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

• 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 (δ18O, δ13C), as proxies of stomatal conductance and water-use efficiency, across 34 Sahelian woody species.
• Dryland species exhibited diverging leaf δ18O and δ13C 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 δ18O and δ13C, 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 δ18O in dryland vegetation that is consistent with core principles of plant physiology.

Introduction

Drylands occupy c. 45% of the Earth’s surface (Práválie, 2016; Maestre et al., 2021) and are defined as regions where evaportranspiration 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 CO2 fixation and reduces CO2 concentration in leaf intercellular spaces (c_l) 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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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\textsubscript{mass} and N\textsubscript{area} acquired through symbiosis with N\textsubscript{2}-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 δ\textsuperscript{13}C, δ\textsuperscript{18}O and δ\textsuperscript{15}N, respectively), along with xylem water isotopic composition (δ\textsuperscript{2}H and δ\textsuperscript{18}O), 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 δ\textsuperscript{13}C and δ\textsuperscript{18}O in dryland plants can provide reliable proxies of time-integrated intrinsic water-use efficiency (WUE\textsubscript{i}) and g\textsubscript{s}, respectively (Ehleringer, 1993; Williams & Ehleringer, 1996; Barbour, 2007). Leaf δ\textsuperscript{13}C in C\textsubscript{3} species is negatively and linearly correlated with the time-integrated δ\textsuperscript{13}C/δ\textsuperscript{18}O ratios during photosynthesis (Farquhar et al., 1989) and reflects the relationship between net photosynthetic rate (A) and g\textsubscript{s}, thereby providing a robust indicator of time-integrated intrinsic water-use efficiency (WUE\textsubscript{i} = A/g\textsubscript{s}) during the growing season (Dawson et al., 2002). Leaf δ\textsuperscript{18}O 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 δ\textsuperscript{18}O a good proxy of time-integrated stomatal conductance (g\textsubscript{s}) and cumulative transpiration in dryland species (Querejeta et al., 2006; Barbour, 2007; Ramirez et al., 2009; Prieto et al., 2018). The enrichment in \textsuperscript{18}O of leaf dry matter above the δ\textsuperscript{18}O value of the source water used by the plant (leaf Δ\textsuperscript{18}O) 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 δ\textsuperscript{18}O/Δ\textsuperscript{18}O and δ\textsuperscript{13}C (hereafter leaf δ\textsuperscript{13}C-WUE\textsubscript{i}) can help in assessing variations in photosynthetic capacity, stomatal conductance, WUE\textsubscript{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\textsubscript{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 δ\textsuperscript{13}C and δ\textsuperscript{18}O 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 (Paillassa et al., 2020); and that higher leaf N\textsubscript{area} will be associated with higher leaf δ\textsuperscript{18}O and δ\textsuperscript{13}C values across dryland species, indicating greater carboxylation capacity, tighter stomatal regulation with lower conductance and higher WUE\textsubscript{i} (Wright et al., 2001, 2003). We further aimed to elucidate whether higher leaf δ\textsuperscript{13}C-WUE\textsubscript{i}, with increasing leaf N\textsubscript{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\textsubscript{area} on both δ\textsuperscript{18}O and δ\textsuperscript{13}C), or primarily through enhanced carboxylation capacity but with little or no impact on stomatal conductance (resulting in weak or no influence of leaf N\textsubscript{area} on leaf δ\textsuperscript{18}O). 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 δ\textsuperscript{18}O and δ\textsuperscript{13}C-WUE\textsubscript{i} values and greater drought tolerance than nonlegumes thanks to higher leaf N\textsubscript{area} achieved through symbiotic atmospheric N\textsubscript{2} fixation. We also hypothesized that dryland legumes would fix large amounts of atmospheric N\textsubscript{2}, resulting in distinct leaf δ\textsuperscript{15}N values near 0‰, whereas co-occurring nonlegumes would show high leaf δ\textsuperscript{15}N values typical of plants using \textsuperscript{15}N-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 Ségo (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 semi-arid 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). Ségo has a continental semi-arid 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) 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 N$_{mass}$ is a surrogate of stomatal conductance ($g_s$) and δ$^{13}$C is a surrogate of WUE. 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 ($R^2$, 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 (e.g. legumes, Table 1). Both legumes and nonlegumes include evergreen species that retain a full canopy throughout the year (e.g. legumes, Table 1). Both legumes and nonlegumes include evergreen species that retain a full canopy throughout the year (e.g. legumes, Table 1). Also included in the study were species from the families Bignoniaceae, Malvaceae, and Sterculiaceae. 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 δ$^{13}$C, δ$^{15}$N and δ$^{18}$O 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 δ$^{18}$O) helps in assessing the approximate depth of soil water uptake by roots in dryland ecosystems where steep vertical gradients in soil water δ$^{18}$O develop during rainy seasons (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 δ$^{18}$O values indicate uptake of isotopically enriched water from shallower soil layers exposed to intense evaporation, whereas lower xylem water δ$^{18}$O values indicate utilization of non-enriched water from deeper, less evaporated water sources (Querejeta et al., 2007; Ding et al., 2021).

