UNIVERSIDAD DE MURCIA

Escuela de Doctorado

TESIS DOCTORAL

Diversidad funcional de estrategias de uso de agua y nutrientes por las plantas en ecosistemas mediterráneos.

Functional diversity of plant water and nutrient use strategies in Mediterranean ecosystems

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Resumen general

El incremento de la aridez provocado por el cambio climático conlleva una serie de cambios a nivel funcional y estructural en las comunidades vegetales que van desde adaptaciones fisiológicas y morfológicas a nivel de especie, como el grado de control estomático o el tamaño de las hojas, hasta reemplazos en los tipos funcionales de las especies de plantas dominantes en las comunidades. Las zonas áridas y semiáridas ocupan un 41% de la superficie terrestre del planeta, por lo que estudiar las adaptaciones y estrategias de uso de recursos que presentan las plantas adaptadas a la aridez es muy importante para diseñar planes de conservación y gestión de estos ecosistemas. Uno de los marcos conceptuales más utilizados para evaluar las estrategias de uso de recursos en ecofisiología vegetal es el espectro de economía foliar (LES, Leaf Economic Spectrum), que representa el balance entre la capacidad de adquirir recursos (concentración de nutrientes y asimilación de carbono) y los costes de construcción a nivel de hoja (área específica foliar, SLA; contenido de materia seca en las hojas, LDMC). En el extremo adquisitivo de este espectro encontramos especies que tienen alta capacidad para asimilar carbono y adquirir nutrientes rápidamente y con costes de construcción foliar bajos, a expensas de una menor longevidad foliar, mientras que en el extremo conservador encontramos plantas con tasas de asimilación de carbono y adquisición de nutrientes más lentas y con costes de construcción foliar más altos, que se compensan mediante mayor longevidad de las hojas. La universalidad de este eje adquisitivo-conservador del LES se ha demostrado a escala global con gran variedad de especies, rasgos y condiciones climáticas, pero su posible vínculo con las estrategias de uso del agua sigue poco explorado.

En ambientes mediterráneos, donde el agua es el principal recurso limitante para el crecimiento de la vegetación, explorar el vínculo entre las estrategias de uso de agua, carbono y nutrientes de las plantas es fundamental para entender el funcionamiento de estos ecosistemas. Estos vínculos están frecuentemente determinados por el carácter estacional del clima mediterráneo, en el que se intercalan largos periodos de sequía con cortos periodos de lluvias torrenciales, generando así una inestabilidad espacial y temporal en la disponibilidad de recursos (agua y nutrientes) para las plantas. Para abordar estas complejas relaciones, el análisis de isótopos estables ofrece una herramienta muy eficaz que permite vincular directamente las estrategias funcionales con el acceso a y el uso del agua. En este sentido, los isótopos estables de carbono y oxígeno en hoja ($\delta^{13}C_L$ y $\delta^{18}O_L$), así como la composición isotópica del agua del suelo y del xilema ($\delta^{18}O$, $\delta^{2}H$, d-excess), permiten obtener una buena aproximación de la

eficiencia en el uso del agua, la conductancia estomática y la profundidad de extracción de agua de las distintas especies, respectivamente. La integración de todos estos rasgos hídricos de las plantas junto con los del LES ofrece la oportunidad de conocer los mecanismos que dominan los balances entre las inversiones en carbono y nutrientes con el uso del agua, así como su respuesta a las condiciones ambientales de una forma más integradora y holística. Además, dado que los principales patrones de coordinación y compensación entre diversos rasgos funcionales son altamente dependientes de la escala, es muy importante abordar estos estudios a distintos niveles, desde el nivel de especie para conocer la diversidad de mecanismos de adaptación existentes, hasta el nivel de comunidad para evaluar cuales son las estrategias dominantes en respuesta a cambios en las condiciones ambientales.

El objetivo principal de esta tesis doctoral es estudiar la diversidad de estrategias de uso del agua, carbono y nutrientes en diferentes comunidades de plantas leñosas mediterráneas, así como su grado de coordinación dentro de cada comunidad y también a lo largo del gradiente de aridez en el que se distribuyen estas comunidades. Estos objetivos generales se han concretado en tres objetivos específicos: I) explorar la posible coordinación entre la profundidad de extracción de agua por las raíces en el perfil edáfico ($\delta^{18}O_{XW}$, $\delta^{2}H_{XW}$, d-excess) y las estrategias de uso de agua a nivel de hoja (conductancia estomática y eficiencia en el uso del agua) dentro de cada sitio y a lo largo del gradiente de aridez (capítulo 1); II) evaluar la posible coordinación de un eje adquisitivo-conservador (LES) de uso de C (fotosíntesis, SLA, LDMC) y nutrientes (N, P y K) con las estrategias de uso de agua a nivel de hoja (conductancia estomática, transpiración, eficiencia en el uso del agua) y de planta (profundidad de extracción) dentro de cada comunidad y a lo largo del gradiente de aridez (capítulo 2); III) explorar los posibles cambios adaptativos de las estrategias de uso de agua, carbono y nutrientes a nivel de comunidad en respuesta al incremento de la aridez, así como evaluar la diversidad funcional de las distintas comunidades de plantas leñosas mediterráneas y su relación con la aridez (capítulo 3).

Para abordar estos objetivos se seleccionaron 6 comunidades vegetales de plantas leñosas (Pulpí, Pliego, Ayna, Majarazán, Yeste y Montejo) distribuidas en un transecto de 600 km desde el litoral del sureste (más cálido y seco) hasta Sistema Central de la Península Ibérica (más frío y húmedo). Para algunos objetivos, se añadieron cuatro comunidades adicionales muestreadas previamente (Calblanque, Los Cuadros Natural, Los Cuadros Repoblación y Venta del Olivo). La precipitación anual media en estos sitios varía entre los 272 mm en las zonas más secas del sureste hasta los 726 mm en las más húmedas, con temperaturas medias anuales entre 18.5ºC y 8.5ºC, respectivamente, lo que representa gran parte del espectro de variación climática en la

región mediterránea. Además, la altitud entre sitios oscila entre los 10 m.s.n.m en las zonas más secas y cercanas a la costa, hasta los 1450 m en las regiones montañosas del centro de la península. Los suelos son predominantemente sedimentarios calcáreos con una mezcla de margas, areniscas y arcillas, con presencia local de esquistos en Montejo y Calblanque. La estructura de la vegetación difiere marcadamente entre sitios, abarcando desde pinares y matorrales abiertos dominados por *Pinus halepensis* y arbustos grandes como *Rhamnus lycioides,* del sureste árido, bosques cerrados dominados por *Pinus pinaster y Quercus rotundifolia* en los sitios más mésicos del interior, hasta bosques dominados por especies caducifolias como *Fagus sylvatica* o *Quercus petraea* en la zona más húmeda de las montañas del centro peninsular.

Los muestreos de los 6 sitios principales se llevaron a cabo en dos años climáticamente contrastados, 2019, ligeramente más seco que la media histórica, y 2022, un año especialmente lluvioso y húmedo. Se midió la abundancia relativa de cada especie en cada comunidad y se muestrearon 6 individuos de las especies más abundantes responsables del 90% de la cobertura en cada zona. En total, se muestrearon 62 especies de plantas leñosas a lo largo del gradiente, resultando en 121 combinaciones especie-sitio. De cada individuo tomamos muestras de hoja para medir rasgos morfológicos (SLA, LDMC), la concentración de nutrientes (N, P, K por masa y área) y rasgos isotópicos ($\delta^{18}O_L$ y $\delta^{13}C_L$) como indicadores de la conductancia estomática y eficiencia en el uso del agua, respectivamente. También tomamos una muestra de tallo por individuo para extraer el agua contenida en el xilema y calcular su composición isotópica ($\delta^{18}O_{XW}$, $\delta^{2}H_{XW}$, d-excess), como indicador de la profundidad de extracción de agua por las raíces en cada especie. Además, se calculó el enriquecimiento isotópico de $\delta^{18}O_L$ sobre la composición isotópica de la fuente de agua ($\delta^{18}O_{XW}$), como medida más fiable de la conductancia estomática al controlar la variación debida a diferencias en la composición isotópica del agua utilizada por cada planta. En el muestreo de primavera de 2019 se tomaron también muestras de suelo a distintas profundidades para elaborar un perfil de la variación de la composición isotópica del agua del suelo, el contenido de humedad y la disponibilidad de nutrientes con la profundidad. En el muestreo de primavera de 2022 también se realizaron medidas de intercambio de gases (conductancia estomática, g_s; transpiración, E; eficiencia intrínseca e instantánea en el uso del agua, WUE_i y WUE_t; y tasa fotosintética, A) en todos los individuos muestreados para obtener medidas instantáneas del estado hídrico de las plantas y para evaluar la validez de los isótopos estables como indicadores de las estrategias de uso de agua de las distintas especies. Todos los muestreos se realizaron durante el periodo de mayor actividad de la vegetación en cada sitio de manera secuencial, empezando a principios

de abril en los sitios más cálidos y áridos y terminando a principios de julio en los sitios más frescos y húmedos.

En el Capítulo 1 de la tesis exploramos la diversidad de las estrategias de uso de agua y el grado de coordinación entre rasgos hídricos en 10 comunidades de plantas leñosas mediterráneas a lo largo del gradiente de aridez. En los dos sitios más frescos y húmedos con mayor riqueza de especies coexistentes se realizaron muestreos específicos para obtener una medida adicional de arquitectura hidráulica como es el ratio entre el área de las hojas y de la albura del tallo que las alimenta. Este ratio es un indicador del área transpirativa de la planta relativa al área de xilema responsable del transporte de agua. Además, evaluamos la fiabilidad del $\delta^{18}O_L$ y $\delta^{13}C_L$ en hoja y del $\delta^{18}O$ en el xilema como rasgos indicadores de la eficiencia en el uso del agua, conductancia estomática y profundidad de extracción de agua, respectivamente, mediante medidas de intercambio de gases en hoja (LICOR) y comparando los valores isotópicos obtenidos entre dos años con características climáticas contrastadas. Los resultados mostraron una amplia diversidad en la segregación vertical en la profundidad de extracción de agua entre las especies coexistentes en cada uno de los sitios, así como en los valores de conductancia estomática y eficiencia en el uso del agua, evidenciando así una amplia diversidad de estrategias en el uso del agua independientemente del grado de aridez al que estén expuestas las diferentes comunidades. También encontramos una fuerte coordinación entre las estrategias de captación de agua en el perfil edáfico y las estrategias de uso del agua a nivel de hoja, así como con el ratio de área de hojas frente a área de albura del tallo. Las especies de menor tamaño (pequeños arbustos), que emplean una mayor proporción de agua almacenada en capas superficiales del suelo, presentaron a su vez una mayor conductancia estomática, una menor eficiencia en el uso del agua y una menor superficie foliar respecto al diámetro de la albura del tallo. Por contra, las especies de mayor tamaño (arbustos grandes y árboles), que usan una mayor proporción de agua almacenada en capas profundas, presentaron un patrón opuesto, con una menor conductancia estomática, mayor eficiencia en el uso del agua y una mayor superficie foliar respecto al diámetro de la albura del tallo. Finalmente, comprobamos que los isótopos estables son buenos indicadores integrados en el tiempo de las estrategias de uso del agua de las plantas, ya que reflejan razonablemente bien los datos instantáneos de intercambio de gases en hoja. Además, hemos podido comprobar que los rasgos isotópicos pueden considerarse en gran medida como rasgos funcionales idiosincráticos de cada especie, ya que las diferencias entre especies para cada isótopo ($\delta^{18}O_L$, $\delta^{13}C_L$, $\delta^{18}O_{XW}$) se mantienen relativamente constantes entre años con condiciones climáticas distintas.

En el Capítulo 2 exploramos la posible coordinación entre las estrategias de uso del C y nutrientes (LES) y las estrategias de uso de agua en los individuos muestreados en 2022, para los que tenemos información sobre rasgos del espectro de economía foliar, isotópicos y de intercambio de gases, a los que añadimos los individuos muestreados en Calblanque. Aquí pudimos confirmar la existencia de un eje adquisitivo-conservador de recursos en los rasgos del LES tanto general entre todos los sitios de estudio como dentro de cada sitio. Nuestros resultados indican que existe una fuerte coordinación entre el LES y las estrategias de uso del agua a lo largo del gradiente de aridez. Las especies de menor tamaño (pequeños arbustos), que invierten menos carbono en la construcción de hojas (hojas menos esclerófilas con alto SLA y bajo LDMC), presentaron una alta concentración de nutrientes (N, P, K) por unidad de masa foliar, así como mayor conductancia estomática, transpiración, tasa fotosintética y eficiencia en el uso del nitrógeno, pero una menor eficiencia en el uso del agua, lo que indica un uso más adquisitivo tanto del carbono como del nitrógeno y el agua, con un uso de agua más superficial. Por su parte las especies de mayor porte (árboles y arbustos grandes) que realizan una mayor inversión de carbono en la producción de hojas más esclerófilas y longevas (con menor SLA y mayor LDMC) presentaron una menor concentración de nutrientes por masa foliar, y también menores tasas fotosintéticas, conductancia estomática, transpiración y eficiencia en el uso del nitrógeno, mientras que presentan una mayor eficiencia en el uso del agua y un uso del agua más profundo en el perfil edáfico. Todas estas relaciones de coordinación entre el LES y el uso del agua fueron mucho más claras y fuertes cuando consideramos solo los sitios estrictamente mediterráneos (excluyendo Montejo). Esto sugiere que la estrecha coordinación entre múltiples rasgos funcionales relacionados con el uso de los recursos podría ser exclusiva de las plantas que habitan en ecosistemas limitados por agua.

En el Capítulo 3 el principal propósito es estudiar, a nivel de toda la comunidad vegetal, la variación de los distintos rasgos funcionales indicadores de las estrategias de uso de carbono, nutrientes y agua de las plantas con el aumento de la aridez que observamos a lo largo del gradiente. Para ello se calcularon tanto las medias ponderadas por la abundancia de cada rasgo como el espacio funcional que ocupa cada una de las comunidades, y se analizó su variación a lo largo del gradiente de aridez. Los resultados indican que, hacia el extremo más árido y cálido del gradiente, las comunidades vegetales inevitablemente aumentaron la conductancia estomática para evitar el sobrecalentamiento de las hojas y el consiguiente daño de la maquinaria fotosintética bajo condiciones ambientales de altas temperaturas y fuerte demanda evaporativa de la atmósfera. Sin embargo, paradójicamente estas comunidades del extremo más cálido y árido del gradiente también lograron mantener una mayor eficiencia en el uso del agua

que las comunidades de zonas más frescas y húmedas. Este fenómeno, aunque aparentemente contradictorio, se puede explicar debido a que hacia el extremo árido las comunidades de plantas también presentan hojas más esclerófilas y con mayor cantidad total de nutrientes por unidad de área foliar, lo que permite aumentar la tasa fotosintética por unidad de área, manteniendo una eficiencia en el uso del agua elevada. Sin embargo, esta tendencia no se ha visto igual para todos los tipos funcionales, ya que mientras que los árboles y arbustos grandes sí consiguen incrementar su eficiencia en el uso del agua pese a la mayor conductancia estomática bajo condiciones ambientales más cálidas y áridas, los arbustos pequeños no consiguen compensar la mayor pérdida de agua mediante ese incremento de la capacidad fotosintética, y por tanto no logran aumentar su eficiencia en el uso del agua bajo condiciones de mayor aridez. También se observó que las comunidades propias de zonas más áridas son más abiertas y presentan una mayor abundancia relativa de especies de arbustos pequeños, mientras que la abundancia relativa de arbustos grandes y árboles aumenta progresivamente hacia el extremo húmedo y fresco del gradiente climático. Además, las distintas comunidades presentes a lo largo del gradiente de aridez ocupan espacios funcionales diferentes y contrastados entre sí, siendo funcionalmente más similares aquellas comunidades más próximas climáticamente.

A modo de conclusión, hemos visto que la capacidad de las distintas especies de acceder a fuentes de agua profundas y más estables, o por el contrario la dependencia exclusiva del agua superficial cuya disponibilidad a lo largo del año es más fluctuante y efímera, determinan fuertemente los patrones de gestión y uso de recursos (agua, carbono y nutrientes). Hemos encontrado que las especies de pequeño porte, que dependen en mayor medida del agua superficial, adoptan una estrategia más oportunista y adquisitiva en el uso de los recursos para adaptarse a los cortos periodos de tiempo en los que el agua y los nutrientes están disponibles en capas superficiales del suelo para ser usados por las plantas. Por su parte, las especies de mayor tamaño, que son capaces de acceder a fuentes de agua profunda que son más estables en el tiempo, adoptan una estrategia más conservadora de los recursos que les permite seguir fotosintéticamente activas durante más tiempo aun cuando atraviesen periodos de sequía. De este modo, el aumento en la aridez restringe la diversidad de posibles estrategias de uso de recursos que resultan factibles para las plantas, favoreciendo la coordinación de estrategias de uso de agua, carbono y nutrientes más adecuada para cada especie como respuesta a las limitaciones espaciales y temporales en la disponibilidad de agua en cada sitio. Finalmente, cabe resaltar que en esta tesis presentamos pruebas convincentes de coordinación funcional entre múltiples rasgos y de la segregación de las especies coexistentes entre estrategias más adquisitivas o

conservadoras de uso de los recursos, incluido el uso del agua, en la vegetación leñosa mediterránea. Estos patrones de coordinación entre rasgos funcionales y de diversidad de estrategias de uso de los recursos se mantienen en una amplia diversidad de especies y comunidades expuestas a diferentes condiciones ambientales en la región mediterránea.

General summary

The increase in aridity driven by climate change entails a series of functional and structural changes in plant communities, ranging from physiological and morphological adaptations at the species level—such as stomatal regulation or leaf size—to shifts in the functional types of the dominant plant species within communities. Arid and semi-arid regions cover 41% of the Earth's terrestrial surface, making it essential to study the adaptations and resource-use strategies displayed by plants adapted to aridity in order to design effective conservation and management plans for these ecosystems. One of the most widely used conceptual frameworks for assessing resource-use strategies in plant ecophysiology is the Leaf Economic Spectrum (LES), which represents the balance between resource acquisition capacity (nutrient concentration and carbon assimilation) and leaf-level construction costs (specific leaf area, SLA; leaf dry matter content, LDMC). At the acquisitive end of this spectrum, species exhibit high rates of carbon assimilation and rapid nutrient uptake with low leaf construction costs, at the expense of shorter leaf lifespan. Conversely, species at the conservative end display slower rates of carbon assimilation and nutrient uptake and higher leaf construction costs, compensated by greater leaf lifespan. The universality of this acquisitive–conservative axis of the LES has been demonstrated globally across a wide variety of species, traits, and climatic conditions, but its potential linkage with water-use strategies remains poorly understood. In Mediterranean environments, where water is the main limiting factor for vegetation growth, exploring the relationships between water-, carbon-, and nutrient-use strategies in plants is fundamental for understanding how these ecosystems function. These linkages are often shaped by the strongly seasonal nature of the Mediterranean climate, characterized by long drought periods interspersed with short episodes of intense rainfall, creating spatial and temporal instability in water and nutrient availability for plants. To address these complex interactions, stable isotope analysis provides a highly effective tool for directly linking functional strategies with water access and use. In this regard, the stable isotopes of carbon and oxygen in leaves ($\delta^{13}C_L$ and $\delta^{18}O_L$), together with the isotopic composition of soil water and xylem water ($\delta^{18}O$, $\delta^{2}H$, d-excess), provide robust estimates of water-use efficiency, stomatal conductance, and rooting depth, respectively. Integrating these plant water-use traits with LES traits offers a unique opportunity to investigate the mechanisms that govern the balance between carbon and nutrient investments and water use, as well as their responses to environmental conditions in a more integrative and holistic manner. Moreover, given that the major patterns of coordination and trade-offs among functional traits are strongly scale-

dependent, it is important to conduct these studies at multiple levels—from the species level, to understand the diversity of existing adaptive mechanisms, to the community level, to identify the dominant strategies under varying environmental conditions.

The main objective of this doctoral thesis is to investigate the diversity of water-, carbon-, and nutrient-use strategies in different Mediterranean woody plant communities, as well as their degree of coordination within each community and along the aridity gradient across which these communities are distributed. These general goals are divided into three specific objectives: I) to explore the potential coordination between rooting depth in the soil profile ($\delta^{18}O_{XW}$, $\delta^{2}H_{XW}$, d-excess) and leaf-level water-use strategies (stomatal conductance and water-use efficiency) within each site and along the aridity gradient (Chapter 1); II) to assess the coordination between an acquisitive–conservative (LES) axis of carbon- (photosynthesis, SLA, LDMC) and nutrient-use (N, P, and K) with leaf- (stomatal conductance, transpiration, water-use efficiency) and plant-level (rooting depth) water-use strategies within each community and along the aridity gradient (Chapter 2); III) to examine potential adaptive changes in community-level water-, carbon-, and nutrient-use strategies in response to increasing aridity, and to evaluate the functional diversity of Mediterranean woody plant communities and its relationship with aridity (Chapter 3).

To achieve these objectives, we selected six woody plant communities (Pulpí, Pliego, Ayna, Majarazán, Yeste, and Montejo) distributed along a 600 km transect from the southeastern coast (warmer and drier) to the Central System of the Iberian Peninsula (cooler and wetter). For some analyses, four additional previously sampled communities were included (Calblanque, Los Cuadros Natural, Los Cuadros Plantation, and Venta del Olivo). Mean annual precipitation across the sites ranges from 272 mm in the driest southeastern localities to 726 mm in the wettest ones, with mean annual temperatures ranging from 18.5°C to 8.5°C, respectively, thus encompassing much of the climatic variation present in the Mediterranean region. Site elevation spans from 10 m above sea level in the driest coastal areas to 1450 m in the mountainous central regions. Soils are predominantly calcareous sedimentary substrates composed of marls, sandstones, and clays, with local schist outcrops in Montejo and Calblanque. Vegetation structure differs markedly among sites, ranging from open pine forests and shrublands dominated by *Pinus halepensis* and large shrubs such as *Rhamnus lycioides* in the arid southeast, to closed forests dominated by *Pinus pinaster* and *Quercus rotundifolia* in mesic inland areas, and deciduous forests dominated by *Fagus sylvatica* or *Quercus petraea* in the humid mountainous regions of the central peninsula.

Sampling in the six main sites took place in two climatically contrasting years: 2019, slightly drier than the historical average, and 2022, an exceptionally rainy and humid

year. We quantified the relative abundance of each species in each community and sampled six individuals of the most abundant species contributing to 90% of total vegetation cover. In total, 62 woody plant species were sampled along the gradient, resulting in 121 site–species combinations. From each individual, we collected leaf samples to measure morphological traits (SLA, LDMC), nutrient concentrations (N, P, and K on a mass and area basis), and isotopic traits ($\delta^{13}C_L$ and $\delta^{18}O_L$) as indicators of stomatal conductance and water-use efficiency, respectively. A stem sample was also collected from each individual to extract xylem water and determine its isotopic composition ($\delta^{18}O_{XW}$, $\delta^{2}H_{XW}$, d-excess), used as an indicator of rooting depth. Additionally, we calculated $\Delta^{18}O$, the isotopic enrichment of $\delta^{18}O_L$ above the isotopic composition of source water ($\delta^{18}O_{XW}$), providing a more reliable measure of stomatal conductance by controlling for differences in source-water isotopic signatures. In spring 2019, soil samples were collected at different depths to characterize vertical profiles of soil-water isotopic composition, moisture content, and nutrient availability. In spring 2022, leaf gas-exchange measurements (stomatal conductance, g_s; transpiration, E; intrinsic and instantaneous water-use efficiency, WUE_i and WUE_t; photosynthetic rate, A) were conducted for all sampled individuals to obtain instantaneous measurements of plant water status and to evaluate the effectiveness of stable isotopes as indicators of water-use strategies. Sampling was carried out during the peak growing season at each site, sequentially from early April in the warmest and driest sites to early July in the coolest and wettest ones.

In Chapter 1, we explored the diversity of water-use strategies and the degree of coordination among water-use traits across 10 Mediterranean woody plant communities along the aridity gradient. In the two coolest and wettest sites, which also had the highest species richness, additional measurements of hydraulic architecture were taken: the ratio of leaf area to stem sapwood area supplying those leaves. This ratio is an indicator of the transpiring area relative to the xylem area responsible for water transport. We also evaluated the reliability of $\delta^{13}C_L$ and $\delta^{18}O_L$ in leaves and $\delta^{18}O_{XW}$ as indicators of water-use efficiency, stomatal conductance, and rooting depth, respectively, by comparing them with instantaneous gas-exchange measurements (LICOR) and by examining interannual consistency in isotope values across two climatically distinct years. Results revealed substantial diversity in vertical water-use partitioning among coexisting species within each site, as well as in stomatal conductance and water-use efficiency, demonstrating a wide range of water-use strategies regardless of community-level aridity. We also found strong coordination between water uptake depth in the soil profile and leaf-level water-use strategies, as well as with the leaf area–to–sapwood area ratio. Smaller species (small shrubs), which rely predominantly on water stored in shallow soil

layers, exhibited higher stomatal conductance, lower water-use efficiency, and lower leaf area relative to stem sapwood area. Conversely, larger species (large shrubs and trees), which use a greater proportion of deep-stored water, showed the opposite pattern: lower stomatal conductance, higher water-use efficiency, and greater leaf area relative to sapwood area. Finally, we confirmed that stable isotopes are useful time-integrated indicators of plant water-use strategies, as they reasonably reflect instantaneous gas-exchange data. Moreover, isotopic traits can be considered largely species-specific functional traits, given that interspecific differences in $\delta^{13}C_L$, $\delta^{18}O_L$ and $\delta^{18}O_{XW}$ remained relatively consistent across years with contrasting climatic conditions.

In Chapter 2, we examined the potential coordination between carbon- and nutrient-use strategies (LES) and water-use strategies in individuals sampled in 2022, for which we had complete trait datasets including LES traits, isotopes, and gas-exchange measurements, supplemented with individuals sampled in Calblanque. Here we confirmed the existence of an acquisitive–conservative axis in LES traits both across all sites and within each site. Our results demonstrate strong coordination between LES traits and water-use strategies along the aridity gradient. Smaller species (small shrubs), which allocate less carbon to leaf construction (less sclerophyllous leaves with higher SLA and lower LDMC), exhibited higher leaf nutrient concentrations (N, P, K), higher stomatal conductance, transpiration, photosynthetic rate, and nitrogen-use efficiency, but lower water-use efficiency, indicating a more acquisitive use of carbon, nitrogen, and water, associated with shallow water use. In contrast, larger species (trees and large shrubs), which invest more carbon in producing more sclerophyllous and longer-lived leaves (lower SLA and higher LDMC), showed lower nutrient concentrations, lower photosynthetic rates, stomatal conductance, transpiration, and nitrogen-use efficiency, but higher water-use efficiency and deeper water uptake. All these coordination patterns between LES and water use became much clearer and stronger when considering only strictly Mediterranean sites (excluding Montejo). This suggests that tight coordination among multiple resource-use traits may be characteristic of plants inhabiting water-limited ecosystems.

In Chapter 3, the main goal was to investigate community-level variation in functional traits related to carbon-, nutrient-, and water-use strategies in response to increasing aridity along the gradient. To this end, we calculated abundance-weighted community means for each trait and quantified the functional space occupied by each community, assessing their variation along the aridity gradient. Results show that toward the drier and warmer end of the gradient, plant communities inevitably increased stomatal conductance to avoid leaf overheating and associated damage to the photosynthetic machinery under conditions of high temperatures and high atmospheric evaporative

demand. However, these warmest and driest communities also maintained higher water-use efficiency than communities from cooler and wetter areas. Although seemingly contradictory, this can be explained by the fact that communities in the arid end present more sclerophyllous leaves with higher total nutrient content per unit leaf area, which allows higher photosynthetic rates per area while maintaining high water-use efficiency. Nonetheless, this tendency did not apply equally across all functional types: while trees and large shrubs increased water-use efficiency despite higher stomatal conductance under warmer and drier conditions, small shrubs were unable to fully compensate for increased water loss through enhanced photosynthetic capacity, and thus did not increase water-use efficiency under greater aridity. Communities in more arid regions also tended to be more open and showed a higher relative abundance of small shrub species, whereas the relative abundance of large shrubs and trees increased progressively toward the cooler and wetter end of the climatic gradient. Furthermore, communities distributed along the aridity gradient occupied distinct and contrasting functional spaces, with functionally more similar communities occurring in climatically closer regions.

In conclusion, we found that the ability of different species to access deep, stable water sources—or, alternatively, their exclusive dependence on shallow soil water, whose availability is more fluctuating and ephemeral, strongly determines patterns of resource acquisition and use (water, carbon, and nutrients). Small-stature species dependent on shallow water adopt more opportunistic and acquisitive resource-use strategies to take advantage of the short periods when water and nutrients are available in surface layers. In contrast, larger species capable of accessing deeper and more stable water reservoirs adopt more conservative resource-use strategies that allow them to remain photosynthetically active for longer periods, even during drought. Thus, increasing aridity restricts the diversity of feasible resource-use strategies available to plants, promoting coordination among water-, carbon-, and nutrient-use strategies that best fit the spatial and temporal constraints on water availability in each site. Finally, this thesis provides strong evidence of functional coordination among multiple traits and of the segregation of coexisting species along acquisitive versus conservative resource-use strategies—including water use—in Mediterranean woody vegetation. These patterns of coordination among functional traits and the diversity of resource-use strategies are maintained across a wide variety of species and communities exposed to different environmental conditions within the Mediterranean region.

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General Introduction

General introduction

Introduction to functional Ecology: the value of functional traits and the Leaf Economic Spectrum as an integrative framework at global scale.

Functional ecology is a discipline that studies the role of species in the ecosystems they inhabit, based on their morphological, physiological, or life history characteristics (Calow 1987). It is a branch of ecology that can be applied to many living organisms from bacteria (Krause et al. 2014) to large mammals (Sebastián-González et al. 2021), and has generated a great deal of attention in the study of plant species and plant communities (McGill et al. 2006). This discipline focuses on understanding how organisms interact with their environment through measurable characteristics, known as functional traits (Shipley et al. 2016). Its core approach involves: i) describing organisms based on trait values rather than taxonomic or phylogenetic relationships, ii) comparing trait values across species and environments to identify general ecological patterns; iii) analysing trait variation along environmental gradients to understand how traits influence and respond to environmental conditions; iv) scaling trait-based understanding from plant organs or organisms to entire ecosystems, under the assumption that ecosystem structure and function emerges from the traits of the species present. The basis for studying these dynamics lies in finding morphological, physiological or phenological features measurable at the individual plant level that influence fitness indirectly through their effects on growth, reproduction and survival. These features are known as functional traits (Violle et al. 2007). Determining which traits are functional and capture ecological information is challenging, because direct links between traits and fitness are difficult to demonstrate (Shipley et al. 2016), prompting some authors to further refine and clarify the concept (Volaire, Gleason, and Delzon 2020; Kearney et al. 2021; Sandra Díaz 2025). Despite these challenges, the functional trait approach represents a major advance in understanding plant form and function. It allows researchers to quantify key dimensions of trait space that reflect plant ecological strategies (Sandra Díaz et al. 2016; Carmona et al. 2021; Wright et al. 2004) and to identify easy measurable traits that can be applied across a large number of individuals, species and communities facilitating the assessment of general patterns and trade-offs in plant ecology.

Understanding how these traits vary is only one part of functional ecology; equally important is exploring the correlations and trade-offs among traits, which reveal the

evolutionary, genetic, and physical constraints that shape plant ecological strategies. Correlations between functional traits can arise in different ways, including evolutionary divergence processes in which certain trait combinations persist across lineages, or as a result of the availability of diverse functional niches, or physically enforced correlations (Westoby et al. 2002). Evolutionary constraints on trait coordination may result from selection, which limits the persistence of poorly-performing trait combinations in a given environment, or from genetic constraints, when the variability required to produce particular trait combinations is lacking (Donovan et al. 2011). Many studies have described the key evolutionary role in coordinating plant functional traits across morphological, physiological and phenological dimensions (Ramírez-Valiente et al. 2020; Donovan et al. 2011; Flores et al. 2014; Sanchez-Martinez et al. 2020; Matesanz, Gianoli, and Valladares 2010). Selective evolutionary pressures imposed by environmental conditions have partly shaped the evolution of plant lineages, driving both long-term structural adaptations and short-term physiological responses (Sanchez-Martinez et al. 2020; Donovan et al. 2011). These processes can operate at different time-scales: slower structural changes in morphology or anatomy, such as shifts in species height distribution within forest canopies, provide long-term advantages, whereas faster physiological adjustments, such as modifications in gas exchange rates or hydraulic conductance, enable rapid responses to environmental perturbations (Westoby et al. 2002; Ramírez-Valiente et al. 2020).

