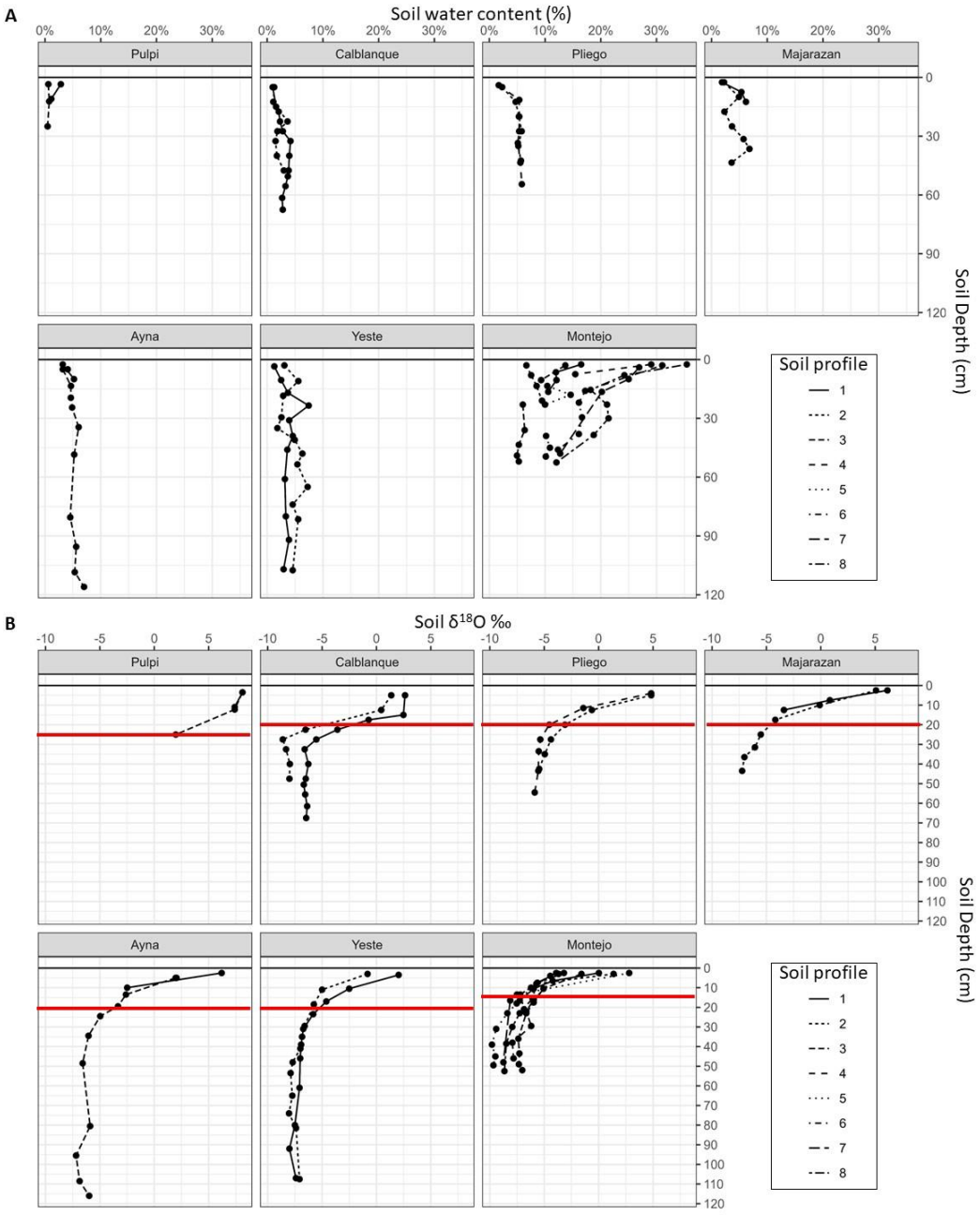
Title: Trait coordination and trade-offs constrain the diversity of water use strategies in Mediterranean woody plants

Supplementary Material

Figures



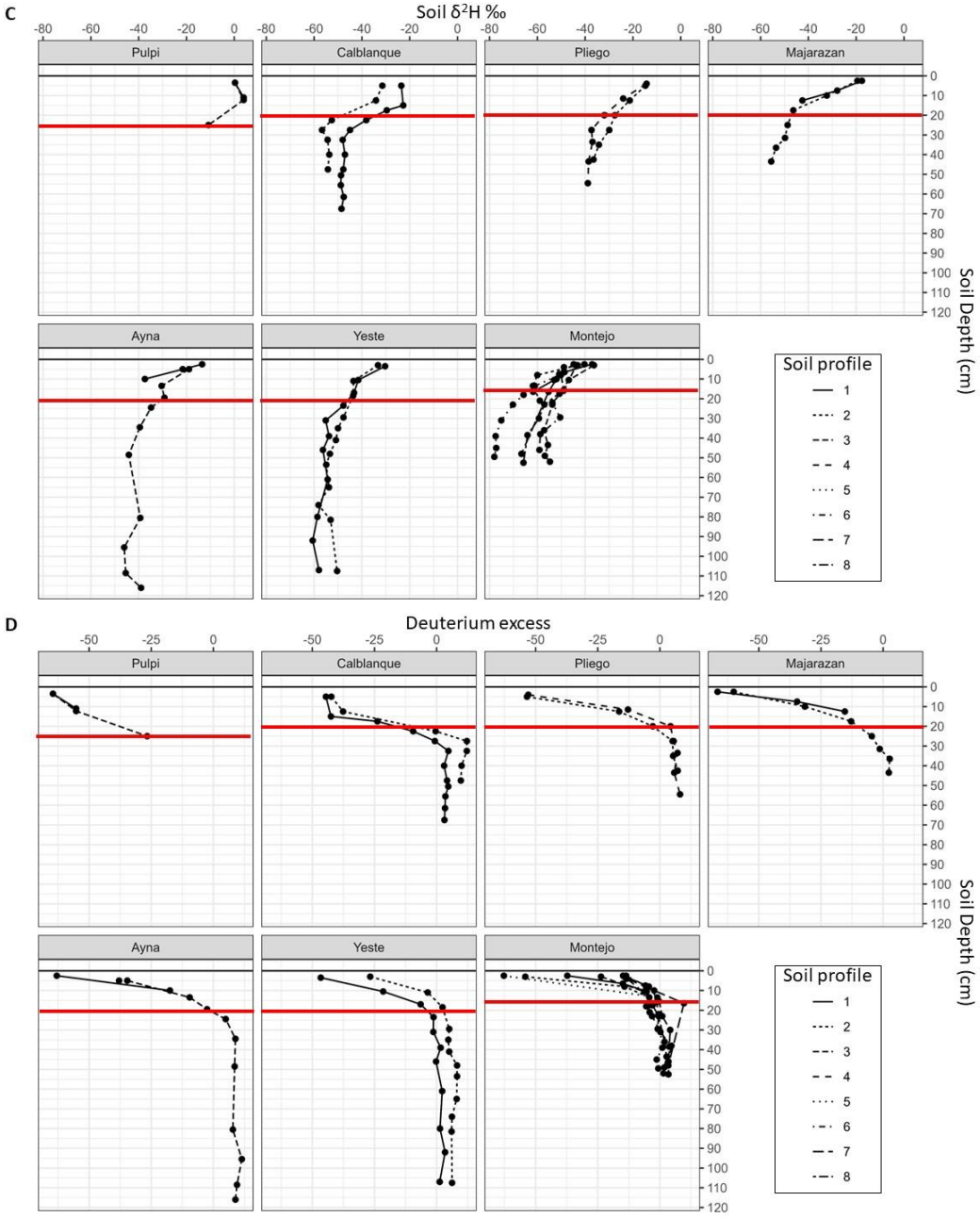


Figure S1. Steep gradients in gravimetric soil water content % (A), $\delta^{18}O$ ‰ (B) $\delta^{2}H$ ‰ (C), and d-excess (D) with soil depth (0 – 110 cm). Dots connected by lines depict the isotopic composition or soil water content of multiple soil profiles at each site. Red lines indicate the limits between shallow and deep water pools, which was set at 20 cm for Ayna, Calblanque, Majarazán, Pliego and Yeste, at 15 cm in Montejo and at 25 cm in Pulpí based on visual inspection of the data. Source data are provided as a Source Data file.

