symbols are for different life forms sensu Raunkiaer (1934;  $\diamond$ : Therophytes;  $\square$ : Hemicryptophytes;  $\blacktriangle$ : Chamaephytes;  $\bullet$ : Deciduous phanerophytes;  $\bullet$ : Evergreen phanerophytes). Solid lines denote significant relationships (the thicker the line, the highest the  $p$  value). SMA slopes and coefficients of determination ( $r^2$ , between brackets) are given for each relationship; significance levels: \*\*\* $p < 0.001$ ; \* $p < 0.05$ ; ns:  $p > 0.05$ . All variables are log transformed. The relationships shown on the panels of Figures 1–4 are numbered from 1 to 38 (next to the letter identifying the panel). These numbers are used in the various comparisons between the slopes of the relationships (see text and Figures S3 and S4).

104  $\mu\text{m}$  for the annual grass *Brachypodium distachyon* and 533  $\mu\text{m}$  for the perennial dicot *Echinops ritro*.

LDMC and LT were both important determinants of LMA (Figure 1d,e), but while the  $r^2$  of both relationships were similar, the SMA slope was higher for LDMC than for LT. There was no evidence that LT and LDMC were related (Figure 1f).

For these six relationships, conclusions were the same when phylogeny was accounted for (Figure S3).

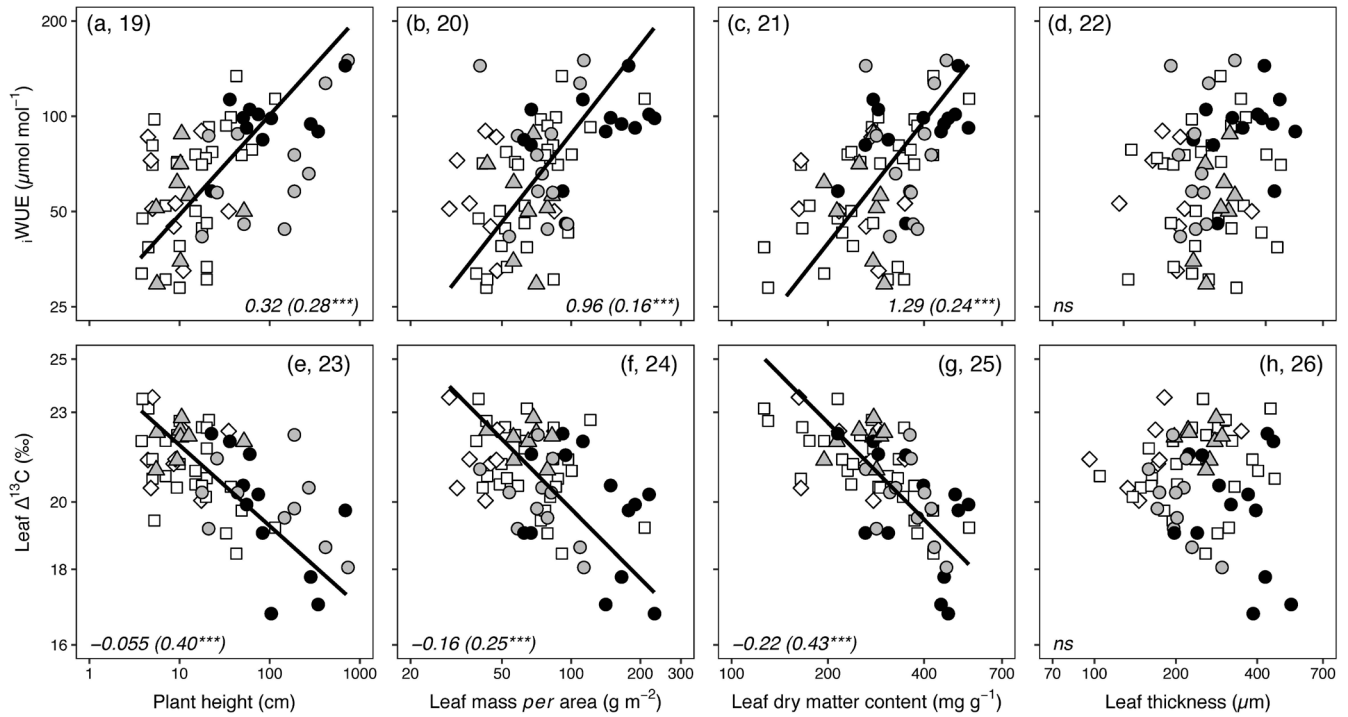
### 3.2 | Photosynthetic rate, stomatal conductance and intrinsic water-use efficiency

Plant height was negatively related to  $A_{\text{area}}$ ,  $g_{\text{sw}}$  and  $A_{\text{mass}}$  (Figure 2a,e,i), while the relationship with  $\text{iWUE}$  was positive

(Figure 3a). The  $r^2$  of the correlation with  $A_{\text{area}}$  was substantially lower than those with the three other traits. There was also strong evidence of a negative relationship between plant height and  $\Delta^{13}\text{C}$  (Figure 3e), indicative of tall plants having a high time-integrated water-use efficiency (note that water-use efficiency relates negatively to  $\Delta^{13}\text{C}$ : Farquhar et al., 1989; and compare Figure 3a with Figure 3e).