Environmental conditions determine resource availability and therefore the diversity of ecological niches at local scales. Selective pressures favour the presence of certain trait values within each niche, so that ecosystems containing a wide diversification of niches promote niche complementarity, enhancing water-saver efficiency by increasing the range of functional traits represented in plant communities (Sandra Díaz and Cabido 2001). Parallel to these adaptive processes, considerable effort has focused on identifying physically enforced trade-offs that constrain plant growth, water-saver and reproductive strategies. One of the first attempts to schematically describe plant functional strategies was Grimes's CSR triangle (Grime 1977, 1974), which identified responses to disturbances (R, ruderal) and to favourable growing conditions (S-C, stress tolerator – competitor) as primary ecological dimensions. Building on this framework, the Leaf-Height-Seed (LHS) scheme (Westoby 1998) translated CSR dimensions into measurable traits, setting a practical ecological strategy scheme globally. Within the LHS scheme, specific leaf area (SLA) captures variation in species responsiveness to opportunities for rapid growth, while height and seed mass reflect strategies for coping

with disturbance, although other dimensions and trade-offs can further expand these initial axes (Westoby 1998; Westoby et al. 2002).

Among these trait based frameworks, one is particularly powerful for its generality and predictive power, the Leaf Economics Spectrum (LES) (Wright et al. 2004). The LES describes a global resource acquisition-conservation gradient in investment returns of nutrient and dry mass, based on leaf morphological and physiological traits linked to the leaf lifespan (Wright et al. 2004; Reich 2014). The formulation of the LES (Wright et al. 2004) captured essential information on leaf economics, linking C uptake (i.e. photosynthetic capacity, A_{max}) with the structural component of leaves, i.e. the ratio between the C investment per light interception capacity (specific leaf area, SLA) and with leaf N concentration as an integral component of the RuBisCO and thus linked to the carboxylation capacity (Wright et al. 2004). Along this spectrum, conservative (slow) species display high leaf C construction costs (low SLA), low leaf N concentration and low C uptake, i.e low photosynthetic rates, but have longer leaf lifespans, while acquisitive species at the other end of the LES display opposite traits and shorter leaf lifespans (Wright et al. 2004; S. Díaz et al. 2004). The LES theory has been expanded to other plant organs, such as stems or roots; Reich (2014) proved that the principles of the LES could be expanded to other plant organs and from individual species to the community and ecosystem scale. Thus, being fast (or acquisitive) at acquiring or using a given resource (carbon or nutrients) at the leaf level requires being fast at acquiring or using resources at any other organ (Reich 2014). Following this theoretical framework, many studies have focused on determining the degree of coordination in resource-use strategies at multiple scales, within individuals (between different plant organs, above and belowground), across communities, and globally (Sandra Díaz et al. 2016; Carmona et al. 2021; Saatkamp et al. 2019; de la Riva et al. 2016, 2021; Prieto et al. 2015; Pérez-Ramos et al. 2012). The leaf and plant economics spectra have proven to be universally valid conceptual framework that are widely applicable globally (Sandra Díaz et al. 2016; Wright et al. 2005), although they have largely overlooked other important resources such as water (but see Garnier et al., 2025; Illuminati et al., 2022; Lloret et al., 2016; Pérez-Ramos et al., 2012; Prieto et al., 2018; Werner and Maguas, 2010). To date, little is still known about how water uptake and use interact with nutrient and carbon economics or about the main drivers and trade-offs underlying these relationships from the individual to the ecosystem scale.

The importance of integrating water in the LES

Water plays a central role in almost every plant function: it provides cell turgor, acts as a solvent for chemical compounds as nutrients or osmolytes and it is the transport medium for organic and inorganic compounds involved in central physiological functions such as photosynthesis or organ signalling. Thus, water is as important a resource as light, oxygen, CO2, or essential macro and micronutrients for plants (Silvertown 2004). Water is taken up by plants through the xylem tension generated by the negative internal pressure gradient created between the atmosphere and the soil through transpiration (Tyree and Sperry 1988; Sperry 2000). Similarly, it is also important to consider the energy with which water is retained in the soil, or matric potential, which increases with depth and as the soil dries out (Hillel 2003). Plant water use and acquisition by plants is thus constrained by three main factors: soil water availability or water potential, biophysical regulation and structural constrains (Silvertown, Araya, and Gowing 2015). Furthermore, water and nutrients are often unevenly distributed along the soil profile in dryland ecosystems, as postulated by the *two water-pool hypothesis* (Ryel et al. 2008). According to this theory, topsoil layers accumulate organic matter and are richer in nutrients, but their water availability varies substantially over time, especially in seasonally dry climates such as the Mediterranean where water is abundant in the topsoil between autumn and spring but is very scarce during the summer drought. On the other hand, deep soil layers are poorer in nutrients than the topsoil, but water availability is higher and more stable through time even in seasonal dry climates (Ryel et al. 2008). The ability of plant species to capture water and nutrients from each of these two pools will then determine their carbon and nutrient use strategies (Querejeta, Ren, and Prieto 2021). The second main constrain to water use is biophysical and related to the trade-off governing plant gas exchange. The uptake of CO_2 by leaves and water loss through transpiration are both regulated by leaf stomata, i.e. the closure of stomata reduces plant water loss but also limits the leaf ability to take up CO_2 (Silvertown, Araya, and Gowing 2015). This balance between water loss control and productivity (CO_2 uptake) has been a central question in plant ecophysiology to understand plant water and C use strategies (Seibt et al. 2008; Cernusak et al. 2013; Moreno-Gutiérrez et al. 2012). The third constrain to water uptake arises from a mechanical trade-off related to the safety-efficiency trade-off in plant internal water transport (Silvertown, Araya, and Gowing 2015; Manzoni et al. 2013), The rate at which water moves through the plant vascular conduits (vessels and leaf veins) is determined by the opening of stomata and the transpiration demand by the atmosphere. Thus, it would be an adaptive strategy to keep the stomata as open as possible, but when water is the soil is scarce and the soil water potential is

low or very low, a high water potential gradient between the soil and the atmosphere may lead to an increased risk of drought-induced embolism, hydraulic failure and plant death (Fernández-de-Uña et al. 2023; Manzoni et al. 2013; McDowell and Allen 2015). Thus, plants need to strike a balance between maximizing their water transport capacity but being less resistant to embolism, or building embolism-resistant xylem vessels, (i.e. by increasing the number of tracheids in the xylem or narrower vessels), albeit at the cost of a lower water transport capacity (Sperry, Meinzer, and McCulloh 2008; Gleason et al. 2016).

Plant size and height strongly influence plant water-use strategies by determining the access to deeper soil water pools while at the same time increasing the hydraulic requirements and risks associated with increasing size. Because plant shoot size is strongly correlated with root system size, taller plants generally develop larger and deeper root systems, although temperature and seasonality can modulate overall plant geometries (Tumber-Dávila et al. 2022). Extensive and deeper rooting systems allow tall species to access more stable and reliable soil water sources (Ryel et al. 2008), but higher plant height entails higher xylem tension and stronger hydraulic limitations imposed by gravity. To mitigate the risk of hydraulic failure, taller species often display compensatory mechanisms such as enhanced stomatal regulation, hydraulic conductance, internal water storage capacity and capacitance (Fernández-de-Uña et al. 2023). Both soil water extraction depth and xylem vulnerability have been identified as two main factors determining species-specific water-use strategies in response to drought (Laughlin et al. 2023). Therefore, developing tools and identifying morphological and physiological functional traits that integrate water use with carbon and nutrient economics is essential for advancing our understanding of plant adaptations to aridity.

Stable isotopes as reliable proxies for water use efficiency and stomatal conductance.

While the theoretical framework linking water, C and nutrient use is clear, quantification at relevant spatial scales remains challenging. Stable isotopes techniques offer a solution for the empirical quantification of water use that, in combination with plant traits linked to C and nutrient use, are powerful tools to empirically test their link. The use of stable isotopes as indicators of biological and biogeochemical processes has been expanding since the early 1990s (Ehleringer and Dawson 1992). Although stable isotopes techniques are applied to many disciplines such as hydrology, geology, and zoology, their use in plant ecophysiology has attracted much interest in recent decades (Dawson and Siegwolf 2007; Dawson et al. 2002). The functional relevance of stable

isotopes lies in the fractionation processes between the light and heavy isotopes of each element, which can be classified into two main types (Dawson et al. 2002): i) equilibrium isotope fractionation occurs when exchange reactions are bidirectional during phase changes (e.g., liquid to gas), in which the degree of fractionation is proportional to the mass difference between isotopes; ii) kinetic isotope fractionation occurs only in unidirectional, mass-dependent reactions in which lighter and heavy isotopes react or diffuse at different rates (e.g., diffusional processes or enzymatic reactions). In plant water relations studies, the most studied isotopes are carbon and oxygen in leaves, cellulose and wood ($\delta^{13}C$ and $\delta^{18}O$), and oxygen and hydrogen in soil and xylem water ($\delta^{18}O$ and $\delta^{2}H$). Isotopic ratios are commonly expressed in delta notation (δ), representing the deviation between the isotope composition of sample to a reference standard (Vienna standard Mean Ocean Water, V-SMOW, for oxygen and hydrogen isotopic composition; or Vienna Pee Dee Belemnite standard, V-PDB, for carbon isotopic composition) and reported in per mill (‰).

The variation in the $^{13}C/^{12}C$ ratio of the leaf organic matter is mainly determined by gas exchange, where discrimination against the heavier isotope ^{13}C by the carboxylation enzyme (RubisCO) during photosynthesis links $\Delta^{13}C$ to the ratio of intercellular to atmospheric CO_2 (c_i/c_a) (Farquhar, O'Leary, and Berry 1982; Cernusak et al. 2013) (Figure 1). When stomatal conductance (g_s) is low relative to the CO_2 assimilation by photosynthesis, intercellular CO_2 (c_i) concentration decreases and leads to a lower c_i/c_a ratio. This decline in c_i/c_a ratios leads to an enrichment in ^{13}C because the partial pressure of ^{12}C decreases more rapidly than that of ^{13}C (Bigeleisen 1965). As a consequence, the organic matter of tissues formed under these conditions will have higher $\delta^{13}C$ values (Siegwolf et al. 2023). Therefore, leaf $\delta^{13}C$ provides an integrative proxy for the balance between CO_2 supply and demand through the relative magnitudes of photosynthetic assimilation and stomatal conductance, and is widely used as a proxy for intrinsic water use efficiency during leaf tissue formation (Dawson et al. 2002; Moreno-Gutiérrez et al. 2012).

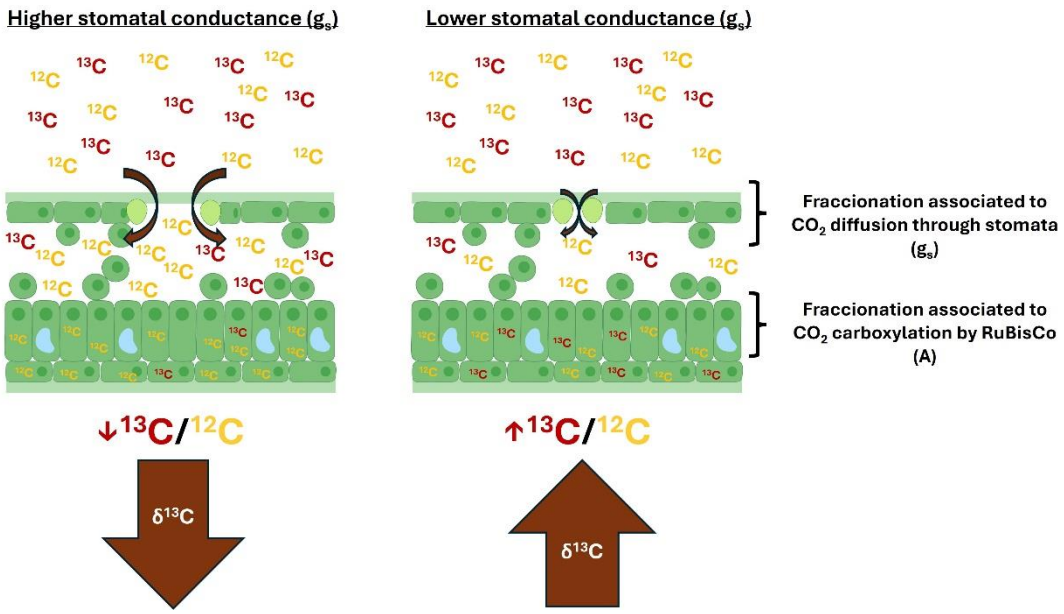

Figure 1. Schematic representation of the isotopic composition of carbon in the intercellular spaces and tissues of leaves under low or high stomatal conductance. Carbon isotopic composition experienced two phases of isotopic fractionation favouring ^{12}C over 1^3C. First is associated to CO_2 diffusion through stomata (g_s) and second associated to CO_2 carboxylation by RuBisCo (A). When stomatal conductance is low relative to assimilation rates in the chloroplasts, intercellular CO_2 concentrations decrease leading to a ^{13}C enrichment in plant tissues and a higher $\delta^{13}C$. This image was made using Biorender.

The oxygen isotopic composition on leaves is mainly due to leaf H^2O exchange during photosynthesis (Barbour, 2007; Cernusak et al., 2016; Song et al., 2022) (Figure 2). As leaf water transpires through the stomata, the lighter ^{16}O evaporates faster than heavier ^{18}O water molecules, leaving the remaining leaf water enriched in $H_2{}^{18}O$ and with this, the compounds synthesised in the leaves. $\delta^{18}O$ is therefore a good indicator of stomatal conductance. Oxygen enrichment above source water ($\Delta^{18}O$) can be calculated to reduce the influence source water isotopic composition and isolate stomatal signal. Oxygen and carbon isotopic composition in organic tissues can be used together to better understand the relationship between stomatal conductance and photosynthetic capacity resulting from environmental changes, as i) both carbon and plant oxygen isotope ratios are modified during gas exchange in the same leaf due to photosynthesis (CO_2) and transpiration (H_2O) and ii) carbon and oxygen are incorporated equally into organic matter so it provides a way to discriminate whether a change in isotopic ratios is the result of a change in photosynthesis or stomatal conductance (Scheidegger et al., 2000; Siegwolf et al., 2023). The combination of stable isotopes with more traditional measurements (LES) thus emerges as a very powerful tool to characterise water-use strategies in plants. It is also a method that allows extensive, large-scale sampling with

less effort than other traditional techniques (e.g., gas exchange or water potentials measurements), although its use and the standardisation of methodologies to make them as comparable as possible still requires further research (Ceperley et al., 2024).

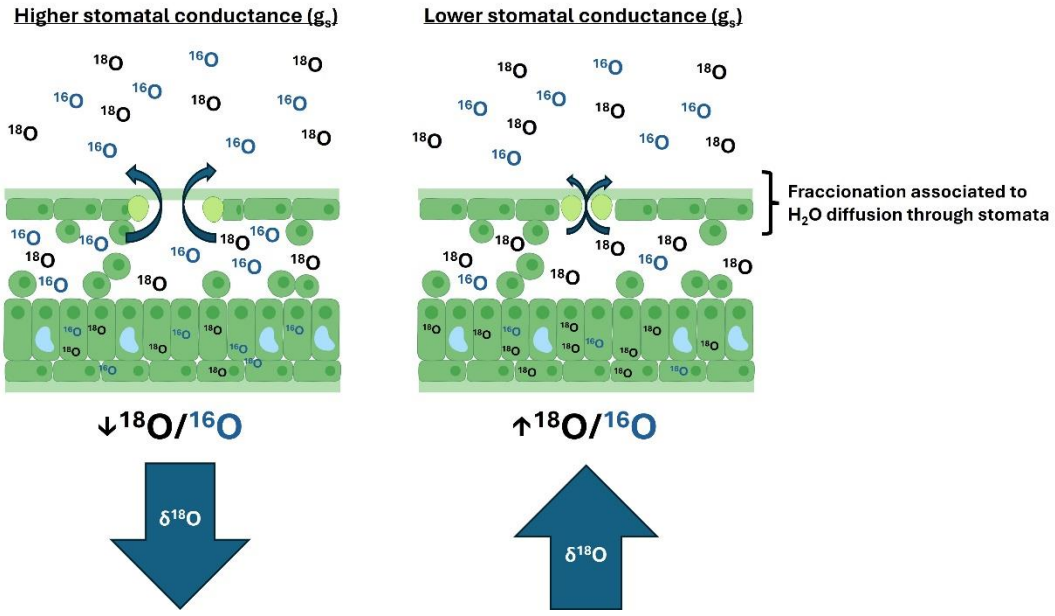

Figure 2. Schematic representation of the isotopic composition of oxygen in the intercellular spaces and tissues of leaves under low or high stomatal conductance. Oxygen isotopic composition in leaves is mainly due to H^2O exchange during photosynthesis. When g_s is low, as ^{16}O evaporates faster than ^{18}O water molecules, the concentration of ^{18}O increases in the intercellular spaces and its incorporation to foliar tissues leading to higher $\delta^{18}O$. This image was made using Biorender.

Beyond leaf level gas exchange, stable isotopes also provide a powerful tool for tracking belowground water acquisition strategies. The variation in the ratio of heavy to light oxygen isotopes ($^{18}O/^{16}O$) throughout the water cycle, from precipitation to soil water uptake by plants, and ultimately to the oxygen fixed in the organic matter of leaves, is influenced by both climatic and physiological factors. The isotopic composition of rainwater varies with factor such as continentality, altitude or temperature, with warmer conditions leading to a greater enrichment in ^{18}O (Bowen and Revenaugh 2003; Bowen, Wassenaar, and Hobson 2005). As precipitation infiltrates into the soil, vertical differences in soil water $\delta^{18}O$ values appear. Topsoil layers typically show higher water $\delta^{18}O$ values due to evaporative enrichment, as the lighter isotope ^{16}O evaporates at faster rates than the heavier ^{18}O because of its lower atomic mass (Allison, Barnes, and Hughes 1983). Similarly, an enrichment of the heavier deuterium isotopes (^2H) in topsoil layers occurs as a result of evaporative fractionation (Allison, Barnes, and Hughes 1983).

When water is absorbed by plant roots, there can be a slight species-specific fractionation of deuterium (^2H) (Ellsworth and Williams 2007), but no isotopic fractionation has been found for oxygen making the latter a more reliable proxy for determining plant water sources, especially when comparing plant uptake across different species (Barbeta et al. 2022). The combined measurement of xylem and soil water isotopic composition has proven to be a reliable method for estimating plant water uptake depth (Ehleringer and Dawson 1992; Bachofen et al. 2024; Barbeta et al. 2020; West et al. 2012). Since xylem water is a mixture of water extracted at different soil depths, its isotopic composition can be used to infer the relative contribution of each soil layer. This can be done using isotope mixing models that allow a precise calculation of the relative contribution of each soil layer at specific moments in time (Stock et al. 2018; Parnell et al. 2010).

The effect of aridity on functional trait coordination and plant community composition.

The patterns of trait coordination and aridity responses described above operate at the species level, but ultimately shape community composition and ecosystem functioning. To understand how these individual-level strategies scale up to determine community structure and functional diversity, a trait-based framework integrated with niche theory provides essential analytical tools. Dryland ecosystems are defined as regions where potential evapotranspiration, the evaporation from soil surfaces and plant transpiration, exceeds precipitation (Maestre et al. 2016, 2021). These ecosystems are characterized by low and irregular rainfall and high solar radiation, resulting in strong water limitations for life (Middleton and Thomas 1997; Nardini et al. 2014). The aridity of a region is often characterized with the aridity index (AI), which the ratio between mean annual precipitation (MAP) and potential evapotranspiration (PET). According to this index, regions with values lower than 0.65 are classified as drylands, which are further divided into hyper-arid (<0.05), arid (0.05 < AI < 0.20), semiarid (0.20 < AI 0.50) or dry subhumid zones (0.50 < AI < 0.65) (Maestre et al. 2021). Drylands currently occupy 45% of global surface area and are home for more than 38% of the global population (Prăvălie 2016). Recent climate projections suggest that the area covered by drylands can increase by 11-23% by the end of the century, driven by global climate change (Huang et al. 2016). Increasing aridity has major effects on multiple ecosystem functions and services, including nutrient cycling, carbon sequestration, soil microbial communities and water dynamics (Maestre et al. 2016; Berdugo et al. 2020). These changes also affect plant physiology and structure at multiple scales. In the short-term, physiological responses include a decrease in the activity of certain enzymes (e.g. Rubisco) under high

temperatures (Scafaro et al. 2023) or increases in stomatal conductance to reduce high leaf temperatures through evaporative leaf cooling (Prieto et al. 2023; León-Sánchez et al. 2018; Prentice et al. 2014; Marchin et al. 2022; Aparecido et al. 2020), while long-term adaptations can include modifications in xylem morphology and architecture and in leaf orientation or leaf size (Evans, Hu, and Michaletz 2025; Diao et al. 2024; Prentice et al. 2014; Scafaro et al. 2023). At the community level, reduced soil water and nutrient availability with increasing aridity causes systemic changes in plant community structure and composition (Berdugo et al. 2020). Numerous studies at both local and global scales, have shown a consistent link between aridity and the acquisition-conservation spectrum (LES), reflected by plant size and leaf morphology. As aridity increases, plant species tend to adopt and exhibit more conservative traits, such as smaller plant size, smaller and thicker leaves and lower SLA (Li and Prentice 2024; Liu et al. 2024; de la Riva et al. 2016; Carrascosa et al. 2023). While a few studies have explored the coordination between carbon, nutrient and water-use traits at the leaf level (Wang and Wen 2022b; Wang et al. 2021; Wang and Wen 2022a), their coordination at the whole plant level is not yet clear and there are still important knowledge gaps, particularly in seasonally dry ecosystems where prolonged droughts may shape unique plant responses.

These general patterns are particularly relevant in Mediterranean-type ecosystems, where pronounced seasonality and prolonged summer droughts impose strong constraints on plant community composition and trait coordination. Mediterranean type ecosystems are characterized by a pronounced seasonality, with intense but infrequent rainfall events interspersed with long dry periods, especially during the hot summer. These conditions drive the evolution of above and belowground strategies to cope with drought, broadly categorized into drought avoidance and drought tolerance (Mooney and Dunn 1970; Ackerly 2004). Drought avoidance is common in shallow-rooted species with loose stomatal control over water loss that rely on a high capacity for carbon fixation during short favourable wet periods, while drought tolerance is found in species with deeper root systems, tighter stomatal control and higher water use efficiency (Pinheiro and Chaves 2011; Zia et al. 2021). Drought-avoiding species with shallow roots are more exposed to longer periods of drought in the topsoil, while drought-tolerant species generally have access to deeper and more stable water pools over time. Most plant species preferentially use water from shallow soil layers when available as this topsoil water pool is richer in nutrients, but during extended drought the shallow water pool is depleted and becomes unavailable and only deep-rooted species can access the more stable deeper soil water pool (Schwinning, Starr, and Ehleringer 2005). To cope with prolonged drought periods, drought-avoidant species shed their leaves, adopting a

drought-deciduous strategy to reduce transpiration demand and prevent irreversible damage to tissues (Pivovaroff et al. 2016), whereas drought-tolerant species retain their leaves throughout the year (evergreen strategy) supported by a strict water loss control through stringent stomatal regulation.

These contrasting strategies create different growth opportunities: smaller and shallow-rooted drought-avoidant species have a narrow window for growth after rainfall events when shallow water is available, while evergreen, deeper rooted species can have a continuous growth throughout the year (Huxman et al. 2004). At the community level, increasing aridity and longer drought periods enhance the competition for water. In this scenario, communities transition from being dominated by perennial trees and shrubs in wetter environments, where retaining leaves and having a photosynthetic activity throughout the year is viable, to assemblages dominated by summer deciduous shrubs when rainfall is too scarce to sustain evergreen strategies but sufficient to trigger episodic growth in drought-deciduous species (Mooney and Dunn 1970). Given the strong sensitivity of Mediterranean ecosystems to global warming, and the increased risk of aridification, understanding how carbon, nutrient and water-use strategies shape plant communities is essential for predicting their responses to climate change and develop adaptive plans for their conservation.

To understand how plant strategies scale up to shape community-level patterns, a functional trait framework provides valuable tools to quantify diversity, coordination and trade-offs across species and environments. Functional diversity is the variation of functional traits between individuals, species or communities, and its study offers a strong potential to evaluate the impact of climate change on biodiversity and community assembly (Carmona et al. 2016). Different components of functional diversity, such as richness, evenness, divergence, and skewness, have been widely studied, with early studies focusing on mean trait values and more recent methods incorporating intraspecific trait variability (Pavoine and Bonsall 2011; Carmona et al. 2019; Blonder et al. 2014). Recent methods in trait-based ecology now enable the construction of a multidimensional functional space, where species or communities are positioned based on their trait values. According to the multidimensional niche concept (Hutchinson 1957), while species can potentially occupy any region of the functional trait space, not all regions confer equal fitness under given environmental conditions or species interactions. Higher functional diversity expands the range of possible trait combinations and strategies within communities, thereby enhancing the efficiency of resource use and promoting niche differentiation and complementarity. Mediterranean climates, where resources are unevenly distributed both spatially (e.g. soil nutrient and water vertical

heterogeneity) and temporally (e.g. variable water availability throughout the year) may enhance plant functional diversity. But as aridity increases, the viable trait combinations decrease and optimal regions of the trait space are defined (de la Riva et al. 2018; Sfair, Rosado, and Tabarelli 2016; Medeiros et al. 2025; Rodríguez-Alarcón, Tamme, and Carmona 2022). Along aridity gradients in Mediterranean ecosystems, community assembly reflects both competition, with acquisitive species capturing resources faster, and facilitation, with deep-rooted species redistributing water to shallow-rooted neighbours overnight, processes that can shift in importance as water becomes increasingly limiting (Gross et al. 2013; Prieto, Armas, and Pugnaire 2012). Understanding how functional water-saver strategies (carbon, water and nutrient use strategies), functional diversity and trait coordination vary with aridity is therefore crucial to predicting community dynamics and ecosystem functioning under climate change.

Objectives and hypothesis

The present doctoral thesis focuses on the key questions posed in the previous sections. We argue that, although significant progress has been made for understanding plant carbon and nutrient strategies and their response to aridity (Leaf and plant economics spectrums), there is still a large knowledge gap about the potential links bridging carbon and nutrient use strategies with water-use strategies, especially in water limited ecosystems such as those in the Mediterranean region, both at the species and community levels. Thus, the main objective of this doctoral thesis is to improve current understanding of the diversity of C and nutrient strategies and water-use strategies and their degree of coordination in Mediterranean woodland and shrubland plant communities, by covering a wide range aridity conditions, and considering both the species and community levels. To address this general objective, we have designed three complementary studies that gradually incorporate various aspects of plant resource economics to achieve this overarching goal. The diversity of water use strategies and how they align with belowground water use are established in Chapter 1. The coordination between water-use strategies and the leaf economics spectrum (carbon- and nutrient-use) is specifically tested in Chapter 2. These species-level patterns are scaled to the community level along an aridity gradient in Chapter 3. Thus, specific objectives are to:

- Assess the diversity and degree of coordination of above and belowground plant water-use traits across plant species and life forms in Mediterranean plant communities (Chapter 1).
    - Coexisting woody species exhibit distinct ecohydrological niche segregation driven by size-related differences in soil water uptake depth ($\delta^{18}O_{XW}$, $\delta^{2}H_{XW}$, d-excess$_{XW}$) between shallow- and deep-rooted species. Also, in leaf level stomatal regulation and water use efficiency ranging ($\delta^{13}C_{L}$, $\Delta^{18}O_{L}$) from acquisitive water-spender to conservative water-saver strategies.
    - Aboveground water-use traits are coordinated with belowground water uptake depth across coexisting woody species and life forms, with water-spender species exhibiting shallower rooting and water uptake depth, while the opposite is expected for water-saver species.
    - Aboveground hydraulic traits are coordinated between them and with water uptake depth across plant species and functional types, so that

shallow-rooted water-spender strategy will be compensated by a lower leaf level area to sapwood area ratio.

- The diversity of whole plant water-use strategies present in upland Mediterranean ecosystems is constrained by the tight coordination and trade-offs between aboveground and belowground water-use traits.
- Interspecific differences in above and belowground water-use traits among coexisting species (water uptake depth, $\delta^{13}C_L$ and $\Delta^{18}O_L$) remain largely stable through time, so that within-site isotopic trait-based species rankings are well preserved between dry and wet years.

- Evaluate the diversity of carbon and nutrient use strategies along an acquisitive-conservative spectrum (Leaf Economics Spectrum) and the degree of coordination between these and plant water-use strategies across species and life forms in Mediterranean plant communities (Chapter 2)
  - Leaf carbon (SLA and LDMC) and nutrient (leaf N, P, K concentrations) use traits converge a single LES- acquisitive-conservative axis across species in Mediterranean woody vegetation.
  - Acquisitive species along the LES (high SLA and nutrient concentrations and low LDMC) are tightly coordinated with to a more profligate water-use strategy at leaf level (higher g_s and E combined with lower $\delta^{13}C_L$, $\Delta^{18}O_L$, WUE_i and WUE_t), while opposing trend occur in coexisting species combining conservative LES traits and more water-saver strategy at leaf level.
  - Species with acquisitive LES traits will need to use a greater proportion of water stored in nutrient-rich topsoil layers to meet their higher nutrient demand, compared to co-occurring species with more conservative leaf traits along the LES and thus lower nutrient demand.
  - Coordination and functional convergence between leaf economic traits and wate use traits should be stronger in more arid environments where nutrients and carbon acquisition by plants is severely constrained by low water availability.
- Explore the impact of aridity on plant variability in carbon, nutrient and water-use traits at community-level, and to identify the dominant functional strategies along the aridity gradient (Chapter 3).
  - Aridity has a strong effect on LES and leaf water-use traits, so that there is a greater dominance of conservative resource-use (C and nutrients) and water-use strategies towards the arid end of the gradient as a response to more severe water and nutrient limitation.

- There is a tight coordination between plant water and leaf economics traits along the aridity gradient.
- Plant life form represents a main driver of functional trait variation across the aridity gradient.
- More climatically distant communities along the aridity gradient should occupy more distinct volumes in functional trait space, indicating that aridity plays a key role in shaping the dominant trait values in each of these plant communities.

Study area

The Mediterranean region is a mosaic of ecosystems where plant communities predominantly occur in arid or semi-arid (Maestre et al. 2021) conditions with summers characterized by high temperatures and low precipitation. Mediterranean ecosystems have been recognized as one of the major biodiversity hotspots, with a particularly high number of endemic species, approximately 13,000 of the 25,000 plant species described in the region are endemic (Myers et al. 2000). The work carried out during this doctoral thesis was conducted in 6 Mediterranean woody plant communities (Pulpí, Pliego, Ayna, Majarazán, Yeste and Montejo) located along a 600 km aridity gradient from the hotter and drier south-east to the colder and wetter centre of the Iberian Peninsula. Other 4 communities sampled in previous fieldwork campaigns following the same procedures (Calblanque, Los Cuadros Natural, Los Cuadros Repoblación and Venta del Olivo) were added in the first and second chapter to broaden the gradient and diversity of species included in the study. According to data compiled from terraclimate dataset (Abatzoglou et al. 2018) for the whole available period (1958-2022), annual precipitation ranged from 272 in the drier location near the coast to 724 mm in the centre of the Iberian peninsula, while mean annual temperatures ranged from 8.6 to 18.5 in these same locations. We also calculated an aridity value as 1-P/PET, being PET, potential evapotranspiration (Figure 3). This climatic range encompasses almost the total climatic range found in Mediterranean ecosystems (Seager et al. 2019), thus offering an extensive approximation of the functioning of Mediterranean plant communities.