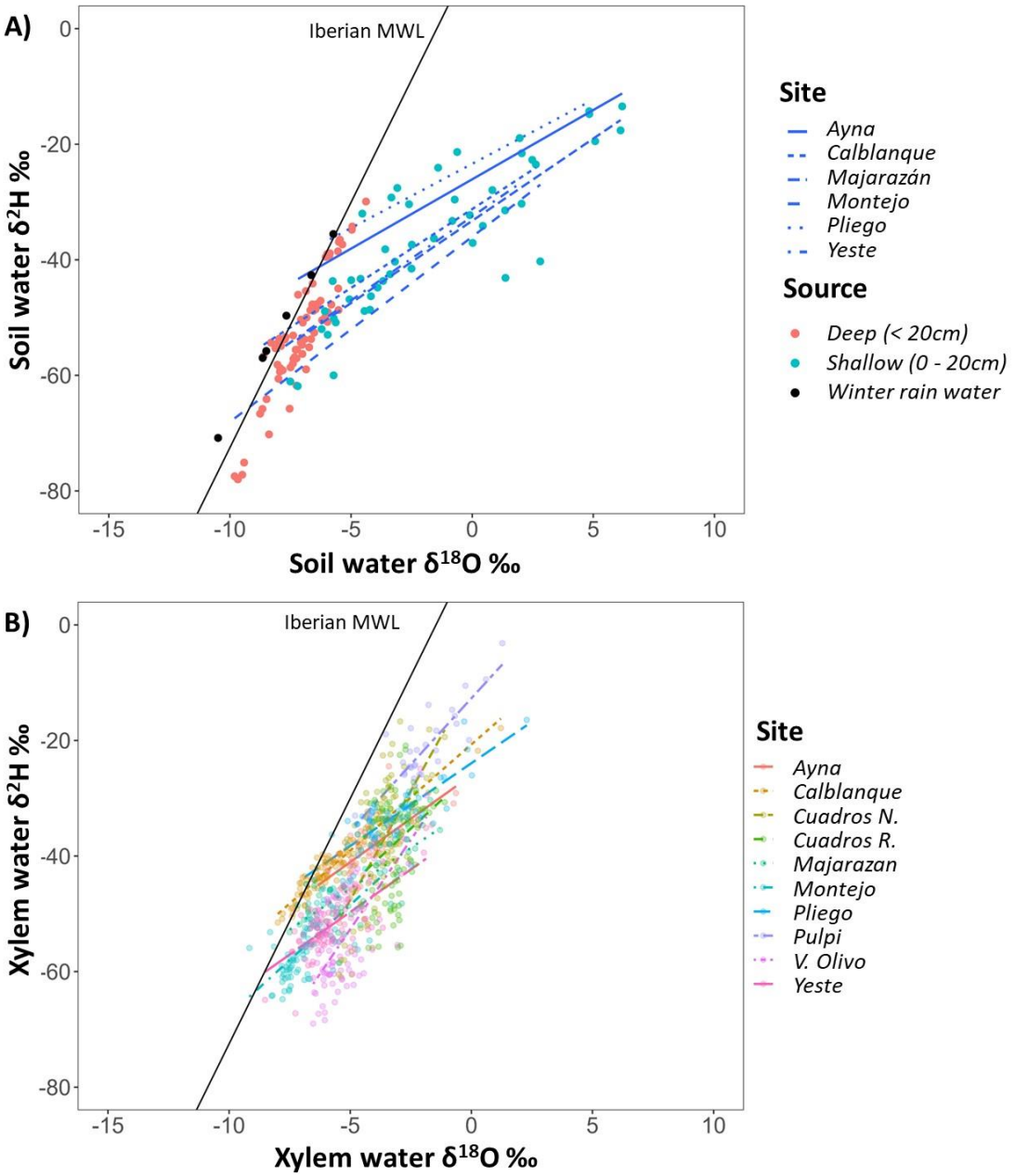


Figure S2. Soil and xylem water isotopic composition for all the soil profiles and plant individuals sampled at each study site. A) Regression lines of soil water isotopic composition ($\delta^{18}O$ and $\delta^{2}H$) values per site and the Iberian Peninsula Meteoric Water Line (solid black line) are shown. Dots represent the isotopic composition of individual shallow (blue) and deep (red) soil water samples. Black dots represent the average winter rain water isotopic composition at each site [1,2]. B) Regression lines of xylem water isotopic composition ($\delta^{18}O$ and $\delta^{2}H$) per site (color lines) and the Iberian Peninsula Meteoric Water Line [3] (black) are shown. Colored dots in panel B represent the isotopic composition of xylem water in one plant individual per site. Source data are provided as a Source Data file**.**

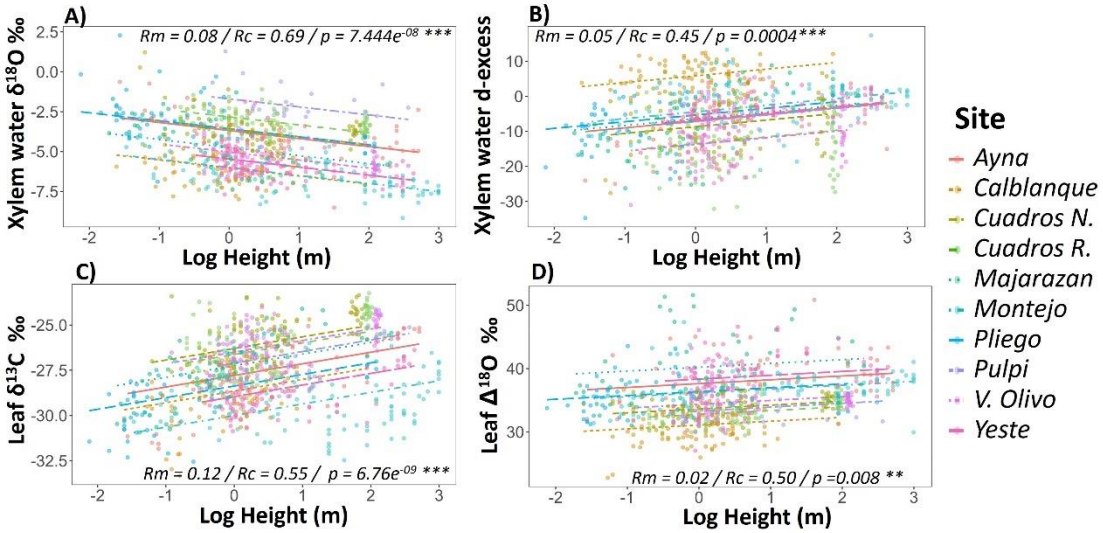


Figure S3. Within site relationships between log transformed plant height (m) and the isotopic composition of xylem water ($\delta^{18}O$, d-excess) (A, B) and leaves ($\delta^{13}C_L$, $\Delta^{18}O_L$) (C,D) across coexisting woody species. Plant height was log-transformed to comply with normality. Regression lines per site from linear mixed regression models are shown. Coloured dots represent individual plants for each site. Marginal and conditional r-square values indicate de proportions of variance explained by fixed (Rm) and by fixed and random (Rc) effects in each linear mixed model and P values indicate the degree of significance based on F-tests with Satterthwaite approximation of degrees of freedom. Source data are provided as a Source Data file**.**