Due to the tight correlation between  $A_{\text{area}}$  and  $g_{\text{sw}}$  ( $r^2 = 0.62$ ,  $p \lll 0.001$ , not shown), the relationships between leaf structural traits and these two traits were similar (compare Figure 2a–d with Figure 2e–h): both  $A_{\text{area}}$  and  $g_{\text{sw}}$  were negatively correlated with LMA and LDMC, but the SMA slopes were steeper and the  $r^2$  were higher for LDMC than for LMA. There was no evidence of a relationship between LT and any of these two traits (Figure 2d,h). The directions of the relationships were similar





**FIGURE 3** Relationships between vegetative plant height (plant height: a, e), leaf mass per area (b, f), leaf dry matter content (c, g), leaf thickness (d, h), and intrinsic water-use efficiency ( $iWUE = A_{area}/g_{sw}$ ; a–d) and leaf carbon isotope discrimination ( $\Delta^{13}C$ ; e–h), for 60 species (70 populations) from three sites in the Mediterranean Basin. Symbols are for different life forms sensu Raunkjær (1934;  $\diamond$ : Therophytes;  $\square$ : Hemicryptophytes;  $\blacktriangle$ : Chamaephytes;  $\bullet$ : Deciduous phanerophytes;  $\bullet$ : Evergreen phanerophytes). Solid lines denote significant relationships (the thicker the line, the highest the  $p$  value). SMA slopes and coefficients of determination ( $r^2$ , between brackets) are given for each relationship; significance levels: \*\*\* $p < 0.001$ ; ns:  $p > 0.05$ . All variables are log transformed. The relationships shown on the panels of Figures 1–4 are numbered from 1 to 38 (next to the letter identifying the panel). These numbers are used in the various comparisons between the slopes of the relationships (see text and Figures S3 and S4).

when photosynthetic rate was expressed either on an area or a mass basis (Figure 2), but the slopes were steeper and the  $r^2$  was higher for the mass-based values. Noticeably, LT was negatively related to  $A_{mass}$  (Figure 2i), while there was no evidence that it was related to  $A_{area}$  (Figure 2d). The relationships between  $A_{area}$  and plant height on the one hand, and between  $A_{area}$  and LDMC on the other hand, were not significant when phylogeny was accounted for (Figure S3).

For both LMA and LDMC, the slopes of the relationships with  $g_{sw}$  were steeper than with  $A_{area}$  (compare Figure 2b,c with Figure 2f,g), resulting in positive associations between these two traits and  $iWUE$  as assessed through gas exchange ( $=A_{area}/g_{sw}$ ; Figure 4b,c; LDMC– $iWUE$  relationship not significant when phylogeny accounted for: Figure S3). As for  $A_{area}$  and  $g_{sw}$ , the slope was steeper and the  $r^2$  was higher for the relationship with LDMC than that with LMA. When assessed through carbon isotope discrimination ( $\Delta^{13}C$ ), the relationship was negative for the two traits (i.e. time-integrated water-use efficiency increase with increasing values of the two traits: Figure 3f,g), and it was also tighter with LDMC than with LMA. There was no evidence that LT and  $iWUE$ , either assessed from gas exchange or carbon isotope discrimination (Figure 3d,h), were related.