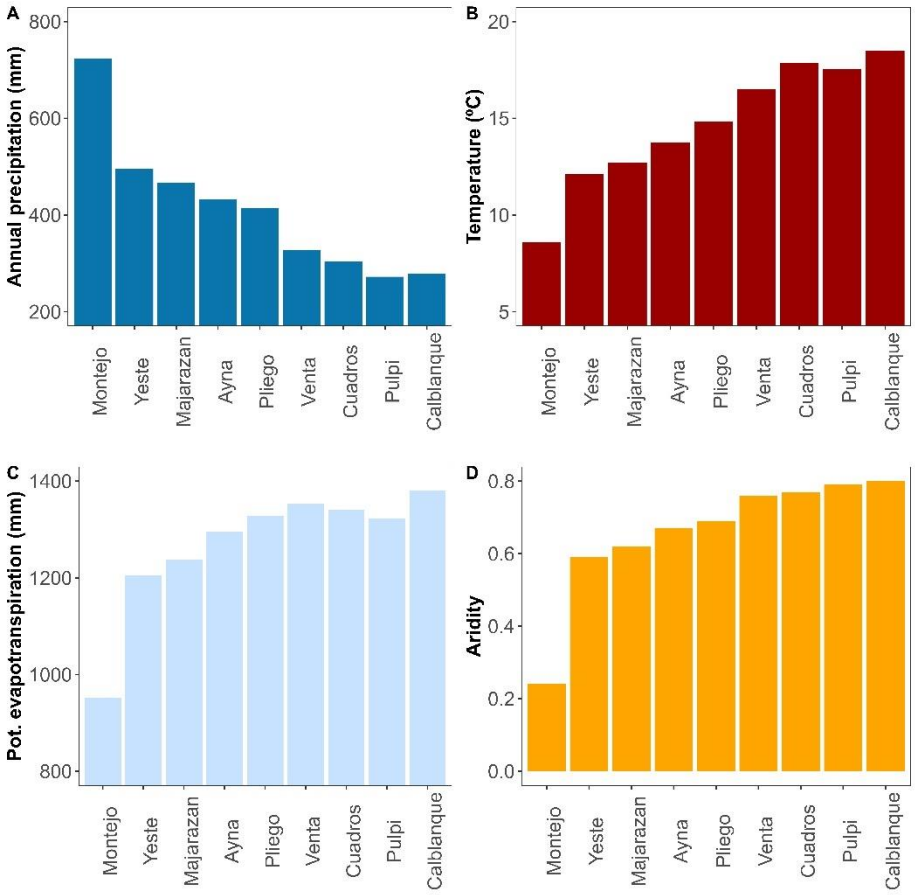

Figure 3. Mean annual (A) precipitation (mm), (B) temperature (ºC), (C) potential evapotranspiration (mm) and (D) aridity (1-P/PET) for the 1958-2022 period. Data were compiled from the terraclimate database (Abatzoglou et al. 2018) for each plant community.

The vegetation structure in the communities studied varies widely along the aridity gradient (Figure 4). The communities located at the arid end of the gradient are open forests and scrublands dominated by *Pinus halepensis,* large shrubs like *Rhamnus lycioides* or *Pistacia lentiscus* and mainly dominated by small shrubs like*, Salvia rosmarinus or Cistus clusii.* In intermediate areas, the communities are predominantly *Pinus halepensis* forests with a greater presence of small shrubs such as *Salvia rosmarinus* or *Thymus spp.* in the driest and warmest areas, and a greater presence of other large trees and shrubs such as *Quercus rotundifolia*, *Juniperus oxycedrus* or *Juniperus phoenicia* in the highest and coldest areas. For its part, at the wettest end of the gradient in the centre of the peninsula, the sampled community was a closed canopy forest dominated by deciduous tree species such as *Fagus sylvatica*, *Quercus petraea* or *Quercus pyrenaica*, and sparse extensions of shrubs such as *Lavandula stoechas,*

Adenocarpus hispanicus or *Juniperus communis* in the clearings and more open areas of the forest.

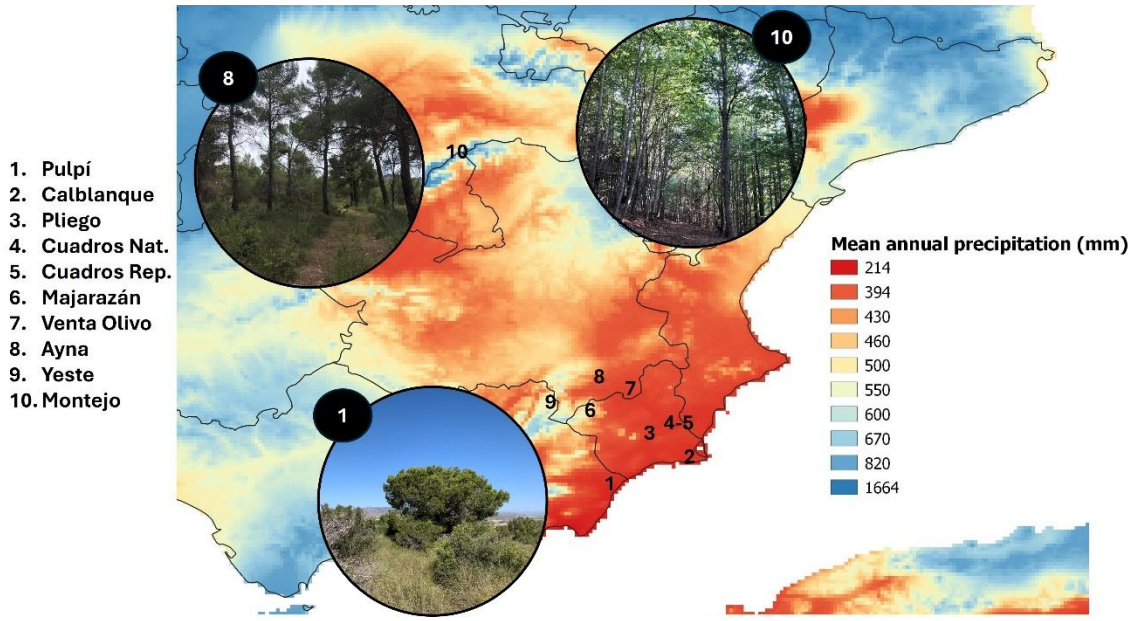

Figure 4. Map of mean annual precipitation (mm) using Terraclimate database (period 1958-2021) (Abatzoglou et al. 2018) in the study area. Numbers indicate the position of each community in the gradient and images showed the different type of plant communities present in each site.

Sampling at the six main sites in this thesis was carried out in two different years, 2019 and 2022 (2021 and 2022 in Montejo), which had different climatic conditions. 2019 and 2021 were years around the historical average for rainfall or slightly below and 2022 had a particularly wet spring (Figure 5). All sampling was conducted to coincide with the period of maximum vegetation activity at each site, thus beginning in early April at the driest sites and ending at the wettest sites in early July. Because recent precipitation can influence the isotopic composition of soil water with depth, as the enrichment gradient may temporarily disappear when top layers are recharged (Allison, Barnes, and Hughes 1983), all sampling was carried out at least two weeks after the last rainfall event in each community. Within each community, 6 individuals of each species matching the 90% of most abundant species were sampled raising up to 62 different plant species in the whole gradient. From each individual we collected leaf and stem samples to obtain data on the isotopic content of dry matter and water, morphological data and nutrient content, which we will break down later in the methodology section of each chapter.

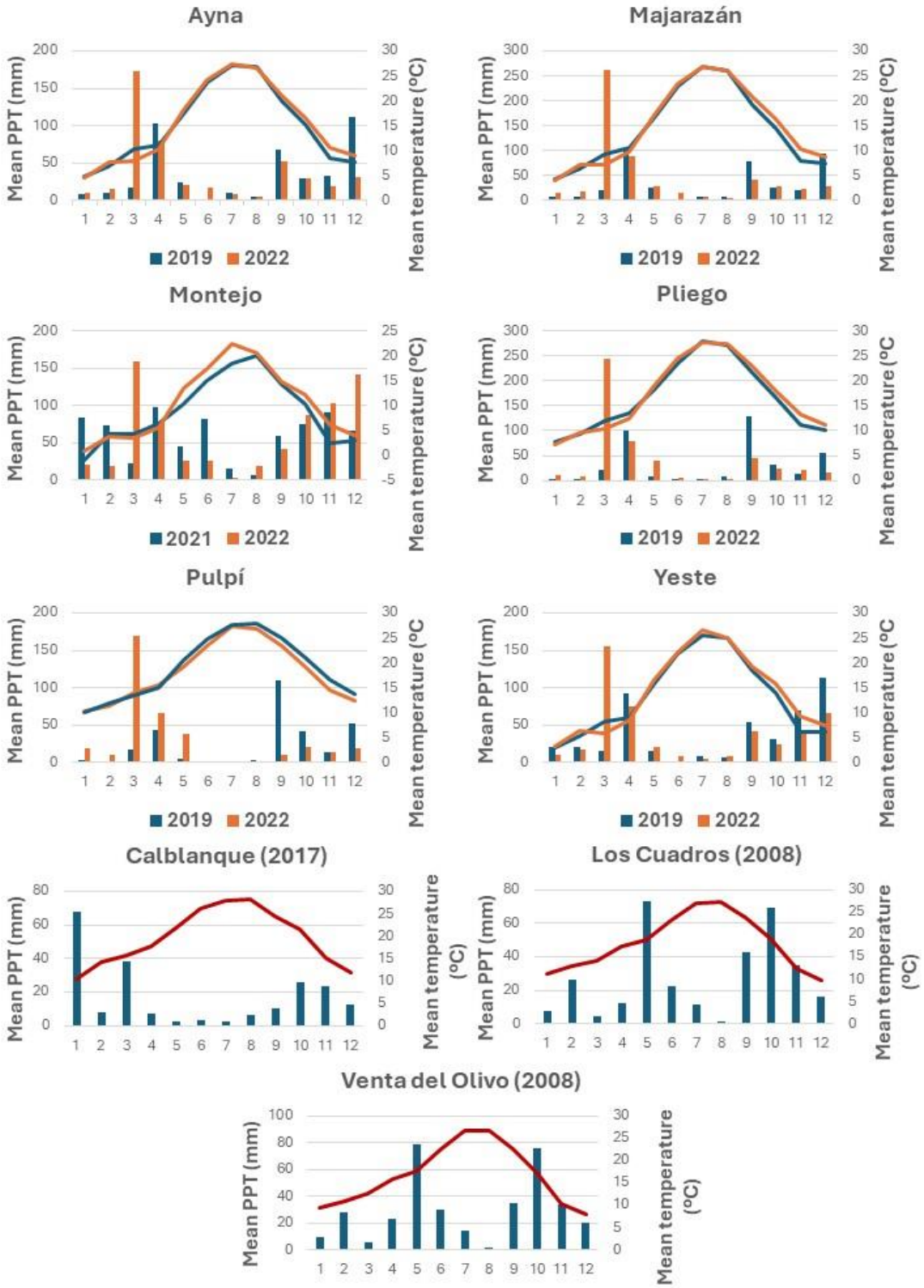

Figure 5. Climate diagram showing monthly precipitation (PPT, mm, depicted by bars) and temperature (ºC, depicted by lines) for the years when the field sampling campaigns were performed at each study site. For sites where two field sampling campaigns were conducted in different years, a different colour was set for each year for temperature (lines) and precipitation (bars).

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Chapter 1

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

Chapter 1: Trait coordination and trade-offs constrain the diversity of water use strategies in Mediterranean woody plants

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Introduction

Understanding the relationship between plant taxonomic and functional diversity has been central to ecology for decades, yet few studies have explored the potential coordination of key below- and aboveground water use traits such as soil water uptake depth by roots and leaf-level stomatal regulation stringency or water use efficiency. Plant species size and position along the Plant Economic Spectrum (acquisitive-conservative continuum) are key indicators of species nutrient and carbon use strategies (Díaz et al. 2016; Bruelheide et al. 2018; Reich 2014; Wright et al. 2004). However, the diversity of water use strategies among coexisting species in dryland plant communities, or the potential coordination and tradeoffs between water uptake depth and leaf-level water use

traits in drought-prone ecosystems, have received comparatively much less research attention (Moreno-Gutiérrez et al. 2012; Araya et al. 2011; Silvertown, Araya, and Gowing 2015; Mooney and Dunn 1970b, 1970a; Ackerly 2004).

A generally good correlation between above and belowground plant component sizes has been described across multiple ecosystem types worldwide (Tumber-Dávila et al. 2022). However, it is important to note that water uptake depth depends not only on plant size but also on multiple other environmental factors such as climate type or groundwater accessibility to roots (Laughlin et al. 2023; Fan et al. 2017). In this context, plant species from humid climates and ecosystems tend to rely more heavily on shallow soil water sources than species from dry climates, as the latter generally develop deeper root systems and are capable of using a greater proportion of deep soil/bedrock water sources (Sohel et al. 2021; Laughlin et al. 2023; Evaristo and McDonnell 2017). Woody plants in Mediterranean and other dryland habitats typically use less than 30% of water from recent precipitation stored in upper soil layers (Miguez-Macho and Fan 2021), so water uptake from deeper soil and bedrock moisture sources has an important role in sustaining vegetation transpiration during prolonged rainless periods. Vertical segregation in plant water uptake patterns among coexisting species can lead to ecohydrological niche partitioning, in which plant species with contrasting water use traits gain preferential access to different soil water pools present along the edaphic profile (Silvertown, Araya, and Gowing 2015). Ecohydrological niche segregation (Araya et al. 2011) and contrasting plant water use strategies among coexisting species and life forms could favor resource partitioning that enhances complementarity and efficiency in the use of the limited water resources in drought-prone ecosystems (Grossiord 2019).

Woody plants in seasonally dry upland Mediterranean ecosystems depend on two distinct water pools available along the edaphic profile: an easily accessible topsoil pool in upper layers with intermittent, erratic and rapidly fluctuating water availability that is recharged mainly by recent precipitation, but is exposed to rapid evaporative loss and competitive depletion by neighbors; and a less accessible, deeper soil/bedrock pool with more temporally stable water storage and availability that is recharged by large winter and autumn precipitation events and is less exposed to direct evaporative losses or competitive depletion by neighbors (Illuminati et al. 2022; Silvertown, Araya, and Gowing 2015; Bachofen et al. 2024). Plant species access to, and utilization of, each of these two moisture pools may be constrained by multiple trade-offs between structural and physiological traits. Traits such as small size or shallow rooting pattern may severely constrain access to the more stable water pool stored in deep soil/bedrock layers (Fernández-de-Uña et al. 2023; Laughlin et al. 2023; Tumber-Dávila et al. 2022).

Whereas physiological traits associated to leaf-level water use patterns, such as stomatal regulation stringency or water use efficiency (Siegwolf et al. 2023) could modulate a faster or slower utilization of the available soil water by each species according to their position along the profligate-conservative water use continuum (Moreno-Gutiérrez et al. 2012; Ding et al. 2021; Prieto et al. 2018). Previous studies investigating the diversity of plant water use strategies present in Mediterranean plant communities largely focused on local spatial scales with relatively few coexisting species (Illuminati et al. 2022; Querejeta, Ren, and Prieto 2021; Palacio, Montserrat-Martí, and Ferrio 2017). More ambitious and comprehensive surveys encompassing larger numbers of plant species growing at multiple sites with contrasting climatic and environmental conditions are thus needed to further advance our current understanding of water use patterns by woody vegetation across the Mediterranean biome. Moreover, such an approach could help elucidate any potential coordination and trade-offs between water uptake depth and leaf-level water use strategies across coexisting plant species and life forms at multiple sites.

Stable isotopes and their variations in plant tissues provide a valuable tool to investigate interspecific differences in water use strategies among coexisting species in natural plant communities. The isotopic composition of xylem water ($\delta^{18}O_{xw}$, $\delta^{2}H_{xw}$, d-excess$_{xw}$) reflects the relative proportion of water taken up from different soil depths by plants, as little or no isotopic fractionation occurs during root water uptake from soil (Barbour, 2007; but see Barbeta et al., 2022 and Ellsworth and Williams, 2007 for issues with $\delta^{2}H$). The relative contributions of shallow vs deep soil water sources to total plant water uptake can be more easily estimated when intense evaporative isotopic enrichment of the shallow soil water pool occurs (Allison, Barnes, and Hughes 1983), which is typically found in Mediterranean ecosystems with frequent hot and rainless periods during which topsoil water is exposed to intense evaporative isotopic fractionation. The stable carbon isotope composition of leaf dry matter ($\delta^{13}C_L$) represents a metabolic set point for the integration and coordination of leaf gas exchange fluxes (Ehleringer and Cerling 1995) that is affected by both CO_2 assimilation during photosynthesis and CO_2 diffusion through stomata, so it provides a good proxy for time-integrated intrinsic water use efficiency (WUE_i) defined as the ratio between net photosynthetic rate (A) and stomatal conductance (g_s) (Siegwolf et al. 2023; Dawson et al. 2002; G D Farquhar, Ehleringer, and Hubick 1989; Prieto et al. 2018; Moreno-Gutiérrez et al. 2012). The oxygen isotopic composition ($\delta^{18}O_L$) of leaf dry matter reflects the isotopic signal of the source water used by the plant ($\delta^{18}O_{xw}$), and is also affected by leaf-level evaporative effects during transpiration (Barbour 2007; Graham D. Farquhar, Cernusak, and Barnes 2007). Thus,

the oxygen isotopic composition of leaf dry matter calculated as enrichment above source water ($\Delta^{18}O_L = \delta^{18}O_L - \delta^{18}O_{xw}$) can provide a time-integrated indication of cumulative g_s and transpiration over the growing season (Siegwolf et al. 2023; Moreno-Gutiérrez et al. 2012). However, the interpretation of interspecific differences in $\delta^{13}C_L$ and $\Delta^{18}O_L$ among coexisting plant species is complicated by multiple uncertainties, due to the myriad complex factors that may affect the carbon and oxygen isotopic composition of leaf organic matter in natural plant communities(Werner and Maguas 2010; Seibt et al. 2008; Dawson et al. 2002; Song et al. 2013; Kahmen et al. 2009; Wang, Yakir, and Avishai 1998; Cornwell et al. 2018). It is thus advisable to simultaneously conduct conventional leaf gas exchange measurements (stomatal conductance, intrinsic water use efficiency) in the field to validate the interpretation of $\delta^{13}C_L$ and $\Delta^{18}O_L$ and to confirm that these isotopic water-use traits actually provide reliable time-integrated proxies of leaf-level water use patterns across coexisting woody species within each plant community.

In this study, we assess the diversity of plant water use strategies among coexisting woody species at ten study sites with contrasting climatic and environmental conditions in the Iberian Peninsula. Our study sites span from warm arid and semiarid areas near the Mediterranean coast where dwarf palms (*Chamaerops humilis*) and Iberian-African endemic shrubs (*Periploca angustifolia, Maytenus senegalensis*) are common, to much cooler and wetter areas located at higher altitudes at the Mediterranean-Temperate transitional border in central Spain, where temperate tree species such as *Fagus sylvatica* or *Quercus petraea* are present. Our 10 study sites were carefully chosen to represent the wide spectrum of precipitation and temperature conditions encountered across the Mediterranean biome (from warmer arid and semiarid climates to cooler dry-subhumid climates, Table S1). Our pool of 62 woody plant species (771 individuals) encompasses contrasting plant life forms: small shrubs (< 1m height), large shrubs (1-3 m height) and trees (3 to 20 m in height) (Tumber-Dávila et al. 2022), and is representative of the large functional and taxonomic diversity of the Mediterranean biogeographical region, which includes evergreen, drought-deciduous/semideciduous and winter-deciduous species (Table S2, Table S6).

We investigate the potential occurrence of vertical niche partitioning of soil water and ecohydrological niche segregation among coexisting woody species at all the 10 study sites. Matching soil water isotopic composition variations along the soil profile with the xylem water isotopic composition of plants ($\delta^{18}O_{xw}$, $\delta^{2}H_{xw}$, d-excess$_{xw}$), we estimate the proportion of water taken up from shallow vs deeper moisture pools by the different species and life forms present in these plant communities during the peak of the growing

season in the Mediterranean biome (Spring). In parallel, we also assess the diversity of leaf-level water use strategies using foliar $\delta^{13}C_L$ and $\Delta^{18}O_L$ as proxies for time-integrated water use efficiency (WUE_i) and stomatal conductance (g_s), respectively. In addition, we measure the leaf area to sapwood area ratio of terminal shoots as a key aboveground trait related to plant hydraulic architecture (i.e. the ratio between the total transpiring leaf area to the cross-sectional sapwood area supplying water to leaves) at two sites with contrasting climatic conditions and high plant species diversity (Yeste and Montejo, see Material and methods section). In a subset of six study sites, the isotopic water use traits of all the coexisting woody species ($\delta^{13}C_L$, $\Delta^{18}O_L$ $\delta^{18}O_{xw}$, $\delta^{2}H_{xw}$, d-$excess_{xw}$) were measured twice on two separate years (2019 and 2022) with sharply contrasting rainfall amounts and patterns, in order to assess whether interspecific differences in water use traits among coexisting species are well preserved through time across years with contrasting climatic conditions (dry vs wet years). During the second sampling year (2022) we also conducted instantaneous leaf gas exchange measurements in the field to confirm that leaf carbon and oxygen isotopes indeed provide reliable proxies of interspecific differences in leaf-level water use patterns among coexisting woody species at each study site. Finally, we investigate the potential coordination and tradeoffs between aboveground water use traits ($\delta^{13}C_L$, $\Delta^{18}O_L$, leaf area to sapwood area ratio) and belowground water uptake depth ($\delta^{18}O_{xw}$, $\delta^{2}H_{xw}$, d-$excess_{xw}$) across coexisting woody plant species and life forms. Our main objective is to address the current knowledge gaps and uncertainty regarding the potential links, interconnectivity and degree of coordination between above- and belowground water use traits across plant species and life forms in Mediterranean ecosystems. More specifically, we aim to identify potential tradeoffs among those traits that might constrain the diversity of whole-plant water use strategies and trait constellations that are ecologically and physiologically feasible and viable for native woody species growing under Mediterranean-type climates. Characterizing the diversity of above and belowground water use traits and their potential coordination and tradeoffs among coexisting plant species at multiple sites with contrasting environmental conditions could provide valuable insights into how native vegetation copes with drought stress in the Mediterranean biome and help improve our current understanding of soil water utilization and partitioning among coexisting individuals in species-rich plant communities (Jiao et al. 2021; Liu et al. 2020).

Based on the earlier preliminary findings of local-scale studies conducted in Mediterranean-type ecosystems (Illuminati et al. 2022; Fernández-de-Uña et al. 2023; Ding et al. 2021; Moreno-Gutiérrez et al. 2012) we hypothesize that: i) Coexisting woody species and plant life forms exhibit distinct ecohydrological niche segregation driven by

size-related differences in soil water uptake depth ($\delta^{18}O_{xw}$, $\delta^{2}H_{xw}$, d-excess$_{xw}$) between shallow- and deep-rooted species (H1); ii) Coexisting species and plant life forms also exhibit large differences in leaf-level stomatal regulation stringency and water use efficiency ($\delta^{13}C_L$, $\Delta^{18}O_L$) ranging from profligate water-spender to conservative water-saving strategies at leaf level (H2); iii) Aboveground hydraulic traits are coordinated across species and plant functional types, so that a more profligate water-use strategy at leaf level (lower $\delta^{13}C_L$, $\Delta^{18}O_L$) will be compensated by a lower leaf area to sapwood area ratio (Hernandez-Santana et al. 2023) (H3); iv) Aboveground water use traits ($\delta^{13}C_L$, $\Delta^{18}O_L$, leaf area to sapwood area ratio) are also coordinated with belowground water uptake depth ($\delta^{18}O_{xw}$, $\delta^{2}H_{xw}$, d-excess) across coexisting woody species and life forms, with water-spenders exhibiting a shallower rooting depth and water uptake pattern than water-saver species (H4); v) The diversity of whole-plant water use strategies present in upland Mediterranean ecosystems is limited and constrained by the tight coordination and tradeoffs between above- ($\delta^{13}C_L$, $\Delta^{18}O_L$, leaf area to sapwood area ratio) and belowground ($\delta^{18}O_{xw}$, $\delta^{2}H_{xw}$, d-excess) water use traits (H5); vi) Interspecific differences in above- and belowground water use traits among coexisting woody species (water uptake depth, leaf $\delta^{13}C_L$, $\Delta^{18}O_L$) remain largely stable through time, so that within-site isotopic trait-based species rankings are well preserved between dry and wet years (H6).

Here, we show that water source partitioning and ecohydrological niche segregation driven by differences in water uptake depth among coexisting species are widespread across Mediterranean plant communities with contrasting climatic conditions. Foliar carbon and oxygen isotopes indicate that leaf-level stomatal regulation stringency and water-use efficiency also differ greatly among coexisting species and are both tightly coordinated with water uptake depth. Larger and taller trees generally use a greater proportion of deeper soil water, display more conservative water use traits at leaf level ("water-savers") and show greater investment in foliage relative to shoots. Conversely, smaller shrubs rely mainly on shallow soil water, exhibit a more profligate water use strategy at leaf level ("water-spenders") and invest more heavily in shoots relative to foliage. Drought and heat stress appear to favor coordination between above and belowground water-use traits, resulting in possibly unavoidable tradeoffs that constrain the diversity of whole-plant water use strategies in Mediterranean plant communities.

Methods

Study sites and sampling design.

We selected 10 different Mediterranean shrubland, open woodland and closed-canopy forest communities with contrasting climate for the present study (Table S1). These 10 sites were distributed along a 600 km transect from the south-eastern (warmer and drier) to the central part (cooler and wetter) of the Iberian Peninsula. Mean annual precipitation ranged from 272 mm near the Mediterranean coast to 726 mm at the Central Ranges of the Castilian Plateau. Mean annual temperature ranged from 18.5 °C at the coastal sites to 8.6 °C at the Central Ranges site (Abatzoglou et al. 2018). Mean altitude above sea level of the study sites ranged from 10 m for sites near the Mediterranean coast to 1450 m at the Central Ranges site. Vegetation structure differs dramatically among study sites, from open pine woodlands and shrublands dominated by *Pinus halepensis* and large shrubs (*Rhamnus lycioides, Periploca angustifolia*) at the southern and drier coastal sites, to closed-canopy forests dominated by *Pinus pinaster* and *Quercus rotundifolia* in more mesic sites located further inland (e.g. Ayna and Yeste), to multi-layer, closed-canopy forests dominated by *Fagus sylvatica* and *Quercus petraea* in the northernmost and more humid location. Soils are predominantly sedimentary calcareous with a mixture of marls, sandstones and clays depending on the site. Schist formations interspersed with calcareous lithologies can also be found at Calblanque and Montejo (Rodríguez-Fernández et al. 2015).

We collected samples from at least 6 individuals of the most dominant woody species in each plant community, i.e. those overall encompassing 90% of the total cover in the community. In total, we sampled 771 individuals of 62 woody species belonging to 21 plant families, resulting in 121 species-site combinations (Table S6). The species were categorized into three major plant life forms according to their aboveground vegetative height using an adaptation from Raunkaier's life form classification (Raunkiær 1934; Tumber-Dávila et al. 2022): small shrubs (SS, <1m height), large shrubs (LS, 1-3 m) and trees (T, 3-20 m). Details about the leaf habit (deciduous, evergreen or semideciduous), plant lifespan (long-lived, > 30 years or short-lived, <30 years) and maximum rooting depth were obtained from the scientific literature and our own expert field knowledge for all species(de la Riva et al. 2018; Navarro et al. 2009; Gavilán et al. 2018; Beccari and Carmona 2024; Tumber-Dávila et al. 2022) (Table S2). Field sampling campaigns were conducted during the phenological and physiological peak at each location during Spring, starting in early April at the drier and warmer sites (Pulpí, Calblanque, Los Cuadros), continuing in May at sites with typical meso-mediterranean climate (Venta Olivo, Pliego, Ayna, Majarazán, Yeste) and ending in early July at the wettest and coolest site with the

latest onset of the growing season (Montejo). The sampling date was carefully chosen at each study site to ensure at least a 2-week period with no rainfall prior to the sampling date. The rationale for this was to ensure the development of a steep soil water isotopic gradient with depth along the edaphic profile, which is typically formed in response to topsoil water evaporation and isotopic fractionation during prolonged rainless periods (Allison, Barnes, and Hughes 1983). Six of the study sites were sampled in two separate years with contrasting climatic conditions and rainfall patterns, as much higher precipitation was recorded in 2022 compared to 2019 (or 2021 in Montejo) across all sites (Figure S10).

Xylem and soil water isotopic analyses

For each plant individual, we sampled one basal 5 cm segment of fully-lignified stem from a terminal branch, to avoid issues related to potential xylem water isotopic enrichment due to backflow from transpiring leaves or green photosynthetic stems (Schwinning 2008). For tree species, we sampled stem samples from fully sun-exposed mid-canopy branches (at least 3-6 m above ground level) using a pole-pruner. The stems were debarked to isolate the xylem, stored in 5 ml glass vials, sealed with parafilm to prevent water loss, and stored at -20ºC until water extraction. At each site, we sampled a minimum of 2 and up to 6 soil water profiles, using a cylindrical hand auger with 4 cm diameter (Eijkelkamp, The Netherlands). Soil samples were taken, every 5 cm in the topsoil layer (down to 20 cm) and every 10cm below 20 cm deep, until we reached impenetrable hard bedrock layers (which were located between 30 and 120 cm deep, depending on soil characteristics at each study site). All freshly collected soil samples were immediately placed in double sealed plastic bags in the field, maintained in a cooler and transported to the laboratory, where a soil subsample was transferred to 5-ml glass vials, sealed with parafilm, and kept at -20°C until water extraction.

We used a cryogenic vacuum distillation line maintained at 100°C and 10 militorr vacuum pressure for at least 2 hours to extract all the water contained in plant stems (xylem water) and soil samples (Ehleringer and Osmond 1989). Extracted water samples were shipped to the Center for Stable Isotope Biogeochemistry of the University of California (CSIB-UCB, Berkeley, USA) for isotopic analyses. Water δ^2H composition was determined in a dual inlet using a hot chromium reactor unit (H/Device™; Thermo Scientific, Waltham, MA, USA) interfaced with a Thermo Delta V Plus mass spectrometer (Thermo Fisher Scientific). Continuous flow using a Thermo Gas Bench II interfaced to a Thermo Delta V Plus mass spectrometer was used for analyzing water $\delta^{18}O$ composition. Hydrogen (δ^2H) and oxygen ($\delta^{18}O$) isotopic composition are expressed in ‰ notation relative to the standard V- SMOW (Vienna standard Mean Ocean Water).

Long-term external precision values for water δ^2H and $\delta^{18}O$ determinations were ± 0.60‰ and ± 0.12‰, respectively. Deuterium excess was calculated as the deviation from the Local Water Meteoric Line (LMLW) for the Iberian Peninsula (d-excess = δ^2H – 8.49 x $\delta^{18}O$; (Diaz-Teijeiro, Rodríguez-Arévalo, and Castaño 2009)).

Leaf isotopic and gas exchange measurements

We sampled sun-exposed, fully expanded and healthy-looking leaves from the same branches collected for xylem water extractions in each individual plant. These leaves were immediately placed in sealed bags containing a piece of wet paper in the field, transported to the lab and stored in the fridge at 4°C for leaf material processing within 24-48 hours. Leaf samples were oven dried at 60°C for 48 hours and finely ground using a ball mill (MM200, Retsh, Germany) before being weighted and encapsuled into tin or silver capsules for leaf $\delta^{13}C$ and leaf $\delta^{18}O$ isotopic analyses, respectively. Leaf $\delta^{13}C$ was measured at CSIB-UCB by continuous flow (CF) dual isotope analysis using a CHNOS Elemental Analyzer interfaced to an IsoPrime100 mass spectrometer, and leaf $\delta^{18}O$ was determined in continuous flow (CF) using an Elementar PYRO Cube interfaced to a Thermo Delta V mass spectrometer. Both leaf $\delta^{13}C$ and $\delta^{18}O$ isotopic composition are expressed in delta notation (‰) relative to the Vienna Pee Dee Belemnite standard (V-PDB) and the Vienna Standard Mean Ocean Water (VSMOW), respectively. Long-term external precision values for leaf $\delta^{13}C$ and $\delta^{18}O$ determinations were ± 0.10‰ and ± 0.20‰, respectively. As a way to isolate the leaf-level evaporative signal from the source water isotopic signal (Barbour 2007), we calculated the oxygen isotopic enrichment of leaf organic matter above the isotopic composition of plant source water, as the difference between the $\delta^{18}O$ value of bulk leaf material and the $\delta^{18}O$ value of xylem water in each individual plant: $\Delta^{18}O_L = \delta^{18}O_{leaf} - \delta^{18}O_{xylemwater}$.