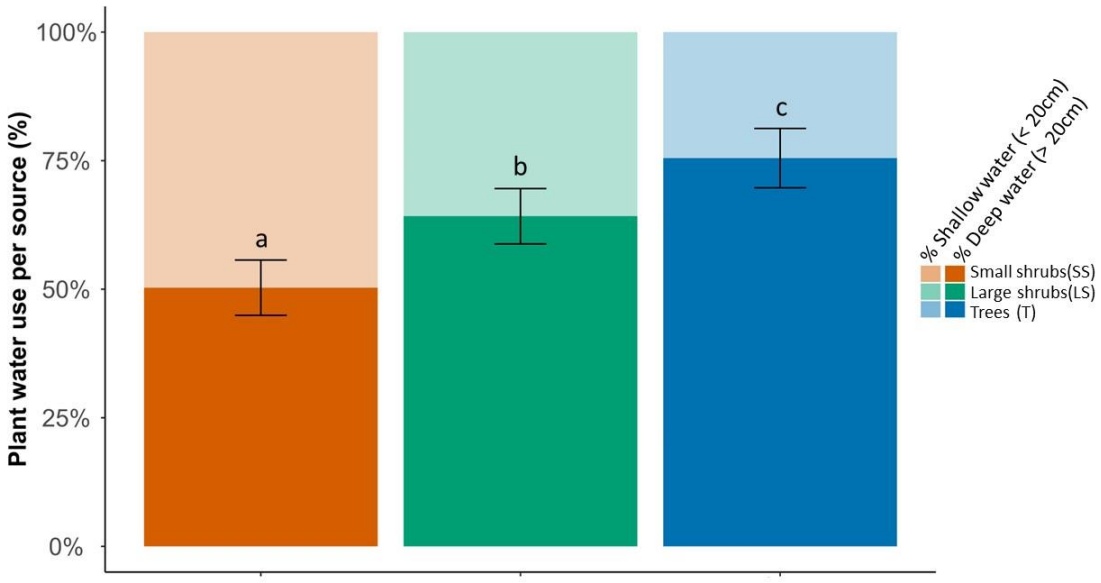


Figure S4. Average proportions (% ± Std.error) of shallow (0 – 20 cm; faded colour in upper part of columns) and deep (> 20 cm; dark colour in lower part of columns) soil water taken up by plants of different life forms; Small shrubs (SS, < 1m height), large shrubs (LS, > 1m height) and trees (T). Different letters denote significant differences between plant life forms in the average proportion of deep vs. shallow water sources used based on post-hoc tests (LSD) in linear mixed models ($p<0.05$). N = 6 individuals per specie in Ayna, Majarazan, Montejo, Pliego, Pulpí and Yeste; n = 3 individuals per specie in Calblanque; and n = 10 individuals per specie in Cuadros Natural, Cuadros Repoblacion and Venta Olivo. Source data are provided as a Source Data file.

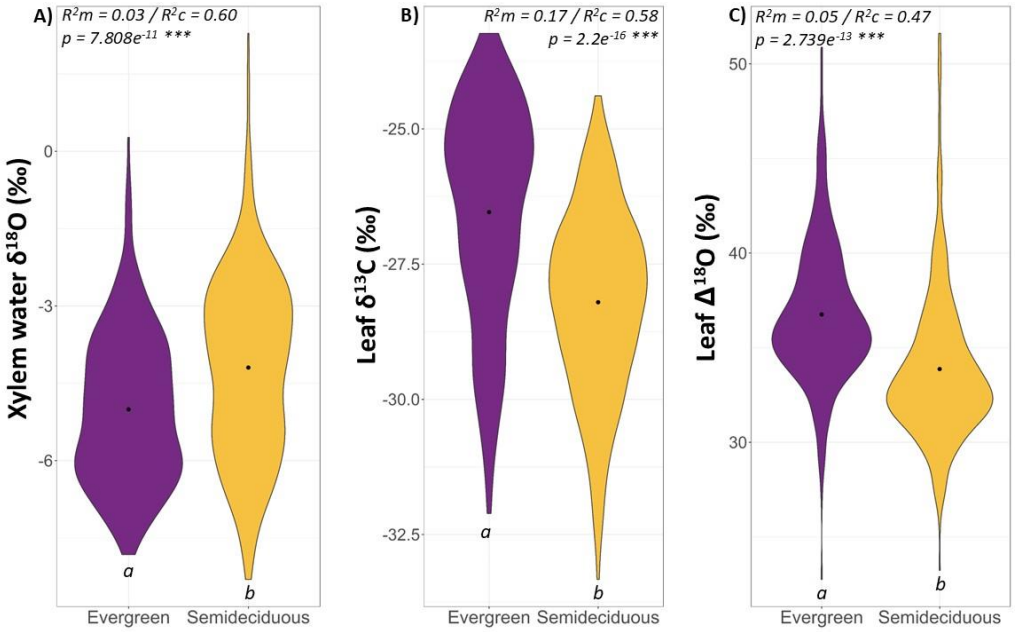


Figure S5. Violin plots depicting the oxygen isotopic composition of xylem water (xylem water $\delta^{18}O$) (A) and leaf carbon and oxygen isotopic composition (leaf $\delta^{13}C$ and $\Delta^{18}O$, B and C respectively) of woody plants grouped by leaf habit (Evergreen or Drought-deciduous/semideciduous) across species and study sites. Winter-deciduous species were not considered for these analyses as they were present only at the most humid site (Montejo) representing the transition from Mediterranean to wet Temperate climate. Black points indicate mean values of each isotopic trait in both groups of species according to leaf habit. Different letters denote significant ($p < 0.05$) differences between leaf habit groups. Marginal (R^2m) and conditional R^2 (R^2c) indicate the proportion of variance explained by fixed factors or by fixed and random factors in the model, respectively; P values indicate the significance of each linear mixed model based on F-tests with Satterthwaite approximation of degrees of freedom. Source data are provided as a Source Data file.

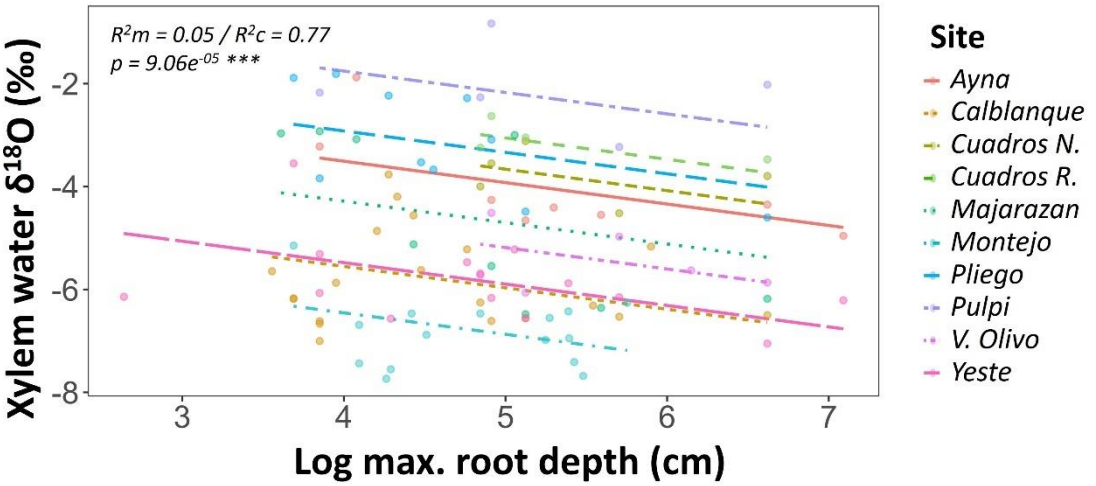


Figure S6. Within-site relationships between mean xylem water oxygen isotopic composition ($\delta^{18}O_{xw}$) and mean log transformed maximum rooting depth across coexisting species. Regression lines per site from linear mixed regression models are shown. Colored dots represent mean species values for each site. Marginal (R^2m) and conditional R^2 (R^2c) indicate the proportion of variance explained by fixed factors or by fixed and random factors in the model, respectively; P values indicate the significance of each linear mixed model based on F-tests with Satterthwaite approximation of degrees of freedom. Source data are provided as a Source Data file.

