

Higher leaf nitrogen content is linked to tighter stomatal regulation of transpiration and more efficient water use across dryland trees

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Summary

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- The least-cost economic theory of photosynthesis shows that water and nitrogen are mutually substitutable resources to achieve a given carbon gain. However, vegetation in the Sahel has to cope with the dual challenge imposed by drought and nutrient-poor soils.
- We addressed how variation in leaf nitrogen per area (N_{area}) modulates leaf oxygen and carbon isotopic composition ($\delta^{18}\text{O}$, $\delta^{13}\text{C}$), as proxies of stomatal conductance and water-use efficiency, across 34 Sahelian woody species.
- Dryland species exhibited diverging leaf $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ values, indicating large interspecific variation in time-integrated stomatal conductance and water-use efficiency. Structural equation modeling revealed that leaf N_{area} is a pivotal trait linked to multiple water-use traits. Leaf N_{area} was positively linked to both $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$, suggesting higher carboxylation capacity and tighter stomatal regulation of transpiration in N-rich species, which allows them to achieve higher water-use efficiency and more conservative water use.
- These adaptations represent a key physiological advantage of N-rich species, such as legumes, that could contribute to their dominance across many dryland regions. This is the first report of a robust mechanistic link between leaf N_{area} and $\delta^{18}\text{O}$ in dryland vegetation that is consistent with core principles of plant physiology.

Introduction

Drylands occupy *c.* 45% of the Earth's surface (Právělie, 2016; Maestre *et al.*, 2021) and are defined as regions where evapotranspiration is greater than precipitation, leading to water deficit (Huang *et al.*, 2016). Low soil fertility is yet another limitation for plant productivity in many drylands, especially regarding soil nitrogen and phosphorus content (Noy-Meir, 1973; Breman & De Wit, 1983). However, dryland plants often show higher leaf nitrogen contents than do species from wetter ecosystems (Wright *et al.*, 2001, 2003). Several hypotheses have been proposed to explain the high leaf N contents typically found in

dryland vegetation (Prentice *et al.*, 2014; Adams *et al.*, 2016). More than 70% of the total N contained in plant leaves is allocated to RuBisCO and other enzymes and proteins involved in photosynthesis (Evans, 1989; Evans & Seemann, 1989; Onoda *et al.*, 2017; Evans & Clarke, 2018). Hence, a higher investment in N uptake and allocation to leaves enhances CO_2 fixation and reduces CO_2 concentration in leaf intercellular spaces (c_i) relative to the atmosphere (c_a), leading to low internal c_i/c_a ratios at the sites of carboxylation. Wright *et al.* (2001, 2003) suggested that plants in low-rainfall environments increase their N content per unit leaf area (N_{area}) so that they can save water while maintaining photosynthetic rates similar to plants from wetter environments. This is achieved by optimizing carboxylation and carbon assimilation capacity thanks to high leaf N_{area} , while at the same time reducing stomatal conductance (g_s) and transpiration (E)

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rates to minimize water loss, given that a steeper CO₂ diffusion gradient helps to maintain supply of CO₂ into the leaves. This general theory has been named the least-cost economic theory of photosynthesis (Wright *et al.*, 2003; Prentice *et al.*, 2014; Wang *et al.*, 2017).

Remote sensing studies have recently raised awareness about the high woody plant cover and large number of tree individuals present across the Sahelian drylands in northwest Africa (Brandt *et al.*, 2020), despite the dual challenge imposed by low soil fertility and drought stress on plant photosynthesis. Legumes (Fabaceae) are often the dominant plant family in terms of cover and species diversity in Sahelian drylands (Felker, 1981; Sprent & Gehlot, 2010) and are also widely used for livestock feeding. In fact, Adams *et al.* (2016) showed that high leaf N_{mass} and N_{area} acquired through symbiosis with N₂-fixing bacteria present in their roots (Powers & Tiffin, 2010; Vitousek *et al.*, 2013; Song *et al.*, 2015), allows legumes to use water more efficiently than non-legumes at global scale. Furthermore, production and accumulation of N-rich osmolytes can also help dryland legumes cope with drought through enhanced internal osmotic adjustment that lowers plant water potentials, thereby increasing plant water uptake from drying soil (Wink, 2013). However, the extent to which optimization of water-use efficiency in dryland woody legumes is achieved primarily through tighter stomatal regulation or via enhanced carbon assimilation capacity (or both) remains unclear (but see Adams *et al.*, 2018a,b for herbaceous and crop legumes).

Carbon, oxygen and nitrogen stable isotopic composition of leaf material (leaf δ¹³C, δ¹⁸O and δ¹⁵N, respectively), along with xylem water isotopic composition (δ²H and δ¹⁸O), have become key traits that integrate information about plant-resource acquisition and how plants interact with and respond to their abiotic and biotic environments (Dawson *et al.*, 2002; Barbour, 2007; Prieto *et al.*, 2018; Querejeta *et al.*, 2018). Leaf δ¹³C and δ¹⁸O in dryland plants can provide reliable proxies of time-integrated intrinsic water-use efficiency (WUE_i) and g_s, respectively (Ehleringer, 1993; Williams & Ehleringer, 1996; Barbour, 2007). Leaf δ¹³C in C₃ species is negatively and linearly correlated with the time-integrated c_i/c_a ratios during photosynthesis (Farquhar *et al.*, 1989) and reflects the relationship between net photosynthetic rate (A) and g_s, thereby providing a robust indicator of time-integrated intrinsic water-use efficiency (WUE_i = A/g_s) during the growing season (Dawson *et al.*, 2002). Leaf δ¹⁸O is influenced by the isotopic composition of the water source used by the plant (Sarris *et al.*, 2013; Ding *et al.*, 2021). The isotopic signal of the water source is thereafter modified by leaf-level evaporative effects, including stomatal responses to changes in atmospheric relative humidity and soil moisture, making leaf δ¹⁸O a good proxy of time-integrated stomatal conductance (g_s) and cumulative transpiration in dryland species (Querejeta *et al.*, 2006; Barbour, 2007; Ramírez *et al.*, 2009; Prieto *et al.*, 2018). The enrichment in ¹⁸O of leaf dry matter above the δ¹⁸O value of the source water used by the plant (leaf Δ¹⁸O) helps to remove the signal of interplant variation in water sources and is thus useful to estimate differences in stomatal regulation among coexisting species exposed to similar environmental conditions (Barbour, 2007). Overall, the

combined measurement of leaf δ¹⁸O/Δ¹⁸O and δ¹³C (hereafter leaf δ¹³C-WUE_i) can help in assessing variations in photosynthetic capacity, stomatal conductance, WUE_i and overall water-use strategy (from conservative to profligate) among coexisting dryland species exposed to similar environmental conditions (Moreno-Gutiérrez *et al.*, 2012; Prieto *et al.*, 2018).

In this study, we addressed how interspecific variation in leaf N_{area} modulates leaf carbon and oxygen isotopic composition across 34 woody species encompassing diverse phylogenies and leaf habits in the Sahel. In particular, we hypothesized that leaf δ¹³C and δ¹⁸O values will be positively correlated across dryland species as a result of the shared dependence of both traits on stomatal conductance, and will converge towards a relatively narrow range of values constrained by the harsh climatic and soil conditions (Paillasa *et al.*, 2020); and that higher leaf N_{area} will be associated with higher leaf δ¹⁸O and δ¹³C values across dryland species, indicating greater carboxylation capacity, tighter stomatal regulation with lower conductance and higher WUE_i (Wright *et al.*, 2001, 2003). We further aimed to elucidate whether higher leaf δ¹³C-WUE_i with increasing leaf N_{area} in dryland species might be achieved through higher carboxylation capacity, allowing lower stomatal conductance for any given photosynthetic rate (resulting in a strong influence of leaf N_{area} on both δ¹⁸O and δ¹³C), or primarily through enhanced carboxylation capacity but with little or no impact on stomatal conductance (resulting in weak or no influence of leaf N_{area} on leaf δ¹⁸O). These hypotheses were analyzed and tested with structural equation modeling (SEM; Fig. 1) using a dataset of leaf and stem traits collected on 34 Sahelian woody species. We thereafter compared legumes (Fabaceae) vs nonlegumes (Table 1) and hypothesized that legumes would exhibit higher leaf δ¹⁸O and δ¹³C-WUE_i values and greater drought tolerance than nonlegumes thanks to higher leaf N_{area} achieved through symbiotic atmospheric N₂ fixation. We also hypothesized that dryland legumes would fix large amounts of atmospheric N₂, resulting in distinct leaf δ¹⁵N values near 0‰, whereas co-occurring nonlegumes would show high leaf δ¹⁵N values typical of plants using ¹⁵N-enriched soil N sources in hot and dry environments (Amundson *et al.*, 2003; Aranibar *et al.*, 2004; Craine *et al.*, 2009, 2015).