According to stable isotope theory, leaf $\delta^{13}C_L$ and $\Delta^{18}O_L$ can be considered proxies for time-integrated intrinsic water use efficiency and stomatal conductance, respectively (Moreno-Gutiérrez et al. 2012; León-Sánchez et al. 2016; Prieto et al. 2018; Dawson et al. 2002; Barbour 2007; Cernusak et al. 2013). To confirm and ensure that this interpretation of leaf C and O isotopic composition as proxies of time-integrated leaf gas exchange was plausible, we also measured photosynthetic rates (A), stomatal conductance (g_s) and transpiration rate (E) at the peak of the growing season at 6 of the 10 study sites during the 2022 sampling campaign using a portable infrared gas analyzer (IRGA, LI-6800, LI-COR Inc. Lincoln, NE, USA). We sampled the 40 woody species present in the 6 study areas, measuring 6 individuals of each species at each site (the same individuals used for isotopic measurements). The LI-6800 equipment had a $2cm^2$ leaf cuvette and a CO_2 injector. Leaf gas exchange was measured on fully sun exposed

leaves between 9:00 and 13:00 AM (GMT) and expressed on a total leaf surface area basis. All leaf gas exchange measurements were made at saturating light intensity of 1500 µmol $mol^{-2}s^{-1}$ and at ambient air temperature and air humidity, with the air flux set to 350 µmol s^{-1}. The CO_2 concentration in the cuvette was maintained at 420 µmol mol^{-1} CO_2. Total leaf area was, for some species, smaller than the leaf cuvette (2cm^2) so we rescaled the measures to the actual leaf area inside the cuvette, which was destructively sampled after IRGA measurements in the field, transported to the lab and measured with a scanner and image software (Photoshop v22.5.0, Tokyo, Japan). Intrinsic water use efficiency (WUE_i) was calculated as the ratio between photosynthetic rate and stomatal conductance (A/g_s), whereas instantaneous water use efficiency (WUE_t) was calculated as the ratio between photosynthetic rate and transpiration (A/E).

Plant hydraulic architecture measurements

We measured the leaf area to sapwood area ratio(Pivovaroff et al. 2021) of terminal shoots in three randomly selected individuals of each woody species present at the two most taxonomically diverse and species-rich study sites (Montejo in 2021, 18 sp; Yeste in 2023, 16 sp). A higher leaf area: sapwood area ratio of terminal branches is indicative of higher total transpiring leaf area per unit of cross-sectional sapwood area supplying water to foliage. The total leaf area of terminal branches was carefully measured in the laboratory within 48h after sampling in the field using a scanner and Adobe Photoshop (Adobe Inc. 2021). Cross-sectional sapwood area was obtained after debarking the distal stem to isolate the xylem and by measuring its diameter with a digital caliper (Storm, Vitoria, Spain). We also measured leaf C and O isotopes and xylem water isotopic composition in these individuals.

Statistical analysis

To estimate the proportion of shallow vs deep-water sources used by each species at the peak of the Spring growing season, we first set the border between both soil depth interval categories at the depth where the soil water $\delta^{18}O$ depletion curve saturated which was between 15 and 30 cm depth depending on the site (Figure S1). At the Pulpi site, the hardness of the ground and shallow bedrock only allowed us to reach 25 cm deep with the hand auger, and the soil water $\delta^{18}O$ depletion curve was not yet saturated at this depth. Soil water isotopic composition data were unavailable for the Venta del Olivo, Los Cuadros Natural and Los Cuadros Repoblacion sites, so we used the average of the top 5% highest individual plant xylem water $\delta^{18}O$ values encountered at each site as a proxy for topsoil water $\delta^{18}O$ values(Allison, Barnes, and Hughes 1983). At these same 3 sites and Pulpí, where no $\delta^{18}O$ data for deep water sources were available, we used the average winter rain isotopic composition as a proxy for the deep water pool, obtained

using geospatial interpolation maps based on GNIP data (Bowen, Wassenaar, and Hobson 2005; Bowen and Revenaugh 2003). The isotopic composition of winter precipitation was used as a proxy for the deep water pool since the isotopic composition of water stored in deep soil layers was very similar to that of winter precipitation at the remaining 6 sites (Figure 1A).

We used Bayesian mixed modelling approach with the MixSIAR package in R(B. C. Stock et al. 2018) to estimate the proportion of deep and shallow soil water used by each woody plant species. We included "site" as a random factor and "species" nested within "site" to account for the variance within a species related to the sites. Given the uncertainties regarding isotopic discrimination against deuterium during water uptake by plant roots (Barbeta et al. 2022, 2020; Ellsworth and Williams 2007) we used only the $\delta^{18}O$ of xylem water of each individual for MixSIAR calculations. The mean $\delta^{18}O \pm$ SD of all shallow or deep soil water samples at each site were included as water source data input in the model. We run the model setting manually the number of iterations, burn-in and thinning to 500000, 200000 and 300 respectively. Model diagnosis was performed via the Gelman-Rubin and Geweke tests (B. C. Stock and Semmens 2013).

This study encompasses 62 woody species belonging to 21 different plant families across 10 sites, so for each trait we assessed the percentage of variance explained by site and species using *calcVarPart* function (package variancePartition (Hoffman and Schadt 2016)). Besides, we used Pagel's Lambda to assess the degree of influence of phylogeny on each trait studied (Münkemüller et al. 2012). We ran phylogenetic linear mixed models (PGLMMs) (Ives and Helmus 2011) to account for phylogeny effect on correlations among traits, using species phylogeny nested within site. A phylogenetic tree with our target species based on GBOTB. extended phylogeny of vascular plants (Smith and Brown 2018; Zanne et al. 2014) was used as an input in the PGLMMs. We also ran general linear mixed models (GLMMs) without considering the phylogeny, including the site and life form as random crossed effects to account for the differences in climate, continentality, altitude and plant community composition between sites along with differences between plant functional types within each site. We analyzed the influence of plant life form and leaf habit on the leaf and xylem water isotopic composition using GLMM, and then explored the relationships between water uptake depth, leaf water-use traits and maximum rooting depth ($\Delta^{18}O_L$ – leaf $\delta^{13}C_L$, leaf $\delta^{13}C_L$ – xylem $\delta^{18}O_{xw}$ and $\Delta^{18}O_L$ – d-excess$_{xw,}$ xylem $\delta^{18}O_{xw}$ – root depth$_{max}$) using general mixed regression models. A principal component analysis (PCA) was performed including $\delta^{13}C_L$, $\delta^{18}O_{xw}$, $\delta^{18}O_L$ and plant height to obtain a multidimensional overview of the coordination of these key plant traits across sites and life forms. The proportion of the total trait variance explained by

plant life form versus site was further evaluated using one-way ANOVAs on the PCA axis 1 and 2 scores of all plant individuals. We also used GLMM to assess the relationships between Leaf area: Sapwood area Ratio and isotopic water use traits ($\Delta^{18}O_L$, leaf $\delta^{13}C_L$, $\delta^{18}O_{xw}$) across species, excluding *Cytisus purgans* from the analysis because it has photosynthetic stems with tiny leaves and exhibits an extreme outlier Leaf area: Sapwood area Ratio. We also analyzed the relationship between Leaf area: Sapwood area Ratio and species height, but as we could not measure individual plant height at time of terminal branch sampling, we ran the model using mean Leaf area: Sapwood area Ratios per species and mean species height in Montejo and Yeste, which had been measured in previous field campaigns. To assess the validity of leaf carbon and oxygen isotopes as proxies for intrinsic water use efficiency and stomatal conductance, respectively, we ran general mixed regression models between leaf $\delta^{13}C_L$ – WUE_i, and WUE_i – g_s, while linear regression was run between $\Delta^{18}O_L$ and g_s. All the analyses were conducted with R (R Core Team 2023) working with packages *nmle* (Pinheiro et al. 2018), *phyr* (Li et al. 2020), *V.phyloMaker* (Jin and Qian 2019) and *MixSIAR* (B. Stock and Semmens 2016). Trait values mentioned throughout the text are always the mean ± standard error (SE).

Results

Vertical soil water source segregation and partitioning among coexisting woody species is widespread in Mediterranean plant communities.

At the peak of the Spring growing season, soil water content increased steeply with soil depth at all the study sites except for the wettest location (Montejo; Figure S1 A). We encountered marked soil water isotopic gradients with depth along the soil profile at all the study sites; topsoil water $\delta^{18}O$ and $\delta^{2}H$ values were always isotopically enriched relative to rainwater, and soil water isotopic values became progressively more depleted (i.e. more negative) with increasing depth along the edaphic profile (Figure S1 B,C). As a result, soil water d-excess values were always more negative near the surface (indicating intense evaporative isotopic fractionation) and became less negative with increasing soil depth at all the study sites (Figure S1 D). Soil water isotopic $\delta^{18}O$ and $\delta^{2}H$ depletion curves generally saturated at 20 cm depth at all the sites (except at the wettest site Montejo, where they saturated at 15 cm), below which soil water isotopic composition remained rather stable throughout the soil profile (Figure S1). Water stored in deeper soil layers generally showed an isotopic composition that was very close to the Local Meteoric Water Line (LWML) for the Iberian Peninsula at all the sites (Figure S2 A),

revealing small or negligible evaporative isotopic enrichment of water stored below 20 cm depth during Spring.

We found evidence of distinct ecohydrological niche segregation and vertical water source partitioning among coexisting woody species at every study site, thereby supporting hypothesis 1 (H1). All the target plant species used varying mixtures of both isotopically enriched topsoil water and deeper, more depleted water sources closer to the Iberian Water Meteoric Line (Figure S2 B). Moreover, a clear water uptake depth continuum ranging from species using predominantly shallow soil water sources to species using mostly deep-water pools was encountered at all the study sites, evidencing a wide variety of coexisting water use strategies leading to distinct ecohydrological niche segregation within Mediterranean plant communities (Figure 1). We encountered a wide range of soil water uptake patterns among woody plant species, ranging from the small, drought semi-deciduous shrub *Teucrium capitatum* (Teu cap) that used 85.7 % of shallow topsoil water (< 20 cm) at the Pliego site, to the winter-deciduous tree *Sorbus aria* using only 6.4 % of shallow topsoil water at the Montejo site (Figure 1). Within each study site, plant size (height) strongly influences the proportion of shallow soil water used by each woody species (Figure S3Figure S3 A, B). Smaller shrubs exhibit much heavier reliance on the topsoil water pool (i.e. higher $\delta^{18}O_{xw}$ values) than neighboring larger shrubs or trees within the same plant community (Figure 2A) at all the sites. Conversely, small shrub species consistently use a smaller proportion of deep-water sources than larger shrub species at all sites, with taller tree species using the largest proportion of water stored in deep soil/bedrock layers (50.3 ± 0.05%, 64.2 ± 0.05% and 75.5 ± 0.06% respectively, Figure S4). Moreover, evergreen species used a greater proportion of water stored in deep soil/bedrock layers (lower $\delta^{18}O_{xw}$ values) than coexisting drought-deciduous or semideciduous species across sites (Figure S5 A). We encountered a tight coordination between species maximum rooting depth (obtained from the scientific literature) and water extraction depth ($\delta^{18}O_{xw}$), with deeper- rooted species consistently using a greater proportion of deep water sources (more negative $\delta^{18}O_{xw}$) (Figure S6).

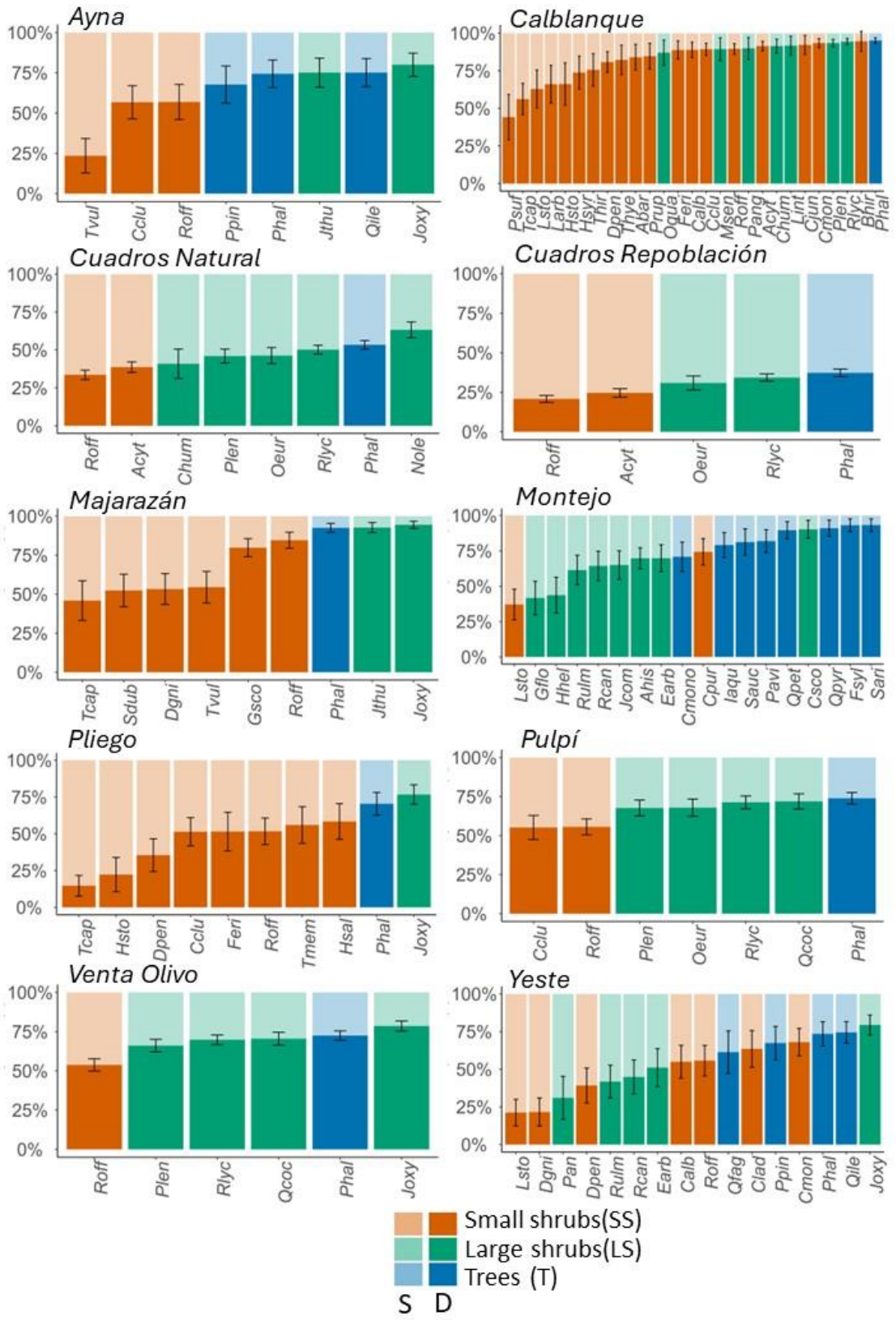

Figure 1. Proportion of shallow vs deep soil water taken up by plants. Average proportions (% ± standard deviation) of shallow (**S;** 0 – 20 cm depth; faded colors in upper part of each column) and deep soil water sources (**D;** > 20 cm depth; solid colors in lower part of each column) taken up by coexisting woody plant species at each study site, estimated using Bayesian mixing models

(see Material and Methods section for specific details on calculations). 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.

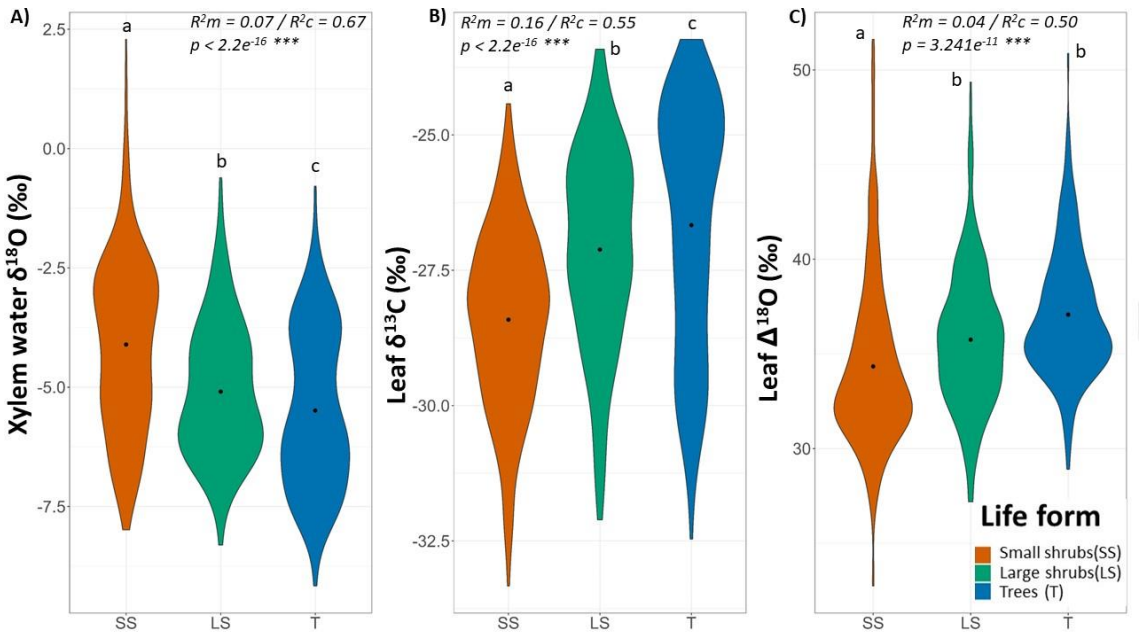

Figure 2. Isotopic differences among plant life forms. Violin plots depicting xylem water isotopic composition (xylem $\delta^{18}O_{xw}$) (A) and leaf isotopic C and O composition (leaf $\delta^{13}C_L$ and $\Delta^{18}O_L$, B,C) by plant life form (small shrubs, large shrubs and trees) across study sites. One-way ANOVA and Tukey's tests were used for multiple comparisons analysis. Black points indicate mean values of each isotope per life form. Different letters denote significant ($p < 0.05$) differences between life forms. 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.

Coordination between belowground water uptake depth and aboveground leaf-level water use traits: the role of phylogeny.

We encountered a remarkably wide range of variation in mean $\delta^{13}C_L$ values among coexisting woody species at every study site, with interspecific variation ($|\delta^{13}C_L$ max - $\delta^{13}C_L$ min|) within sites ranging between 2.1 and 7.3 ‰ (Figure 3). Our findings demonstrate that large interspecific differences in leaf-level intrinsic water use efficiency among coexisting woody species are widespread in Mediterranean ecosystems, regardless of the contrasting climates among study sites, thereby supporting H2. Within-

site variation in mean leaf $\Delta^{18}O_L$ values among coexisting woody species (|$\Delta^{18}O_L$ max - $\Delta^{18}O_L$ min|) was even larger, ranging between 3.1 and 14.7 ‰ (Figure S7). Moreover, we found a strong positive relationship between leaf $\Delta^{18}O_L$ and $\delta^{13}C_L$ values across coexisting woody species (Figure 4 A,B) for both the 2019 and 2022 datasets, which we interpret as evidence that species with tighter stomatal regulation of plant water flux (higher $\Delta^{18}O_L$) generally achieve higher intrinsic water use efficiency within each site (higher $\delta^{13}C_L$). Both $\Delta^{18}O_L$ and $\delta^{13}C_L$ showed a strong phylogenetic signal (Pagel's λ = 0.56, $p < 0.001$ and Pagel's λ = 0.55, $p < 0.01$ respectively). However, the positive relationship between $\Delta^{18}O_L$ and $\delta^{13}C_L$ held robust and remained significant when phylogeny was explicitly considered in the model for both study years ($z = 2.145$, $p < 0.05$ in 2019; $z = 3.274$, $p < 0.01$ in 2022).

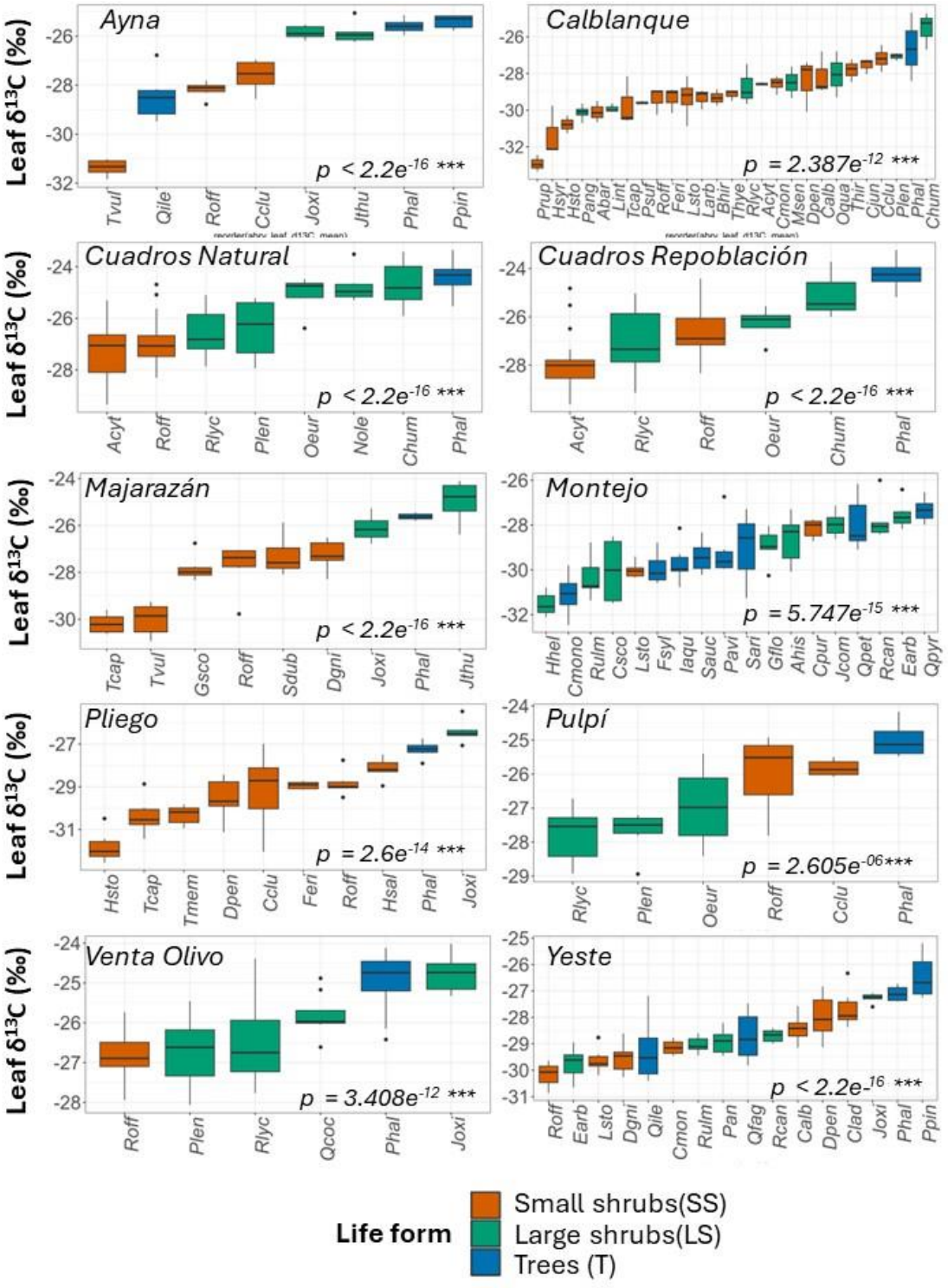

Figure 3. Differences in leaf $\delta^{13}C$ among coexisting plant species. Variation of mean leaf carbon isotopic composition ($\delta^{13}C_L$) among coexisting woody plant species growing at 10 contrasting study sites. Boxplots represent median (line) species values for $\delta^{13}C_L$ from linear models within each site. Lower and upper hinges correspond to the 25th and 75th percentiles and whiskers depicts 1.5*IRQ (interquartile ranges); outlier values are also shown. P values depict significant differences between species based on F-test analysis. Different colors depict different plant life forms. Interspecific variation in average $\delta^{13}C_L$ values among coexisting species was remarkably

large within sites (e.g. -25.6 to -32.9 ‰, in Calblanque), often encompassing much of the full range of $\delta^{13}C_L$ values observed at global scale in C3 plants (-20 to -33 ‰) (Kohn 2010). 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.

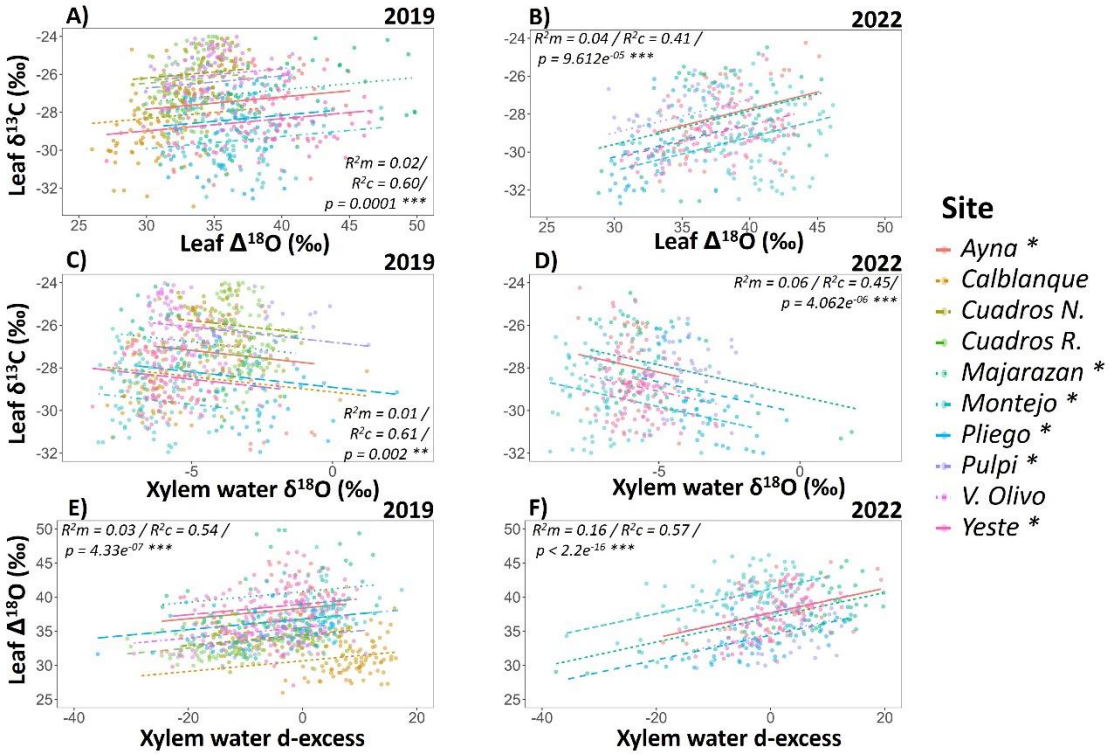

Figure 4. Relationships between leaf isotopes and xylem water isotopes. Within-site relationships between leaf carbon isotopic composition ($\delta^{13}C_L$) and leaf oxygen isotopic composition ($\Delta^{18}O_L$; a, b); between leaf $\delta^{13}C_L$ and xylem water oxygen isotopic composition ($\delta^{18}O_{xw}$; c, d); and between leaf $\Delta^{18}O_L$ and deuterium-excess in xylem water ($d\text{-}excess_{xw}$, e, f) across individuals of coexisting woody plant species in two different years (2019 and 2022) at multiple sites. Regression lines per site from linear mixed regression models are shown. Colored dots represent individual plants 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. Asterisks indicate the six sites that were sampled in both sampling campaigns (2019 and 2022). Source data are provided as a Source Data file.

Principal component analysis (PCA) based on plant height and isotopic water use traits of all 62 woody species across sites revealed a tight coordination among multiple above- and belowground traits, lending support for H4 and H5. For most of these traits, species explained a larger proportion of variance than site, excepting $\delta^{18}O_{xw}$ for which site absorbed a higher proportion of variance. (due to geographically driven variation in rainwater $\delta^{18}O$; Figure S8). The first two PCA axes explained 63.6% of the total trait variability across species and sites. The first axis explained 32.8% of the plant trait variation, with height, $\delta^{18}O_{xw}$ and $\delta^{13}C_L$ contributing substantially to this axis (Table S3). Plant life forms absorbed most of the variance along axis 1 (Table S4), as tree species showed larger size, higher $\delta^{13}C_L$ indicating higher water use efficiency and lower $\delta^{18}O_{xw}$ indicating a deeper water extraction pattern, while small shrub species exhibited opposite traits (Figure 5 A). The second axis of the PCA explained 30.8% of total trait variation. largely reflecting differences in environmental conditions among study sites, as site absorbed the largest proportion of variance along this axis (Table S4). Cooler and wetter sites like Montejo or Yeste plot on the positive side of the second PCA axis, whereas warmer and drier sites like Pulpi or Los Cuadros plot on the negative side of this axis (Figure 5 B). However, one of the warmest and driest sites, Calblanque, showed positive scores in the second PCA axis, likely due to its constant exposure to humid marine winds, given that this coastal site is located very near the Mediterranean shore (200-300 m distance only).