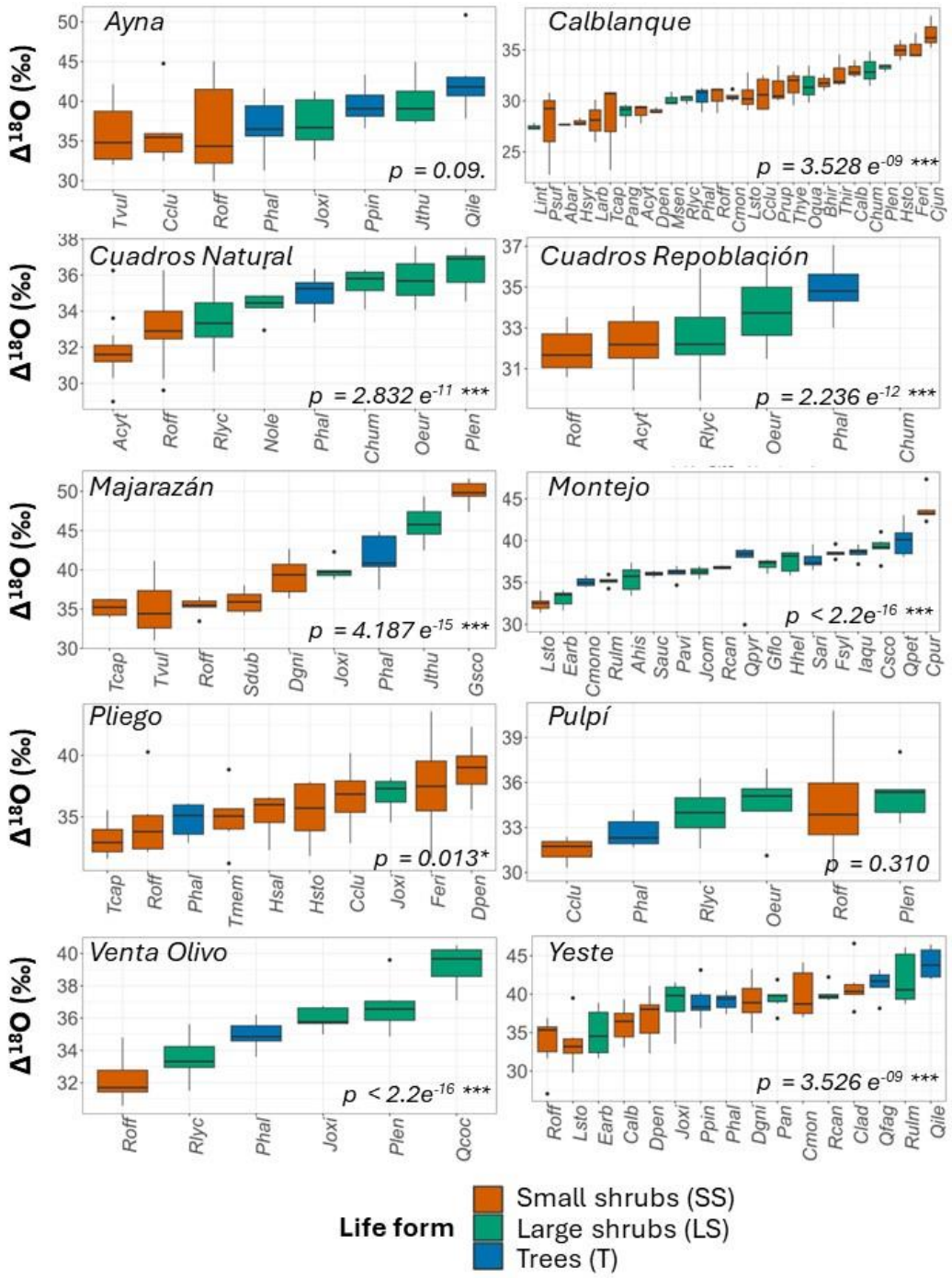


Figure S7. Variation of leaf oxygen isotope enrichment above source water ($\Delta^{18}O$ ‰) across 62 woody species growing at 10 study sites. Boxplots represent median (line) species values from linear models within each site. Lower and upper hinges correspond to the 25^{th} and 75^{th} percentiles and whiskers depicts 1.5*IRQ (interquartile ranges); outlier values are also shown. P values depict significant differences between species.Different colours represent plant life forms (small shrubs, < 1 m; large shrubs > 1-3 m; trees > 3 m). P values indicate significant differences among coexisting species within each site based on F-test analysis. The two species exhibiting the highest mean leaf $\Delta^{18}O$ values in Majarazán and Montejo (*Genista scorpius* and *Cytisus purgans*, respectively) are both nitrogen-rich legume shrubs with photosynthetic stems and very small leaves, which may be capable of exhibiting exceptionally stringent stomatal regulation of plant water flux [4]. N = 6 individuals per specie in Ayna, Majarazan, Montejo, Pliego, Pulpí and Yeste; n = 3 individuals per specie in Calblanque; and n = 10 individuals per specie in Cuadros Natural, Cuadros Repoblacion and Venta Olivo. Source data are provided as a Source Data file.

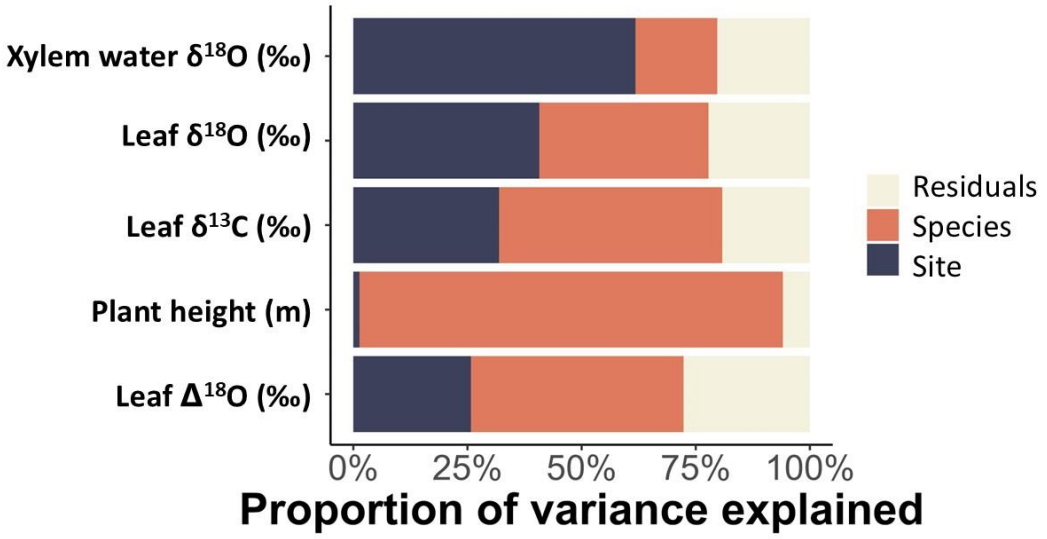


Figure S8. Variance partitioning for each plant trait, with stacked bars showing the percentage of variation explained by each explanatory variable (*species* versus *site*) in different colours from linear regressions. Source data are provided as a Source Data file**.**