### 3.3 | Leaf nitrogen and photosynthetic nitrogen-use efficiency

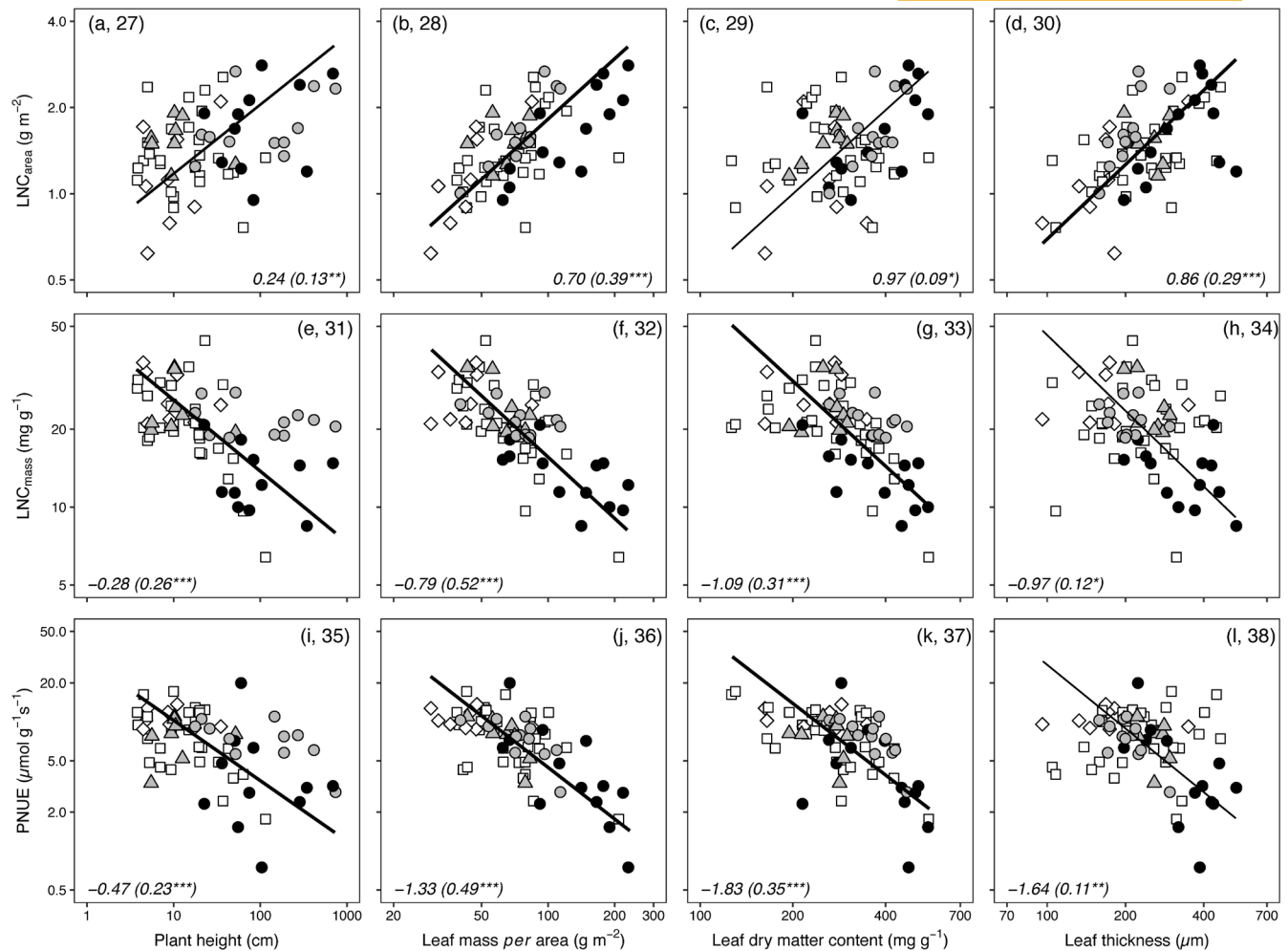
$LNC_{area}$  was positively related to plant height (Figure 4a) and to all three leaf structural traits (Figure 4b–d), with higher  $r^2$  for LMA and LT than for plant height and LDMC. Relationships were negative when leaf nitrogen was expressed on a mass-based basis (Figure 4e–h), with the lowest  $r^2$  value obtained for LT. PNUE was negatively related to plant height, LMA, LDMC and LT (Figure 4i–l), with the strongest relationships (higher  $r^2$ ) obtained for LMA and LDMC.

Finally, leaves with a high PNUE also had a low water-use efficiency: there was a negative relationship between PNUE and  $iWUE$  (SMA slope =  $-0.129$ ,  $r^2 = 0.18$ ,  $p < 0.001$ ; not shown) and a positive relationship between PNUE and  $\Delta^{13}C$  (SMA slope =  $2.59$ ,  $r^2 = 0.31$ ,  $p < 0.001$ ; not shown).

GLS regressions accounting for phylogeny were significant as well for these 12 relationships (Figure S3).