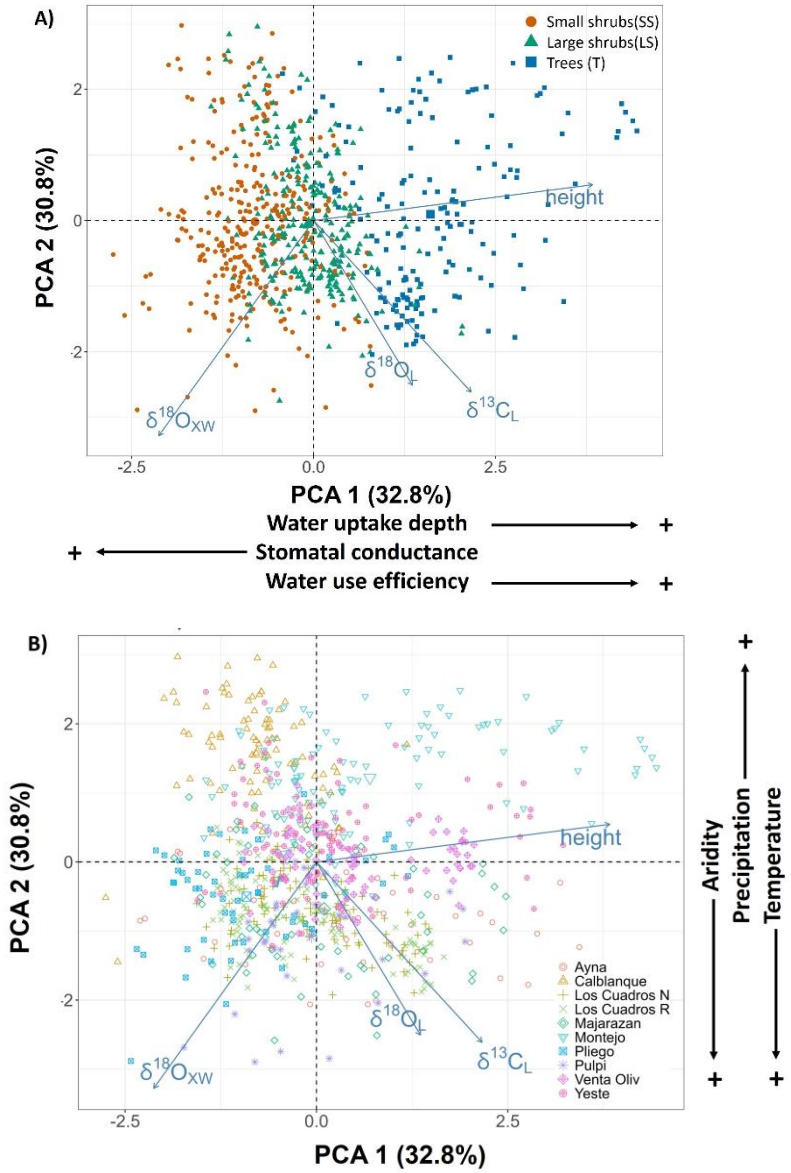

Figure 5. Principal component analysis of key plant traits**.** Principal Component Analysis (PCA) of plant leaf isotopic composition ($\delta^{13}C_L$ and $\delta^{18}O_L$), xylem water oxygen isotopic composition ($\delta^{18}O_{xw}$) and plant height from 63 plant species. Points depict individual plants, colors indicate plant individual plant classification across according to life forms (A) and study site (B). Source data are provided as a Source Data file**.**

The large within-site variation in $\delta^{13}C_L$ and $\Delta^{18}O_L$ among coexisting woody species is strongly modulated by plant size (Figure S3 C,D) and life form, as smaller shrubs generally show lower $\delta^{13}C_L$ and $\Delta^{18}O_L$ values (lower time-integrated WUEi driven by looser stomatal regulation of plant water flux) than coexisting larger shrubs or trees at all the sites (Figure 2 B,C). The strong within-site negative relationships observed between $\delta^{13}C_L$ and $\delta^{18}O_{xw}$ across coexisting species for both the 2019 and 2022 datasets revealed

lower leaf-level intrinsic water use efficiency in smaller-sized species with shallower soil water uptake depth (Figure 4 C, D). This interpretation was further supported by the strong positive relationships found between leaf $\Delta^{18}O_L$ and d-excess$_{xw}$, which indicates looser stomatal regulation of plant water flux in smaller-sized species with a shallower water uptake pattern (Figure 4 E, F). Deuterium-excess$_{xw}$ did not show phylogenetic signal (Pagel's $\lambda = 6.64\ 10^{-5}$, $p = 1.0$) although $\delta^{18}O_{xw}$ did (Pagel's $\lambda = 0.49$, $p < 0.05$). The strong relationship between foliar $\Delta^{18}O_L$ and d-excess$_{xw}$ across coexisting woody species was very robust and was not modified when phylogenetic effects were considered ($z = 6.819$, $p < 0.001$ in 2019; $z = 8.931$, $p < 0.001$ in 2022). In contrast, phylogeny appears to play an important role in the negative relationship between leaf $\delta^{13}C_L$ and $\delta^{18}O_{xw}$ in both study years ($z = 0.242$, $p = 0.808$ in 2019; $z = 0.002$, $p = 0.99$ in 2022). Leaf habit also had a strong influence on $\delta^{13}C_L$ and $\Delta^{18}O_L$ variation among coexisting woody species, as drought-deciduous or semideciduous species showed significantly lower $\delta^{13}C_L$ and $\Delta^{18}O_L$ values than their coexisting evergreen species (Figure S5 B,C).

Mixed models revealed a positive relationship between species height and the leaf: sapwood area ratio, indicating a larger transpiring foliage area per unit cross-sectional sapwood area supplying water to leaves (i.e. lower Huber values) in larger and taller species (Figure 6A). Small shrub species exhibited lower leaf: sapwood area ratios (i.e. higher Huber values) than their coexisting large shrub and tree species, while the latter did not differ from each other (Figure S9). Mixed models also revealed a strong negative correlation between leaf: sapwood area ratio and $\delta^{18}O_{xw}$ across coexisting species (Figure 6B) that was maintained when accounting for phylogenetic effects ($z = 4.824$; $p < 0.001$), which indicates a smaller foliage area per cross-sectional sapwood area in species with a shallower water uptake pattern. Finally, we also found positive relationships between leaf: sapwood area ratio and both leaf $\delta^{13}C_L$ and $\Delta^{18}O_L$ across coexisting woody species (Figure 6 C, D), indicating that a sparser foliage with smaller transpiring leaf area per sapwood area is linked to looser stomatal regulation and lower time-integrated water use efficiency (thus supporting H3). These relationships were significantly influenced by evolutionary history ($z = -1.050$, $p = 0.30$; $z = -1.770$, $p = 0.07$, respectively, when considering phylogenetic effects).

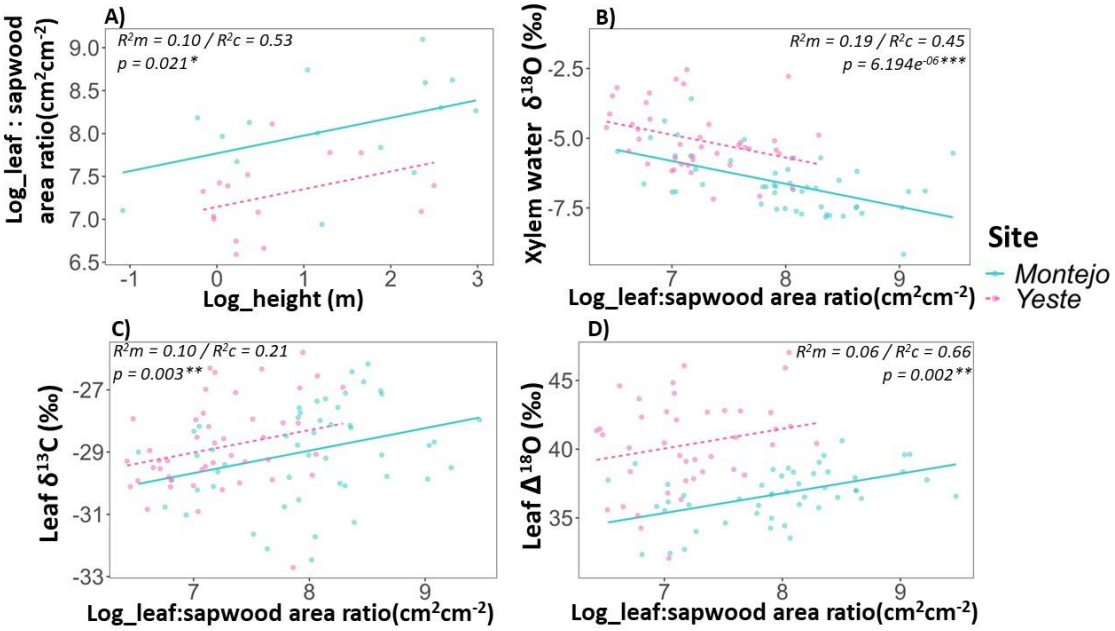

Figure 6. Relationships of leaf area: sapwood area ratio with other key plant traits. Within-site relationships between leaf area to cross-sectional sapwood area (cm^2 m^{-2}) of terminal shoots and plant height (A), xylem water $\delta^{18}O_{xw}$ (B), leaf $\delta^{13}C_L$ (C) and $\Delta^{18}O_L$ (D) for individual plants from the Montejo and Yeste sites (N = 151 individuals of 29 species). Models are linear mixed regression models with site as random effect. Colored dots represent individual plants at each site except for (A) where mean values per species were shown. 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.

Isotopic water-use traits provide reliable proxies for species water use strategies in Mediterranean plant communities

In a subset of six study sites, isotopic water-use traits were measured in two years with sharply contrasting rainfall patterns: In 2019 (2021 in Montejo), rainfall amounts during the winter and spring months preceding sampling were below or near the historical average across all sites, whereas in 2022, the winter-spring months were exceptionally wet, with record-breaking rainfall amounts received during March across all the study sites (200-300% or more above the historical average; Figure S10). As a result, here was a marked offset in leaf isotopic values between study years, with consistently lower $\delta^{13}C_L$ values in the wet year (2022) relative to the dry year (2019) across species and sites, as shown by a slopes lower than 1 in the mixed models (slope = 0.78, Figure 7; see also Table S5. This pattern confirmed that most plants were operating at lower water use efficiencies (lower $\delta^{13}C_L$) in the wetter year (2022) in response to greater soil water

availability and more humid ambient conditions, as expected from physiological theory. This interpretation was further supported by the much lower leaf $\delta^{18}O_L$ values encountered in the exceptionally rainy year of 2022 at all the 5 strictly Mediterranean sites, which indicates greater stomatal aperture with higher stomatal conductance and transpiration rates across species (relative to the previous drier year of 2019; slope = 0.86, Figure 7; Table S5). Interestingly, the isotope trait-based species rankings within Mediterranean plant communities were remarkably well preserved through time and remained rather concordant between separate years with sharply contrasting climatic conditions at multiple sites (supporting H6). Within-site species rankings in mean $\delta^{13}C_L$ were particularly well preserved between separate years with sharply contrasting rainfall patterns at all the six study sites (Figure 7A), thereby supporting the view that the mean leaf carbon isotopic composition of each coexisting woody species reflects a species-specific metabolic set-point informing about the trade-off between carbon gain and water loss [30]. Within-site species rankings regarding mean $\delta^{18}O_{xw}$ (proxy of soil water uptake depth) were also reasonably well preserved between two separate years with sharply contrasting rainfall patterns, indicating that vertical water source partitioning and ecohydrological niche segregation among neighboring woody species during late spring are relatively stable across years at all the study sites (Figure 7C).

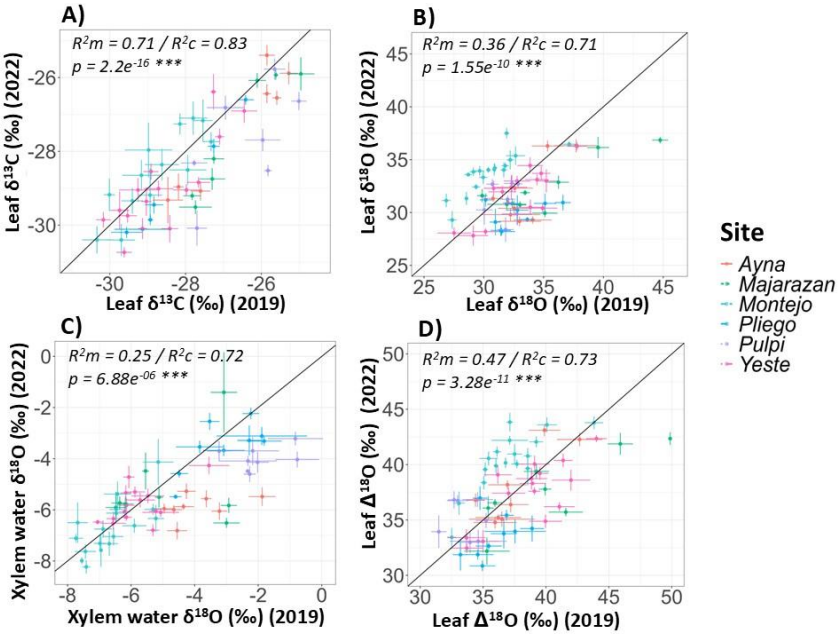

Figure 7. Isotopic rankings among coexisting species are conserved between years with contrasting climatic conditions. Mean leaf carbon isotopic composition ($\delta^{13}C_L$) (A), leaf oxygen isotopic composition (bulk $\delta^{18}O_L$ and calculated as enrichment above source water, $\Delta^{18}O_L$) (B and D) and xylem water oxygen isotopic composition ($\delta^{18}Oxw$) (C) for each *species x site* combination in two separate years with contrasting climatic conditions and rainfall patterns (2019 vs 2022, except for Montejo: 2021 vs 2022). N = 6 individuals per species x site combination in

each year. Vertical and horizontal error bars indicate standard errors of each species mean value in 2019 and 2022 respectively. Solid lines indicate the 1:1 regression line and dashed lines indicate the general regression line from each linear mixed model. 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 based on F-tests with Satterthwaite approximation of degrees of freedom. Source data are provided as a Source Data file.

Instantaneous leaf gas exchange measurements in the field revealed that, within each site, smaller shrub species exhibit higher stomatal conductance and transpiration rates and thus operate at lower water use efficiency than neighboring tree or large shrub species (Figure S11). Furthermore, we found a significant positive correlation between mean species $\delta^{13}C_L$ and WUE_i across coexisting woody species within sites (Figure 8 A), which is remarkable considering the very different timescales involved in both measurement types (time-integrated over several months for carbon isotopes versus snapshot instantaneous measurements for IRGA-based WUEi). This finding confirmed that, within each plant community, a higher species mean $\delta^{13}C_L$ value is indeed linked to higher intrinsic water use efficiency, as expected from stable isotope theory. Analogous to the strong relationship encountered between $\delta^{13}C_L$ and $\Delta^{18}O_L$ across coexisting species within sites, we found a strong within-site association between IRGA-measured WUE_i and g_s that further indicates that species exhibiting lower stomatal conductance can achieve higher intrinsic water use efficiency at leaf level (Figure 7B). A significant negative correlation was also found between $\Delta^{18}O_L$ and IRGA-measured g_s, as expected from stable isotope theory (Figure S12).

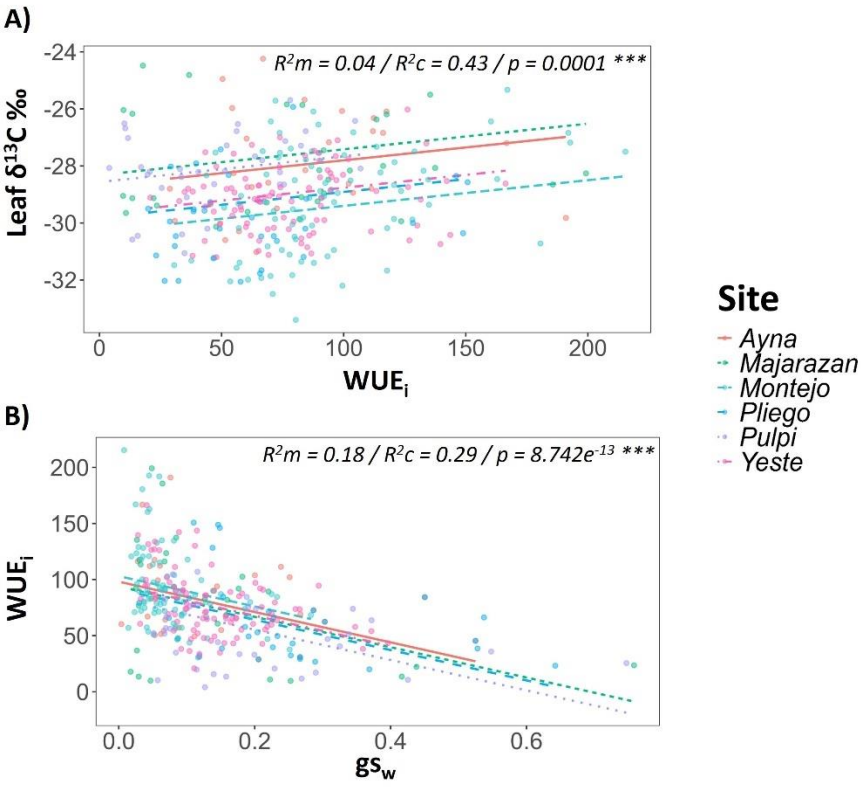

Figure 8. Relationships between leaf $\delta^{13}C$, water use efficiency and stomatal conductance. Within-site relationships between leaf carbon isotopic composition ($\delta^{13}C_L$) and instantaneous intrinsic water use efficiency (WUE_i) measured in the field in the same individuals on the same day in late spring 2022 at each study site (A). Also shown is the relationship between stomatal conductance (gs_w) and WUEi (B). Regression lines per site obtained from linear mixed regression models are shown. 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.

Discussion

Our study demonstrates that distinct vertical ecohydrological niche segregation driven by differences in soil water uptake depth among coexisting woody species is widespread and ubiquitous during the peak of the growing season across Mediterranean plant communities with contrasting climates (Figure 1, H1). Smaller shrub species are heavily dependent on shallow water sources present in the topsoil layer (0-20 cm) at all sites, while larger woody species (large shrubs and trees) use a greater proportion of deeper (> 20 cm) soil water sources that are more reliable and stable through time (Figure S4). The shallow and deep soil water pools differ markedly in their exposure to direct evaporative loss, their stability and reliability through time, their accessibility to plant

roots, and in the contrasting intensity of inter-plant competition for each resource pool (Schenk and Jackson 2002; Ogle, Wolpert, and Reynolds 2004; Ward, Wiegand, and Getzin 2013). Distinct vertical water niche segregation among coexisting woody species should enhance the drought resistance, resilience and overall productivity of diverse plant communities through complementarity effects, a more exhaustive utilization of the most limiting resource along the full soil/bedrock profile, and the attenuation of interspecific competition for soil water (Isbell et al. 2015; Urgoiti et al. 2022). Several local-scale studies have reported that moisture stored deep in the soil/bedrock profile is mainly recharged by winter precipitation and represents an important water source for many evergreen and winter-deciduous tree species during the subsequent growing season in Mediterranean-type ecosystems and elsewhere (Brinkmann et al. 2018; Allen et al. 2019; Goldsmith et al. 2022; Hahm et al. 2020). It is noteworthy that we found marked ecohydrological niche segregation among coexisting woody species during the Spring growing season when soil water availability and climatic conditions are relatively favorable, and vegetation is most physiologically active at all the study sites (Figure 1). Vertical niche segregation in soil water uptake depth among coexisting species would be expected to become even more pronounced during the summer hot drought period, as deep-rooted species progressively shift to deeper water uptake due to topsoil desiccation (Palacio, Montserrat-Martí, and Ferrio 2017; Davis and Mooney 1986; Redtfeldt and Davis 1996).

As noted by several recent studies, the considerable hydraulic pathway length for internal water transport in large trees can lead to substantial time delays between soil water uptake by roots and the isotopic signature of this xylem sap reaching the canopy branches sampled for isotope measurement (Schwendenmann et al. 2010; Evaristo et al. 2019; von Freyberg et al. 2020; Bernhard et al. 2024). This so-called “height effect” may complicate the interpretation of the xylem water isotopic composition encountered in upper canopy branches, thereby compromising the correct identification of the soil water sources that tall trees are using at any given time (de Deurwaerder et al. 2020; Magh et al. 2020; Marshall et al. 2020; Mennekes et al. 2021; Jacobsen et al. 2008). However, the occurrence of long-time lags between soil water uptake by roots and internal water transport to the sites of stem sampling in trees seems very unlikely in our study, for the following reasons. First, the average height of the trees sampled in our study was only 8.5 m (ranging from 3 to 20 m height), which significantly limits the length of the hydraulic pathway and travel time for water transport from roots to the sites of sampling (sun-exposed mid canopy branches). Second, the high xylem sap velocity typically encountered in native trees during the spring growing season under

Mediterranean conditions (characterized by high ambient temperature and VPD) favours rapid internal water transport and travel times in trees, thereby leading to relatively short or negligible time lags between root water uptake and transport to the mid canopy branches sampled in our study. For example, Cohen et al (2007) (Cohen et al. 2008) reported that xylem sap velocity, measured with the heat-pulse method, reached 22-68 cm per hour in Aleppo pine (*Pinus halepensis*) and 33-108 cm per hour in holm oak trees (*Quercus ilex ssp. rotundifolia*) growing under semiarid or dry-sub-humid Mediterranean climates in Israel. Such high xylem sap velocities would translate into water travel times from roots to mid canopy branches of 1-2 days or even less in our Aleppo pine and holm oak trees, which were by far the most abundant and widespread tree species across study sites. Seeger and Weiler (2021) recently concluded that the potential impacts of hydraulic path length and the so-called "height effect" on tree source water identification can be safely neglected when xylem water transport velocities and canopy transpiration rates are high combined with short transport distances between roots and the sites of sampling, as was always the case in our own study. Moreover, distinct ecohydrological niche segregation between trees and co-occurring small shrubs was strongly supported in our study by the highly significant correlation encountered between the maximum rooting depth of the different species and their xylem water isotopic composition across all plant life forms (Fig S8).

Overall, our findings support our assumption that leaf carbon and oxygen isotopes provide valuable time-integrative proxies for interspecific variations in intrinsic water use efficiency and stomatal conductance among coexisting woody species, and thus can be considered as useful integrative physiological traits, at least for water-limited Mediterranean plant communities (Moreno-Gutiérrez et al. 2012; Illuminati et al. 2022; Prieto et al. 2018). We found that the belowground water uptake depth pattern of each species has a marked influence on its aboveground isotopic traits involved in leaf-level water use and flux regulation (Guerrieri et al. 2019; Mathias and Thomas 2021). Within each study site, leaf-level stomatal regulation stringency and intrinsic water use efficiency vary widely (H2) and are both coordinated with soil water uptake depth across coexisting woody plant species (Figure 4; H4). Larger and taller woody species, mostly of evergreen leaf habit (and some winter deciduous) that use a greater proportion of deeper water sources generally display more conservative water use traits at leaf level (higher $\Delta^{18}O_L$ and $\delta^{13}C_L$ in these "water-saver" species). In contrast, smaller shrub species, which are mostly drought deciduous or semideciduous, rely mainly on shallow soil water sources and exhibit a much more profligate and acquisitive water use strategy at leaf level (lower $\Delta^{18}O_L$ and $\delta^{13}C_L$ in these "water-spender" species) within each study

site. Water use by small shrub species with a shallow water uptake pattern is thus more tightly coupled to the rapidly fluctuating dynamics of the topsoil water pool than that of neighboring trees or large shrubs with access to deeper and more temporally stable water pools. In this context, the profligate, water-spender strategy (high g_s and low WUE_i) displayed by smaller shrub species with shallow water uptake pattern likely allows them to optimize water capture and use during the narrow windows of opportunity provided by the irregular rainfall events that recharge the topsoil layer in Mediterranean ecosystems. However, this profligate water-spender strategy could lead to rapid soil moisture depletion within their shallow rooting zone and therefore to a faster onset of soil water deficit, drought stress and enhanced risk of drought-induced cavitation and hydraulic failure during subsequent rainless periods(Henry et al. 2019). Interestingly, we found that smaller shrubs compensate their high stomatal conductance rates per unit leaf area during wet periods (high g_s, low $\Delta^{18}O_L$ and $\delta^{13}C_L$) by reducing their whole-canopy hydraulic demand through lower leaf area to sapwood area ratios (i.e. a smaller transpirative leaf area relative to the cross-sectional sapwood area supplying water to foliage; H3). Small leaves and sparsely foliated shoots and canopy in small shrubs may favor high sapwood water supply rates to foliage, which are needed to sustain high stomatal conductance rates per unit leaf area during favorable periods (Figure 6). Moreover, small shrub species with predominantly shallow soil water uptake pattern often exhibit a drought-deciduous or semi-deciduous leaf habit in Mediterranean ecosystems that further reduces total foliage transpirative area during the summer hot drought period (drought-avoider strategy). Our findings are in good agreement with those of another recent study conducted under semiarid Mediterranean conditions (Hernandez-Santana et al. 2023), but differ in part from those of global scale studies that have linked lower leaf area to sapwood area ratio to higher water use efficiency ($\delta^{13}C_L$) (Hu et al. 2024) and more conservative traits in stems (lower xylem hydraulic conductance) (Mencuccini et al. 2019). This disparity could be due to the rather unique constraints that the Mediterranean climate imposes on plants during the prolonged hot and rainless summer drought period, which is the most distinct feature of the Mediterranean biome relative to other dryland ecosystems worldwide.

We found that larger shrub and tree species (mostly evergreen but also including some winter-deciduous trees) in Mediterranean plant communities use a sizeable proportion of deep soil/bedrock water sources even at the peak of the Spring growing season. Deep soil water sources are less easily accessible to roots than topsoil water but are subject to less intense direct evaporative losses and competitive depletion by neighbors, and thus remain more stable over time (Ryel et al. 2008). Lower g_s and transpiration rates

per unit leaf area and higher time-integrated WUE_i with more conservative water use pattern at leaf level (higher $\Delta^{18}O_L$ and $\delta^{13}C_L$) probably allow tall overstory species with deep rooting patterns to sustain a denser and more abundant foliage per sapwood area (higher leaf area: sapwood area ratio, Figure 6). Furthermore, the emergent canopies of tall overstory woody species are often exposed to harsher and more extreme microclimatic conditions characterized by higher irradiance, temperature, VPD and wind exposure (compared to the more mesic microclimatic conditions for understory species of smaller size), which may force trees, especially evergreens (Figure S4) to adopt more conservative leaf-level water use strategies to withstand these adverse microclimatic conditions (Fernández-de-Uña et al. 2023; McDowell and Allen 2015). Having a tighter stomatal regulation with lower time-integrated stomatal conductance and higher water use efficiency may enable taller tree species to mitigate the drop in xylem water potential in the upper canopy during dry periods associated to their longer hydraulic path-lengths, along with other adaptations in hydraulic architecture and trunk capacitance (Fernández-de-Uña et al. 2023; Medrano, Flexas, and Galmés 2009). Interestingly, large evergreen and winter-deciduous species with a predominantly deep water uptake pattern in Mediterranean plant communities are often capable of maintaining growth and physiological activity over longer periods that even extend into the dry summer season (drought-tolerant strategy), and can thus afford a later phenology compared to their neighboring smaller woody species with shallower rooting pattern and drought-deciduous leaf habit (Máguas et al. 2011).

Interestingly, in our extensive survey of 62 woody plant species across 10 study sites, not every possible combination of above and belowground water use traits was encountered in Mediterranean plant communities. Severe water limitation in upland Mediterranean ecosystems may favour a tight coordination between above and belowground traits (Mueller, Kray, and Blumenthal 2024), resulting in strong trade-offs among multiple water use traits (Figures 4, 6) that may limit the feasibility of certain trait combinations (H5). Interestingly, the combination of a shallow water uptake pattern belowground with a conservative, water-saver leaf-level strategy aboveground was not encountered in any of the target woody species, which suggests that such a combination of water-use traits may not be adaptative or even feasible in Mediterranean dryland environments. In support of this interpretation, a recent study (Blanco-Sánchez et al. 2022) pointed out that acquisitive resource use strategies are preferentially selected over more conservative ones in dryland plant species with short life spans as a way to maximize resource capture and utilization. On the other hand, the combination of a deep-water uptake pattern with a water-spender strategy based on loose stomatal regulation

of plant water flux was not encountered in our survey either, as this could also be a maladaptive strategy for large woody plants growing in upland Mediterranean ecosystems. Evergreen or winter deciduous trees deploying a water-spender strategy at leaf level would increase the risk of drought-induced hydraulic failure of long-lived and carbon costly woody tissues during seasonal summer hot droughts or multi-year droughts when little or no recharge of deep moisture reservoirs takes place (Nardini, Battistuzzo, and Savi 2013). Therefore, the two most widespread and ubiquitous whole-plant water use trait syndromes found in Mediterranean upland ecosystems appear to be either the combination of shallow rooting pattern with a profligate, water-spender leaf-level strategy (mostly in small drought-deciduous or semideciduous shrubs), or the opposite combination of a deep water uptake pattern with a conservative, water-saver leaf-level strategy (in large evergreen shrubs and evergreen and winter deciduous trees; Figure 9 (Mooney and Dunn 1970b, 1970a)).

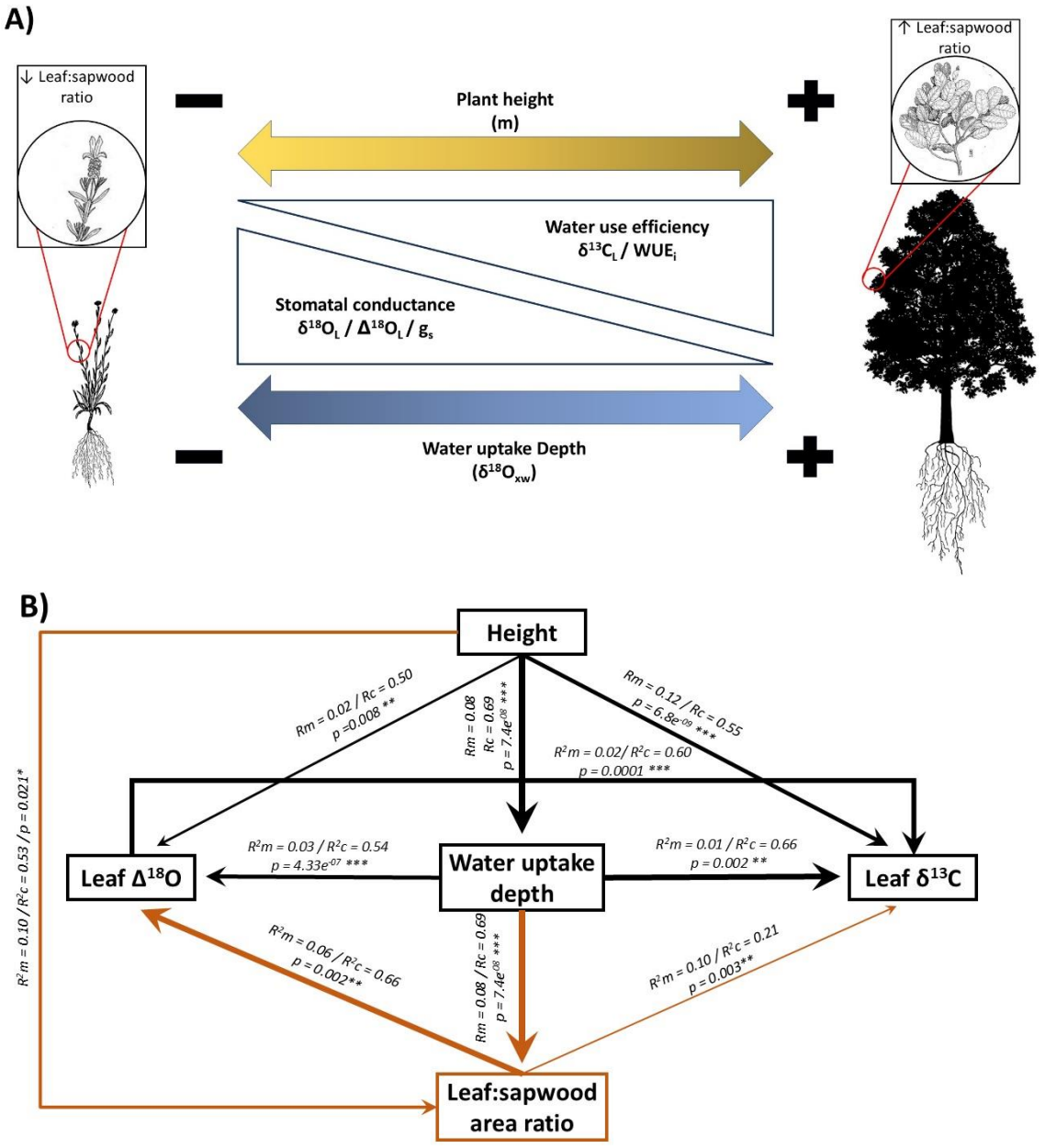

Figure 9. Conceptual model of water-use trait coordination in Mediterranean woody plants. **A)** Conceptual model summarizing the acquisitive-conservative water- use trait continuum encompassed by coexisting woody plant species in Mediterranean plant communities. At one end of the spectrum, we encounter small-sized species with shallow soil water uptake patterns exhibiting a profligate, water-spender strategy at leaf level (high stomatal conductance and low water use efficiency) and smaller leaf area to sapwood area ratios. At the opposite end of the functional spectrum, we encounter larger-sized species with deeper soil water uptake patterns displaying a more conservative water-saver strategy at leaf level (low stomatal conductance and high water use efficiency) and higher leaf area to sapwood area ratios. Images adapted from Flora Iberica (Castroviejo, n.d.). **B)** Correlation network between traits measured in woody species across study sites resulting from performing bivariate linear mixed models with site and life form as crossed random factors. Solid arrows indicate positive relationships between traits, and

marginal and conditional r-squared values along with the p-values for each model based on t-test analysis are shown next to arrows. Water uptake depth is based on $\delta^{18}O_{xw}$ data in all models where it is involved, except for its relationship with $\Delta^{18}O_{L;}$ in this particular case, for which xylem water deuterium excess has been used instead of $\delta^{18}O_{xw}$ as proxy of water uptake depth, since $\Delta^{18}O_L$ and $\delta^{18}O_{xw}$ are mathematically correlated. Black arrows indicate results from linear mixed models including 10 sites (62 species, n = 775 individuals) and brown arrows indicate results from linear mixed models including only 2 sites (Montejo and Yeste, 33 species, n = 151 individuals). 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. Arrows widths are represented according to conditional R^2squared values. Source data are provided as a Source Data file.