Foliar δ$^{13}$C can be used to estimate long-term ratios of the intercellular to ambient CO$_2$ values ($e/c_a$) if the carbon isotope ratio of atmospheric CO$_2$ (δ$^{13}$C$_{a}$) is known (Farquhar et al., 1989). To calculate long-term $e/c_a$ ratios, we first calculated δ$^{13}$C as:
Table 1 General characteristics of the tree and shrub species sampled in two semiarid agroforestry systems in the Sahel (Mali and Senegal).

| Species                          | Growth type | Leaf habit | DBH (m)      | Height (m) | Mali (no. of trees) | Senegal (no. of trees) |
|---------------------------------|-------------|------------|--------------|------------|---------------------|------------------------|
| *Acacia nilotica* (L.) Willd. ex Delile | Fabaceae    | T          | 0.71 ± 0.11  | 5.80 ± 0.28| 1                   | 6                      |
| *Acacia senegal* (L.) Willd.    | Fabaceae    | T          | 0.19 ± 0.01  | 7.25 ± 1.75| 2                   |                        |
| *Acacia seyal* Delile           | Fabaceae    | T          | 0.52 ± 0.08  | 4.94 ± 0.30| 6                    | 2                      |
| *Acacia tortilis* spp. Raddiana (Forssk.) | Fabaceae | T          | 0.27 ± 0.03  | 5.92 ± 1.05| 6                    |                        |

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*Adansonia digitata* L.                     Malvaceae  T  D  2.46 ± 0.64  10.43 ± 0.88  7  6

*Anonna senegalensis* Pers.                Annonaceae S  D  0.97 ± 0.05  6  6

*Anogeissus leiocarpus* (DC.) Guill. & Perr. Combreteaceae T  E  1.54 ± 0.07  10.18 ± 1.19  6  6

*Aphania senegalensis* (Juss. ex Poir.) Raddlk. Sapindaceae T  E  0.29 ± 0.04  4.13 ± 0.88  2  2

*Balanites aegyptiaca* (L.) Dill. Zygophyllaceae T  E  0.69 ± 0.12  5.98 ± 0.70  6  6

*Bauhinia rufescens* Lam.                  Fabaceae   T  D  0.14 ± 0.00  4.25 ± 0.52  3  3

*Boscia senegalensis* (Pers.) Lam. ex Poir. Capparaceae S-T E  1.71 ± 0.23  6  6

*Celius integrifolia* Lam.                Ulmaceae   T  E  0.55 ± 0.08  11.42 ± 1.04  6  6

*Combretum glutinosum* Perr. ex DC.        Combreteaceae T (S-T) E  0.75 ± 0.17  7.11 ± 0.75  6  6

*Combretum micranthum* G. Don.             Combreteaceae T  S  D  0.17 ± 0.06  2.68 ± 0.28  6  6

*Cordia sinensis* Lam.                    Boraginaceae T  E  0.11 ± 0.06  6.00  1  1

*Crateva religiosa* Forst. f.              Fabaceae   T  E  0.33 ± 0.02  8.08 ± 0.96  3  3

*Diospyros mespiliformis* Hochst. ex A. DC. Ebenaceae T  D  1.34 ± 0.13  6.91 ± 0.81  7  7

*Faidherbia albida* (Delile) A. Chev.       Fabaceae   T  D  0.83 ± 0.17  9.04 ± 0.70  6  6

*Ficus gnaphalocarpa* (Miq.) Steud.        Moraceae   T  D  2.11 ± 0.43  8.53 ± 1.11  6  6

*Guiera senegalensis* J. F. Gmel.          Combretaceae S-T D  0.12 ± 0.02  3.02 ± 0.10  6  6