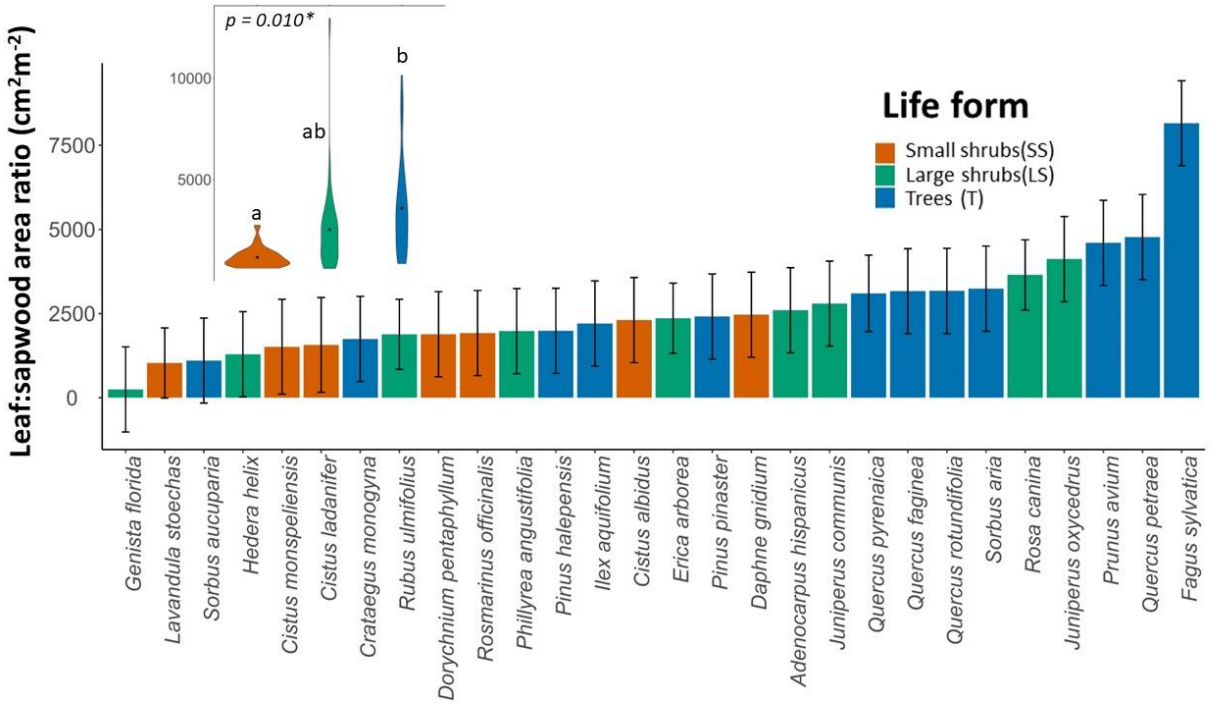


Figure S9. Variation in leaf area to sapwood area ratio (Leaf:sapwood area ratio) across 29 woody species present at two study sites (Yeste and Montejo). Bars represent the mean and whiskers the standard deviation per species. The inset depicts the violin plots of leaf to sapwood are ratio by plant life form. Different letters denote significant differences between life forms (at $p<0.05$) using a post-hoc analysis with Tukey adjustment. P-value of the corresponding linear mixed model (LMM) based on F-tests with Satterthwaite approximation of degrees of freedom is shown in the violin plot. Source data are provided as a Source Data file.

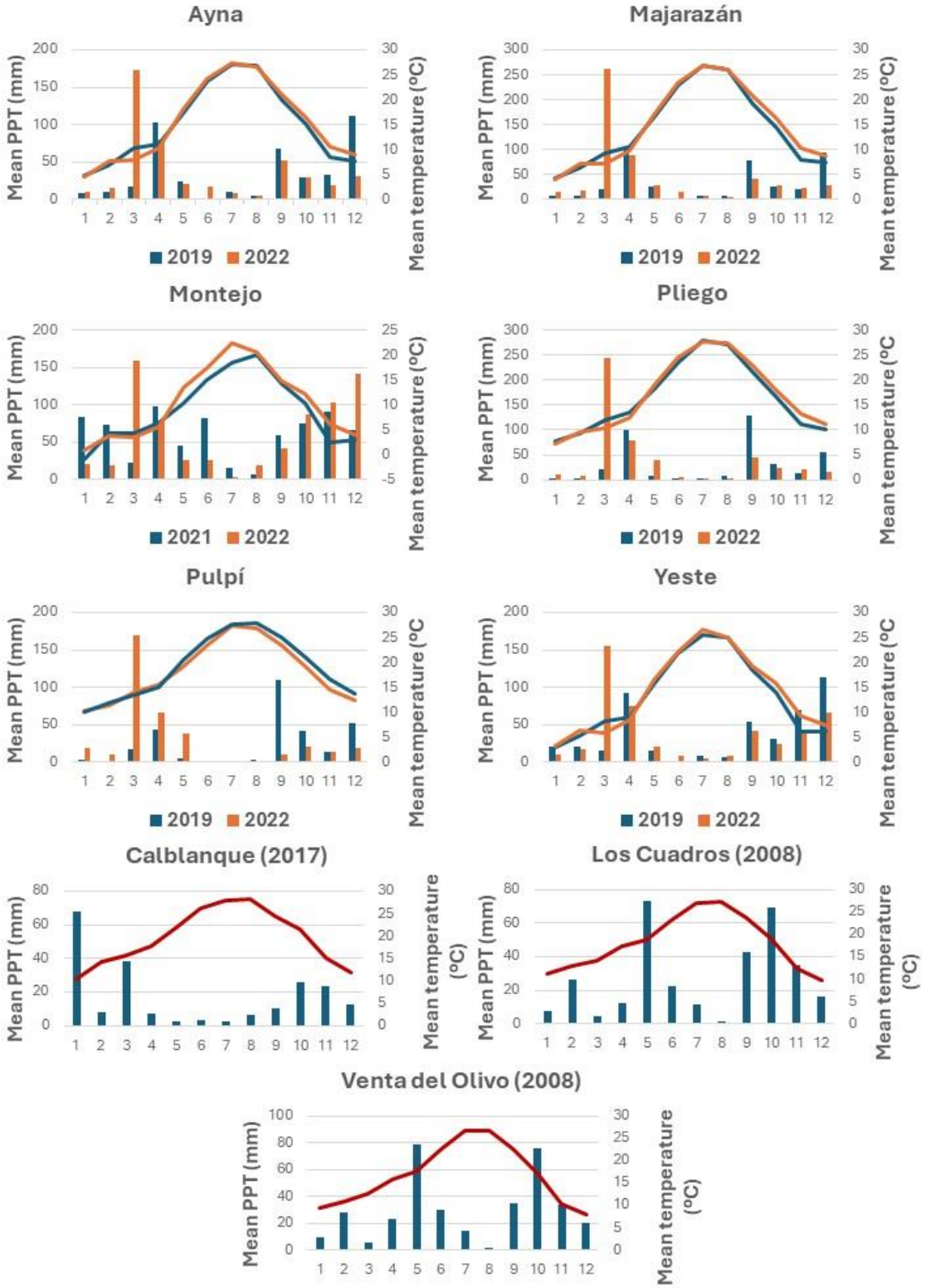


Figure S10. Climograms showing monthly precipitation (PPT, mm, depicted by bars) and temperature (ºC, depicted by lines) for the years when the field sampling campaigns were performed at each study site. For sites where two field sampling campaigns were conducted in different years, a different colour was set for each year for temperature (lines) and precipitation (bars). Source data are provided as a Source Data file.