Materials and Methods

Study sites

The study was conducted in silvopastoral and agroforestry ecosystems of the western Sahel region. Sampling sites were selected near Louga (15°37'N, 16°13'W) in northwest Senegal (seven sites), and near Ségou (13°27'N, 6°16'W) in south-central Mali (eight sites). In this region, rain falls mainly during the monsoon season (June–October), followed by a dry season between November and June (Supporting Information Fig. S1). Louga has a semiarid sub-Canarian climate (Wade, 1997) with a mean temperature of 24.9°C and average annual rainfall of 330 mm (1950–2000). Both rain and the warmest temperatures occur mostly during the monsoon season (Edmunds & Gaye, 1994; NOAA, 2015). Ségou has a continental semiarid climate with

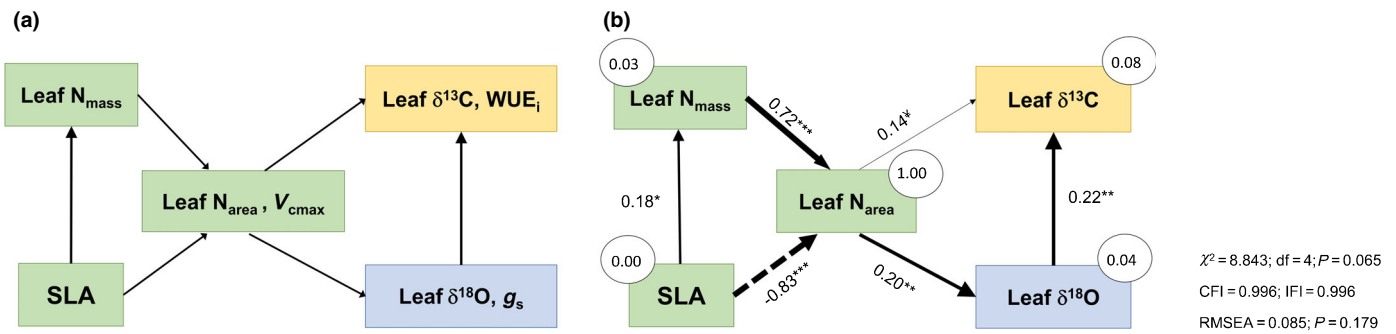


Fig. 1 Structural equation model based on the hypothesized effects of leaf N per area on time-integrated stomatal conductance (g_s) and intrinsic water-use efficiency (WUE_i) at leaf level in dryland woody species (Wright *et al.*, 2001; Adams *et al.*, 2016) in the Sahel. *A priori* set of hypotheses and relationships among variables (a) are described in the 'Materials and Methods' section. In this model, leaf N_{area} is considered as a surrogate of carboxylation capacity (V_{cmax}), leaf δ¹⁸O is a surrogate of stomatal conductance (g_s) and δ¹³C is a surrogate of WUE_i. Continuous and dashed black arrows (b) indicate positive and negative relationships between variables, respectively. Numbers adjacent to arrows indicate the effect size (standardized path coefficients, analogous to partial regression weights) and significance (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; [‡], marginally significant, $P = 0.074$) of the path; arrow thickness is proportional to the effect size ($n = 168$). Numbers within circles indicate squared multiple correlations for the variables. Overall goodness-of-fit tests (χ^2 , comparative fit index (CFI), incremental fit index (IFI) and root mean square error of approximation (RMSEA)) are shown at the bottom of the model. SLA, specific leaf area.

lower relative humidity than Louga, but has higher mean annual temperature (27.8°C) and precipitation (566 mm; NOAA, 2015; Fig. S1). Daily maximum temperatures in both areas frequently reach above 50°C. Soils in Louga and Ségou are highly weathered acidic sands on ancient dunes, inter-dune depressions or plains that typically show low water-holding capacity and organic matter content, and are particularly poor in phosphorus and other nutrients (Bitchibaly *et al.*, 2012). Relatively shallow groundwater can be found in both the Louga and Ségou sites studied. Vegetation of these agroforestry ecosystems is an open savannah with sparse trees and shrubs scattered across a grassland matrix and interspersed with croplands where typical management practices include harvesting of trees and shrubs, grazing and farming (IER, 2010; Konaté, 2010).

Sampling and trait data collection

We sampled leaves and stems from 230 individual trees and shrubs of 34 species, including 11 species from the Fabaceae family (legumes, Table 1). Both legumes and nonlegumes include evergreen species that retain a full canopy throughout the year and drought-deciduous species that remain leafless or partially leafless for several months during the dry season. The only exception is *Faidherbia albida*, a deciduous legume that sheds leaves during the rainy season (Diémé *et al.*, 2018). Species were assigned to leaf habit categories based on local expert knowledge (Diémé *et al.*, 2018, 2019) and descriptions in Arbonnier (2004). Plant samples were collected in the early dry season of 2011 before leaf senescence of deciduous species. Sampled trees and shrubs were healthy-looking and were at least 15 m far apart from each other (ranging between 0.015 and 60 km apart within each country; <http://wms.nina.no/FunciTree/>) and with their crowns fully exposed to sunlight.

From each plant, we sampled two sun-exposed branches from the eastern side of the canopy before dawn. One branch was placed in a sealed plastic bag within a dark hermetic bucket and was used for measuring stem predawn water potential (Ψ_{pd}) with a Scholander-type pressure bomb. Leaf thickness (mm), specific

leaf area (SLA; $m^2 kg^{-1}$), and leaf relative water content (RWC; $g g^{-1}$) were measured in fully expanded, mature, damage-free fresh leaves. Thickness was measured in three points in each leaf with a digital caliper and the mean value was recorded. The leaf collected to measure RWC was first weighed (FW), then fully rehydrated overnight in the dark, weighed again (hydrated weight, HW) and then scanned, and leaf area was then measured with IMAGEJ. Leaves were then oven-dried at 60°C for 72 h and weighed again (DW). SLA is the one-sided area (leaf area, LA) of the fully rehydrated leaf divided by its dry mass ($SLA = LA / DM$), while leaf RWC is the difference between leaf FW and DW divided by the difference between fully hydrated weight and leaf DW (i.e. $RWC = (FW - DW) / (HW - DW)$). Dry leaves were then ground using a ball-mill to determine C and N concentrations (mass based) and δ¹³C, δ¹⁵N and δ¹⁸O composition. Leaf N_{area} ($mg cm^{-2}$) was calculated as the ratio between leaf N_{mass} and SLA. From the second branch, we cut a terminal 8 cm leafless woody stem which was immediately placed in a screw-cap polypropylene vial and sealed with Parafilm. Vials were transported in a cooler to the laboratory within 4 h and stored frozen. Xylem water was extracted using cryogenic vacuum distillation (Ehleringer & Osmond, 1989). The oxygen isotopic composition of xylem water (xylem water δ¹⁸O) helps in assessing the approximate depth of soil water uptake by roots in dryland ecosystems where steep vertical gradients in soil water δ¹⁸O develop during rainless periods (Moreno-Gutiérrez *et al.*, 2012). Evaporation from upper soil during hot, dry periods leads to heavy isotopic enrichment of the remaining soil water near the surface, which decreases steeply with depth (Allison & Hughes, 1983). Higher xylem water δ¹⁸O values indicate uptake of isotopically enriched water from shallower soil layers exposed to intense evaporation, whereas lower xylem water δ¹⁸O values indicate utilization of non-enriched water from deeper, less evaporated water sources (Querejeta *et al.*, 2007; Ding *et al.*, 2021).

Foliar δ¹³C can be used to estimate long-term ratios of the inter-cellular to ambient CO₂ values (c_i/c_a) if the carbon isotope ratio of atmospheric CO₂ (δ¹³C_{air}) is known (Farquhar *et al.*, 1989). To calculate long-term c_i/c_a ratios, we first calculated Δ¹³C as:

Table 1 General characteristics of the tree and shrub species sampled in two semiarid agroforestry systems in the Sahel (Mali and Senegal).