*Maytenus senegalensis* (Lam.) Exell       Celastraceae S-T D  0.12 ± 0.00  2.39 ± 0.44  5  5

*Neocarya macrophylla* (Sабine) Prance     Chrysobalanaceae T  E  0.38 ± 0.04  6.08 ± 0.98  6  6

*Piliostigma reticulatum* (DC.) Hochst.    Fabaceae   T  E  0.79 ± 0.13  4.99 ± 0.26  6  6

*Prosopsis africana* (Guill. & Perr.) Taub. Fabaceae   T  E  2.00 ± 0.24  9.53 ± 1.36  6  6

*Prosopis juliflora* (Sw.) DC.             Fabaceae   T  E  0.26 ± 0.03  8.17 ± 0.76  6  6

*Pterocarpus erinaceus* Poir.              Fabaceae   T  D  1.38 ± 0.11  7.78 ± 0.36  6  6

*Saba senegalensis* (A. DC.) Pichon         Apocynaceae T  E  4.20 ± 0.72  6  6

*Sclerocarya birrea* (A. Rich.) Hochst.    Anacardiaceae T  D  0.77 ± 0.13  7.98 ± 0.40  6  6

*Tamarindus indica* L.                     Fabaceae   T  E  1.20 ± 0.23  8.78 ± 0.91  6  6

*Tamarix senegalensis* DC.                 Tamaricaceae T(S-T) E  2.20 ± 0.21  6  6

*Terminalia laeviflora* Engl.              Combreteaceae T  D  1.70 ± 0.23  9.23 ± 1.14  6  6

*Vitellaria paradoxa* C.F. Gaertn.         Sapotaceae T  D  1.70 ± 0.14  9.83 ± 1.40  6  6

*Vitex doniana* Sweet.                     Labiatae   T  D  2.02 ± 0.12  8.62 ± 0.62  5  5

*Ziziphus mauritiana* Lam.                 Rhamnaceae S  E  0.43 ± 0.18  5.42 ± 0.44  3  3

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 (± SE) diameter at breast height (DBH) and plant vegetative height (height) and number of sampled individuals.

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 δ¹³C, δ¹⁵N 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 δ¹⁸O was measured with a Finnigan TC/EA IRMS. The δ¹⁸O 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 δ¹⁸O and δ¹³C-WUE, in dryland vegetation, we built an *a priori* structural

\[
\Delta^{13}C = \frac{1000(\delta^{13}C_{air} - \delta^{13}C_{leaf})}{1000 - \delta^{13}C_{leaf}}
\]

Eqn 1

where \( \delta^{13}C_{air} \) is the C isotopic composition of atmospheric CO₂ (~8.45‰, Mauna Loa records; http://www.esrl.noaa.gov/gmd/ dvyftpdata.html) and \( \delta^{13}C_{leaf} \) is the C isotopic composition of leaf material. Then, from \( \Delta^{13}C \) values, we calculated \( c1/c2 \) as:

\[
\frac{c1}{c2} = \frac{\Delta^{13}C - a_1}{b - a_1}
\]

Eqn 2

where \( a \) 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).
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_{max}) 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 δ^{18}O is considered a proxy of time-integrated stomatal conductance (inversely related, 1/g); Barbour, 2007), and leaf δ^{13}C is a proxy of WUE, (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 leaves (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 χ² 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 χ² and RMSEA indices (Schermelleh-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 were the main fixed factor was Fabaceae/ nonFabaceae. Geographical 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 (Galecki & 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 SrsS (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_{max}, δ^{18}O and δ^{13}C values, thereby revealing large interspecific differences in carboxylation capacity, stomatal regulation of transpiration, c_i/c_a ratios and WUEi (Table 2). Sahelian trees and shrubs also showed large interspecific variability in SLA, leaf thickness, leaf RWC, δ^{15}N and Δ^{18}O, xylem water δ^{18}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 δ^{18}O was unrelated to xylem water δ^{18}O across species and sites, indicating that variation in leaf δ^{18}O was not primarily driven by inter-plant differences in source water isotopic composition or root water uptake depth. Instead, both foliar δ^{18}O and Δ^{18}O were positively related to leaf δ^{13}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 δ^{18}O/Δ^{18}O and δ^{13}C correlated positively with leaf N_{area}, revealing tighter stomatal regulation and higher WUE, with increasing carboxylation capacity (i.e. leaf N_{area}; Fig. 3; marginally significant for leaf N_{area}– δ^{13}C relationship). Furthermore, leaf δ^{18}O and Δ^{18}O correlated closely with RWC (Fig. 3; Table 3), suggesting that tight 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_{max}) 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/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 δ^{13}C-WUEi in dryland species (Fig. 1b). A main SEM pathway linking leaf N_{area}, δ^{18}O and δ^{13}C highlighted a strong influence of leaf N_{area} on foliar δ^{18}O (β = 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, through leaf δ^{18}O/stomatal effects on Δ^{13}C-WUEi (β = 0.22, P < 0.01). Leaf N_{area} also had a smaller direct positive effect on leaf δ^{13}C (β = 0.14, P = 0.07; marginally significant; Fig. 1b) that...
was unrelated to stomatal effects, which we interpret as an indication of enhanced carboxylation capacity with increasing Narea across species (as predicted by theory) that further contributes to increase WUEi.