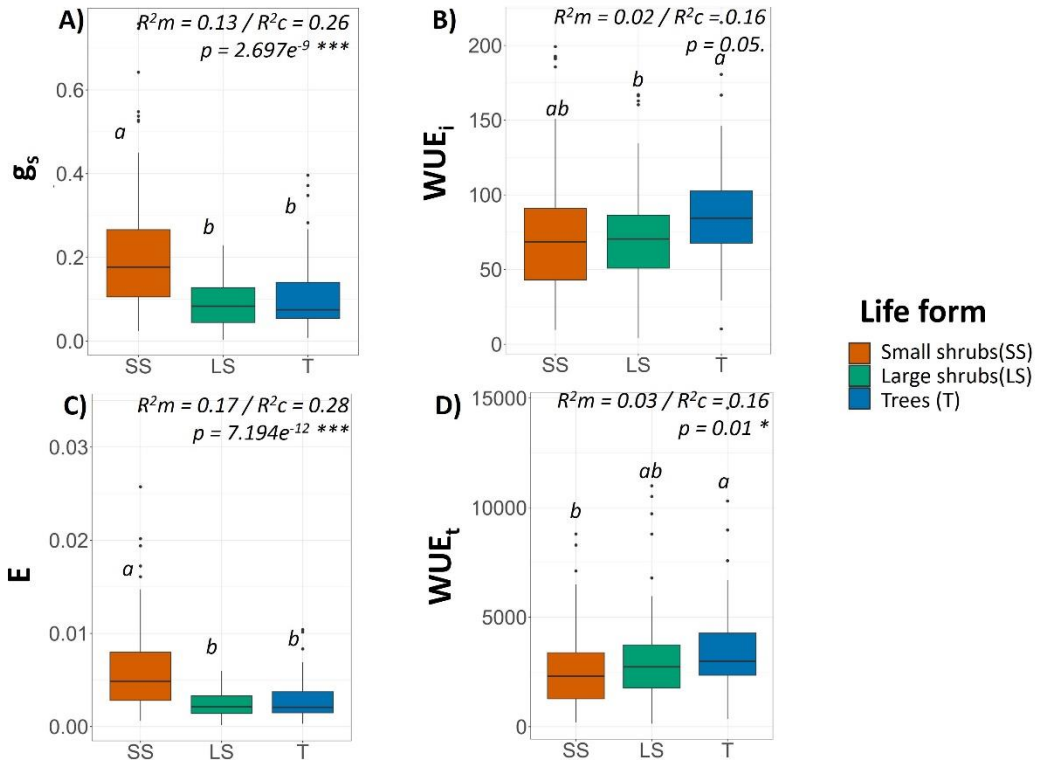


Figure S11. Variation of leaf gas exchange traits (g_s, E, WUE_i and WUE_t; A, B, C, D respectively by plant life form across study sites. Boxplots represent median (line) values from linear models. Lower and upper hinges correspond to the 25^{th} and 75^{th} percentiles and whiskers depicts 1.5*IRQ (interquartile ranges); outlier values are also shown. Different colours were used to represent plant life forms (small shrubs, <1m; large shrubs > 1m and trees). Different letters denote significant differences between life forms (at $p<0.05$) using post-hoc analysis with Tukey adjustment. P-values, marginal r-square (R^2m) and conditional r-square (R^2c) of the corresponding linear mixed models (LMMs) based on F-tests with Satterthwaite approximation of degrees of freedom are shown. Source data are provided as a Source Data file.

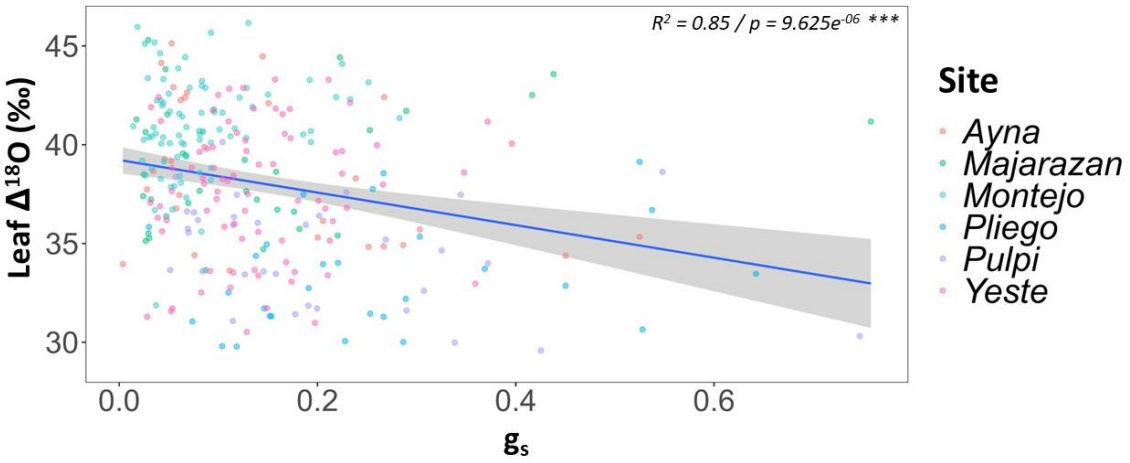


Figure S12 Relationship between leaf oxygen isotope enrichment above source water ($\Delta^{18}O$) and LICOR-measured stomatal conductance (g_s). A linear model approach was used instead of a linear mixed model approach because $\Delta^{18}O$ is strongly influenced by environmental factors such as rainwater $\delta^{18}O$, mean annual temperature, VPD or altitude that differ considerably among study sites. All data were collected during a field campaign performed during the Spring of 2022 (or early Summer in Montejo), in which both leaf isotopic and gas exchange measurements were conducted on the same plant individuals. P-values and r-square (R^2) of the corresponding linear models based on F-test analysis are shown. Source data are provided as a Source Data file.

Tables

Table S1. Location (latitude, longitude), year of field sampling, altitude above sea level (m.a.s.l), mean annual temperature (MAT, ºC), mean annual precipitation (MAP, mm), mean annual potential evapotranspiration (PET, mm), aridity index (AI = 1 – P/PET), and maximum and minimum xylem water oxygen isotopic composition values ($\delta^{18}O$ ‰) measured in plant individuals at each of the ten study sites.