### 3.4 | Network analysis

The trait correlation networks shown in Figure 5 give an overview of the relationships found between the 12 traits assessed in this study

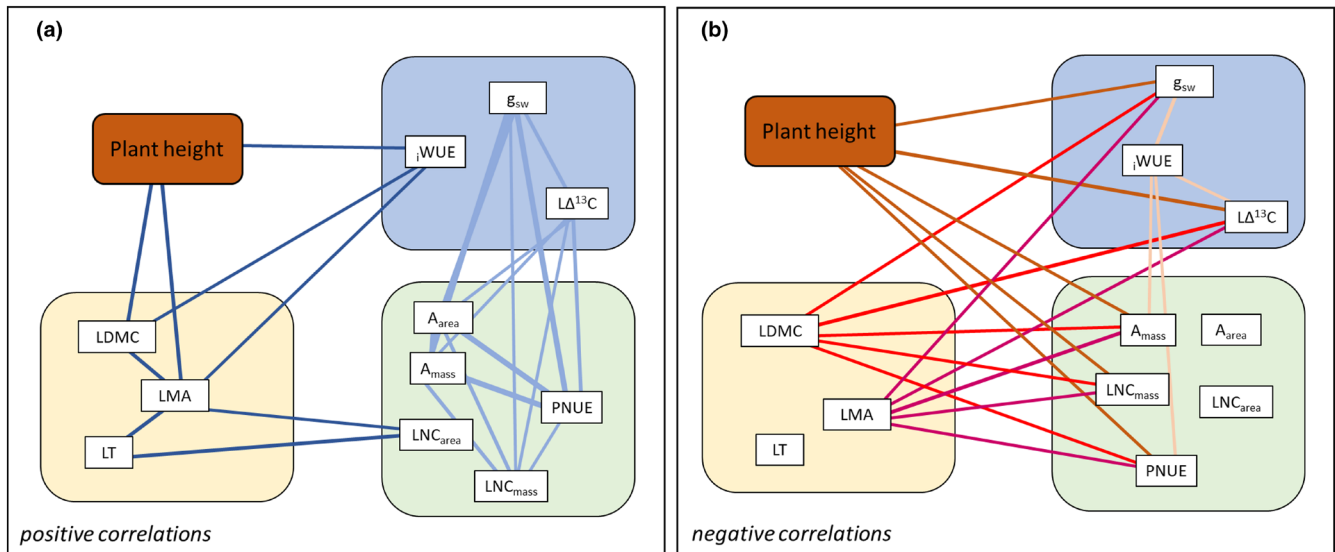


**FIGURE 4** Relationships between vegetative plant height (plant height: a, e, i), leaf mass *per area*: (b, f, j), leaf dry matter content (c, g, k), leaf thickness (d, h, l), and leaf nitrogen content *per unit leaf area* (LNC<sub>area</sub>: a–d), leaf nitrogen content *per unit leaf mass* (LNC<sub>mass</sub>: e–h), and photosynthetic nitrogen-use efficiency (PNUE: i–l), for 60 species (70 populations) from three sites in the Mediterranean Basin. Symbols are for different life forms sensu Raunkiær (1934;  $\diamond$ : Therophytes;  $\square$ : Hemicryptophytes;  $\blacktriangle$ : Chamaephytes;  $\bullet$ : Deciduous phanerophytes;  $\bullet$ : Evergreen phanerophytes). Solid lines denote significant relationships (the thicker the line, the highest the  $p$  value). SMA slopes and coefficients of determination ( $r^2$ , between brackets) are given for each relationship; significance levels: \*\*\* $p < 0.001$ ; \*\* $p < 0.01$ ; \* $p < 0.05$ . All variables are log transformed. The relationships shown on the panels of Figures 1–4 are numbered from 1 to 38 (next to the letter identifying the panel). These numbers are used in the various comparisons between the slopes of the relationships (see text and Figures S3 and S4).

(see also Figure S1), split between positive (Figure 5a) and negative (Figure 5b) relationships. Beyond the examination of individual trait pairs as presented so far, the network analysis enables the identification of overarching patterns across the entire set of traits. Here, we found that traits were not well-organized into distinct groups within the networks, as indicated by the low modularity  $Q$  scores (average of 0.066; cf. Newman, 2006). Accordingly, no distinct group of traits were identified by clique percolation. Both LMA and plant height were central traits in the network, as indicated by high betweenness values (Table S4).  $\Delta^{13}\text{C}$  also tended to occupy a central place in the network. LMA,  $A_{\text{mass}}$ , PNUE and  $g_{\text{sw}}$  were the four traits with the highest connectedness, as indicated by high strength values. On the contrary, LNC<sub>area</sub>, leaf thickness and  $\text{WUE}$  had the lowest strength values and were therefore peripheral to the network.