Species	Family	Growth type	Leaf habit	DBH (m)	Height (m)	Mali (no. of trees)	Senegal (no. of trees)
<i>Acacia nilotica</i> (L.) Willd. ex Delile	Fabaceae	T	D	0.71 ± 0.11	5.80 ± 0.28	1	6
<i>Acacia senegal</i> (L.) Willd.	Fabaceae	T	D	0.19 ± 0.01	7.25 ± 1.75		2
<i>Acacia seyal</i> Delile	Fabaceae	T	D	0.52 ± 0.08	4.94 ± 0.30	6	2
<i>Acacia tortilis</i> spp. Raddiana (Forssk.) Hayne (Savi) Brenan	Fabaceae	T	D	0.27 ± 0.03	5.92 ± 1.05		6
<i>Adansonia digitata</i> L.	Malvaceae	T	D	2.46 ± 0.64	10.43 ± 0.88	7	6
<i>Annona senegalensis</i> Pers.	Annonaceae	S	D		0.97 ± 0.05		6
<i>Anogeissus leiocarpus</i> (DC.) Guill. & Perr.	Combretaceae	T	E	1.54 ± 0.07	10.18 ± 1.19	6	
<i>Aphania senegalensis</i> (Juss. ex Poir.) Radlk	Sapindaceae	T	E	0.29 ± 0.04	4.13 ± 0.88		2
<i>Balanites aegyptiaca</i> (L.) Delile	Zygophyllaceae	T	E	0.69 ± 0.12	5.98 ± 0.70	6	6
<i>Bauhinia rufescens</i> Lam.	Fabaceae	T	D	0.14 ± 0.00	4.25 ± 0.52		3
<i>Boscia senegalensis</i> (Pers.) Lam. ex Poir.	Capparaceae	S-T	E		1.71 ± 0.23		6
<i>Celtis integrifolia</i> Lam.	Ulmaceae	T	E	0.55 ± 0.08	11.42 ± 1.04		6
<i>Combretum glutinosum</i> Perr. ex DC.	Combretaceae	T (S-T)	E	0.75 ± 0.17	7.11 ± 0.75	6	6
<i>Combretum micranthum</i> G. Don.	Combretaceae	S	D	0.17 ± 0.06	2.68 ± 0.28	6	
<i>Cordia sinensis</i> Lam.	Boraginaceae	T	E	0.11	6.00		1
<i>Crateva religiosa</i> Forst. f.	Capparaceae	T	E	0.33 ± 0.02	8.08 ± 0.96		3
<i>Diospyros mespiliiformis</i> Hochst. ex A. DC.	Ebenaceae	T	D	1.34 ± 0.13	6.91 ± 0.81	7	
<i>Faidherbia albida</i> (Delile) A. Chev.	Fabaceae	T	D	0.83 ± 0.17	9.04 ± 0.70	6	6
<i>Ficus gnaphalocarpa</i> (Miq.) Steud.	Moraceae	T	D	2.11 ± 0.43	8.53 ± 1.11	6	
<i>Guiera senegalensis</i> J. F. Gmel.	Combretaceae	S-T	D	0.12 ± 0.02	3.02 ± 0.10	6	
<i>Maytenus senegalensis</i> (Lam.) Exell	Celastraceae	S-T	D	0.12 ± 0.00	2.39 ± 0.44		5
<i>Neocarya macrophylla</i> (Sabine) Prance	Chrysobalanaceae	T	E	0.38 ± 0.04	6.08 ± 0.98		6
<i>Piliostigma reticulatum</i> (DC.) Hochst.	Fabaceae	T	E	0.79 ± 0.13	4.99 ± 0.26	6	2
<i>Prosopis africana</i> (Guill. & Perr.) Taub.	Fabaceae	T	D	2.00 ± 0.24	9.53 ± 1.36	6	
<i>Prosopis juliflora</i> (Sw.) DC.	Fabaceae	T	E	0.26 ± 0.03	8.17 ± 0.76		6
<i>Pterocarpus erinaceus</i> Poir.	Fabaceae	T	D	1.38 ± 0.11	7.78 ± 0.36	6	
<i>Saba senegalensis</i> (A. DC.) Pichon	Apocynaceae	T	E		4.20 ± 0.72	6	
<i>Sclerocarya birrea</i> (A. Rich.) Hochst.	Anacardiaceae	T	D	0.77 ± 0.13	7.98 ± 0.40	6	6
<i>Tamarindus indica</i> L.	Fabaceae	T	E	1.20 ± 0.23	8.78 ± 0.91	6	7
<i>Tamarix senegalensis</i> DC.	Tamaricaceae	T(S-T)	E		2.20 ± 0.21		6
<i>Terminalia laxiflora</i> Engl.	Combretaceae	T	D	1.70 ± 0.23	9.23 ± 1.14	6	
<i>Vitellaria paradoxa</i> C.F. Gaertn.	Sapotaceae	T	D	1.70 ± 0.14	9.83 ± 1.40	6	
<i>Vitex doniana</i> Sweet	Labiatae	T	D	2.02 ± 0.12	8.62 ± 0.62	5	
<i>Ziziphus mauritiana</i> Lam.	Rhamnaceae	S	E	0.43 ± 0.18	5.42 ± 0.44	3	6
Total						124	106

Species ($N = 34$), families, growth type (T, tree; S, shrub; S-T, spp. that can grow as a shrub or tree depending on the environmental conditions), leaf habit (E, evergreen ($N = 15$ spp.); D, deciduous ($N = 19$ spp.)), mean (\pm SE) diameter at breast height (DBH) and plant vegetative height (height) and number of sampled individuals.

$$\Delta^{13}\text{C} = \frac{1000(\delta^{13}\text{C}_{\text{air}} - \delta^{13}\text{C}_{\text{leaf}})}{1000 - \delta^{13}\text{C}_{\text{leaf}}} \quad \text{Eqn 1}$$

where $\delta^{13}\text{C}_{\text{air}}$ is the C isotopic composition of atmospheric CO_2 (-8.45‰ , Mauna Loa records; <http://www.esrl.noaa.gov/gmd/dv/ftpdata.html>) and $\delta^{13}\text{C}_{\text{leaf}}$ is the C isotopic composition of leaf material. Then, from $\Delta^{13}\text{C}$ values, we calculated c_i/c_a as:

$$\frac{c_i}{c_a} = \frac{\Delta^{13}\text{C} - a_s}{b - a_s} \quad \text{Eqn 2}$$

where a_s is the fractionation factor of gaseous diffusion (4.4‰) and b represents effective fractionation as a result of carboxylation (27‰), estimated empirically (Farquhar *et al.*, 1982).

Cryogenic vacuum distillation and stable isotope analyses of leaf and water samples were conducted at the Stable Isotope Ratio Facility for Environmental Research, University of Utah (USA). Leaf N and C concentrations and $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ were measured with an isotope ratio mass spectrometer (Finnigan Mat Delta+ IRMS, Waltham, MA, USA) coupled to an elemental analyzer (EA; Carlo Erba CHN EA1110, Waltham, MA, USA). Leaf $\delta^{18}\text{O}$ was measured with a Finnigan TC/EA IRMS. The $\delta^{18}\text{O}$ isotopic composition of xylem water was measured using a laser water isotope analyzer (Picarro L2130i, Santa Clara, CA, USA).

Data analysis

To investigate the influence of leaf N_{area} on $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ -WUE_i in dryland vegetation, we built an *a priori* structural

equation model (SEM) of hypothesized relationships within a path diagram (see the 'Introduction' section and Fig. 1a), allowing a causal interpretation of the model outputs (Grace, 2006). This *a priori* set of hypothesized relationships were as follows: high leaf N concentration (N_{mass}) and low SLA (i.e. higher LMA) both increase leaf N_{area} , which in turn enhances carboxylation capacity, allowing tighter stomatal regulation of transpiration and lower time-integrated stomatal conductance (Wright *et al.*, 2001, 2003). A key underlying assumption in this SEM model is that a higher leaf N_{area} enhances the leaf carboxylation capacity (V_{cmax}) and thus enables the leaf to achieve a given carbon assimilation rate with lower stomatal aperture and conductance under dry environmental conditions. In our *a priori* SEM model, leaf N_{area} is considered a proxy of V_{cmax} (Wright *et al.*, 2001, 2003), leaf $\delta^{18}\text{O}$ is considered a proxy of time-integrated stomatal conductance (inversely related, $1/g_s$; Barbour, 2007), and leaf $\delta^{13}\text{C}$ is a proxy of WUE_i (Farquhar *et al.*, 1989). The SEM was first tested with the experimental data collected in 34 dryland tree and shrub species from the Sahel ($n = 168$ individuals for which both SLA and N_{area} data were available; Fig. 1). Then, to rule out any potential confounding effects as a result of pooling together species of different leaf habits (deciduous vs evergreens) and with different proportions of legumes (eight legume species were deciduous and two were evergreen), we reanalyzed the dataset excluding evergreen species (Powers & Tiffin, 2010). Goodness of fit of the SEM model was assessed using the traditional χ^2 goodness-of-fit test, but because of its sensitivity to sample size, the Bentler comparative fit index (CFI) and the incremental fit index (IFI) and root mean square error of approximation (RMSEA) were also considered (Grace, 2006). For the SEM analysis, contrary to other statistical analyses, model P -values > 0.05 in the χ^2 and RMSEA indices (Schermerle-Engel *et al.*, 2003), and values close to 1 (> 0.90) for CFI and IFI indices are required to guarantee an acceptable fit (Hu & Bentler, 1999).