Finally, we point out that the tight coordination and strong trade-offs among multiple above- and belowground water use traits encountered in this study may (or may not) be unique and exclusive of the native Mediterranean flora, due to the severe abiotic filters and strong evolutionary pressures imposed by the prolonged hot drought conditions during the summer combined with a winter rainfall pattern. Alternatively, tight coordination and trade-offs among above- and belowground water use traits could be widespread and prevalent across the floras of other water limited biomes around the world (e.g. drylands with warm-season monsoonal precipitation), even though evolutionary pressures may differ significantly from those operating under a Mediterranean-type climate with a hot dry season (Oliveira et al. 2021; López et al. 2021). This is an intriguing, open question that remains to be elucidated by future work in other drought-prone ecosystems beyond the Mediterranean biome.

Data availability

The data generated in this study are provided in the Figshare repository with DOI 10.6084/m9.figshare.27939669 (https://doi.org/10.6084/m9.figshare.27939669). Source Data are provided with this paper.

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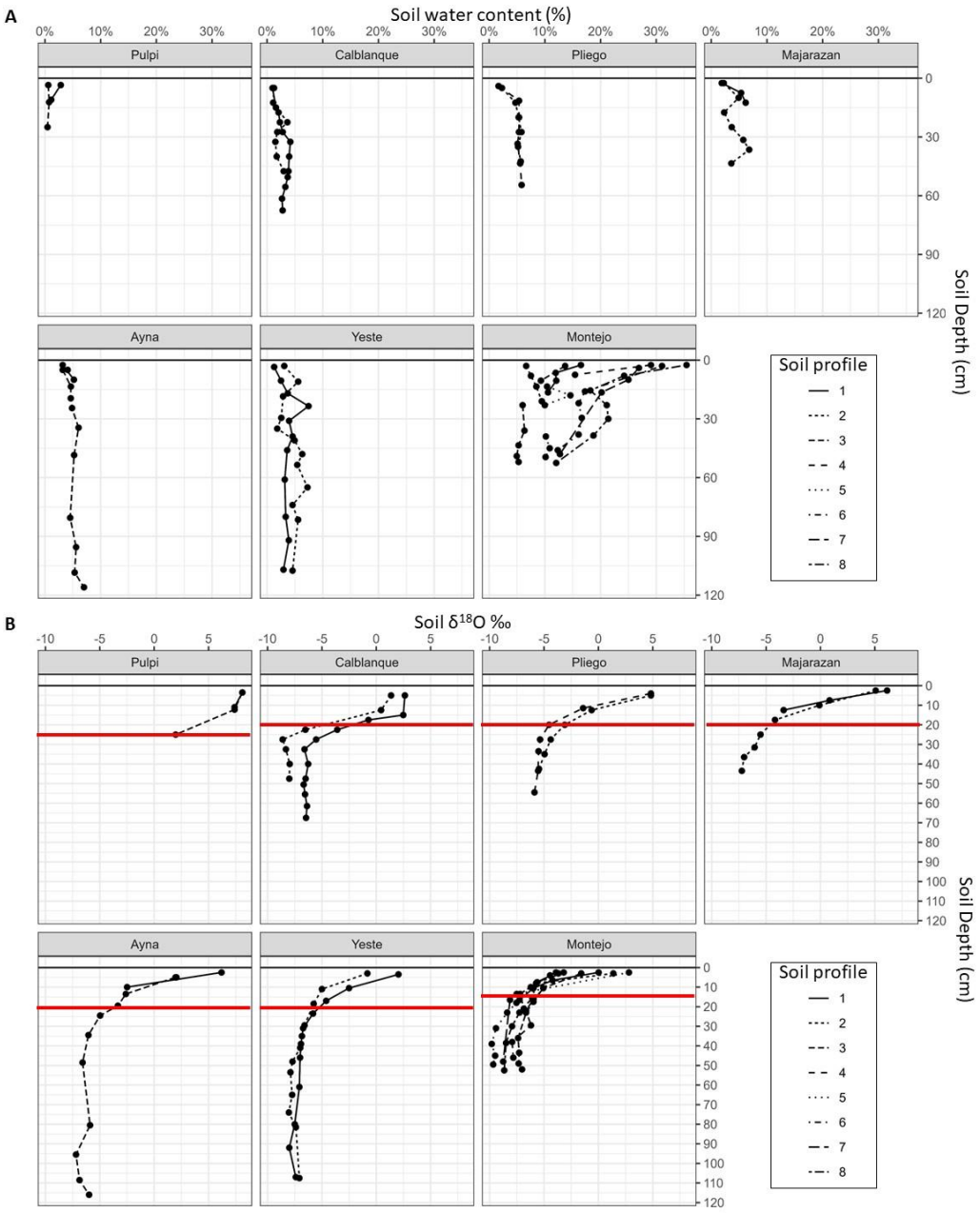
Supplementary Material



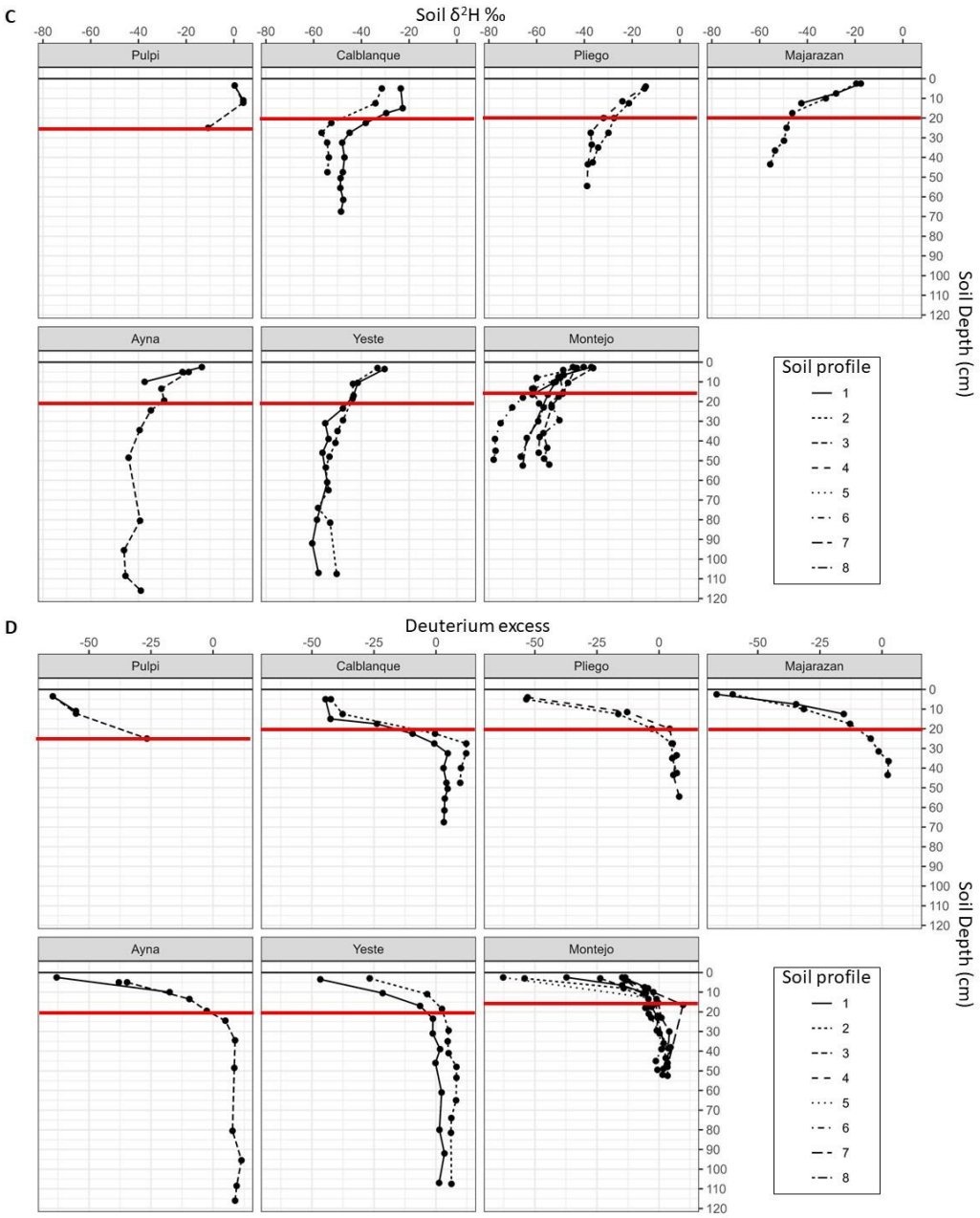


Figure S1. Steep gradients in gravimetric soil water content % (A), $\delta^{18}O$ ‰ (B) $\delta^{2}H$ ‰ (C), and d-excess (D) with soil depth (0 – 110 cm). Dots connected by lines depict the isotopic composition or soil water content of multiple soil profiles at each site. Red lines indicate the limits 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.

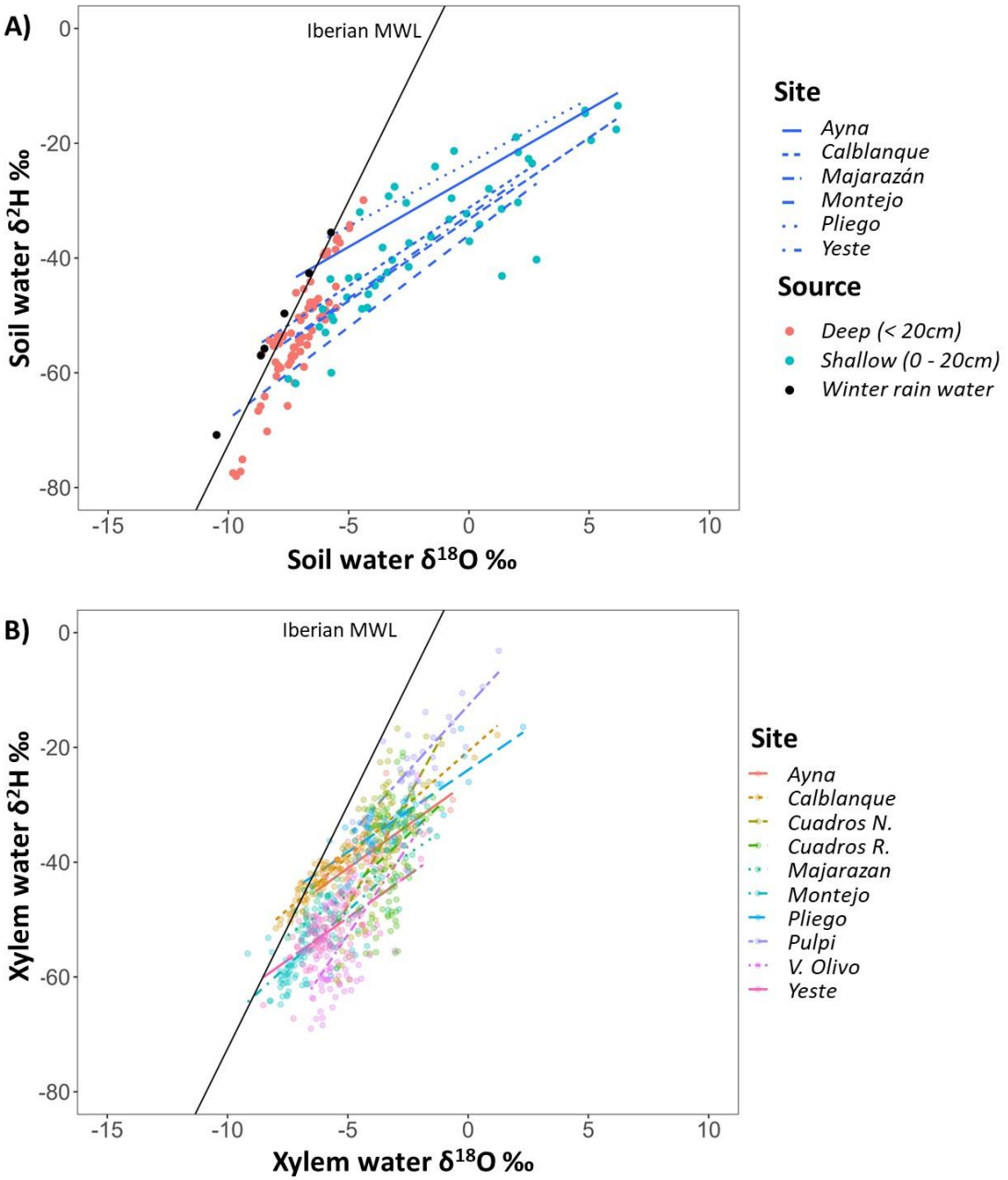


Figure S2. Soil and xylem water isotopic composition for all the soil profiles and plant individuals sampled at each study site. A) Regression lines of soil water isotopic composition ($\delta^{18}O$ and $\delta^{2}H$) values per site and the Iberian Peninsula Meteoric Water Line (solid black line) are 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 (Bowen, Wassenaar, and Hobson 2005; Bowen and Revenaugh 2003). 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 (Diaz-Teijeiro, Rodríguez-Arévalo, and Castaño 2009) (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

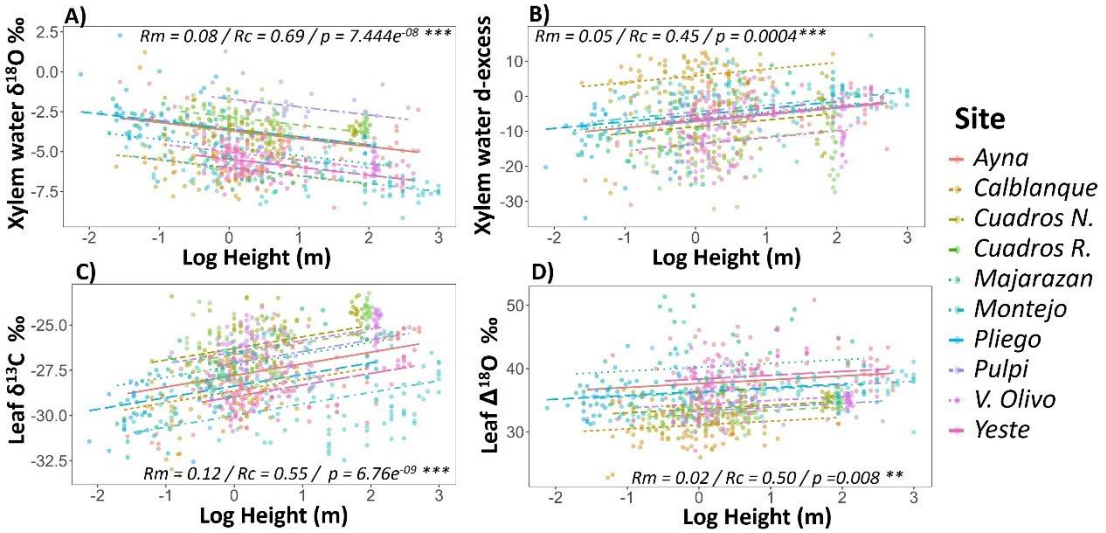


Figure S3. Within site relationships between log transformed plant height (m) and the isotopic composition of xylem water ($\delta^{18}O$, d-excess) (A, B) and leaves ($\delta^{13}C_L$, $\Delta^{18}O_L$) (C,D) across coexisting woody species. Plant height was log-transformed to comply with normality. 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.

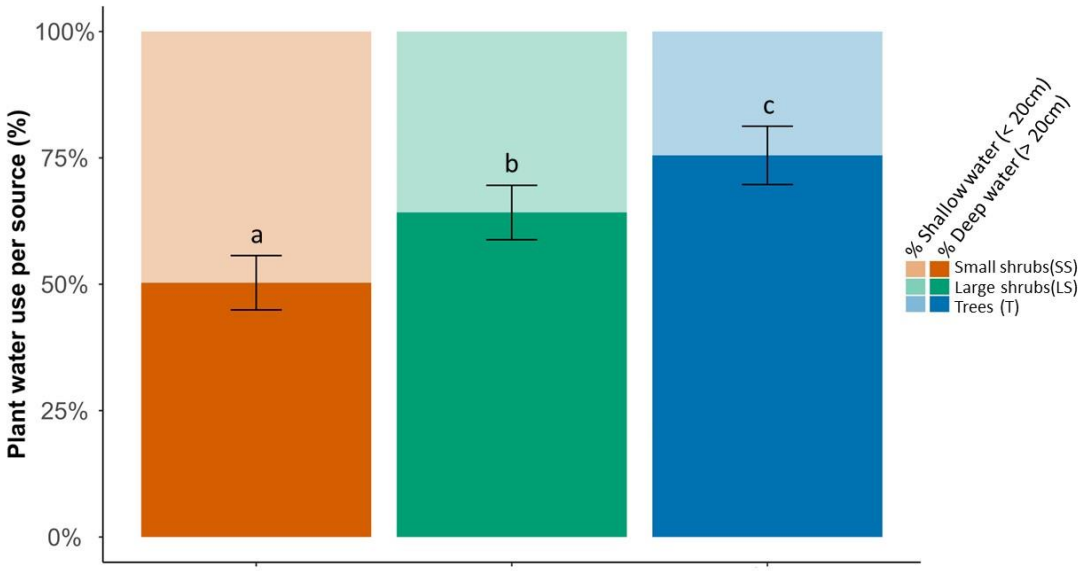


Figure S4. Average proportions (% ± Std.error) of shallow (0 – 20 cm; faded colour in upper part of columns) and deep (> 20 cm; dark colour in lower part of columns) soil water taken up by plants of different life forms; Small shrubs (SS, < 1m height), large shrubs (LS, > 1m height) and trees (T). Different 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.

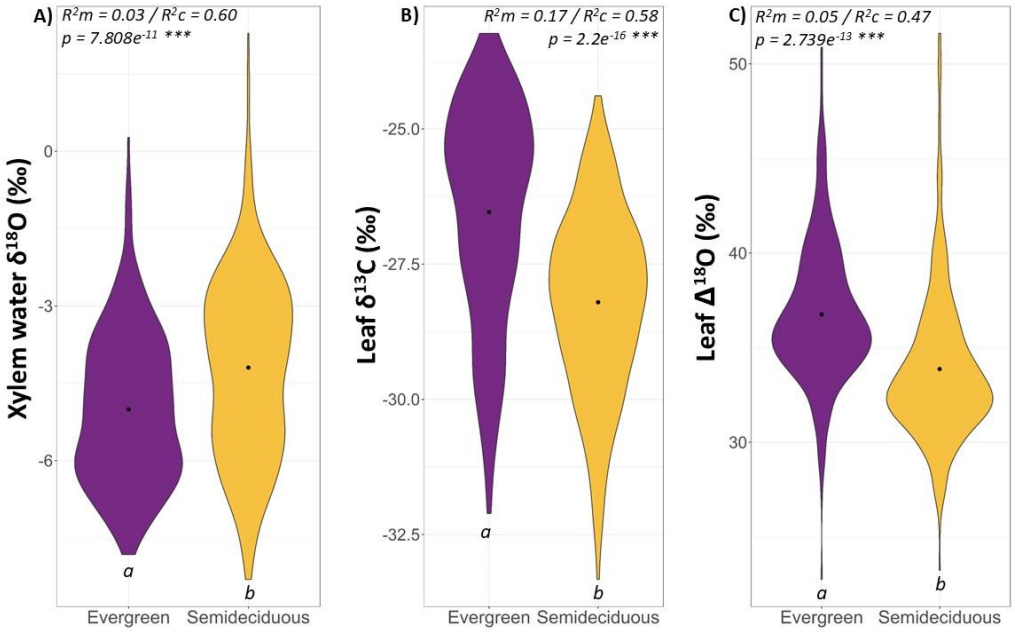


Figure S5. Violin plots depicting the oxygen isotopic composition of xylem water (xylem water $\delta^{18}O$) (A) and leaf carbon and oxygen isotopic composition (leaf $\delta^{13}C$ and $\Delta^{18}O$, B and C respectively) of woody plants grouped by leaf habit (Evergreen or Drought-deciduous/semideciduous) 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.

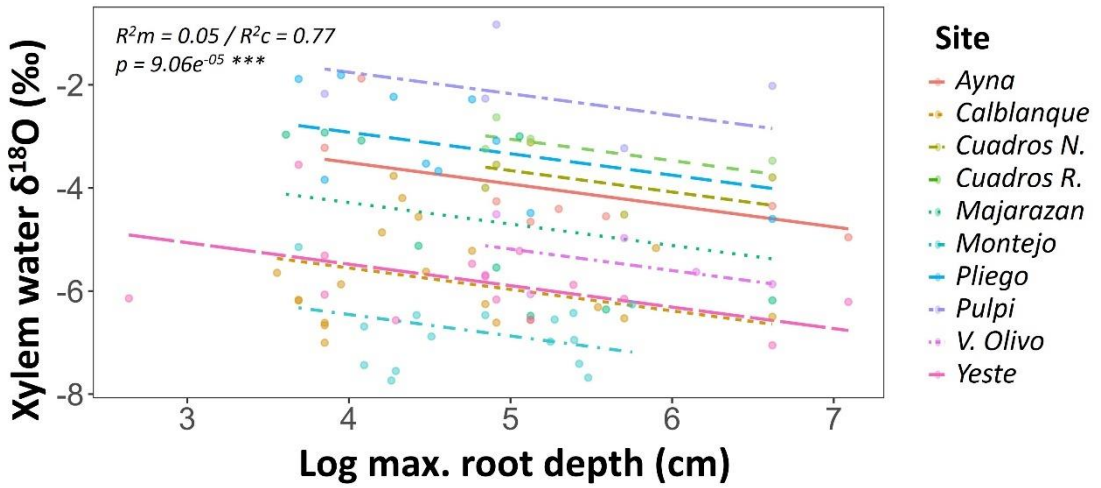


Figure S6. Within-site relationships between mean xylem water oxygen isotopic composition ($\delta^{18}O_{xw}$) and mean log transformed maximum rooting depth across coexisting species. Regression lines per site from linear mixed regression models are shown. Colored dots represent mean species values 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.

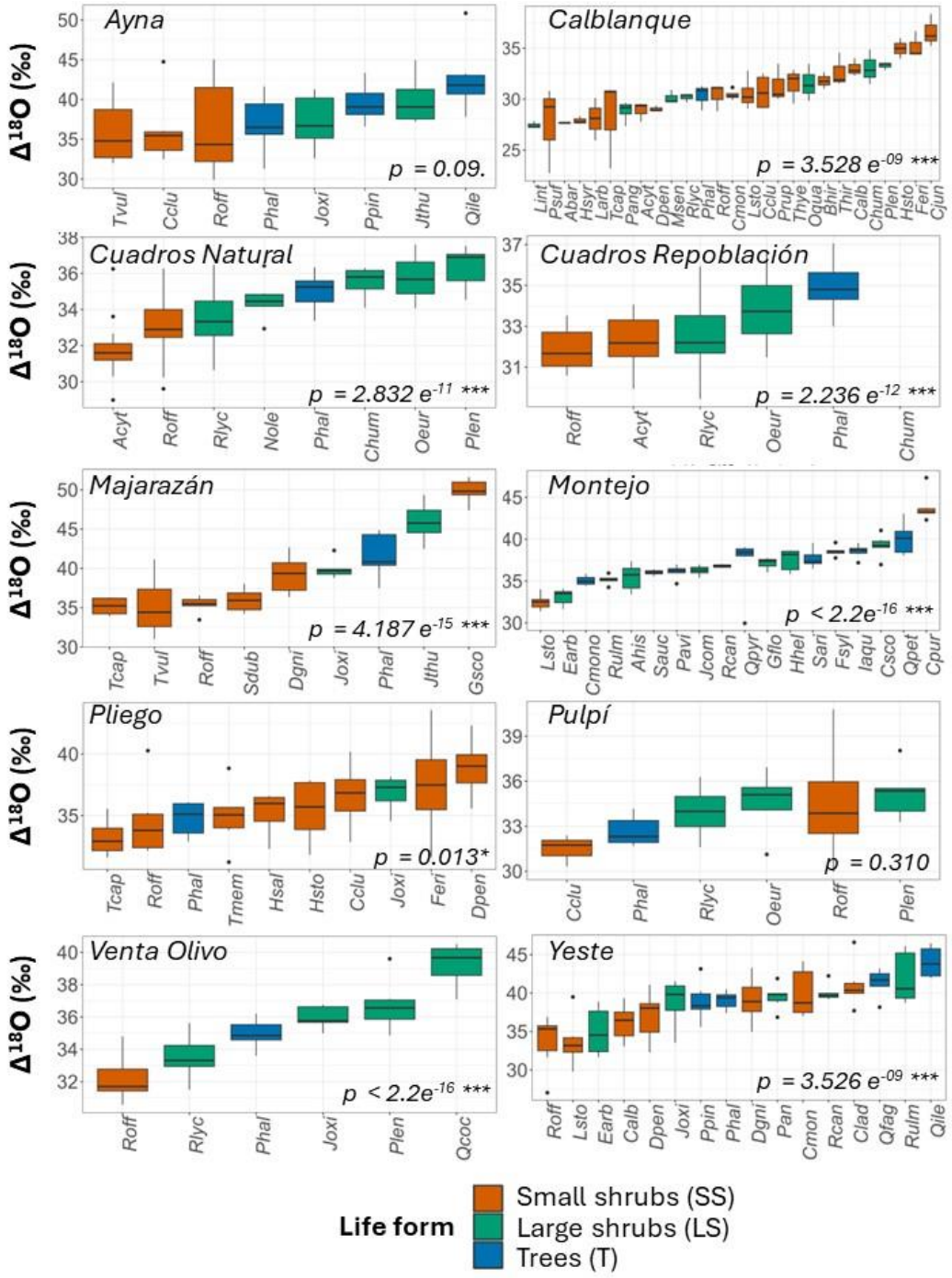


Figure S7. Variation of leaf oxygen isotope enrichment above source water ($\Delta^{18}O$ ‰) across 62 woody species growing at 10 study sites. Boxplots represent median (line) species values from linear models within each site. Lower and upper hinges correspond to the 25^{th} and 75^{th} percentiles 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 (Querejeta et al. 2022). 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.

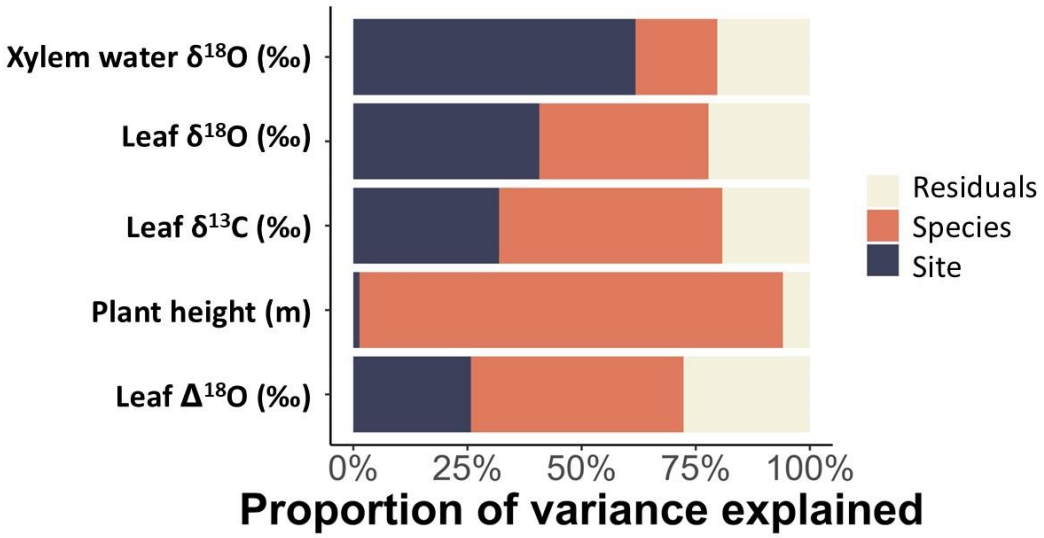


Figure S8. Variance partitioning for each plant trait, with stacked bars showing the percentage of variation explained by each explanatory variable (*species* versus *site*) in different colours from linear regressions. Source data are provided as a Source Data file.

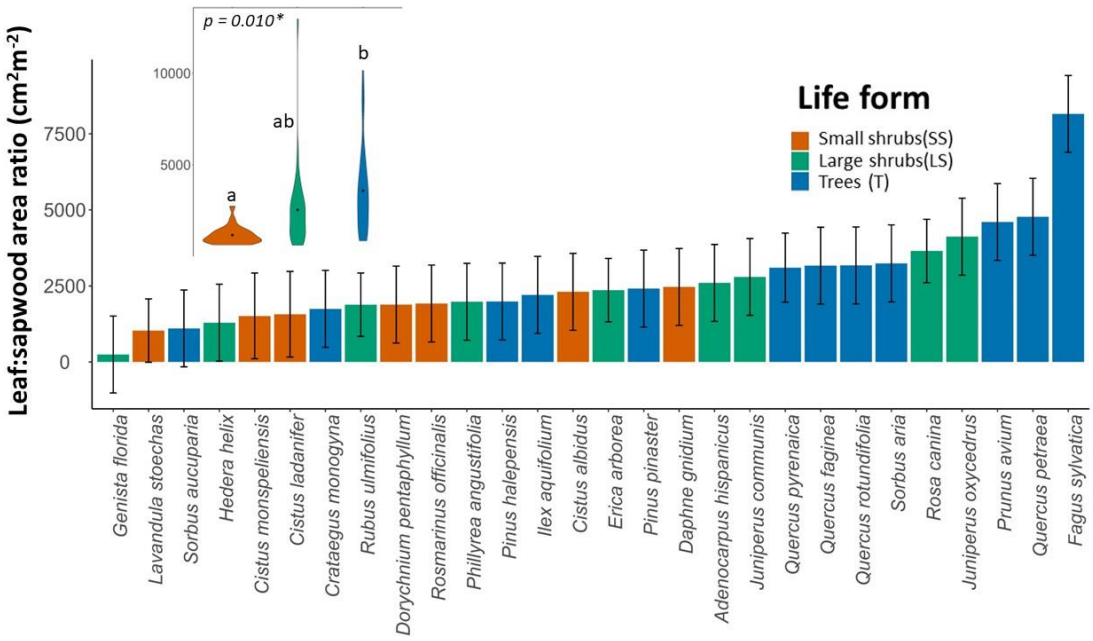


Figure S9. Variation in leaf area to sapwood area ratio (Leaf:sapwood area ratio) across 29 woody species present at two study sites (Yeste and Montejo). Bars represent the mean and whiskers the standard deviation per species. The inset depicts the violin plots of leaf to sapwood are ratio by plant life 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.