## 4 | DISCUSSION

Overall, differences among life forms agree with previous findings on larger sets of species sampled in the Mediterranean (Garnier et al., 2019; Gulías et al., 2003; Navas et al., 2010), as well as with conclusions from data syntheses (Poorter et al., 2009; Vile et al., 2005; but see Flexas et al., 2014, for the noticeable exceptions of  $A_{\text{area}}$  and  $g_{\text{sw}}$ ). Here, we used these variations to assess (1) whether plant height relates to leaf structure and gas exchange across species, which would suggest that the hydraulic constraints identified within individuals and/or species also operate across species within a given habitat type, and (2) which underlying components of leaf mass *per area* relate to gas exchange and leaf nitrogen. The confrontation between the results found here (synthesized in



**FIGURE 5** Trait correlation networks showing relationships among vegetative plant height (plant height), leaf structural traits and resource-use traits, organized by type of trait and resource: Brown: Plant height; pale yellow: Leaf structure; blue: Water; green: Carbon and nitrogen. (a) Positive correlations; (b) negative correlations. Only significant correlations with  $r^2 > 0.25$  are shown. Darker lines correspond to relationships shown as bivariate plots on [Figures 1–4](#), and whose expected direction of variation is summarized in [Table 2](#). Line thickness is proportional to  $r$  value. To improve clarity, the position of labels in the green boxes has been slightly modified between the two panels. In (b), brown lines show relationships with vegetative plant height, red lines with leaf dry matter content (LDMC) and purple lines with leaf mass per area (LMA). See [Table 1](#) for abbreviations.

[Figure 5](#)) and the findings from the literature (cf. [Table S1](#)) shows confirmations, novel findings for previously understudied relationships, and also conspicuous mismatches, which form the core of the discussion below.

#### 4.1 | Plant height, water-use and leaf economics traits

Plant height was consistently related to the 11 leaf traits assessed in this study, albeit with different strengths. The trends observed are not the consequences of changes in light availability with height, since traits were measured on sunlit leaves in all species, including trees (outer canopy leaves). Numerous studies have assessed height-related variations in leaf traits, but for only a fraction of these is it possible to sort out the effects of height per se from those of light. The following discussion considers only this fraction of relevant studies.

First, stomatal conductance,  $A_{area}$  and  $A_{mass}$  all decreased with height, as found within individuals and/or species of trees (for  $g_{sw}$ : e.g. [Ambrose et al., 2009](#); [McDowell et al., 2011](#); [Schäfer et al., 2000](#); for photosynthesis: [Ambrose et al., 2009](#); [Koch et al., 2004](#); but see [Sendall & Reich, 2013](#)). As a result of  $A_{area}$  decreasing less rapidly than  $g_{sw}$ ,  $WUE (= A_{area}/g_{sw})$  increased with height. Consistent with this result,  $\Delta^{13}C$  decreased with height, suggesting higher time-integrated water-use efficiency in taller plants, as previously found in trees, both within individuals and/or species ([Ambrose et al., 2009](#); [Koch et al., 2004](#); [McDowell](#)

[et al., 2011](#)) and across species ([Fernández-de-Uña et al., 2023](#); [McDowell et al., 2011](#)). Overall, our results therefore provide a generalization across growth and life forms of the height dependence of gas exchange and water use previously demonstrated in trees.

A decrease in stomatal conductance with height, which is key to explain the relationships discussed above, agrees with the hydraulic limitation hypothesis ([McDowell et al., 2011](#); [Ryan et al., 2006](#)) as formalized by Darcy's law ([McDowell et al., 2011](#); [Whitehead, 1998](#)). It is the consequence of an increasing soil-to-leaf pathlength for water, of which height is a surrogate. Interspecific differences in other factors involved in Darcy's law such as xylem hydraulic conductivity and the sapwood-to-leaf area ratio may have also played a role beyond height to explain differences in stomatal conductance in our study. As these traits were not measured here, we screened the TRY database ([Kattge et al., 2020](#)) and could find values of xylem vulnerability to cavitation ( $P_{50}$ : the water potential at 50% loss of conductivity), a trait which relates negatively to xylem conductivity ([Gleason et al., 2016](#); [Liu et al., 2019](#)), for 21 of our species (6 herbaceous and 11 woody). For this limited data set, we found that xylem vulnerability was lower (more negative  $P_{50}$  values) in taller species ( $p < 0.05$ ), suggesting a lower stem hydraulic conductivity in these species. Pending further simultaneous measurements of the different variables involved in Darcy's law beyond this coarse preliminary assessment, our results suggest that, as demonstrated within tree individuals and/or species, plant height is an important driver of differences in stomatal conductance among species differing in growth and life forms.

Beyond gas exchange, other key traits involved in the leaf economics spectrum vary with plant height: the higher LMA in taller plants is in line with previous results found in woody species, both within (LMA: Ambrose et al., 2009; Koch et al., 2004; Poorter et al., 2009 for a review) and across species (Cavaleri et al., 2010; Jin et al., 2014), and goes with a higher  $LNC_{area}$ , as also found within tree species (e.g. Koyama et al., 2021; Sendall & Reich, 2013). This higher  $LNC_{area}$  goes with a decrease in  $A_{area}$  (and in  $LNC_{mass}$ ), resulting in lower PNUE values in taller plants. Few studies have assessed the effect of height on leaf nitrogen economy in general and on PNUE in particular (but see Sendall & Reich, 2013), and whether these height-related patterns in nitrogen economy are general both within species and across life forms therefore requires further investigations.