We did not account for spatial variability among sampling sites in the SEM, but performed pairwise regression analyses between traits using linear mixed regression models with sampling site included as a random factor. Differences in plant traits between legumes and nonlegumes were analyzed with linear mixed models where the main fixed factor was Fabaceae/nonFabaceae. Geographic area (i.e. sampling site) was included as a random factor. Normality of residuals and homogeneity of variances assumptions were assessed by graphical inspection of residuals, and when these assumptions were not met (i.e. for Ψ_{pd}), we used a model correction for heterogeneity of variance (*varExp*), which represents an exponential structure of the variance-covariance matrix (Gałeczki & Burzykowski, 2013). *Post hoc* differences were tested with Fisher least significant difference tests.

All statistical analyses were performed with R software (R Core Team, 2019) interfaced by INFOSTAT statistical software v.2020 (Di Rienzo *et al.*, 2020) using the packages LME4 (Bates *et al.*, 2015) and NLME (Pinheiro *et al.*, 2014). Structural equation modeling analyses were carried out with the AMOS extension in SPSS (Arbuckle, 2014).

Results

Trait coordination across dryland woody species

Our pool of 34 woody species (mean height = 7.3 m; Table 1) encompassed a remarkably wide range of leaf N_{area} , N_{mass} , $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ values, thereby revealing large interspecific differences in carboxylation capacity, stomatal regulation of transpiration, c_i/c_a ratios and WUE_i (Table 2). Sahelian trees and shrubs also showed large interspecific variability in SLA, leaf thickness, leaf RWC, $\delta^{15}\text{N}$ and $\Delta^{18}\text{O}$, xylem water $\delta^{18}\text{O}$, and predawn water potentials (Fig. 2; Table S1). Whereas interspecific differences accounted for most of the variability in trait values (Fig. 2), intraspecific variability across sites was also rather high for some traits (Table S1 and indirectly shown in Fig. 2), suggesting large phenotypic trait variability in response to environmental heterogeneity. We did not detect any significant phylogenetic signal (λ) in any of the measured traits (Table S2).

Leaf $\delta^{18}\text{O}$ was unrelated to xylem water $\delta^{18}\text{O}$ across species and sites, indicating that variation in leaf $\delta^{18}\text{O}$ was not primarily driven by inter-plant differences in source water isotopic composition or root water uptake depth. Instead, both foliar $\delta^{18}\text{O}$ and $\Delta^{18}\text{O}$ were positively related to leaf $\delta^{13}\text{C}$ (Fig. 3; Table 3), indicating that foliar oxygen isotopic composition primarily reflected leaf-level evaporative effects related to stomatal regulation and time-integrated stomatal conductance and cumulative transpiration (Fig. 3). Moreover, both foliar $\delta^{18}\text{O}/\Delta^{18}\text{O}$ and $\delta^{13}\text{C}$ correlated positively with leaf N_{area} , revealing tighter stomatal regulation and higher WUE_i with increasing carboxylation capacity (i.e. leaf N_{area} ; Fig. 3; marginally significant for leaf $N_{\text{area}}-\delta^{13}\text{C}$ relationship). Furthermore, leaf $\delta^{18}\text{O}$ and $\Delta^{18}\text{O}$ correlated closely with RWC (Fig. 3; Table 3), suggesting that tighter stomatal regulation and lower time-integrated stomatal conductance are linked to improved leaf hydration during the early dry season in dryland species.

Modulation of water-use efficiency by leaf N_{area} is achieved through both enhanced carboxylation capacity and tighter stomatal regulation in dryland trees

The SEM analysis (Fig. 1) indicated that both higher leaf N concentration on a mass basis (N_{mass}) and lower SLA (i.e. higher LMA) contributed to enhance leaf N_{area} in dryland trees and shrubs. As predicted by theory and global datasets, leaf N_{mass} and SLA ($1/\text{LMA}$) were positively related to each other across species (e.g. Wright *et al.*, 2004). Furthermore, the SEM analysis revealed two simultaneous pathways that explained the positive relationship between leaf N_{area} and $\delta^{13}\text{C}$ - WUE_i in dryland species (Fig. 1b). A main SEM pathway linking leaf N_{area} , $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ highlighted a strong influence of leaf N_{area} on foliar $\delta^{18}\text{O}$ ($\beta = 0.20$, $P < 0.01$), indicating tighter stomatal regulation and lower time-integrated stomatal conductance with increasing leaf N_{area} , which in turn contribute to enhanced WUE_i through leaf $\delta^{18}\text{O}$ /stomatal effects on $\delta^{13}\text{C}$ - WUE_i ($\beta = 0.22$, $P < 0.01$). Leaf N_{area} also had a smaller direct positive effect on leaf $\delta^{13}\text{C}$ ($\beta = 0.14$, $P = 0.07$; marginally significant; Fig. 1b) that

Table 2 Description, number of trees and range of values (min to max) for the 10 functional traits measured in 34 woody species in the Sahel (Mali and Senegal) and their key role in plant functioning.

Trait	Abbreviation	Units	<i>N</i>	Range	Key role in plant functioning/interpretation
Leaf carbon isotopic composition	Leaf $\delta^{13}\text{C}$	‰	217	−31.5 to −24.6	Time-integrated water-use efficiency
Ratio of intercellular and ambient CO_2 concentrations	c_i/c_a	Unitless	217	0.57 to 0.91	CO_2 carboxylation capacity, stomatal conductance
Leaf nitrogen concentration	Leaf N_{mass}	%	216	1.32 to 4.80	Photosynthetic and carboxylation capacity, osmoregulation, nutritional quality
Leaf nitrogen area	Leaf N_{area}	mg cm^{-2}	169	0.08 to 0.98	Carboxylation capacity
Leaf oxygen isotopic composition	Leaf $\delta^{18}\text{O}$	‰	211	19.02 to 34.53	Time-integrated stomatal conductance, cumulative transpiration
Leaf oxygen isotopic enrichment above source water	Leaf $\Delta^{18}\text{O}$	‰	199	23.58 to 42.08	Time-integrated stomatal conductance, cumulative transpiration
Predawn stem water potential	Ψ_{pd}	MPa	224	−5.59 to −0.28	Soil water availability, rooting depth, osmolyte accumulation in plant tissues
Relative water content	RWC	%	221	61.1 to 96.4	Leaf water status and hydration
Leaf thickness	Leaf Thickness	mm	207	0.12 to 0.62	Leaf gas exchange, water retention
Specific leaf area	SLA	$\text{m}^2 \text{kg}^{-1}$	175	4.25 to 18.63	Photosynthetic rate, light capture, relative growth rate
Leaf nitrogen isotopic composition	Leaf $\delta^{15}\text{N}$	‰	217	3.42 to 12.9	Atmospheric N_2 fixation, soil N sources
Oxygen isotopic composition of xylem water	Xylem water $\delta^{18}\text{O}$	‰	211	−8.13 to 1.20	Water sources, depth of soil water uptake

Description of traits and key role in plant function are based on Wright *et al.* (2001, 2005); Barbour (2007); Bernard-Verdier *et al.* (2012); Perez-Harguindeguy *et al.* (2013), and Craine *et al.* (2015).