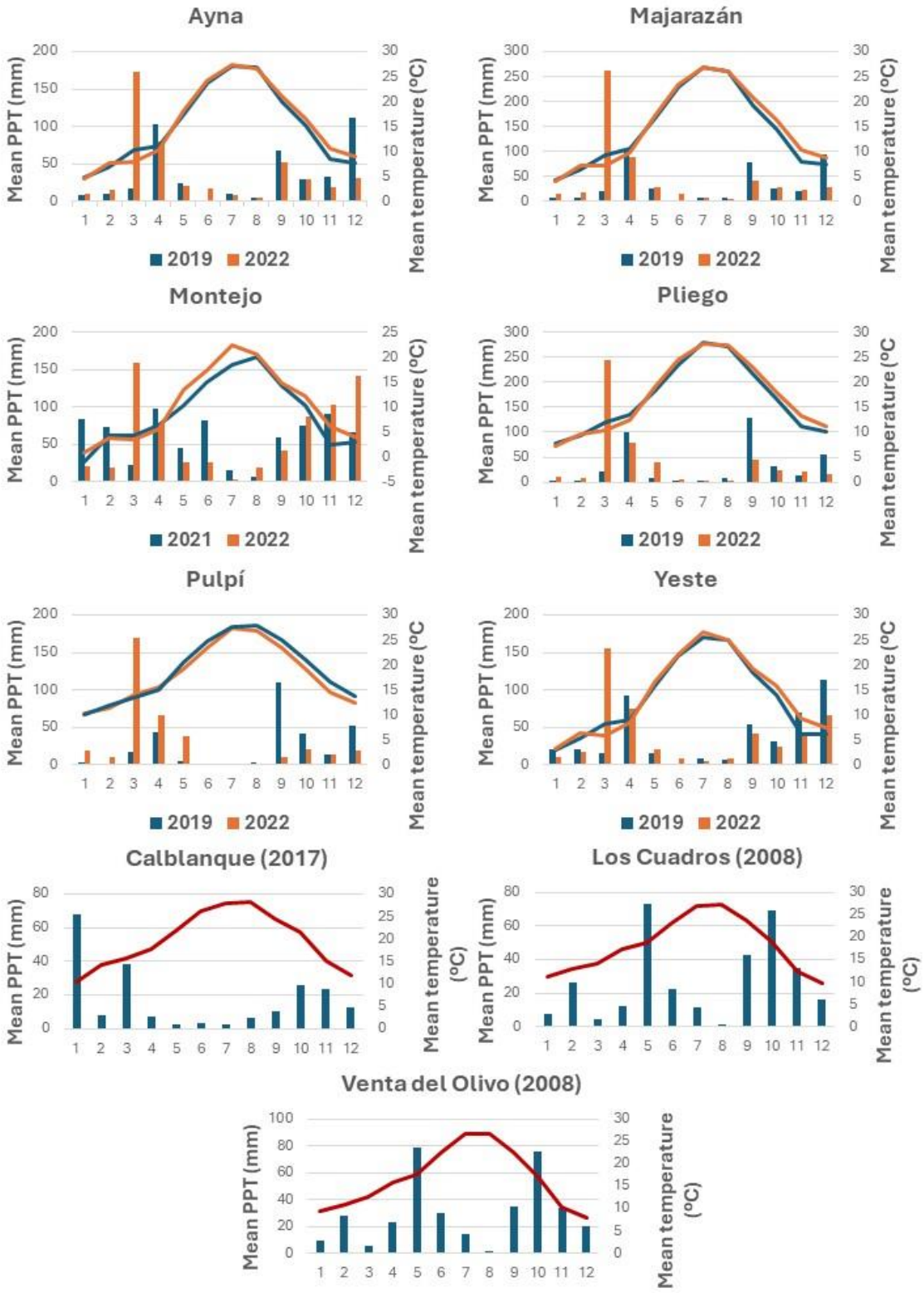


Figure S10. Climograms showing monthly precipitation (PPT, mm, depicted by bars) and temperature (ºC, depicted by lines) for the years when the field sampling campaigns were performed at each study site. For sites where two field sampling campaigns were conducted in different years, a different colour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

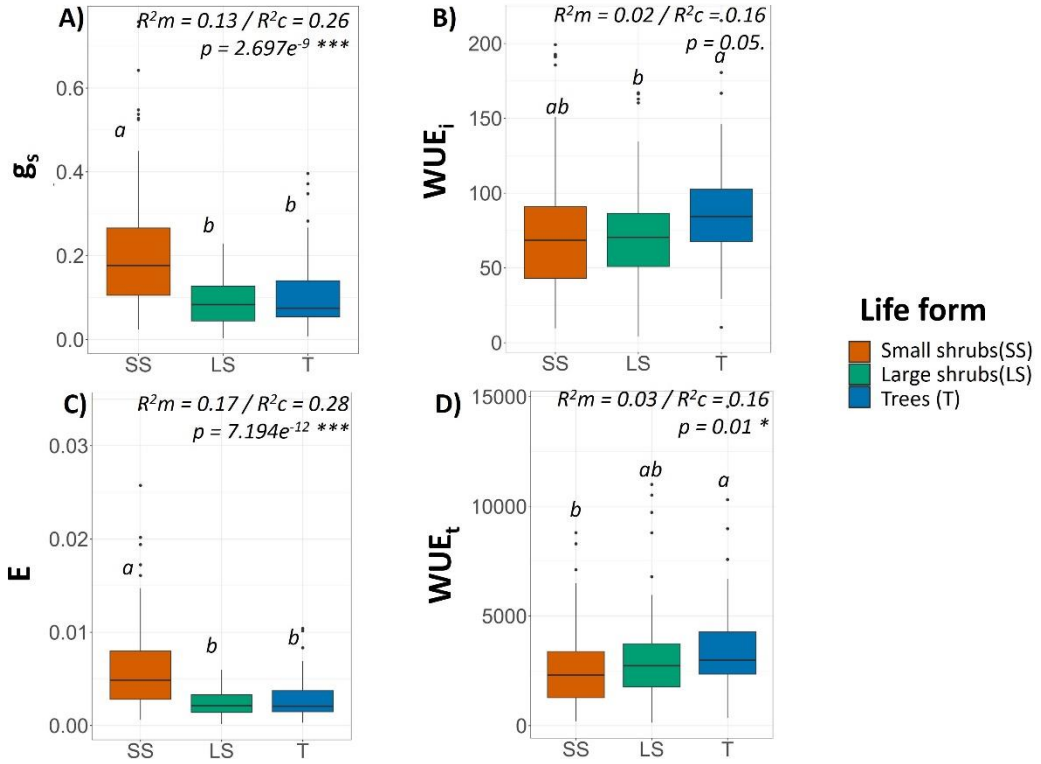


Figure S11. Variation of leaf gas exchange traits (g_s, E, WUE_i and WUE_t; A, B, C, D respectively by plant life form across study sites. Boxplots represent median (line) values from linear models. Lower and upper hinges correspond to the 25^{th} and 75^{th} percentiles and whiskers depicts 1.5*IRQ (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.

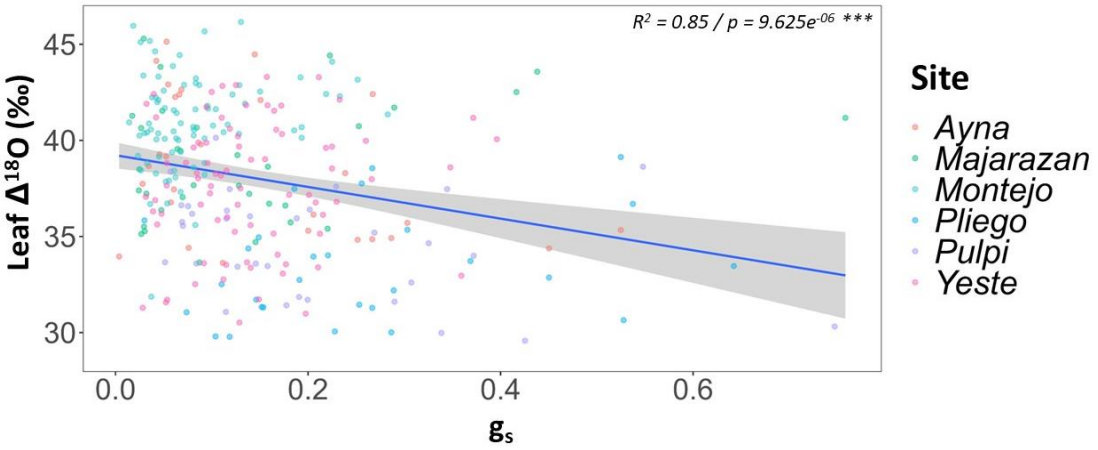


Figure S12. Relationship between leaf oxygen isotope enrichment above source water ($\Delta^{18}O$) and LICOR-measured stomatal conductance (g_s). A linear model approach was used instead of a linear mixed model approach because $\Delta^{18}O$ is strongly influenced by environmental factors such as rainwater $\delta^{18}O$, 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.

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 (de la Riva et al. 2018; Navarro et al. 2009) (E: evergreen; WD: winter-deciduous; DD: summer drought-deciduous or semideciduous), plant lifespan (Talhouk, Fabian, and Dagher 2015) (SL: short-lived, <30 years; LL: long-lived, >30 years; or U: uncertain lifespan) and maximum rooting depth (cm) (Guerrero-Campo et al. 2006; Cutini, Chianucci, and Manetti 2013; Tumber-Dávila et al. 2022; Beccari and Carmona 2024) 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*

Chapter 2

Coping with drought: How woody plants balance water use and leaf economics in Mediterranean ecosystems

Chapter 2: Coping with drought: How woody plants balance water use and leaf economics in Mediterranean ecosystems

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Introduction

Plant functional traits encompass morphological, physiological and phenological characteristics that impact plant fitness by shaping growth, reproduction and survival (Hisano et al., 2024; Pérez-Harguindeguy et al., 2013; Violle et al., 2007). Over the past decade, there has been increasing interest in using plant traits to assess different expressions of organisms' functions and how they respond to climate and environmental changes (Garnier et al., 2016; Kühn et al., 2021; Reich et al., 1997). Understanding plant community responses to such changes, and their cascading effects on ecosystem properties and services like carbon storage and water cycle regulation, requires synthesizing multi-trait information across a wide range of species (Garnier et al., 2016). The leaf economic spectrum (LES hereafter (Wright et al., 2004)) provides a widely accepted framework describing variations in plant form and function, encompassing a continuum from fast-growing, resource acquisitive species to slow-growing, resource conservative species. Acquisitive species with higher specific leaf area (SLA, Table 1) and foliar nutrient concentrations combined with lower leaf dry matter content (LDMC) tend to exhibit higher carbon fixation rates (net photosynthetic rates, A) and faster growth. Conversely, conservative species with lower SLA and foliar nutrient

concentrations combined with higher LDMC generally exhibit lower carbon fixation rates and slower growth (Galmés et al., 2005; Poorter and Bongers, 2006; Shipley, 2006; Wright et al., 2005). Although the LES represents a major axis of variation in plant form and function globally (Díaz et al., 2016; Smart et al., 2017), there is at present much uncertainty about the potential coordination between leaf construction costs and nutrient investment (LES traits) and water use strategies at the leaf or whole-plant levels. Water-use traits have not traditionally been incorporated into the LES framework, despite their critical role in drier environments where water availability is a major constraint on soil nutrient availability, carbon assimilation and plant growth. Addressing this pressing knowledge gap could provide novel insights into how plants balance carbon and nutrient investments and water use, particularly in water-limited Mediterranean ecosystems.

Table 1. Definitions of symbols used repeatedly in the main text.

Trait	Abbreviation	Unit
Leaf dry matter content	LDMC	mg g^{-1}
Specific leaf area	SLA	cm^2 g^{-1}
Leaf nutrient concentration per mass	C, N, P, K	mg g^{-1}
Carbon isotopic composition of leaves	$\delta^{13}C_L$	‰
Oxygen isotopic composition of leaves	$\delta^{18}O_L$	‰
Leaf oxygen isotopic composition enrichment above source water	$\Delta^{18}O_L$	‰
Oxygen isotopic composition of xylem water	$\delta^{18}O_{XW}$	‰
Stomatal conductance to H2O	g_s	mol m^{-2} s^{-1}
Transpiration rate	E	µmol mol^{-1}
Net photosynthesis rate	A	µmol m^{-2} s^{-1}
Intrinsic water use efficiency	WUE_i	µmol CO_2 mol H_2O
Instantaneous water use efficiency	WUE_t	µmol CO_2 mmol H_2O
Photosynthetic nitrogen use efficiency	PNUE	µmol CO_2 g N^{-1} s^{-1}

Regarding the potential coordination between LES and water use traits, Reich (2014) (Reich, 2014) proposed that being acquisitive at acquiring and using carbon or nutrients at any organ level (leaf, stem or root) necessarily requires being acquisitive for all other resources across all organs. Thus, acquisitive species along the LES (which invest less carbon in leaf construction and have higher leaf nutrient concentrations and photosynthetic rates combined with shorter leaf lifespans) should also display acquisitive leaf-level water use strategies (higher stomatal conductance and lower water use

efficiency) due to the unavoidable coupling between carbon, nutrient and water fluxes within plants (Brodribb et al., 2007; Pérez-Ramos et al., 2012; Prentice et al., 2014, 2011). Leaf carbon isotopic composition ($\delta^{13}C_L$) provides a complex signal influenced by both morphological and physiological leaf traits, serving as a time-integrated proxy and for the ratio of intracellular to ambient CO_2 concentrations (c_i/c_a), or more specifically, chloroplast to ambient CO_2 concentration (c_c/c_a) (Farquhar et al., 1989; Siegwolf et al., 2023). This ratio is modulated by: 1) leaf structural traits that influence CO_2 diffusion from the stomatal pores to the carboxylation sites within the chloroplasts, and determine how resources are allocated between construction costs and photosynthetic machinery (Chen et al., 2023; Hultine and Marshall, 2000; Li et al., 2017; Vitousek et al., 1990); 2) net photosynthetic rate (*A*) determined by the ratio of CO_2 fixation by Rubisco; and 3) stomatal conductance (g_s), which regulates leaf water and CO_2 exchange with the atmosphere (Prentice et al., 2011). $\delta^{13}C_L$ has proven to be a reliable proxy for time-integrated intrinsic water use efficiency at the leaf level ($WUE_i = A / g_s$) (Farquhar et al., 1989; Moreno-Gutiérrez et al., 2012). Many studies have shown a trade-off between $\delta^{13}C_L$ and SLA, a key LES trait (de la Riva et al., 2019; Prieto et al., 2018), suggesting that species with acquisitive carbon and nutrient use strategies along the LES (high SLA) may also exhibit acquisitive water use strategies characterized by low intrinsic water use efficiency (more negative $\delta^{13}C_L$) at leaf level. Leaf $\delta^{13}C_L$ may thus be a crucial trait for assessing the links between structural leaf characteristics and leaf-level water use across plant species.

However, additional traits must be considered to fully capture the diversity of whole-plant water use strategies and their potential coupling with the LES across species-rich plant communities. The oxygen isotopic composition of leaves ($\delta^{18}O_L$) reflects the isotopic composition of the plant source water present in the xylem vessels ($\delta^{18}O_{xw}$) and is also influenced by the isotopic evaporative enrichment of leaf water and the isotopic exchange between local water and organic molecules (Barbour, 2007; Farquhar et al., 2007). $\delta^{18}O_L$ is thus strongly influenced by g_s but also by the isotopic composition of the source water in the xylem ($\delta^{18}O_{xw}$), which can vary due to differences in rooting depth and site conditions (Illuminati et al., 2022; Moreno-Gutiérrez et al., 2012; Muñoz-Galvez et al., 2025). Therefore, calculating leaf dry matter oxygen isotopic composition enrichment above source water ($\Delta^{18}O_L = \delta^{18}O_L - \delta^{18}O_{xw}$) helps isolate the leaf-level evaporative signal and offers a time-integrated proxy of cumulative g_s and transpiration (E) over the growing season (Moreno-Gutiérrez et al., 2012; Siegwolf et al., 2023). The combined use of $\delta^{13}C_L$ and $\Delta^{18}O_L$ provides a robust approach for assessing water use strategies at the leaf level (Muñoz-Galvez et al., 2025; Prieto et al., 2018). However,

aboveground water use by leaves is inherently linked to belowground root water uptake strategies and moisture availability along the soil profile (Ding et al., 2021; Illuminati et al., 2022; Muñoz-Galvez et al., 2025). Water stored in the shallow fertile topsoil layer is generally richer in nutrients but is also more prone to rapid evaporative loss and competitive depletion as most species can access it, so this water pool is only available for short periods after rainfall events in dryland ecosystems. Conversely, water stored in deeper subsoil/bedrock layers is poorer in nutrients but is more stable over time and less prone to evaporative loss or depletion by competitors (Ryel et al., 2008). A few studies have explored the links between plant carbon, nutrient and water use strategies at local scales (Illuminati et al., 2022; Prieto et al., 2018; Querejeta et al., 2018), but the generality of these links remains to be tested through a more comprehensive assessment of LES coordination with both above- and belowground water use traits and strategies across a larger set of species inhabiting multiple sites in water-limited ecosystems. The present study aims to bridge this critical knowledge gap.

In this study, we assess the degree of coordination between the LES and water use strategies above (leaf-level) and belowground (i.e. water uptake depth) across multiple woody species inhabiting six typical Mediterranean-type dryland ecosystems with contrasting environmental conditions and one wetter sub-Mediterranean site (Figure S1). We first characterised the distribution and availability of nutrients (N, P, K) and water isotopic composition (soil water $\delta^{18}O$) along the soil profile. We also measured five key leaf morphological and nutrient traits in the LES (SLA, LDMC and foliar N, P, K concentrations) (Prieto et al., 2018; Reich, 2014; Wright et al., 2005), as well as key water-use traits at both the leaf level ($\delta^{13}C_L$, $\Delta^{18}O_L$) and belowground ($\delta^{18}O_{xw}$ as a proxy for water uptake depth along the vertical soil profile (Muñoz-Galvez et al., 2025)). We also conducted conventional leaf gas exchange measurements of stomatal conductance (g_s), transpiration (E) and net photosynthetic rate (A) in the field and calculated intrinsic and instantaneous water use efficiency (WUE_i and WUE_t) to validate the interpretation of $\delta^{13}C_L$ and $\Delta^{18}O_L$ variations across coexisting species and sites. Second, we assessed the potential coordination between multiple leaf economic traits across species and sites, to confirm the existence of a single major axis of trait covariation that encompasses the diversity of carbon and nutrient use strategies at leaf level (i.e. LES) across Mediterranean plant communities. Third, we explored the relationships between the diversity of C and nutrient strategies (LES axis) and isotopic water use traits ($\delta^{13}C_L$, $\Delta^{18}O_L$), gas exchange rates at leaf level (A, g_s, E, WUE_i and WUE_t) and water uptake depth belowground ($\delta^{18}O_{xw}$). We hypothesized that: 1) leaf carbon (SLA and LDMC) and nutrient (leaf N, P, K concentrations) use traits converge along a single LES acquisitive-

conservative axis across species in Mediterranean woody vegetation; 2) acquisitive strategies along the LES (high SLA and nutrient concentrations and low LDMC) are necessarily coupled to a more profligate water use pattern at leaf level (higher g_s and E combined with lower $\delta^{13}C_L$, $\Delta^{18}O$, WUE_i and WUE_t), in opposition to co-occurring species combining conservative LES traits and more water-saver strategy at leaf level; 3) species with acquisitive leaf traits along the LES will need to acquire and use a greater proportion of water stored in nutrient-rich topsoil layers to meet their higher nutrient demand, compared to co-occurring species with more conservative leaf traits along the LES and thus lower nutrient demand; 4) Coordination and functional convergence between leaf economic traits and water use traits should be stronger in more arid environments where nutrients and carbon acquisition by plants is more severely constrained by low water availability.

Materials and methods

Study site and sampling design

The study was conducted in six typical Mediterranean shrubland and woodland communities with contrasting climatic conditions and one sub-Mediterranean forest located at the transition between the Mediterranean and Eurosiberian temperate climates (Figure S1, Table S1). The sites were distributed along a linear 600 km transect from the south-eastern part (most arid) to the Central Range Mountains (wettest) of the Iberian Peninsula. Mean annual precipitation and mean annual temperatures ranged from 272 mm and 18.5ºC, respectively, at the warmest and most arid sites near the Mediterranean coast, to 726 mm and 8.6ºC at the coolest and wettest sub-Mediterranean site in the Central Range Mountains of the Castilian Plateau (Abatzoglou et al., 2018). Altitude above sea level of the study sites ranged from 10 m near the Mediterranean coast to 1450 m at the Central Ranges site. The structure of vegetation changes along this geographical transect from shrub-dominated communities (*Cistus albidus, Thymus vulgaris, Rosmarinus officinalis*) with sparse Aleppo pine trees (*Pinus halepensis*) in the southern and drier coastal sites (e.g. Pulpí), to closed-canopy forests dominated by Maritime pine (*Pinus pinaster*) with larger understory shrub species (*Juniperus communis, Juniperus oxycedrus, Erica arborea*) in more mesic sites further inland (e.g. Ayna and Yeste), to multi-layer mature closed canopy forest dominated by beech (*Fagus sylvatica)*, sessile oak (*Quercus petraea*) and Pyrenean oak *(Quercus pyrenaica)* in the northernmost and more humid sub-Mediterranean site. Except for Montejo, soils were originated predominantly from sedimentary calcareous materials like limestone, marl and sandstone, as well as Quaternary sediments coming from calcareous materials

depending on the site. Schist formations interspersed with calcareous lithologies can also be found at Montejo (Rodríguez-Fernández et al., 2015).

We sampled 6 individuals of the most dominant woody species at each location, i.e. those encompassing 90% of the total plant cover in the community (Table S2). In total, we collected samples from 471 individuals from 57 woody species belonging to 21 plant families (Table S3). The species were categorized into three plant functional groups (life forms) according to their vegetative aboveground height using an adaptation from Raunkaier's life form classification (Raunkiær, 1934; Tumber-Dávila et al., 2022): small shrubs (SS, <1m), large shrubs (LS, 1-3 m) and trees (T, > 3 m), which includes evergreen, winter-deciduous and drought-deciduous species (Table S3). The sampling campaign was conducted in 2022 during the phenological and physiological peak during spring at each location, starting in April at the drier and warmer sites (Pulpí and Pliego), during May-June at sites with meso-mediterranean climate (Majarazán, Ayna and Yeste) and in July at the wettest and coolest sub-Mediterranean site with the latest onset of the growing season (Montejo). Data from Calblanque site was collected in a previous sampling campaign in 2017 following the same procedures. The sampling date was carefully chosen at each study site to ensure at least a 2-week period with no rainfall prior the sampling date. The rationale for this was to ensure the development of a steep isotopic soil water gradient with depth along the edaphic profile, which is formed in response to topsoil water evaporation and isotopic fractionation during prolonged rainless periods (Allison et al., 1983).

Plant and soil water isotopic measurements

For each individual, one basal 5cm segment of fully-lignified stem from a terminal branch was sampled to avoid issues related to potential xylem water isotopic enrichment due to backflow from transpiring leaves or green photosynthetic stems (Schwinning, 2008). We debarked the stems to isolate the xylem, placed them in 5ml glass vials sealed with parafilm to prevent water loss and stored them at -20ºC until water extraction. Soil samples were collected in previous field campaigns conducted in 2017 (Calblanque), 2019 (Pulpí, Pliego, Majarazán, Ayna and Yeste) and 2021 (Montejo). Soil samples were taken, using a cylindrical hand auger with 4 cm diameter (Eijkelkamp, The Netherlands), every 5 cm in the topsoil layer (down to 20 cm) and every 10cm below 20 cm deep, until we reached impenetrable bedrock layers (which were located between 30 and 120 cm deep, depending on soil characteristics at each site). All freshly collected soil samples were immediately double packed in airtight zip-lock plastic bags in the field, maintained in a cooler and transported to the laboratory where subsamples were transferred to 5ml glass vials, sealed with parafilm and stored at -20ºC until water extraction.

We used a cryogenic vacuum distillation line at 85°C and 10 militorr vacuum pressure to extract all the water contained in plant stems (xylem water) and soil samples for at least 2 hours (Diao et al., 2022; Ehleringer and Osmond, 1989). Water extraction efficiency (Ceperley et al., 2024) was above 97% for all samples. All water extractions and isotopic analyses were performed at the Stable Isotope Research Center (SIRC/WSL, Birmensdorf, Switzerland). The $\delta^{18}O$ of water samples were measured with a high temperature conversion elemental analyser coupled to a Delta^{Plus} XP isotope ratio mass spectrometer (TC/EA-IRMS; Finnigan MAT, Bremen, Germany). Oxygen ($\delta^{18}O$) isotopic composition is expressed in ‰ notation relative to the standard V-SMOW (Vienna standard Mean Ocean SMOW (Vienna standard Mean Ocean Water). Long-term external precision values for water $\delta^{18}O$ determinations was ± 0.12‰.

Leaf isotopic, nutrient and morphological traits and soil nutrients

We sampled sun-exposed, fully expanded, and healthy-looking leaves from the same branches used for xylem water extractions in each individual plant. These leaves were immediately placed in sealed bags containing a piece of wet paper in the field to ensure proper hydration, transported to the lab and stored in the fridge at 4ºC for leaf material processing within 24-48 hours. To characterize leaf morphology, we measured specific leaf area (SLA, $cm^2\ g^{-1}$) and leaf dry matter content (LDMC, $mg\ g^{-1}$) using between 4-6 leaves per individual and between 20-40 leaves for species with very small leaves (e.g. *Thymus vulgaris*). We first hydrated leaves overnight at 4ºC in the dark and weighted them (saturated weight, SW, g). After weighing, a picture of the leaves was taken with a Nikon 90D (Tokyo, Japan) and the resulting image processed with Photoshop (v22.5, Tokyo, Japan) to determine leaf area (LA, cm^2). Leaves were then oven dried at 60ºC for 48 hr and weighted again to obtain their dry weight (DW, g). Specific leaf area (SLA, $cm^2\ g^{-1}$) was calculated as SLA = (LA / DW) x 1000 and leaf dry matter content (LDMC, $mg\ g^{-1}$) was calculated as LDMC = ((DW / SW) x 1000).

The remaining leaves were dried following the same protocol and finely ground using a ball mill (MM200, Retsch, Germany) before weighting (4 mg for $\delta^{13}C_L$ and 0.3 mg for $\delta^{18}O_L$) and encapsulating into tin or silver capsules for $\delta^{13}C_L$ and $\delta^{18}O_L$ isotopic analysis, respectively. $\delta^{13}C_L$ was measured by continuous flow (CF) dual isotope analysis using a CHNOS Elemental Analyzer interfaced to an IsoPrime100 mass spectrometer $\delta^{18}O_L$ was determined in continuous flow (CF) using an Elementar PYRO Cube interfaced to a Thermo Delta V mass spectrometer at the Center for Stable Isotope Biogeochemistry, University of California-Berkeley (USA). Both leaf $\delta^{13}C_L$ and $\delta^{18}O_L$ are expressed in delta notation (‰) relative to the Vienna Pee Dee Belemnite standard (V-PDB) and the Vienna Standard Mean Ocean Water (VSMOW), respectively. Long-term external precision

values for leaf $\delta^{13}C$ and $\delta^{18}O$ determinations were ± 0.1‰ and ± 0.2‰, respectively. As a way to isolate the leaf-level evaporative signal from the source water isotopic signal (Barbour, 2007), we calculated the oxygen isotopic enrichment above source water of leaf dry matter ($\Delta^{18}O$) as: $\Delta^{18}O = \delta^{18}O_L - \delta^{18}O_{XW.}$, where $\delta^{18}O_{XW.}$ is the oxygen isotopic composition of xylem water.

Leaf and soil nutrient concentrations per mass (C, N, P and K; mg g^{-1}) were measured, for all sites but Calblanque, on the remaining dry ground leaf tissue and on soil samples dried at 60ºC for 48h. C and N concentrations were determined using an elemental analyser (Thermo- Finnigan EA1112, Milan, Italy); while leaf K and P were measured using inductively coupled plasma optical emission spectrometry (ICP-OES, Thermo Elemental Iris Intrepid II XDL, Franklin, MA, USA) after a microwave-assisted digestion with HNO_2:H_2O_2 (4:1, v:v) at the Ionomics Facility at CEBAS-CSIC (Spain). Foliar N, P, K contents per leaf area were calculated dividing the nutrient concentration per mass by SLA ($Nut_{area} = Nut_{mass} / SLA$).

Leaf gas exchange measurements

We measured net photosynthetic rate (A, µmol m^{-2} s^{-1}), stomatal conductance (g_s mol m^{-2} s^{-1}) and transpiration rate (E, µmol mol^{-1}) in 307 of the 471 individuals sampled in 2022 using a portable infrared gas analyser (IRGA, LI-6800, LI-COR Inc., NE, USA). The LI-6800 equipment had a 2 cm^2 leaf cuvette and a CO_2 injector. Leaf gas exchange was measured on healthy, fully sun-exposed leaves between 9:00 and 13:00 AM (GMT) an expressed on a total leaf surface area basis. All leaf gas exchange measurements were made at a saturating light intensity of 1500 µmol $mol^{-2}s^{-1}$ and at ambient air temperature and humidity, with the air flux set to 350 µmol s^{-1}. The CO_2 concentration in the cuvette was maintained at 420 µmol mol^{-1} CO_2. For some species, total leaf area was smaller than the leaf cuvette so we rescaled the measurements to the actual leaf area inside the cuvette following leaf trait protocols. Measuring leaf gas exchange in *Juniperus oxycedrus, Thymus vulgaris, Lavandula stoechas* and *Fumana ericoides* was not feasible because their leaves were too small to achieve stable A or g_s values. Intrinsic water use efficiency (WUE_i, □□mol CO_2 mol H_2O) was calculated as the ratio between photosynthetic rate and stomatal conductance (A/g_s) and instantaneous water use efficiency (WUE_t, □□mol CO_2 mmol H_2O) was calculated as the ratio between photosynthetic rate and transpiration (A/E). We also calculated the photosynthetic nitrogen use efficiency (PNUE, µmol CO_2 g N^{-1} s^{-1}) as follows (Onoda et al., 2017; Warren and Adams, 2006): $P_{Nut}UE = A \times Nut_{area}$.

Statistical analysis

We set the difference between the deep and shallow soil water pools at the depth where the soil water $\delta^{18}O$ depletion curve saturated, which was between 15 and 25 cm depth depending on the site (Figure S2 A). At the Pulpí site, the hardness of the ground and shallow petrocalcic layer only allowed us to reach 25 cm deep with the hand auger, and the soil water $\delta^{18}O$ depletion curve was not yet saturated at this depth. For this site, we used the average winter rain isotopic composition as a proxy for the deep-water pool, obtained using geospatial interpolation maps based on GNIP data (Bowen et al., 2005; Bowen and Revenaugh, 2003). We used mixed models with soil depth (shallow or deep) as a fixed factor and soil profile nested within site as random factors to test differences in soil nutrient concentrations and soil $\delta^{18}O$ between shallow and deep soil layers.

We assessed the coordination between LES traits (SLA, LDMC, leaf N, leaf P, leaf K) by carrying out a Principal Components Analysis for the whole dataset. Individual PCA axis 1 scores were used as a continuous variable defining the LES. We tested differences between life forms along PCA axis 1 using linear mixed models using site as a random factor. To test the coordination between LES and water use traits or leaf gas exchange rates ($\delta^{13}C$, $\Delta^{18}O_L$, A, g_s, E, WUE_i, WUE_t, PNUE), we used linear mixed regression models with site as random factor to control for the influence of differences in climatic conditions, altitude and plant community composition among sites. We ran linear mixed regression models with and without data from Montejo, which is the most humid site with Sub-Mediterranean climate, to ensure that the relationships between LES and water use traits hold both across the six drier Mediterranean sites and across all sites including the wettest Sub-Mediterranean site. We also ran linear regressions to further assess the coordination between LES and water use traits or leaf gas exchange across sites. All analyses were conducted with R 12.1(R Core Team, 2023) working with prcomp function to make PCA analysis, and packages nlme (Pinheiro et al., 2018) and MuMIn (Barton, 2025).

Results

Soil water isotopic composition and soil nutrient distribution with depth.

We found steep soil water isotopic gradients with depth driven by surface evaporation at all the study sites (Figure S2 A). Topsoil water $\delta^{18}O$ was always isotopically enriched relative to rainwater, and soil water became progressively more depleted (i.e. more negative $\delta^{18}O$ values) with depth along the soil profiles at each site. Soil water ^{18}O-depletion curves saturated at 20 cm depth at all sites, below which soil water isotopic

composition remained rather constant with depth (except at the wettest site, Montejo, where it saturated at 15 cm depth). Soil water was always significantly more evaporatively ^{18}O-enriched (i.e. less negative) in the shallow topsoil water pool (0-20 cm depth) than in the deeper soil water pool (>20 cm depth) (Figure S3 A, $p<0.001$). We also encountered marked vertical gradients in soil nutrient availability (N, P, K) with depth at all the study sites (Figure S2 B, C, D), as soil N, K and P contents were always higher in topsoil than in deeper soil layers (Figure S3 B,C,D; $p<0.001$ for N and $p<0.01$ for P and K). As a result, we found a tight coupling between soil N and P content and soil water $\delta^{18}O$ enrichment along the soil profile ($F = 27.76$, $p < 0.001$ and $F = 8.60$, $p < 0.01$ for soil N and P, respectively; Figure S4). Topsoil N, P and K contents ranged from 0.5 ± 0.10 to 9.6 ± 1.20 g kg $^{-1}$, 0.20 ± 0.01 to 0.80 ± 0.04 g kg^{-1} and 5.90 ± 1.10 to 10.30 ± 0.60 g kg^{-1}, respectively across sites (Table S1).

Coordination among leaf economic spectrum (LES) traits.