Site	Location	Year	Altitude (m.a.s.l)	MAT	MAP	PET	Aridity Index	Xylem $\delta^{18}O$ (max/min)
Calblanque	37.6040, - 0.7471	2017	10	18.5	278	1380.30	0.80	1.28 / -4.80
Pulpí	37.3601, - 1.7331	2019, 2022	195	17.6	272	1323.28	0.79	1.22 / -7.99
Cuadros Natural	38.0864, -1.1044	2008	130	17.9	303	1340.35	0.77	-1.10 / -5.47
Cuadros Repoblación	38.0864, -1.1044	2008	130	17.9	303	1340.35	0.77	-1.10 / -4.38
Venta del Olivo	38.372, -1.4931	2008	563	16.5	327	1352.90	0.76	2.29 / -6.89
Pliego	37.9546, -1.5013	2019, 2022	473	14.8	414	1328.71	0.69	-1.29 / -7.50
Ayna	38.5224, -2.0874	2019, 2022	900	13.7	432	1296.31	0.67	-1.85 / -6.53
Majarazán	38.0997, -2.0709	2019, 2022	1235	12.7	466	1238.18	0.62	-0.63 / -6.34
Yeste	38.3899, -2.4575	2019, 2022	1151	12.1	496	1205.84	0.59	-1.87 / -8.52
Montejo	41.1114, -3.5011	2021, 2022	1450	8.6	726	951.34	0.24	-3.58 / -9.20

Table S2 List of 62 woody plant species including full name, taxonomic family, plant life form (Small shrub, SS, <1 m; Large shrub, LS, 1-3 m; Trees, T, > 3 m), leaf habit [5,6] (E: evergreen; WD: winter-deciduous; DD: summer drought-deciduous or semideciduous), plant lifespan [7] (SL: short-lived, <30 years; LL: long-lived, >30 years; or U: uncertain lifespan) and maximum rooting depth (cm) [8–11] according to the scientific literature and completed by expert knowledge when possible. (n.a. = no available information in the literature). For species marked as "U" we also added a tentative lifespan category based on our own expert field knowledge.

Species	Family	Abb	Life Form	Leaf habit	Plant lifespan	Maximum rooting depth (cm)
Adenocarpus hispanicus	Fabaceae	Ahis	LS	E	LL	n.a.
Anthyllis cytisoides	Fabaceae	Acyt	SS	DD	U/SL	168
Artemisia barrelieri	Asteraceae	Abar	SS	DD	U/SL	35
Ballota hirsuta	Lamiaceae	Bhir	SS	DD	U/SL	n.a.
Chamaerops humilis	Arecaceae	Chum	LS	E	LL	n.a.
Cistus albidus	Cistaceae	Calb	SS	DD	SL	47
Cistus clusii	Cistaceae	Cclu	SS	DD	SL	47
Cistus ladanifer	Cistaceae	Clad	SS	DD	SL	14
Cistus monspeliensis	Cistaceae	Cmon	SS	DD	SL	47
Coronilla juncea	Fabaceae	Cjun	SS	DD	U/SL	n.a.
Crataegus monogyna	Rosaceae	Cmono	T	WD	LL	195
Cytisus purgans	Fabaceae	Cpur	SS	WD	U/SL	60
Cytisus scoparius	Fabaceae	Csco	LS	DD	U/LL	60
Daphne gnidium	Thymelaeaceae	Dgni	SS	E	U/SL	157
Dorychnium pentaphyllum	Fabaceae	Dpen	SS	DD	U/SL	117
Erica arborea	Ericaceae	Earb	LS	E	LL	219
Fagus sylvatica	Fagaceae	Fsyl	T	WD	LL	240
Fumana ericoides	Cistaceae	Feri	SS	DD	U/SL	40
Genista florida	Fabaceae	Gflo	LS	DD	LL	n.a.
Genista scorpius	Fabaceae	Gsco	SS	DD	U/SL	84
Hedera helix	Araliaceae	Hhel	LS	E	LL	n.a.
Helianthemum cinereum	Cistaceae	Hcin	SS	DD	U/SL	95
Helianthemum syriacum	Cistaceae	Hsyr	SS	DD	U/SL	67
Helichrysum stoechas	Asteraceae	Hsto	SS	DD	U/SL	52
Ilex aquifolium	Aquifoliaceae	Iaqui	T	E	LL	91
Juniperus communis	Cupressaceae	Jcom	LS	E	LL	315
Juniperus oxycedrus	Cupressaceae	Joxy	LS	E	LL	168
Juniperus phoenicea	Cupressaceae	Jpho	LS	E	LL	268
Launaea arborescens	Asteraceae	Larb	SS	DD	U/SL	84
Lavandula stoechas	Lamiaceae	Lsto	SS	DD	SL	40
Lycium intricatum	Solanaceae	Lint	LS	DD	LL	255
Maytenus senegalensis	Celastraceae	Msen	LS	DD	LL	n.a.
Nerium oleander	Apocynaceae	Nole	LS	E	LL	n.a.
Olea europaea	Oleaceae	Oeur	LS	E	LL	n.a.
Osyris quadripartita	Santalaceae	Oqua	LS	E	LL	n.a.
Paronychia suffruticosa	Caryophyllaceae	Psuf	SS	E	U/SL	76
Periploca angustifolia	Apocynaceae	Pang	LS	E	LL	n.a.
Phagnalon rupestre	Asteraceae	Prup	SS	DD	U/SL	n.a.
Phillyrea angustifolia	Oleaceae	Pan	LS	E	LL	n.a.
Pinus halepensis	Pinaceae	Phal	T	E	LL	750

Pinus pinaster	Pinaceae	Ppin	T	E	LL	300
Pinus pinea	Pinaceae	Ppine	T	E	LL	200
Pistacia lentiscus	Anacardiaceae	Plen	LS	E	LL	300
Prunus avium	Rosaceae	Pavi	T	WD	LL	190
Quercus coccifera	Fagaceae	Qcoc	LS	E	LL	468
Quercus faginea	Fagaceae	Qfag	T	WD	LL	73
Quercus petraea	Fagaceae	Qpet	T	WD	LL	227
Quercus pyrenaica	Fagaceae	Qpyr	T	WD	LL	73
Quercus rotundifolia	Fagaceae	Qrot	T	E	LL	1200
Rhamnus lycioides	Rhamnaceae	Rlyc	LS	DD	LL	127
Rosa canina	Rosaceae	Rcan	LS	WD	LL	83
Rosmarinus officinalis	Lamiaceae	Roff	SS	DD	LL	136
Rubus ulmifolius	Rosaceae	Rulm	SS	E	LL	127
Sorbus aria	Rosaceae	Sari	T	WD	LL	71
Sorbus aucuparia	Rosaceae	Sauc	T	WD	LL	220
Staehelina dubia	Asteraceae	Sdub	SS	E	U/SL	47
Teucrium capitatum	Lamiaceae	Tcap	SS	DD	U/SL	72
Teucrium leonis	Lamiaceae	Tleo	SS	DD	U/SL	37
Thymelaea hirsuta	Thymelaeaceae	Thir	SS	DD	U/SL	365
Thymus hyemalis	Lamiaceae	Thye	SS	DD	SL	88
Thymus membranaceus	Lamiaceae	Tmem	SS	DD	SL	88
Thymus vulgaris	Lamiaceae	Tvul	SS	DD	SL	59

Table S3. Trait loadings for the principal component analysis (PCA) performed using four traits (plant height, leaf $\delta^{18}O$, leaf $\delta^{13}C$ and xylem water $\delta^{18}O$). The percentages of the total trait variance explained by each axis in the PCA are shown in parentheses.

Traits	PC1 (32.8%)	PC2 (30.8%)
Plant height	0.75	0.11
Leaf $\delta^{18}O$	0.27	-0.51
Leaf $\delta^{13}C$	0.43	-0.53
Xylem water $\delta^{18}O$	-0.42	-0.67

Table S4. Summary statics for one-way ANOVAs of the scores of individual plants along PCA axes 1 and 2 according to plant life form and site across all the 62 species included in this study. F-values (F), significance (p-value) and adjusted R^2 values obtained based on F-test analysis. Source data are provided as a Source Data file**.**

	Life form		Site		R^2 adjusted
	F	p-value	F	p-value	
PCA Axis1 scores	634.6	$2.2e^{-16}$***	50.6	$2.2e^{-16}$***	0.73
PCA Axis 2 scores	0.66	0.52	157.2	$2.2e^{-16}$***	0.68

Table S5. Cross-species mean ± standard error per site for all isotopic traits in 2019 and 2022 (2021-2022 in Montejo). Total mean ± standard error across sites are also shown for each isotopic trait and year.