One step further, our study shows that changes in LMA with height were due to changes in LDMC rather than in leaf thickness, which agrees with the work conducted on redwoods by Oldham et al. (2010). Leaves with high LDMC/and or tissue density have been found to have a low osmotic potential at turgor loss point (Májeková et al., 2021; Nadal et al., 2023), which is considered key for drought tolerance. These height-related changes in LMA and LDMC are not likely to be related to water constraints induced by gravity (Cavaleri et al., 2010; Oldham et al., 2010) however. The difference in the gravimetric component of water potential between the shortest (*Convolvulus arvensis*, 3.8 cm) and the tallest species included in our study (*Quercus pubescens*, 738 cm) is about  $-0.07$  MPa ( $-0.01$  MPa/m: cf. Fernández-de-Uña et al., 2023), which is certainly too small to account for the differences in LMA and LDMC values observed across species. Another hypothesis that can be put forward involves trait coordination among organs in relation to height. In their global analysis, Díaz et al. (2016) have shown that stem-specific density (SSD) was related to plant height, both within and across herbaceous and woody growth forms. This suggests that a stiff stem is needed to grow tall. Combining SSD from this study (Díaz et al., 2022) with LDMC and LMA from the present study, we found positive associations between SSD and both LDMC ( $r^2=0.62$ ,  $p<0.0001$ ,  $n=43$ ) and LMA ( $r^2=0.43$ ,  $p<0.0001$ ,  $n=43$ ). We can thus make the hypothesis that the high LMA and LDMC values of tall plants are the consequence of trait coordination between stems and leaves, tall plants showing a combination of high values for the three traits.

Are these relationships between plant height and leaf traits solely the consequence of comparing species whose height differ among growth forms? To answer this question, we compared the slopes of the different relationships obtained separately for the 31 herbaceous and 29 woody species of our data set. For the 11 relationships involving height, these slopes compared well between the two groups of species, whether phylogeny was accounted for or not (Figures S4 and S5). Some relationships remained significant in one group only, but for key relationships (with LMA, LDMC,  $g_{sw}$ ,  $\Delta^{13}C$ ,  $LNC_{area}$  and  $LNC_{mass}$ ), these remained consistent within the two groups. The slopes of each of these 11 relationships were further

compared within each life form (Figure S5): although the statistical power of these analyses is low due to the small number of species within some of the categories, in 82% of the cases (45 relationships out of 55), the sign of the relationship within each life form was the same as that found for the relationship involving all species. Of the 10 exceptions, eight were found for therophytes or chamaephytes (Figure S5), which were represented by only eight species each. We thus conclude that most relationships between plant height and leaf traits are conserved whatever the scale (within or between growth and life forms) at which the comparison is conducted.

The studies mentioned above have been conducted within one or a few sites, and the height dependence of many leaf traits they show is at variance with the conclusions put forward at a global scale (cf. Table S1). These discrepancies between global and local scales were actually pointed out by Price et al. (2014): when they split their data set by 'habitat' (meadow tundra, chaparral, tropical rainforest, etc.), the likelihood of detecting an effect of height on leaf traits raised substantially (not always in the same direction as in the present study, however). Two hypotheses may be put forward to explain these discrepancies: (1) the relationships between height and leaf traits are context dependent, that is, their strength and direction might vary under 'different biotic, abiotic, spatiotemporal, or observational circumstances' (Catford et al., 2022). To assess this context-dependency would require testing the values of slopes and elevations of these relationships across sites and species pools, which should be possible with the increasing amount of relevant trait data collected worldwide in different, well-identified sites; (2) in global analyses, trait values are aggregated at the species level for each trait, which raises at least two issues: (i) for a given species, all traits have not necessarily been measured on the same individuals in the same location (Albert et al., 2011), and (ii) the number of original data points used to calculate an average value for each species  $\times$  trait combination might vary substantially (cf. Borgy et al., 2017). Our study, together with many of those reviewed in this section, tends to show that when all traits are measured on the same individuals in the same location, leaf traits relate to plant height across species, in the same way as they do within species or individuals.

#### 4.2 | The effects of LMA and its underlying components on leaf functioning

In addition to, or in combination with plant height, we found moderate to strong evidence that all gas exchange characteristics were related to LMA. For all relationships, LDMC was the component for which relationships were stronger, with weak to undetectable effects of leaf thickness. High LMA/LDMC leaves have low photosynthetic rates and stomatal conductance, high instantaneous and time-integrated water-use efficiency, and low PNUE. We further discuss these findings below, using the literature synthesized in Table S1 as a background.