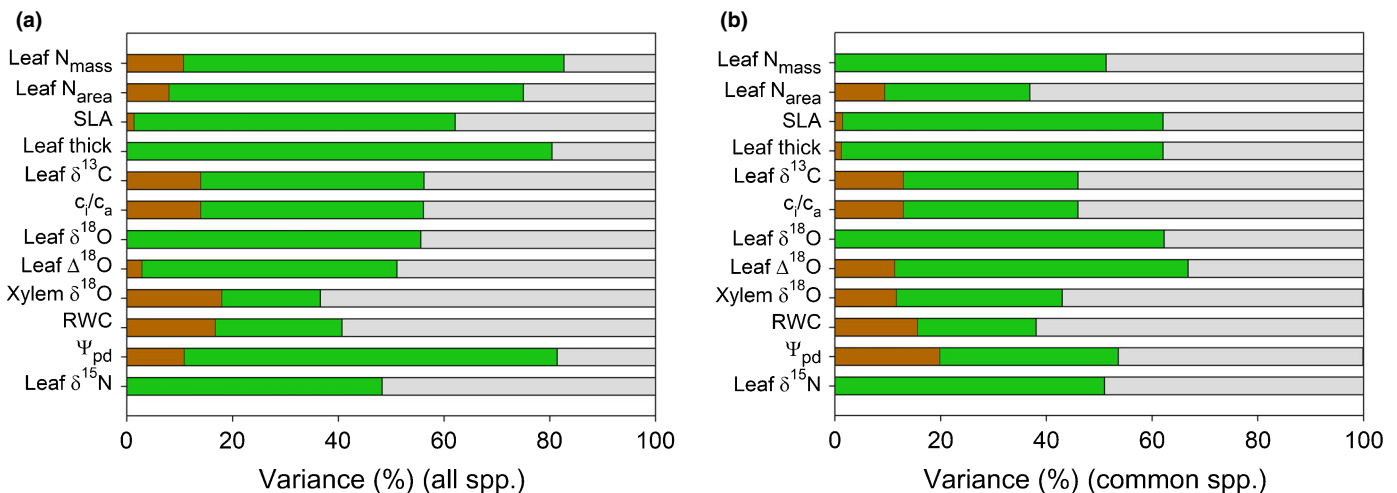


Fig. 2 Estimated variance decomposition for 12 functional traits measured in woody species in the Sahel: (a) in the 34 woody species measured; and (b) in nine species common to the two sites sampled (Ségou, Mali, and Louga, Senegal; see Table 1 and Supporting Information Table S1). Variance partitioning between sites (15 sample sites, brown) and species (interspecific, green) is shown; residual variance is also shown (light gray). Leaf trait abbreviations and units are as follows: leaf N_{mass} , leaf nitrogen concentration on a mass basis (%); leaf N_{area} (mg cm^{-2}), leaf N content per leaf area; SLA ($\text{m}^2 \text{kg}^{-1}$), specific leaf area; leaf thick, leaf thickness (mm); leaf $\delta^{13}\text{C}$, leaf carbon isotopic composition (‰); c_i/c_a , ratio of intercellular and ambient CO_2 concentrations (unitless); leaf $\delta^{18}\text{O}$, leaf oxygen isotopic composition (‰); leaf $\Delta^{18}\text{O}$, leaf ^{18}O enrichment above source (xylem) water (‰); xylem $\delta^{18}\text{O}$, xylem water oxygen isotopic composition (‰); RWC, leaf relative water content (g g^{-1}); Ψ_{pd} , predawn stem water potential (MPa); leaf $\delta^{15}\text{N}$, leaf nitrogen isotopic composition (‰).

was unrelated to stomatal effects, which we interpret as an indication of enhanced carboxylation capacity with increasing N_{area} across species (as predicted by theory) that further contributes to increase WUE_i .

Pairwise regression analyses between leaf traits further supported the key role of a high leaf N_{area} in enhancing carboxylation capacity, thereby allowing a tighter stomatal regulation of transpiration (i.e. lower time-integrated stomatal conductance) and higher WUE_i . It is worth highlighting that, in addition to

the positive pairwise relationship between leaf N_{area} and $\delta^{13}\text{C}$ (Figs. 1, 3a; marginally significant), there was also a stronger positive pairwise relationship between leaf N_{mass} and $\delta^{13}\text{C}$ (Table 3). Leaf N_{area} was also positively correlated with leaf $\delta^{15}\text{N}$, and negatively correlated with predawn water potential (Table 3), the latter suggesting greater tree ability to sustain more negative water potentials probably through internal plant osmoregulation linked to accumulation of N-based osmolytes (Fig. 3c). Pairwise regression analyses also revealed a strong

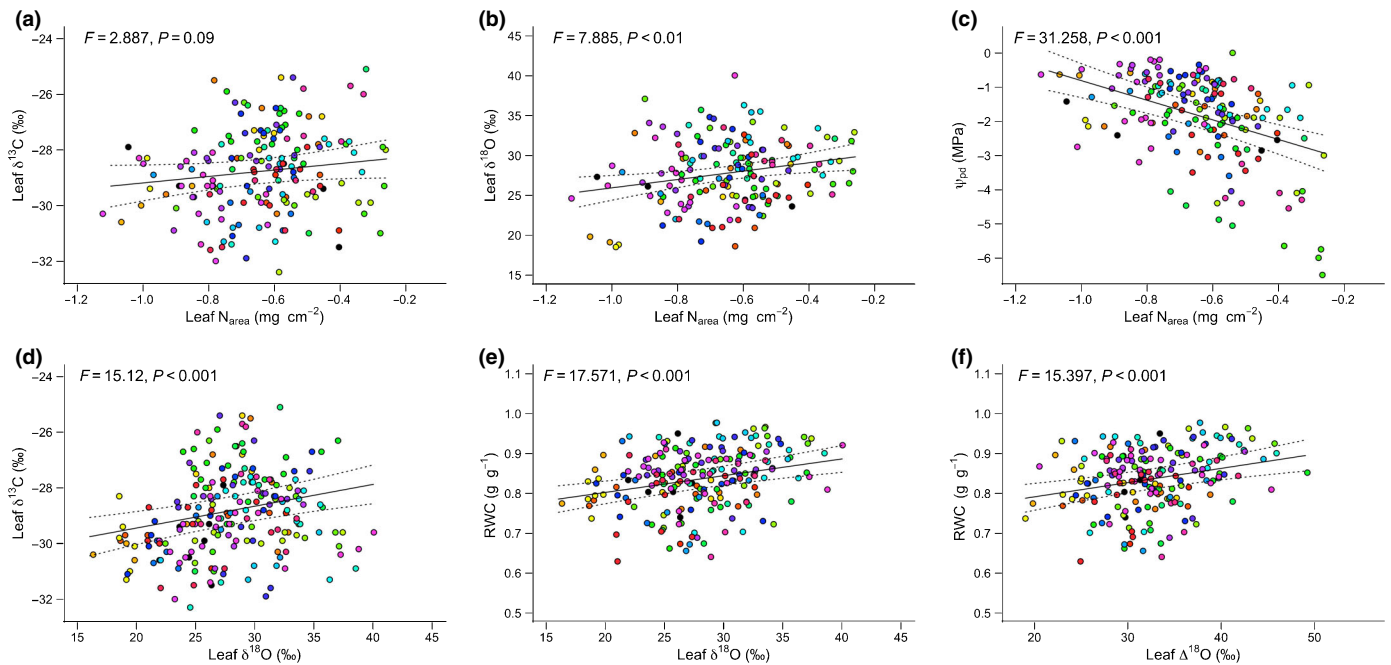


Fig. 3 Relationships between different pairs of traits measured in 34 woody species in the Sahel ($n = 168\text{--}230$ individuals): relationship between leaf N_{area} and leaf carbon isotopic composition ($\delta^{13}\text{C}$) (a), leaf oxygen isotopic composition ($\delta^{18}\text{O}$) (b) and predawn stem water potential (Ψ_{pd}) (c), between leaf $\delta^{13}\text{C}$ and leaf $\delta^{18}\text{O}$ isotopic composition (d), and between relative water content (RWC) and leaf $\delta^{18}\text{O}$ (e) and leaf oxygen isotopic enrichment above source (xylem) water ($\Delta^{18}\text{O}$) (f). Solid lines represent the model-predicted relationships, and dotted lines the 95% confidence intervals. Colored dots depict different species. Log-transformed N_{area} values (negative scale) are shown in panels (a–c).

Table 3 Results from linear mixed regression models for pairwise relationships between key traits analyzed in this study (many of them included in the *a priori* structural equation modeling; see Fig. 1) for the whole dataset ($N = 34$ species).