Site	Leaf $\delta^{13}C$		Leaf $\delta^{18}O$		Xylem water $\delta^{18}O_{xw}$		Leaf $\Delta^{18}O$	
	2019	2022	2019	2022	2019	2022	2019	2022
Pulpi	-26.53 ± 0.37	-27.69 ± 0.54	32.08 ± 0.44	31.13 ± 0.65	-1.99 ± 0.29	-3.99 ± 0.16	33.99 ± 0.55	35.05 ± 0.67
Pliego	-29.18 ± 0.54	-29.93 ± 0.60	32.73 ± 0.69	29.89 ± 0.39	-3.08 ± 0.36	-3.53 ± 0.33	35.87 ± 0.58	33.51 ± 0.64
Majarazán	-27.10 ± 0.55	-28.19 ± 0.76	35.49 ± 1.68	32.60 ± 0.91	-4.84 ± 0.56	-5.14 ± 0.57	40.40 ± 1.86	37.74 ± 1.20
Ayna	-27.27 ± 0.73	-27.78 ± 0.68	33.53 ± 0.76	31.86 ± 1.04	-3.99 ± 0.36	-5.58 ± 0.17	37.47 ± 0.92	37.73 ± 1.15
Yeste	-28.60 ± 0.28	-28.99 ± 0.31	32.75 ± 0.69	31.74 ± 0.63	-5.08 ± 0.21	-5.81 ± 0.18	38.63 ± 0.78	37.45 ± 0.75
Montejo	-29.21 ± 0.30	-29.16 ± 0.37	30.54 ± 0.58	33.76 ± 0.47	-6.63 ± 0.20	-6.71 ± 0.25	37.05 ± 0.65	40.39 ± 0.64
Total	-28.24 ± 0.21	-28.77 ± 0.22	32.52 ± 0.37	32.07 ± 0.31	-4.82 ± 0.23	-5.43 ± 0.18	37.34 ± 0.42	37.49 ± 0.43

Table S6. List of woody plant species present at each study site. These species encompass over 90% of the total plant cover in each plant community.

Site	Species
Ayna	*Cistus clusii, Juniperus oxycedrus, Juniperus phoenicea, Pinus halepensis, Pinus pinea, Quercus rotundifolia, Rosmarinus officinalis, Thymus vulgaris*
Calblanque	*Anthyllis cytisoides, Artemisia barrelieri, Ballota hirsuta, Chamaerops humilis, Cistus albidus, Cistus clusii, Cistus monspeliensis, Coronilla juncea, Dorychnium pentaphyllum, Fumana ericoides, Helianthemum syriacum, Helichrysum stoechas, Launaea arborescens, Lavandula stoechas, Lycium intricatum, Maytenus senegalensis, Osyris quadripartita, Paronychia suffruticosa, Periploca angustifolia, Phagnalon rupestre, Pinus halepensis, Pistacia lentiscus, Rhamnus lycioides, Rosmarinus officinalis, Teucrium capitatum, Thymelaea hirsuta, Thymus hyemalis*
Cuadros Natural	*Anthyllis cytisoides, Chamaerops humilis, Nerium oleander, Olea europaea, Pinus halepensis, Pistacia lentiscus, Rhamnus lycioides, Rosmarinus officinalis*
Cuadros Repoblación	*Anthyllis cytisoides, Chamaerops humilis, Olea europaea, Pinus halepensis, Rhamnus lycioides, Rosmarinus officinalis*
Majarazán	*Daphne gnidium, Genista scorpius, Juniperus oxycedrus, Juniperus phoenicea, Pinus halepensis, Rosmarinus officinalis, Staehelina dubia, Teucrium leonis, Thymus vulgaris*
Montejo	*Adenocarpus hispanicus, Crataegus monogyna, Cytisus purgans, Cytisus scoparius, Erica arborea, Fagus sylvatica, Genista florida, Hedera helix, Ilex aquifolium, Juniperus communis, Lavandula stoechas, Prunus avium, Quercus petraea, Quercus pyrenaica, Rosa canina, Rubus ulmifolius, Sorbus aria, Sorbus aucuparia*
Pliego	*Cistus clusii, Dorychnium pentaphyllum, Fumana ericoides, Helianthemum cinereum, Helichrysum stoechas, Juniperus oxycedrus, Pinus halepensis, Rosmarinus officinalis, Teucrium capitatum, Thymus membranaceus*
Pulpí	*Cistus clusii, Olea europaea, Pinus halepensis, Pistacia lentiscus, Rhamnus lycioides, Rosmarinus officinalis*
Venta Olivo	*Juniperus oxycedrus, Pinus halepensis, Pistacia lentiscus, Quercus coccifera, Rhamnus lycioides, Rosmarinus officinalis*
Yeste	*Cistus albidus, Cistus ladanifer, Cistus monspeliensis, Daphne gnidium, Dorychnium pentaphyllum, Erica arborea, Juniperus oxycedrus, Lavandula stoechas, Phillyrea angustifolia, Pinus halepensis, Pinus pinaster, Quercus faginea, Quercus rotundifolia, Rosa canina, Rosmarinus officinalis, Rubus ulmifolius*

References

1. Bowen, G. J., Wassenaar, L. I. & Hobson, K. A. Global application of stable hydrogen and oxygen isotopes to wildlife forensics. *Oecologia* **143**, 337–348 (2005).

2. Bowen, G. J. & Revenaugh, J. Interpolating the isotopic composition of modern meteoric precipitation. *Water Resour. Res.* **39**, 1299 (2003).

3. Diaz-Teijeiro, M. F., Rodríguez-Arévalo, J. & Castaño, S. La Red Española de Vigilancia de Isótopos en la Precipitación (REVIP): distribución isotópica espacial y aportación al conocimiento del ciclo hidrológico. *Ing. Civ.* **155**, 87–97 (2009).

4. Querejeta, J. I. *et al.* Higher leaf nitrogen content is linked to tighter stomatal regulation of transpiration and more efficient water use across dryland trees. *New Phytol.* **235**, 1351–1364 (2022).

5. de la Riva, E. G. *et al.* Relationships between leaf mass per area and nutrient concentrations in 98 Mediterranean woody species are determined by phylogeny, habitat and leaf habit. *Trees - Struct. Funct.* **32**, 497–510 (2018).

6. Navarro, T., Pascual, V., Cabezudo, B. & Alados, C. Architecture and functional traits of semi-arid shrub species in Cabo de Gata Natural Park, se Spain. *Candollea* **64**, 69–84 (2009).

7. Talhouk, S. N., Fabian, M. & Dagher, R. Landscape Plant Database. *Department of Landscape Design & Ecosystem Management, American University of Beirut.* (2015).

8. Guerrero-Campo, J., Palacio, S., Pérez-Rontomé, C. & Montserrat-Martí, G. Effect of root system morphology on root-sprouting and shoot-rooting abilities in 123 plant species from eroded lands in north-east Spain. *Ann. Bot.* **98**, 439–447 (2006).

9. Cutini, A., Chianucci, F. & Manetti, M. C. Allometric relationships for volume and biomass for stone pine (Pinus pinea L.) in Italian coastal stands. *IForest* **6**, 331–335 (2013).

10. Tumber-Dávila, S. J., Schenk, H. J., Du, E. & Jackson, R. B. Plant sizes and shapes above and belowground and their interactions with climate. *New Phytol.* **235**, 1032–1056 (2022).

11. Beccari, E. & Carmona, C. P. Aboveground and belowground sizes are aligned in the uni fi ed spectrum of plant form and function. *Nat. Commun.* (2024). doi:10.1038/s41467-024-53180-x