#### 4.2.1 | Leaf thickness

Thicker leaves had a higher leaf nitrogen content *per unit area*, as found in a number of other studies (e.g. Castro-Díez et al., 2000; Garnier et al., 1999; Niinemets, 1999). This probably corresponds to a higher amount of mesophyll *per unit leaf area* (de la Riva et al., 2016; Garnier et al., 1999; Niinemets, 1999), and thus, a larger amount of leaf nitrogen involved in the processes of CO<sub>2</sub> fixation (Evans, 1989; Field & Mooney, 1986). This does not translate into a higher photosynthetic rate *per unit area* however, and the trend is even negative when photosynthetic rate is expressed *per unit mass*, as found for LMA (see Onoda et al., 2017). This might be the consequence of light extinction in deeper mesophyll layers (e.g. Vogelmann & Evans, 2002), and/or to a longer path length for CO<sub>2</sub> within the leaf, inducing a higher resistance to CO<sub>2</sub> diffusion in the gaseous phase (cf. Parkhurst, 1994).

#### 4.2.2 | Leaf dry matter content

In our data set, LDMC and stomatal conductance were negatively related. A first hypothesis is that this relationship is mediated through plant height, as discussed in the previous section. A second, non-exclusive hypothesis, pertains to the anatomy of the leaf itself. High-density leaves have a high fraction of the leaf volume occupied by dense tissues such as vascular bundles and sclerenchyma as well as small mesophyll cells (Castro-Díez et al., 2000; Garnier & Laurent, 1994) and thus a high amount of cell walls *per unit leaf volume*. Leaves with a more robust structure (i.e. dense leaves) exhibit lower mesophyll conductances ( $g_m$  hereafter) and photosynthetic rates as a consequence of a larger CO<sub>2</sub> drawdown from substomatal cavities to chloroplasts (Flexas et al., 2008; Niinemets et al., 2009; Onoda et al., 2017). This agrees with results from Niinemets et al. (2009) and Hassiotou et al. (2010) showing that the negative relationship between  $g_m$  and LMA was driven by the negative scaling between  $g_m$  and leaf density, with no noticeable effect of leaf thickness on  $g_m$ . The low photosynthetic rate of high LDMC leaves can also be due to their low water content (LDMC is actually functionally equivalent to leaf-saturated water content:  $LSWC = 1 - LDMC$ ): two recent studies have indeed found positive relationships between LSWC and photosynthetic rate (Nadal et al., 2023; Wang et al., 2022), which would be the consequence of water content being positively related to metabolic rates in general. The low  $A_{area}$  and  $A_{mass}$  of high LDMC leaves may thus be due to both their low stomatal and mesophyll conductances to CO<sub>2</sub> diffusion and/or to their low water content resulting in slow metabolic rates.

An important finding of our study is that LDMC was the leaf trait most tightly related to instantaneous and time-integrated water-use efficiencies. This might be due to a combination of whole plant (height) and local effects related to leaf anatomy. At the leaf level, it might be the consequence of LDMC influencing both mesophyll conductance (see above) and leaf chemical composition. Differences in LDMC values indeed reflect differences in the proportion of

different tissues within leaves (mesophyll, vascular bundles, sclerenchyma, etc.), hence differences in their composition in, for example, lipids, lignin, proteins, etc. (Niinemets, 1999; Poorter et al., 2009, and references therein) whose isotopic signatures differ (cf. Badeck et al., 2005; Hobbie & Werner, 2004). How LDMC and/or leaf density relates to diffusional conductances and leaf chemical composition would help understand the impact of this trait(s) on leaf carbon isotopic composition.

The higher fraction of leaf mass invested in cell walls in high LDMC leaves also has consequences for the allocation of nitrogen. Since cell walls contain 2%–10% of nitrogen and account for a large fraction of leaf mass (Onoda et al., 2017 and references therein), the proportion of leaf nitrogen in non-photosynthetic components is likely to be higher in high LDMC leaves. A negative relationship has indeed been found between leaf density and the fraction of leaf nitrogen invested in Rubisco for 17 woody species (Niinemets, 1999; see also the negative relationships between LMA and the proportion of nitrogen found in Rubisco in Onoda et al., 2017), which is probably at the basis of the negative relationship between LDMC and PNUE found in our data set. The lower investment in the photosynthetic machinery in high LDMC leaves therefore further explains the negative relationship between LDMC and photosynthetic rate, which occurs in spite of these high LDMC leaves having a higher amount of nitrogen *per unit leaf area* (see also Niinemets, 1999).