Pairwise regression analyses between leaf traits further supported the key role of a high leaf Narea in enhancing carboxylation capacity, thereby allowing a tighter stomatal regulation of transpiration (i.e. lower time-integrated stomatal conductance) and higher WUEi. It is worth highlighting that, in addition to the positive pairwise relationship between leaf Narea and δ13C (Figs. 1, 3a; marginally significant), there was also a stronger positive pairwise relationship between leaf Nmass and δ13C (Table 3). Leaf Narea was also positively correlated with leaf δ15N, 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

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       | N     | Range          | Key role in plant functioning/interpretation                                                                 |
|--------------------------------------------|--------------|-------------|-------|----------------|----------------------------------------------------------------------------------------------------------------|
| Leaf carbon isotopic composition           | Leaf δ13C    | ‰           | 217   | −31.5 to −24.6 | Time-integrated water-use efficiency                                                                         |
| Ratio of intercellular and ambient CO2 concentrations | c/i          | Unitless    | 217   | 0.57 to 0.91   | CO2 carboxylation capacity, stomatal conductance                                                            |
| Leaf nitrogen concentration                | Leaf Nmass   | %           | 216   | 1.32 to 4.80   | Photosynthetic and carboxylation capacity, osmoregulation, nutritional quality                            |
| Leaf nitrogen area                          | Leaf Narea   | mg cm⁻²     | 169   | 0.08 to 0.98   | Carboxylation capacity                                                                                     |
| Leaf oxygen isotopic composition           | Leaf δ18O    | ‰           | 211   | 19.02 to 34.53 | Time-integrated stomatal conductance, cumulative transpiration                                           |
| Leaf oxygen isotopic enrichment above source water | Leaf Δ18O | ‰           | 199   | 23.58 to 42.08 | Time-integrated stomatal conductance, cumulative transpiration                                           |
| Predawn stem water potential               | Ψpd          | 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          | m² kg⁻¹     | 175   | 4.25 to 18.63  | Photosynthetic rate, light capture, relative growth rate                                                  |
| Leaf nitrogen isotopic composition         | Leaf δ15N    | ‰           | 217   | 3.42 to 12.9   | Atmospheric N2 fixation, soil N sources                                                                  |
| Oxygen isotopic composition of xylem water | Xylem δ18O  | ‰           | 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).
positive relationship between leaf $\delta^{13}C$ and $\delta^{18}O$ across dryland species (Fig. 3d), further evidencing a shared stomatal control on both traits. Moreover, tighter stomatal regulation and lower time-integrated stomatal conductance (infected from higher leaf $\delta^{18}O$ and $\Delta^{18}O$ 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 where 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 $N_{\text{area}}$ and $N_{\text{mass}}$ 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 $N_{\text{mass}}$, SLA and $\delta^{18}O/\Delta^{18}O$ values, and thinner, less sclerophyllous leaves compared with nonlegumes (Fig. 4). By contrast, legumes and nonlegumes did not differ significantly in leaf $N_{\text{area}}$, $\delta^{13}C$ (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 \( \delta^{18}O \) 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 \( \delta^{13}C \)-WUEi (Fig. S2) than nonlegumes, along with higher leaf \( N_{\text{mass}} \) and \( \delta^{18}O/\Delta^{18}O \) values and thinner leaves. Differences in leaf \( N_{\text{mass}} \) 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 \( N_{\text{mass}} \)), leaf N content per leaf area (leaf \( N_{\text{area}} \)), specific leaf area (SLA), leaf thickness, leaf oxygen isotopic composition (leaf \( \delta^{18}O \)), leaf oxygen enrichment above source (xylem) water (leaf \( \Delta^{18}O \)), leaf carbon isotopic composition (leaf \( \delta^{13}C \)), and leaf N isotopic composition (leaf \( \delta^{15}N \)) 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 δ^{18}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 (δ^{13}C, δ^{18}O, ∆^{18}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_{area}–δ^{18}O and N_{area}–δ^{13}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 δ^{13}C-WUE_{i} was driven by an enhanced carboxylation capacity but even more strongly by a tight stomatal conductance 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 high leaf δ^{18}O and ∆^{18}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; Aparicido 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 (sahaven trees; Lin et al., 2015). The high leaf N_{area} combined with low leaf δ^{18}O and
δ¹³C 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 Nmass 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 N₂ fixation in dryland legumes**