Predictor variable	Response variable	Slope	SE	F-value	P-value
Leaf N_{area}	Leaf $\delta^{13}\text{C}$	1.162	0.684	2.887	<i>0.091</i>
Leaf N_{area}	Leaf $\delta^{18}\text{O}$	5.247	1.869	7.885	0.005
Leaf N_{area}	Leaf $\Delta^{18}\text{O}$	5.614	2.362	5.648	0.019
Leaf N_{area}	Ψ_{pd}	-2.869	0.513	31.258	< 0.001
Leaf N_{area}	RWC	0.020	0.033	0.368	0.545
Leaf N_{area}	Leaf $\delta^{15}\text{N}$	1.762	0.520	11.46	< 0.001
Leaf N_{mass}	Leaf $\delta^{13}\text{C}$	0.312	0.116	7.269	0.007
Leaf N_{mass}	Leaf $\delta^{18}\text{O}$	1.674	0.533	9.883	0.002
Leaf N_{mass}	Leaf $\Delta^{18}\text{O}$	1.377	0.654	4.437	0.036
Leaf N_{mass}	Ψ_{pd}	-0.514	0.125	17.048	0.000
Leaf N_{mass}	RWC	-0.008	0.008	-0.923	0.357
Leaf N_{mass}	Leaf $\delta^{15}\text{N}$	0.447	0.205	4.756	0.030
Leaf $\delta^{18}\text{O}$	Ψ_{pd}	-0.007	0.015	0.177	0.674
Leaf $\delta^{18}\text{O}$	RWC	0.004	0.001	17.57	< 0.001
Xylem water $\delta^{18}\text{O}$	Leaf $\delta^{18}\text{O}$	-0.057	0.122	0.218	0.641
Leaf $\delta^{13}\text{C}$	Ψ_{pd}	-0.053	0.052	1.048	0.307
Leaf $\delta^{13}\text{C}$	RWC	-0.002	0.003	0.342	0.559
Leaf $\delta^{13}\text{C}$	Leaf $\delta^{18}\text{O}$	0.758	0.213	12.641	< 0.001

Shown are the slope and standard error of the slope (SE) and F - and P -values (significant relationships, i.e. $P < 0.05$, are in bold, and marginal significant relationships are in italics). Trait abbreviations are as in Table 2.

positive relationship between leaf $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ across dryland species (Fig. 3d), further evidencing a shared stomatal control on both traits. Moreover, tighter stomatal regulation

and lower time-integrated stomatal conductance (inferred from higher leaf $\delta^{18}\text{O}$ and $\Delta^{18}\text{O}$ values) were linked to improved leaf hydration and higher leaf relative water content in dryland woody species (Fig. 3e,f).

The majority of N-fixing species in our dataset were drought deciduous (Table 1), so we thereafter tested the robustness of the described links among traits within the subset of drought deciduous species only, in order to ascertain that the potentially confounding effects of contrasting leaf phenology (evergreen/deciduous) were not biasing the results of the SEM analyses (while at the same encompassing the full range of N_{area} and N_{mass} values present in the dataset). Within the subset of deciduous species (i.e. excluding evergreens), SEM analyses revealed very similar links among traits; however, the overall fit of the SEM analysis was poorer as a result of low statistical power ($n = 87$; data not shown). Anyhow, pairwise relationships between variables for deciduous species were also similar to those found in the whole dataset (Table S3), which further confirms the robustness of the described patterns.

Differences in plant traits between dryland legumes and nonlegumes

Dryland legumes (Fabaceae) had, on average, higher leaf N_{mass} , SLA and $\delta^{18}\text{O}/\Delta^{18}\text{O}$ values, and thinner, less sclerophyllous leaves compared with nonlegumes (Fig. 4). By contrast, legumes and nonlegumes did not differ significantly in leaf N_{area} , $\delta^{13}\text{C}$ (Fig. 4) or leaf RWC ($F_{1,212} =$

0.67; $P = 0.414$), predawn water potential of stems ($F_{1,215} = 1.35$; $P = 0.247$), xylem water $\delta^{18}\text{O}$ values ($F_{1,202} = 1.64$; $P = 0.202$) or average tree height ($F_{1,219} = 1.19$; $P = 0.276$).

When considering only deciduous species ($n = 19$ spp.), legumes had marginally higher leaf $\delta^{13}\text{C}$ -WUE_i (Fig. S2) than nonlegumes, along with higher leaf N_{mass} and $\delta^{18}\text{O}/\Delta^{18}\text{O}$ values and thinner leaves. Differences in leaf N_{mass} between legumes

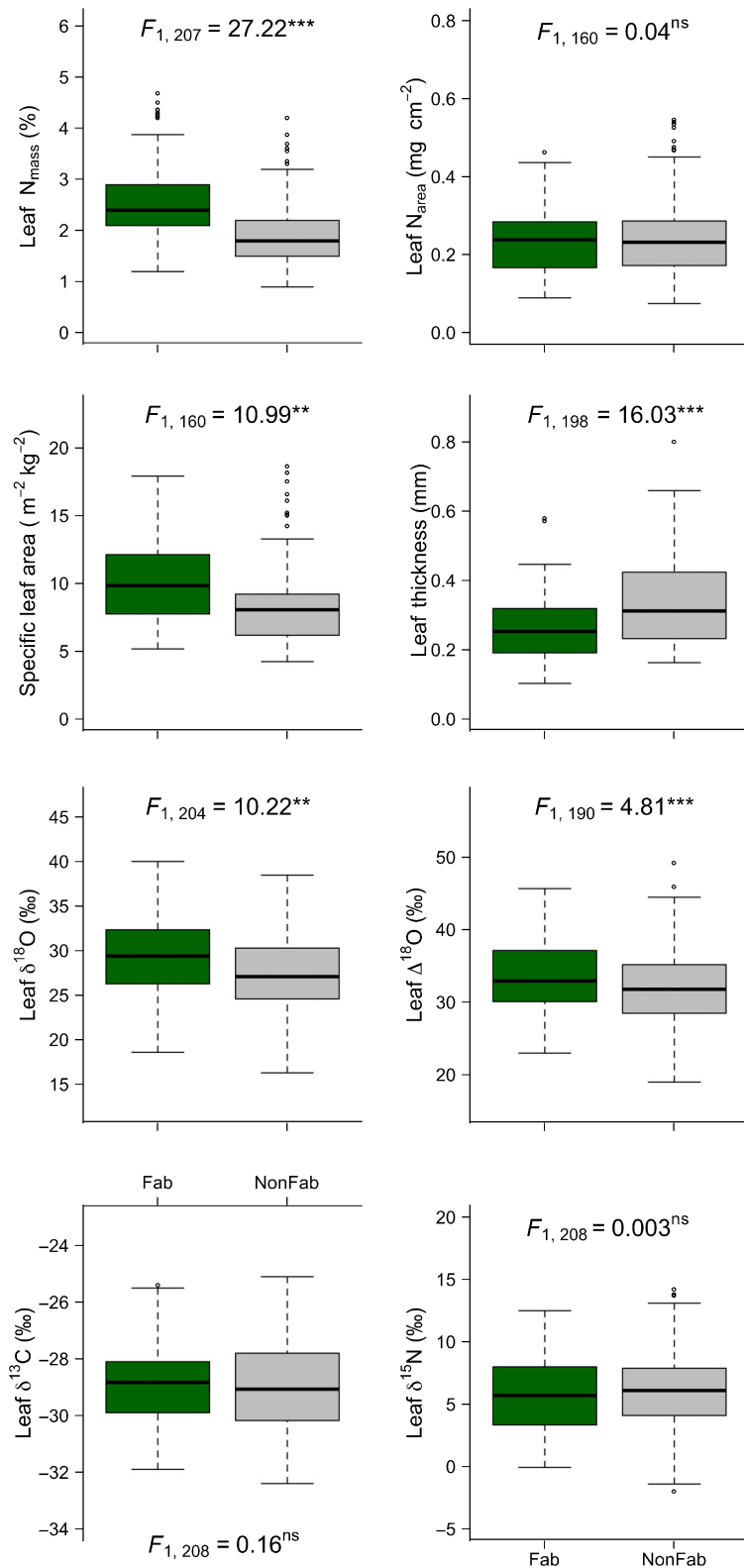


Fig. 4 Differences in average trait values between legume and nonlegume species from the Sahel. From left to right panels and from the top to the bottom panels: leaf N content on a mass basis (leaf N_{mass}), leaf N content per leaf area (leaf N_{area}), specific leaf area (SLA), leaf thickness, leaf oxygen isotopic composition (leaf $\delta^{18}\text{O}$), leaf oxygen enrichment above source (xylem) water (leaf $\Delta^{18}\text{O}$), leaf carbon isotopic composition (leaf $\delta^{13}\text{C}$), and leaf N isotopic composition (leaf $\delta^{15}\text{N}$) measured in nonlegumes (nonFabaceae, nonFab) and legumes (Fabaceae species, Fab). Values included in each panel are F -Fisher results (F_{n_1, n_2} , where n_1 and n_2 are degrees of freedom) of the linear mixed models testing differences between nonFab and Fab species and model significance (**, $P < 0.01$; ***, $P < 0.001$; ns, nonsignificant differences). Results are shown as boxplots that include different components: midline, median; upper and lower box edges, first and third quartiles; whiskers, 5% and 95% confidence intervals; points, outliers.

and nonlegumes were larger in deciduous species than in the whole dataset, which includes evergreens (0.75% vs 0.39% higher N_{mass} in Fabaceae, deciduous vs all spp., respectively). Compared with deciduous nonlegumes (nonFab), deciduous legumes (Fab) also had significantly lower predawn water potentials (-1.41 ± 0.36 vs -2.02 ± 0.36 MPa, nonFab vs Fab; $F_{1,114} = 22.82$, $P < 0.0001$) and higher xylem water $\delta^{18}\text{O}$ values (-4.75 ± 0.47 vs -3.78 ± 0.51 ‰, nonFab vs Fab; $F_{1,106} = 4.04$, $P = 0.047$), suggesting colonization of drier microsites and use of shallower, more evaporatively enriched soil water sources by deciduous legumes.