### 4.3 | Ecological implications

The vegetation of the three Mediterranean sites studied results from a strong and long-lasting human imprint and is engaged in a highly dynamic process of secondary succession following different types of disturbances: grazing and agriculture in Cazarils (Le Floc'h et al., 1998) and Les Agros (Garnier, Laurent, et al., 2001), agriculture and fire in Garraf (Lloret & Vilá, 2003). Species selected for this study are abundant at the sites and representative of the species pool found in different successional stages, from recently disturbed to mature forest (Lloret & Vilá, 2003; Navas et al., 2010). The observed differences in trait values among species actually reflect their position along the succession, especially in Cazarils where all stages are well-represented (cf. Table S3). Small-statured species with high stomatal conductance and related traits (high photosynthetic rates, low LMA, high  $LNC_{mass}$  and PNUE) but low water-use efficiency are progressively replaced by tall species with opposite trait values as succession proceeds (see also Navas et al., 2010). This pattern is consistent with the shift of species along the size and leaf economics dimensions as documented in other secondary successional series (Bazzaz, 1996; Huston & Smith, 1987). Changes in water use can be best interpreted in relation to species phenology in the Mediterranean. In early stages, small-statured annual species with high photosynthetic rates and stomatal conductance but low water-use efficiency can escape drought by completing their life cycle during the drought-free period (see Navas et al., 2010). By contrast,

most woody species of late stages which are tall and maintain their leaves during summer, tend to have both high water-use efficiency and LDMC, allowing them to tolerate drought during the driest part of the year. The coordination between plant height and leaf traits shown here within and among growth forms suggests that functional shifts along the successional gradient should be considered as a gradual, continuous process, rather than a discrete process of life form replacement.

## 5 | CONCLUSIONS

Combining whole-plant and leaf-level perspectives allowed us to show that both height and leaf properties impact leaf gas exchange across species. Our study is among the first to demonstrate that the height dependence of leaf traits observed within individuals and/or tree species is also found among species from different life forms. At the leaf level, leaf dry matter content appears to be the underlying component with the strongest impact on gas exchange, water-use and chemical composition, which warrants further investigations aiming at understanding the underlying anatomical and biochemical causes and consequences of variations of this fundamental trait. The discrepancy between our results obtained locally and syntheses conducted at the global level with respect to the influence of plant height points to the urgent need to address context dependence in the study of plant form and function.

### AUTHOR CONTRIBUTIONS

Eric Garnier and Catherine Roumet conceived the study and designed methodology. Catherine Roumet, Eric Garnier, Sandrine Debain, Denis Vile, Lorraine Bottin and Gérard Laurent collected the data. Eric Garnier, Denis Vile and Catherine Roumet analysed the data. Eric Garnier led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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### CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

### DATA AVAILABILITY STATEMENT

The values of vegetative plant height and leaf traits averaged for each species–site combination (70 values per variable) are archived at the CNRS institutional repository InDoRES (<https://data.indores.fr:443/dataverse/CEFE>) with the following DOI: <https://doi.org/10.48579/PRO/PLNU9F> (Garnier et al., 2024).

### STATEMENT ON INCLUSION

Our study was conducted in southern France and northern Spain, in the context of the Laboratoire Européen Associé 'Mediterranean Ecosystems in a Changing World'. The main scientist involved in Spain was a co-author of two previous publications reporting other aspects of the work conducted. Wherever relevant, literature published by this scientist and/or his group was cited.

### ORCID

Eric Garnier  <https://orcid.org/0000-0002-9392-5154>

Denis Vile  <https://orcid.org/0000-0002-7948-1462>

Lorraine Bottin  <https://orcid.org/0000-0002-4062-5203>

Catherine Roumet  <https://orcid.org/0000-0003-1320-9770>

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

**Table S1:** Synthesis of a literature survey conducted at the *interspecific* level showing the direction of variation of relationships between plant height and leaf traits on the one hand, and between leaf morpho-anatomical traits and gas exchange, water-use and leaf nitrogen on the other hand.

**Table S2:** List of references used for the literature survey presented in Table S1.

**Table S3:** List of species measured in the threes site, with their botanical family, life form sensu Raunkiaer and growth form and successional stage indicator value.

**Table S4:** Centrality measures of the network built with the 12 traits.

**Figure S1:** Bivariate relationships between the 12 traits assessed in this study.



**Figure S2:** Distribution of log-transformed values of the 12 traits assessed for 60 species (70 populations) from the Mediterranean belonging to different life forms sensu Raunkiaer.

**Figure S3:** Comparisons of slope values from bivariate relationships between traits obtained with different regression methods.

**Figure S4:** Comparisons of slope values from bivariate relationships between traits obtained for herbaceous and woody species.

**Figure S5:** SMA slopes of the relationships between plant height and the 11 leaf traits at different scales of comparisons.

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