The wide range of leaf δ¹⁵N 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 δ¹⁵N 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 ¹⁵N enrichment of leaf nitrogen pools despite vegetation dominance by N₂-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 ¹⁵N-enriched livestock manure in Sahelian silvopastoral systems may further stimulate heavy gaseous N losses and ¹³N enrichment of the remaining soil and vegetation N pools. Interestingly, highly enriched plant δ¹⁵N 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 δ¹⁵N values found in legumes were indistinguishable from those of nonlegumes, which suggests that atmospheric N₂ fixation may not be their primary source of nitrogen, possibly as a result of the strong water and P limitation imposed on biological N₂ fixation by the weathered sandy soils of the Sahel (Breman & 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 δ¹⁸O and Δ¹⁸O 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; Spret & Gehlot, 2010; Pellegrini et al., 2016; Gei et al., 2018). However, dryland legumes as a group exhibited higher leaf N concentrations (Nmass) but did not exhibit significantly higher average leaf Narea, or δ¹³C-WUE; values than nonlegumes, even though higher leaf Nmass was strongly linked to higher δ¹³C-WUE 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 δ¹⁸O and Δ¹⁸O values) would be expected to also lead to higher δ¹³C-WUE. Moreover, higher leaf Nmass in legumes would be expected to enhance carboxylation capacity and thus lead to greater drawdown of CO₂ concentrations at the sites of carbon fixation, thereby widening the δ ∆c₂/c₈ gap (i.e. lower δc₂/δc₈ ratios) and further enhancing δ¹³C-WUE, (Wright et al., 2001, 2003; Prentice et al., 2011, 2014; Onoda et al., 2017). However, it is important to note that average Narea 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 Nmass, thereby leading to converging and similar Narea and δ¹³C-WUE values between these two plant functional groups. In other words, Sahelian legumes and nonlegumes achieved similar average Narea and δ¹³C-WUE 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 δ¹³C-WUE 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 CO₂ (gₘ) favoring high CO₂ concentration at the sites of carboxylation (δc₂) (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 $\delta^{13}C$ 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 $\delta^{18}O$ and $\Delta^{18}O$) with the unexpected lack of differences in $\epsilon_1/\epsilon_2$ ratios between legumes and nonlegumes, along with the similar $N_{\text{area}}$ between both functional groups (Warren & Adams, 2006). Nonetheless, it should be noted that the subset of deciduous legumes still had marginally higher mean leaf $\delta^{13}C$-WUE value and higher $N_{\text{area}}$, as a group compared with deciduous nonlegumes (Fig. S2), as previously reported at a global scale (Adams et al., 2016). Achieving any given leaf $N_{\text{area}}$ value through high SLA and $N_{\text{mass}}$ (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 $N_{\text{mass}}$ (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 $\delta^{18}O$ and $\delta^{13}C$ 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 $N_{\text{area}}$ is a pivotal trait linked to multiple other traits related to water use in Sahelian woody vegetation. In particular, higher leaf $N_{\text{area}}$ is linked to both higher carboxylation capacity and tighter stomatal regulation of transpiration, which in turn is associated with higher WUE, 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 $\delta^{18}O$ and $\delta^{13}C$ measurements as useful functional traits for screening the water-use strategies of multiple species in remote dryland locations where expensive gas exchange measurements are often not feasible.

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Author contributions

GMR and FIP conceived the FUNGITEE 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 FUNGITEE 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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