Discussion

Variation in SLA, leaf N_{mass} , N_{area} and water-related isotope traits ($\delta^{13}\text{C}$, $\delta^{18}\text{O}$, $\Delta^{18}\text{O}$) among Sahelian woody plants was strikingly large, revealing large interspecific differences in the relative costs of nitrogen and water acquisition. This finding can be interpreted in light of the least-cost economic theory of photosynthesis, showing that nitrogen and water are mutually substitutable resources to achieve a given photosynthetic carbon gain, so that the cost associated with acquiring one of them can be alleviated by increased supply of the other (Wright *et al.*, 2001, 2003; Prentice *et al.*, 2014; Wang *et al.*, 2017). Therefore, a high photosynthesis can be achieved by either high leaf N_{area} or high stomatal conductance, so that the total cost per unit photosynthetic carbon gain associated with carboxylation and transpiration is minimized under the environmental conditions prevailing in the plant's habitat (Paillassa *et al.*, 2020). Within this optimality framework, the positive foliar $N_{\text{area}}-\delta^{18}\text{O}$ and $N_{\text{area}}-\delta^{13}\text{C}$ correlations found across Sahelian plants are in agreement with the theory's prediction that a high leaf N_{area} should allow higher carboxylation capacity, tighter stomatal regulation of transpiration for any given carbon assimilation rate and more efficient use of water in dryland vegetation (Wright *et al.*, 2001, 2003). The structural equation model analysis revealed that the link between leaf N_{area} and $\delta^{13}\text{C}$ -WUEi was driven by an enhanced carboxylation capacity but even more strongly by a tighter stomatal regulation of transpiration (inferred from leaf $\delta^{18}\text{O}$) with increasing leaf N_{area} . To our knowledge, this is the first study demonstrating a robust mechanistic link between leaf N content per area and foliar oxygen isotope composition in dryland vegetation, which is fully consistent with first principles of plant physiology (Wright *et al.*, 2001, 2003; Dong *et al.*, 2017).

Our pool of 34 phylogenetically diverse tree and shrub species spanned *c.* 70% of the range of $\delta^{13}\text{C}$ values commonly encountered in C3 vegetation at global scale (-22 to -32 ‰; Kohn, 2010; Maire *et al.*, 2015; Cornwell *et al.*, 2018; Paillassa *et al.*, 2020). The hot and dry climate with a short rainy season and the nutrient-poor sandy soils and flat terrain of low elevation (< 300 m asl) that prevail across our sampling sites appear to offer limited potential for environmental heterogeneity and niche segregation that could favor such wide c_i/c_a divergence among coexisting Sahelian woody species. However, more subtle environmental heterogeneity regarding microtopography, groundwater accessibility to roots, soil fertility or microclimatic conditions across

and within sampling sites may create opportunities for niche segregation and physiological divergence among species (Álvarez-Yépez *et al.*, 2017). Environmental heterogeneity could also explain the large within-species variability encountered for some key traits, including leaf N_{area} , $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ (Donovan *et al.*, 2007). Nonetheless, the strikingly large interspecific differences in trait values encountered in this study primarily reflect idiosyncratic differences in leaf physiology and resource use strategies among coexisting species, and must therefore have an evolutionary basis (Fig. 2).

The average c_i/c_a ratio of Sahelian woody species (0.74) was unexpectedly high considering their rather high leaf N concentrations ($> 2\%$ on average), N_{area} values and the high vapor pressure deficit (VPD) prevailing across sampling sites (for comparison, see the average c_i/c_a ratios typically found in drylands as reported by Prentice *et al.* (2014) and Paillassa *et al.* (2020)). High leaf N_{area} and carboxylation capacity combined with high VPD would be expected to favor tight stomatal regulation of leaf gas exchange, leading to very low c_i/c_a ratios (Cernusak *et al.*, 2013; Cornwell *et al.*, 2018; Grossiord *et al.*, 2020; Paillassa *et al.*, 2020). However, extremely hot temperatures combined with the high O_2 partial pressure at low elevations may boost photorespiration in C₃ plants, thereby constraining photosynthetic nitrogen-use efficiency and preventing further decreases in c_i (Paillassa *et al.*, 2020; but see also Terashima *et al.*, 1995). Moreover, low availability of soil P or other essential nutrients for photosynthesis as a result of poor soil fertility may further constrain carbon assimilation capacity despite high leaf N_{area} (Augusto *et al.*, 2017; Du *et al.*, 2020; Paillassa *et al.*, 2020). Alternatively, the high c_i/c_a ratios observed in our study may simply reflect that most of the annual carbon assimilation in Sahelian species takes place during the short rainy season when high soil water availability may help to sustain high stomatal conductance, but with little additional carbon assimilation during the drier part of the year (Choat *et al.*, 2006). Finally, taproot access to relatively shallow groundwater may have further contributed to higher-than-expected average c_i/c_a ratios, particularly for some phreatophytic species that can behave as profligate water users under hot and high VPD conditions (Gries *et al.*, 2003).

The leaf $\delta^{18}\text{O}$ and $\Delta^{18}\text{O}$ values of Sahelian woody species were generally lower than those of trees and shrubs from other drylands with less extreme temperature, VPD and more fertile soils (e.g. semiarid Mediterranean ecosystems; Cernusak *et al.*, 2005; Moreno-Gutiérrez *et al.*, 2012, 2015). This suggests rather high stomatal conductance during the short growing season, possibly linked to the need for evaporative leaf cooling to prevent leaf overheating and damage of the photosynthetic machinery under extremely hot conditions in the Sahel (Helliker & Richter, 2008; Aparecido *et al.*, 2020). Moreover, high stomatal conductance may be needed to enhance nutrient harvesting through transpiration-driven mass flow of soil nutrients to roots in these low-fertility sandy soils (Cramer *et al.*, 2009; Matimati *et al.*, 2014; Salazar-Tortosa *et al.*, 2018). Modeling studies at a global scale have shown that stomatal conductance should reach peak levels in dry tropical vegetation (savannah trees; Lin *et al.*, 2015). The high leaf N_{area} combined with low leaf $\delta^{18}\text{O}$, $\Delta^{18}\text{O}$ and

$\delta^{13}\text{C}$ values of Sahelian woody plants suggests that they are capable of achieving high rates of transpiration and carbon assimilation during the short rainy season (Cornwell *et al.*, 2018; Sibret *et al.*, 2021). Sahelian woody vegetation may thus contribute substantially to global primary productivity, despite the severe water and nutrient limitations typical of these drylands (Ahlström *et al.*, 2015; Smith *et al.*, 2019; Brandt *et al.*, 2020).

High foliar N content can reflect the production and accumulation of N-based osmolytes, such as proline, that enable plants to endure and sustain more negative internal water potentials during drought (Wink, 2013; Adams *et al.*, 2016), as suggested by the strong negative correlation found between foliar N_{mass} and predawn water potential across species (Table 3). Accumulation of N-based osmolytes enhances internal plant osmotic adjustment and thus the ability of N-rich species to extract water held at progressively lower soil moisture potentials during dry periods, potentially conferring a competitive advantage in drylands (Giordano *et al.*, 2011). Lower predawn water potentials with increasing foliar N content may thus indicate greater drought tolerance and a superior ability to establish in drier microsites in N-rich species such as legumes.

Limited N_2 fixation in dryland legumes

The wide range of leaf $\delta^{15}\text{N}$ values encountered among Sahelian woody species revealed the existence of a wide diversity of N-acquiring mechanisms and N sources (Craine *et al.*, 2008). Sahelian trees and shrubs exhibited remarkably high foliar $\delta^{15}\text{N}$ values, suggesting high rates of gaseous N losses from the soil system (i.e. through ammonia volatilization, nitrification and denitrification) that could explain the heavy ^{15}N enrichment of leaf nitrogen pools despite vegetation dominance by N_2 -fixing legumes (Craine *et al.*, 2008, 2015; Ruiz-Navarro *et al.*, 2016). High rates of gaseous N losses from the soil system may be greatly favored by hot temperatures and transiently moist soil conditions during the short Sahel rainy season, which are known to stimulate the abiotic (e.g. ammonia volatilization) and biotic (e.g. microbial denitrification) processes driving gaseous N losses from soil (Craine *et al.*, 2015). The large inputs of ^{15}N -enriched livestock manure in Sahelian silvopastoral systems may further stimulate heavy gaseous N losses and ^{15}N enrichment of the remaining soil and vegetation N pools. Interestingly, highly enriched plant $\delta^{15}\text{N}$ values were also reported in the Kalahari drylands dominated by woody legumes growing on nutrient-poor sandy soils (Aranibar *et al.*, 2004). Contrary to expectations, the remarkably high leaf $\delta^{15}\text{N}$ values found in legumes were indistinguishable from those of nonlegumes, which suggests that atmospheric N_2 fixation may not be their primary source of nitrogen, possibly as a result of the strong water and P limitation imposed on biological N_2 fixation by the weathered sandy soils of the Sahel (Bremner & De Wit, 1983; Henao & Baanante, 1999; Vitousek *et al.*, 2010). Sahelian legumes may instead deploy other effective N-acquiring mechanisms that may contribute to their higher average leaf N concentration as a group (McKey, 1994; Dovrat *et al.*, 2020). Moreover, the livestock habit of seeking shelter under the shade of large trees and shrubs in this hot climate may provide an

abundant external N subsidy of animal origin from the surrounding grasslands in the silvopastoral systems of the Sahel. This 'fertility' island effect could be even greater under legume trees and shrubs producing nutritious pods or fodder that are attractive for livestock (Casals *et al.*, 2014; Hoosbeek *et al.*, 2018).

Differences in water-use traits between legumes and other species

The higher average leaf $\delta^{18}\text{O}$ and $\Delta^{18}\text{O}$ values of legumes compared with nonlegumes revealed a tighter stomatal regulation of transpiration with lower time-integrated stomatal conductance, which may represent a key physiological advantage of Fabaceae that could contribute decisively to their dominance across the Sahel and other tropical dryland ecosystems (Felker, 1981; Sprent & Gehlot, 2010; Pellegrini *et al.*, 2016; Gei *et al.*, 2018). However, dryland legumes as a group exhibited higher leaf N concentrations (N_{mass}) but did not exhibit significantly higher average leaf N_{area} or $\delta^{13}\text{C}$ -WUE_i values than nonlegumes, even though higher leaf N_{mass} was strongly linked to higher $\delta^{13}\text{C}$ -WUE_i across the whole dataset (Table 3). This was an unexpected result, as tighter stomatal control of transpiration in N-rich legumes (inferred from their high leaf $\delta^{18}\text{O}$ and $\Delta^{18}\text{O}$ values) would be expected to also lead to higher $\delta^{13}\text{C}$ -WUE_i. Moreover, higher leaf N_{mass} in legumes would be expected to enhance carboxylation capacity and thus lead to greater draw-down of CO_2 concentration at the sites of carbon fixation, thereby widening the $c_a - c_c$ gap (i.e. lower c_c/c_a ratios) and further enhancing $\delta^{13}\text{C}$ -WUE_i (Wright *et al.*, 2001, 2003; Prentice *et al.*, 2011, 2014; Onoda *et al.*, 2017). However, it is important to note that average N_{area} values did not differ significantly between legumes and nonlegumes, because much higher average LMA in nonlegumes (i.e. thicker leaves with lower SLA) compensated for their lower leaf N_{mass} , thereby leading to converging and similar N_{area} and $\delta^{13}\text{C}$ -WUE_i values between these two plant functional groups. In other words, Sahelian legumes and nonlegumes achieved similar average N_{area} and $\delta^{13}\text{C}$ -WUE_i values through contrasting trait combinations: high SLA and leaf N concentrations in legumes vs lower N concentrations with lower SLA values (i.e. higher LMA) in nonlegumes.

Several other nonmutually exclusive hypotheses could further contribute to explain the unexpected lack of differences in average $\delta^{13}\text{C}$ -WUE_i values between legumes and nonlegumes: a substantial proportion of leaf N in Sahelian legumes may be invested in nonphotosynthetic proteins, such as N-based osmolytes and/or defensive compounds against herbivores (Adams *et al.*, 2016); the thin, high-SLA, nitrogen-rich leaves of legumes may exhibit high mesophyll conductance to CO_2 (g_m) favoring high CO_2 concentration at the sites of carboxylation (c_c) (Seibt *et al.*, 2008; Niinemets *et al.*, 2009; Onoda *et al.*, 2017; Ma *et al.*, 2021); legumes could be more prone to photorespiration burdens under hot conditions if their tighter stomatal regulation of transpiration and lower time-integrated stomatal conductance hamper evaporative leaf cooling aimed at alleviating overheating. A higher vulnerability to leaf overheating and photorespiration in legumes would constrain carbon assimilation capacity and impede any

further decreases in c_i despite their high leaf N concentrations (Paillassa *et al.*, 2020). All this would help to reconcile the lower stomatal conductance of legumes as a group (inferred from their higher leaf $\delta^{18}\text{O}$ and $\Delta^{18}\text{O}$) with the unexpected lack of differences in c_i/c_a ratios between legumes and nonlegumes, along with the similar N_{area} between both functional groups (Warren & Adams, 2006). Nonetheless, it should be noted that the subset of deciduous legumes still had marginally higher mean leaf $\delta^{13}\text{C}$ -WUE_i value and higher N_{area} as a group compared with deciduous nonlegumes (Fig. S2), as previously reported at a global scale (Adams *et al.*, 2016). Achieving any given leaf N_{area} value through high SLA and N_{mass} (as in dryland legumes) could be more effective for enhancing carboxylation and photosynthetic nitrogen-use efficiency than achieving it through lower SLA (i.e. higher LMA) with lower N_{mass} (as in nonlegumes), given that a larger proportion of leaf N is usually invested in nonphotosynthetic structural tissues and compounds in low SLA leaves (e.g. cell walls; Poorter & Evans, 1998; Onoda *et al.*, 2017).

In conclusion, the Western Sahelian drylands harbor woody species with strikingly diverse leaf $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ values and water-use strategies that appear to be similarly adaptive to cope with the dual challenge imposed by drought and nutrient-poor soils (Wright *et al.*, 2001). Our findings provide strong experimental support for the least-cost economic theory of photosynthesis in which water and nitrogen are mutually substitutable resources to achieve a given carbon assimilation gain in dryland ecosystems (Wright *et al.*, 2003). Structural equation modeling revealed that foliar N_{area} is a pivotal trait linked to multiple other traits related to water use in Sahelian woody vegetation. In particular, higher leaf N_{area} is linked to both higher carboxylation capacity and tighter stomatal regulation of transpiration, which in turn is associated with higher WUE_i and a more conservative water-use strategy in dryland trees. Legumes possess several key functional traits that may help them thrive under the harsh environmental conditions of the Sahel. Their efficient N-acquiring ability enables them to achieve both high carboxylation capacity and tight stomatal control of transpiration and may also enhance their drought tolerance through improved osmotic adjustment and ability to extract water from drying soil. Finally, our study highlights the promise offered by leaf $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ measurements as useful functional traits for screening the water-use strategies of multiple species in remote dryland locations where extensive gas exchange measurements are often not feasible.

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This paper is dedicated to the memory of the late Prof. Harouna Yossi, who pioneered plant physiology work in the arid west African region, and who sadly passed away in 2020 during preparation of this manuscript. Dr Yossi worked for more than 40 yr for the advancement of forestry scientific research and was one of the backbones of the Malian national forestry research, teaching and development programs.

Author contributions

GMR and FIP conceived the FUNCiTREE project and designed the general field campaign. JIQ designed the hypotheses to be tested with the help of IP and CA, and proposed new variables to be measured. IP led the statistical analyses with the help of FC and CA. Field data collection was led by GMR, CA, MD, JSD, HY and BK, and CA organized the laboratory analyses, and assembled and curated the database. JIQ, IP and CA wrote the first draft of the manuscript, and all authors contributed substantially to revisions. JIQ, IP and CA contributed equally to this work.

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Data availability

The data that support the findings of this study are available from the corresponding author (cris@eeza.csic.es) or Graciela M. Rusch (graciela.rusch@nina.no) upon reasonable request. Trait data per species (mean and SE) are available in the Table S1 and number of replicates per species and site (n) are in Table 1. The data are deposited in the FUNCiTREE database (<https://doi.org/10.15468/nye57x>) at the Norwegian Institute for Nature Research (NINA).

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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 Climograms for the studied regions in the Sahel.

Fig. S2 Differences in trait values between deciduous legume and deciduous nonlegume woody species.

Table S1 Summary statistics of 12 functional traits measured in 34 woody species in the Sahel (Mali and Senegal).

Table S2 Phylogenetic tree for the 34 target species and phylogenetic signals in measured traits.

Table S3 Pairwise relationships between all traits analyzed in legumes and deciduous tree species.

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