Principal component analysis based on 5 leaf morphological (LDMC and SLA) and nutrient (N, P, K) traits across 57 mediterranean woody species showed strong trait coordination along the first PCA axis (i.e., the LES axis) (Figure 1). All leaf traits showed high loadings on this LES axis, which alone explained 55.9% of the overall leaf trait variance across species and sites. In contrast, PCA axis 2, which reflects a secondary variation trend only explained 18.6% (Table S4). The LES axis highlights the large variation in resource use strategies present in Mediterranean plant communities, with acquisitive species showing high leaf nutrient concentrations (N, P and K) and high SLA values (i.e. negative scores along the LES axis), and conservative species showing high LDMC combined with low SLA and leaf nutrient concentrations (i.e. positive scores along the LES axis; Figure 1). We found remarkably wide interspecific diversity in carbon and nutrient use strategies (i.e. species scores along the LES axis) among coexisting species within each study site (Figure 2). Small shrub species (such as *Thymus spp., Lavandula stoechas, Genista scorpius* or *Hellichrysum stoechas)* exhibited more acquisitive traits (negative scores along the LES axis), while larger shrubs and trees (such as *Juniperus spp., Olea europaea*, *Pistacia lentiscus*, *Pinus spp.)* showed more conservative traits (positive scores along the LES axis) (Figure 2). This pattern was encountered at all the 6 dryland sites with Mediterranean climate *sensu stricto*, but less so at the wetter Sub-Mediterranean site (Montejo), where some large shrubs and trees like *Genista florida* or *Prunus avium* showed more acquisitive leaf traits than their coexisting small shrubs (*Cytisus purgans* or *Lavandula stoechas*; Figure 2). Nonetheless, significant differences between life forms across all sites, including Montejo, were found along the LES axis ($F = 38.17$, $p < 0.001$); small shrubs had more acquisitive traits, i.e., lower scores in the

LES (mean ± SE = -0.31 ± 0.4), while large shrubs (mean score ± SE = 0.64 ± 0.4) and trees (mean score ± SE = 1.03 ± 0.4) displayed more conservative traits (Figure 1).

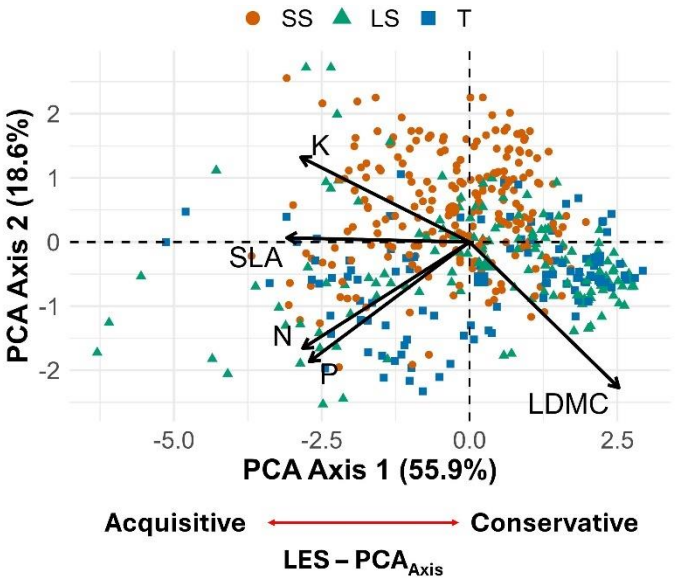

Figure 1. Principal component analysis (PCA) of leaf morphological and nutrient traits for 57 species across 7 study sites with contrasting climatic conditions. The first PCA axis represents the species variation across the leaf economic spectrum. Points depict individual plants, shape and color indicate plant individual plant classification according to life forms (SS, small shrubs (< 1 m height); LS, large shrubs (1-3 m); T, trees (> 3 m).

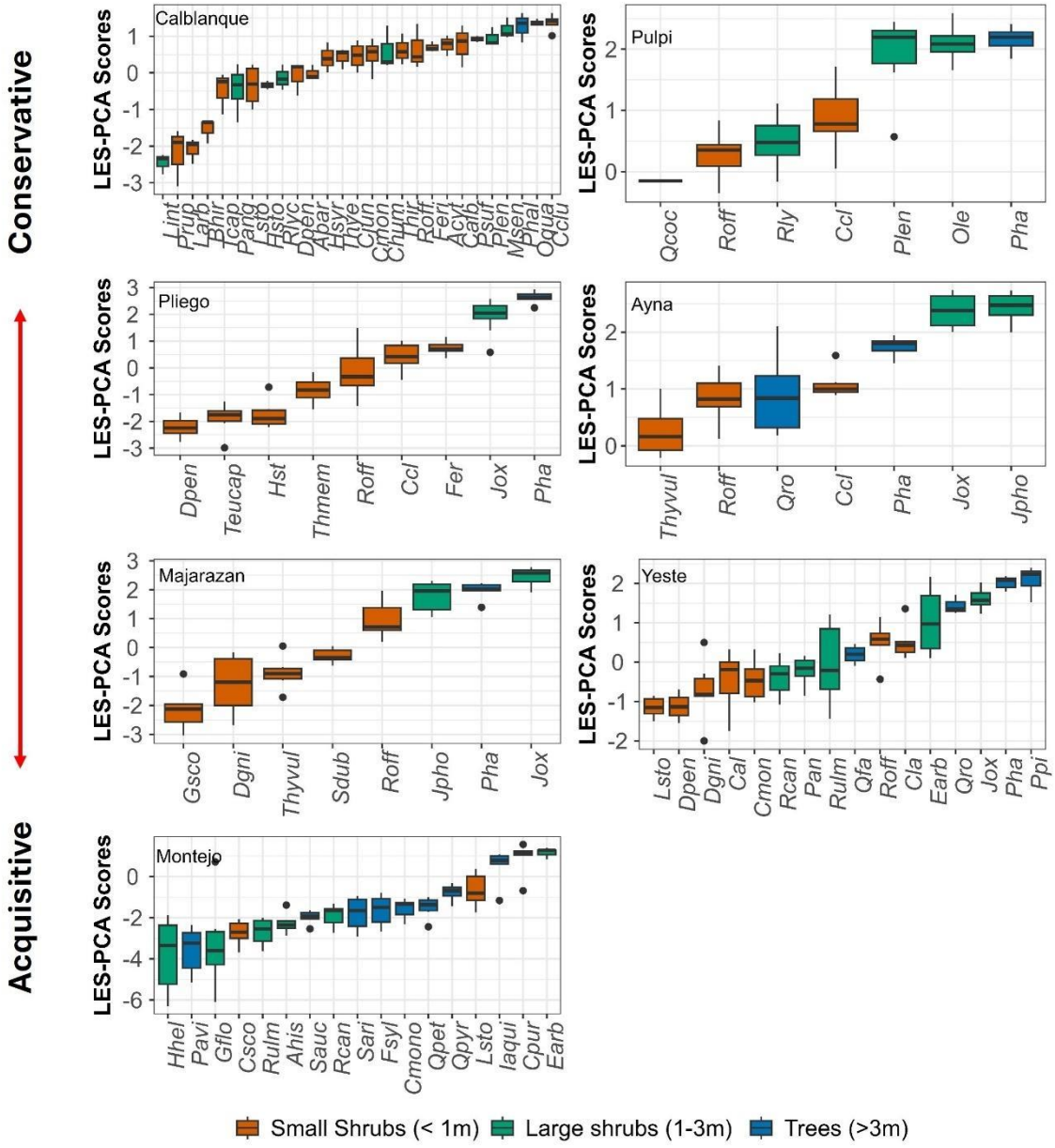

Figure 2. Interspecific variation in mean scores along the LES-PCA axis among coexisting woody species growing at 7 study sites with contrasting climatic conditions (see Table S1). Bars within boxplots indicate species mean LES-PCA axis scores. Different colours depict different plant life forms (legend). Abbreviations for species are as in table S3.

Coordination between LES traits, water use traits and root water uptake patterns.

We found strong relationships between leaf-level carbon and nutrient use strategies (LES) and water use strategies in Mediterranean woody vegetation (Figure 3). Plant species with more acquisitive leaf traits (negative scores along the LES axis) have also more acquisitive water use strategies ("water-spenders"), showing both looser time-

integrated stomatal regulation of plant water flux (lower $\Delta^{18}O_L$) and lower time integrated water use efficiency (low $\delta^{13}C_L$), especially when only strictly Mediterranean sites are considered (Figure 3 A and C). The opposite is found for conservative species along the LES (more positive scores) that exhibit a more conservative water use strategy at leaf level (i.e. "water-savers" with higher $\Delta^{18}O_L$ and $\delta^{13}C_L$ values denoting more stringent stomatal regulation of plant water flux and higher WUE_i). Regarding plant water uptake patterns, we found that the LES axis was negatively related to xylem water $\delta^{18}O$ values across coexisting plants in Mediterranean sites (Figure 3 E), indicating that species with more acquisitive leaf traits (higher nutrient concentrations and SLA) use a greater proportion of water from shallower soil layers (less negative $\delta^{18}O_{xw}$ values). Conversely, species with more conservative leaf traits (lower nutrient concentrations and higher LDMC) use a greater proportion of water from deeper soil layers (more negative $\delta^{18}O_{xw}$ values). The relationships between the LES and $\delta^{13}C_L$ and $\delta^{18}O_{XW}$ held when including the wetter Sub-Mediterranean site (Montejo, Figure 3 B, F), but did not hold for $\Delta^{18}O_L$ (Figure 3 D).

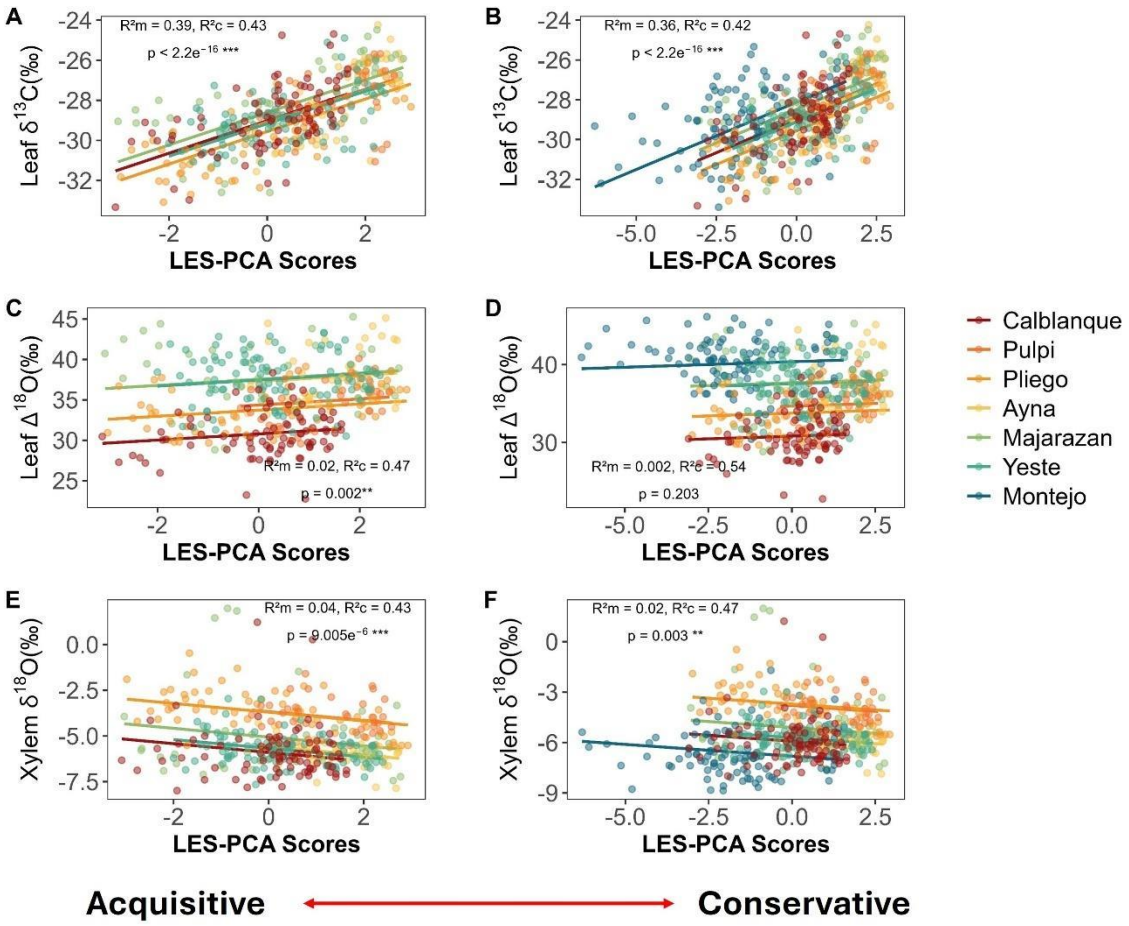

Figure 3. Across site relationships between isotopic water use traits and LES-PCA axis scores obtained from the general PCA (see Figure 1, PCA axis 1) for the six strictly Mediterranean sites

(A, C and E), or with the whole dataset that also includes the Sub-mediterranean site (Montejo) with cooler and wetter climatic conditions (B, D and E). Negative scores along the LES-PCA axis indicate acquisitive leaf traits, whereas positive scores indicate conservative resource use traits and strategy at leaf level. Results for each linear mixed model are shown within each panel: P values indicate the significance of each linear mixed model and marginal (R^2m) and conditional R^2 (R^2c) indicate the proportion of variance explained by the fixed factor (R^2m) or by the fixed and random factor in the model (R^2c), respectively. For clarity, instead of the global regression line across sites, individual regression lines per site are shown in each panel to represent the different intercepts for each site. Leaf carbon isotopic composition ($\delta^{13}C_L$) reflects time integrated water use efficiency; oxygen isotopic enrichment above source water ($\Delta^{18}O_L$) reflects time-integrated stomatal conductance; xylem water oxygen isotopic composition ($\delta^{18}O_{XW}$) reflects water uptake depth.

Acquisitive species along the LES axis also showed higher stomatal conductance (g_s) and transpiration rates (E) than coexisting species with more conservative traits (Figure 4 A and B) and thus lower intrinsic and instantaneous water use efficiency at leaf level (WUE_i and WUE_t, respectively). Acquisitive species also showed higher net photosynthetic rates (A) and photosynthetic nitrogen efficiencies (PNUE), compared to coexisting species with more conservative traits along the LES axis (Figure 4 C, D, E, F). The coordination observed between LES and water use traits was also consistent across sites using linear regressions that did not include site variability (except for WUEi, Figure S5), but only when considering the six Mediterranean sites *sensu stricto* (Table S5). When the sub-Mediterranean site was included in the linear regression analysis, only the relationships of the LES with $\delta^{13}C_L$ and PNUE remained significant and with same sign.

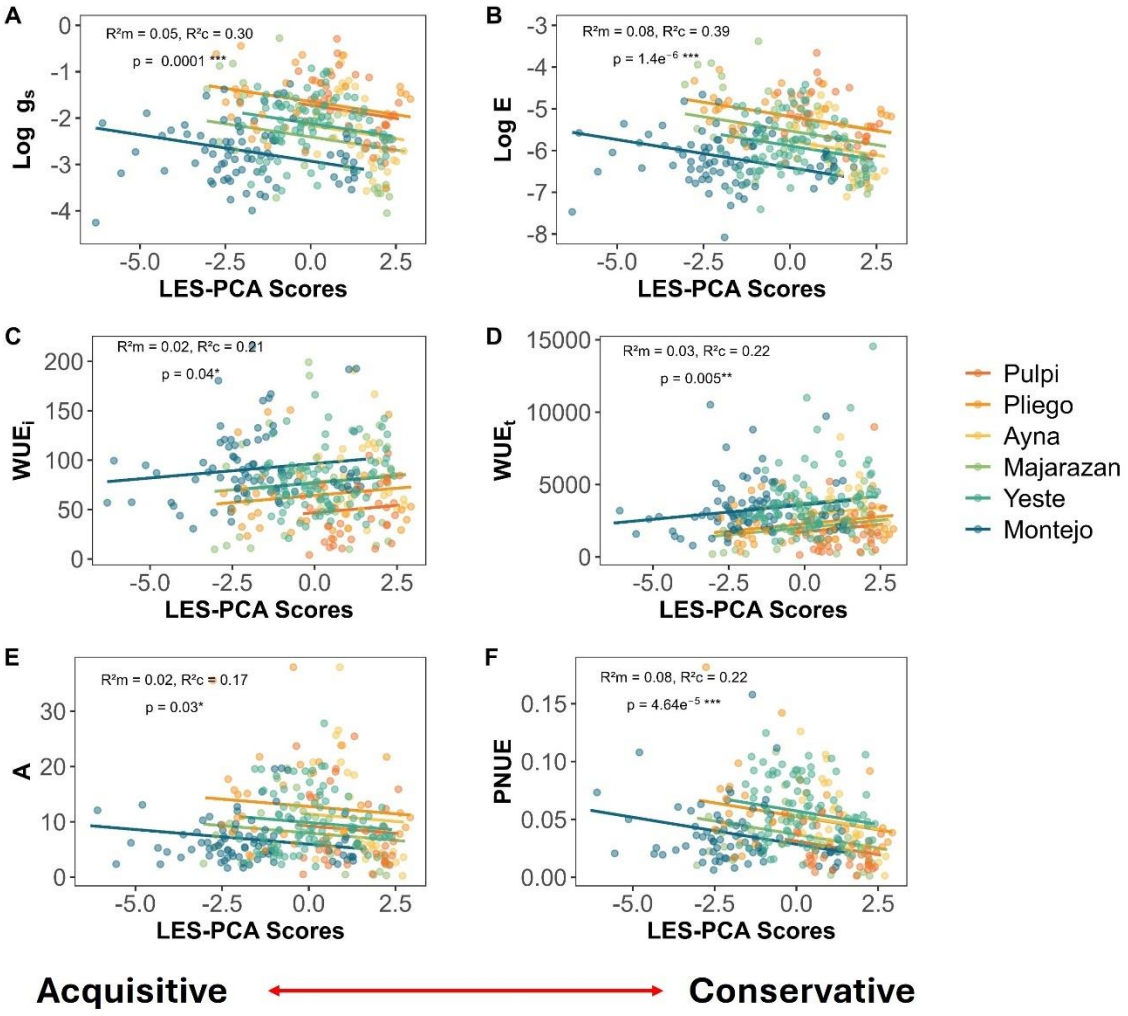

Figure 4. Across site relationships between leaf gas exchange traits and LES-PCA axis scores obtained from general PCA (see Figure 1, PCA axis 1) for the whole dataset (gas exchange data was not available for Calblanque). Negative scores along the LES-PCA axis indicate acquisitive leaf traits, whereas positive scores indicate conservative resource use traits and strategy at leaf level. Results for each linear mixed model are shown within each panel: P values indicate the significance and marginal (R^2m) and conditional R^2 (R^2c) indicate the proportion of variance explained by the fixed factor or by the fixed and random factor in the model, respectively. For clarity, instead of the global regression line across sites, individual regression lines per site are shown in each panel to represent the different intercepts for each site. Coloured dots represent individual plants for each site. Stomatal conductance, g_s (mol m^{-2} s^{-1}); transpiration rate, E (µmol mol^{-1}); intrinsic water use efficiency, WUE_i (µmol CO_2 mol H_2O); instantaneous water use efficiency, WUE_t (µmol CO_2 mmol H_2O); net photosynthetic rate, A (µmol m^{-2} s^{-1}); photosynthetic nitrogen use efficiency, PNUE (µmol CO_2 g N^{-1} s^{-1}).

Discussion

We report a remarkably large diversity of soil resource-use strategies among Mediterranean woody species both within sites, under similar climatic conditions, and across sites with contrasting climatic conditions. Our study reveals a significant coupling and functional convergence between leaf economic (LES) traits, water use traits and root water uptake depth across multiple Mediterranean woody species of different life forms (Figures 3, 4). Plant species with acquisitive traits along the LES (high SLA, leaf N, P, K and low LDMC) exhibit higher photosynthesis combined with higher g_s and E (i.e. lower $\Delta^{18}O_L$), which results in lower intrinsic and instantaneous water use efficiencies (WUE_i and WUE_t, respectively) and thus lower $\delta^{13}C_L$ (compensated by higher PNUE). Moreover, these resource-acquisitive species are also more heavily dependent on water uptake from the nutrient-rich topsoil layers (higher $\delta^{18}O_{xw}$; Figure 3 E,F). Conversely, other species with more conservative resource use traits along the LES (low SLA, foliar N, P, K and high LDMC) exhibit lower photosynthesis and a markedly lower g_s and E (higher $\Delta^{18}O_L$), and thus higher WUE_i, WUE_t and $\delta^{13}C_L$ (but lower PNUE) at leaf-level. These resource conservative species also exhibit a deeper water uptake pattern along the soil profile (lower $\delta^{18}O_{xw}$). Our findings thus highlight the significant interdependence, coordination and alignment among classic leaf economic traits, water use traits and water uptake depth across woody plant species in Mediterranean plant communities (Figure 5). In particular, the remarkably tight coordination of $\delta^{13}C_L$ with the LES axis across multiple woody plant species and sites suggests that leaf $\delta^{13}C_L$ is a pivotal trait that links carbon and nutrient use strategies with water use strategies in Mediterranean woody plant communities (Ding et al., 2021; Pérez-Ramos et al., 2012; Prieto et al., 2018).

First, we encountered a remarkably wide range of leaf $\delta^{13}C_L$ values (from -24 ‰ to -33 ‰) encompassing almost 65% of the global variation in this physiological trait (Kohn, 2010). Variation in $\delta^{13}C_L$ was tightly coordinated with LES axis scores, thus highlighting both the generality of this coordination across Mediterranean ecosystems and the wide diversity of water use strategies present within the scope of this study. Leaf anatomy has a strong influence on carbon isotopic discrimination, as thicker and more robust sclerophyllous leaves (high LDMC, low SLA) are linked to thicker cell walls and lower mesophyll conductance to carbon dioxide. This reduces CO_2 concentrations at the sites of carboxylation in the chloroplast (Chen et al., 2023; Hultine and Marshall, 2000; Maire et al., 2015; Vitousek et al., 1990), thereby reducing internal c_c/c_a ratios during photosynthetic carbon assimilation and resulting in higher leaf $\delta^{13}C_L$ values (Hultine and Marshall, 2000; Seibt et al., 2008; Tomás et al., 2013). A high carbon investment in cell

wall structural components (high C construction costs) reduces CO_2 diffusion through the leaf lamina mesophyll (Chen et al., 2023; Onoda et al., 2017)and thus contributes to high $\delta^{13}C_L$ in conservative species with sclerophyllous leaves. Low SLA (high LMA) in sclerophyllous leaves also increases leaf N content on a leaf area basis (N_{area}) despite low N concentration on a mass basis (N_{mass}), which may further contribute to lower internal c_c/c_a ratios through stimulation of CO_2 drawdown at the sites of carboxylation (Wright et al., 2003), thereby further increasing $\delta^{13}C_L$ values in resource-conservative species. Conversely, in resource-acquisitive species with low $\delta^{13}C_L$ values, high SLA is linked to higher foliar area per unit investment of C for capturing light and lower mesophyll resistance to CO_2 diffusion through the leaves, whereas high leaf N concentration for investment in Rubisco enzyme enables high carboxylation and photosynthetic capacity during wet periods (Wright et al., 2003). Likewise, high foliar P is needed to achieve a fast metabolism with high photosynthesis and growth rate, given that P is an essential component of energy source molecules such as ATP (Aerts and Chapin, 1999), phospholipids in cell membranes, and RNA in ribosomes to build the proteins needed to produce new stems and leaves (Veneklaas et al., 2012). High leaf K accumulation in shallow rooted species with acquisitive strategies also enhances photosynthesis and helps endure severe drought stress through osmotic adjustment and more efficient stomatal functioning during the frequent periods of topsoil drying in Mediterranean climates (Ren et al., 2024; Sardans et al., 2013).

Second, plant species' position along the LES axis is coordinated with several other leaf-level water use traits related to stomatal regulation of plant water flux in Mediterranean woody plants (Figures 3 and 4). Resource-acquisitive species along the LES axis show a "water-spender" strategy at leaf level characterized by high stomatal conductance and transpiration rates and thus low $\Delta^{18}O_L$, $\delta^{13}C_L$, WUE_i and WUE_t. Conversely, resource conservative species along the LES axis exhibit "water-saver" strategies characterized by opposite water-use trait values (low gs and E, and high $\Delta^{18}O_L$, $\delta^{13}C_L$, WUE_i and WUE_t) at leaf level. Foliar morphology plays an important role in shaping leaf hydraulic conductivity and water diffusion through the leaf lamina, given that the combination of high SLA and low LDMC tends to reduce water flow path length and tortuosity within leaves (Ferrio et al., 2012; Flexas et al., 2013). The looser stomatal regulation of plant water flux (high g_s, E and low $\Delta^{18}O_L$, $\delta^{13}C_L$) in resource-acquisitive species likely requires a high leaf hydraulic conductivity to achieve continuous and abundant flow of water through the leaf lamina. Higher g_s favours a faster flux of water from leaves to the atmosphere, resulting in evaporative leaf cooling and lower leaf temperatures (Siegwolf et al., 2023), and thus lower $\Delta^{18}O_L$ values in acquisitive species. Moreover, in resource-

acquisitive plant species, maintaining high g_s and E during favourable wet periods could help maximize nutrient acquisition through enhanced diffusion and mass flow of soil nutrients to roots and fast internal nutrient transport to leaves with the transpiration flux (Cramer et al., 2009; Querejeta et al., 2018; Wang et al., 2021). At the same time, higher g_s reduces stomatal constraints on carbon uptake and assimilation, favouring a higher c_c/c_a ratio and leading to lower leaf ^{13}C values in acquisitive species. However, this water-spender strategy at leaf level comes at the unavoidable cost of operating at lower water use efficiency during carbon assimilation (lower WUE_i, WUE_t, which is traded off for higher PNUE, Figure 4) in acquisitive species.

Interspecific differences in diffusive limitations to photosynthesis (i.e. stomatal conductance to water and mesophyll conductance to CO_2, which are often tightly coupled across Mediterranean plants (Flexas et al., 2008)) appear to be the key drivers of the observed variation in time-integrated c_c/c_a ratios and leaf $\delta^{13}C_L$ across species. Both stomatal and non-stomatal (mesophyll) diffusive constraints are thought to be the main factors co-limiting net photosynthesis rates across coexisting Mediterranean species (Flexas et al., 2008; Medrano et al., 2009). Our leaf gas exchange data suggest that, for dryland species, the combination of high SLA and high N_{mass} coupled with high g_s may be more effective for achieving high photosynthetic rates than a low SLA, high N_{area} coupled with low g_s (Querejeta et al., 2022). We find that, in Mediterranean plant communities, resource acquisitive species along the LES axis prioritize photosynthetic N use efficiency (PNUE) over WUE_i, whereas resource conservative species instead prioritize WUE_i over PNUE (Figure 4). Thus, we see how acquisitive species with low $\delta^{13}C_L$ values prioritize rapid water and nutrient uptake and fast carbon assimilation at leaf level, while conservative species with high $\delta^{13}C_L$ instead prioritize a high efficiency in the use of the most limiting resource (water), although at the unavoidable cost of a lower PNUE (Prieto et al., 2023; Wright et al., 2003). The “fast/slow” theory postulates that being fast (or slow) at acquiring or using any given resource (i.e. carbon, nutrients or water) necessarily involves being also fast (or slow) at the acquisition and use of other essential resources (Reich, 2014). Consistent with this theory, we find that acquisitive species with low $\delta^{13}C_L$ show a more profligate water-spender strategy characterized by high g_s and E that reduces stomatal constraints on photosynthesis (Onoda et al., 2017; Terashima et al., 2011; Tomás et al., 2013), coupled with higher carbon assimilation rates (A) linked to higher leaf N_{mass} and higher SLA. Our findings are thus in good agreement with the “fast-slow” plant economic spectrum theory (Lerdau et al., 2023; Reich, 2014) and previous local-scale studies reporting some degree of coordination between LES traits and leaf-level water use traits (Chen et al., 2023; Jin et al., 2016;

Prieto et al., 2018; Song et al., 2022; Wang et al., 2021; Yin et al., 2018). However, in contrast to earlier work, the present study covers a much broader number of plant species and sites with contrasting environmental conditions, and encompasses a larger number of traits, thus providing a more robust and comprehensive demonstration of multiple leaf trait coordination across diverse plant life forms and spatial scales.

Here we show that woody plant species with diverse and sharply contrasting leaf economic and water use traits coexist within Mediterranean plant communities, and we find that this coordination is dependent on the ability of each species to access different soil water pools stored at different depths in the soil profile. Since water and nutrients are unevenly distributed along the soil profile and are often spatially decoupled from each other during dry periods when the nutrient-rich topsoil layer is dry (Querejeta et al., 2021), the ability of each plant species to access and use water stored in different pools determines their resource use strategies (Fan et al., 2017). According to the "two water pool hypothesis" proposed by Ryel et al., (2008), there is a marked difference between the nutrient-rich "growth water pool" stored in the fertile topsoil layers and the nutrient-poor "maintenance water pool" stored in deeper soil/bedrock layers. The availability of the shallow "growth water pool" to plants fluctuates sharply through time in drylands, because it is quickly recharged by rainfall events but is also exposed to fast evaporation losses and rapid depletion by neighbours during subsequent rainless periods. Conversely, the deeper "maintenance pool" is more temporally stable because it is less exposed to evaporation and less accessible (or unreachable) to shallow-rooted plants. We encounter a negative relationship between $\delta^{18}O_{xw}$ and species scores along the LES across plant species in Mediterranean sites (Figure 3), which indicates that species exhibiting a more acquisitive resource use strategy at leaf level tend to use a greater proportion of ^{18}O-enriched topsoil water stored in the shallow "growth pool" that is rich in dissolved nutrients. Conversely, species exhibiting more conservative resource-use traits at leaf level tend to use a greater proportion of the nutrient-poor water stored in the deep "maintenance pool" (i.e. lower $\delta^{18}O_{xw}$). In these Mediterranean dryland environments where water is supplied during few and irregular rainfall pulses, resource-acquisitive species that mainly rely on the shallow and nutrient-rich "growth water pool" appear to prioritize and maximize rapid soil resource acquisition and use. This is achieved by a loose stomatal control of the transpiration flow that allows fast water uptake from topsoil layers and rapid internal flux of water and dissolved nutrients during favourable wet periods, thereby reducing both nutritional and stomatal constraints on photosynthesis. Along with a low leaf construction cost (high SLA and low LDMC), this strategy allows shallow rooted species to grow faster in a highly competitive understory

environment during the short windows of opportunities when the topsoil is wet after rainfall events (Ding et al., 2021; Ogle and Reynolds, 2004; Reynolds et al., 2004). Moreover, the shorter leaf lifespans and higher leaf turnover rates typical of acquisitive species (which are often drought-deciduous in Mediterranean environments) may increase their overall demand for soil nutrients to sustain high photosynthesis during the short favourable wet periods, which can only be met through heavy utilization of the nutrient-rich “growth water pool” stored in topsoil.

A sharply contrasting soil resource-use strategy is displayed by larger woody species with evergreen leaf habit and deeper taproots that can reach the “maintenance water pool” stored in deeper soil/bedrock layers (Muñoz-Galvez et al., 2025). Access to a more temporally stable and reliable moisture source allows them to build more carbon-costly, thicker and sclerophyllous leaves with lower nutrient concentrations and photosynthetic capacity but longer lifespans and investment return periods that make a more efficient use of water through tighter stomatal control. When topsoil desiccation occurs during long rainless periods, deep-rooted species can rely on the more temporally stable deep maintenance pool for water supply (Guber et al., 2008; Palacio et al., 2017) and are thus capable of maintaining a better hydration status and physiological activity throughout the prolonged summer dry season in the Mediterranean drylands (Granda et al., 2022; Pivovaroff et al., 2021). However, using a greater proportion of deep soil water sources often comes at the cost of a reduction in nutrient uptake, transpiration and photosynthetic rates (Grossiord et al., 2017; Querejeta et al., 2021). The longer leaf lifespans of evergreen species with sclerophyllous leaves enhances nutrient conservation and may significantly reduce their overall demand for soil nutrients, which allows them to use a greater proportion of water from the nutrient-poor deep maintenance pool. This conservative use of water at leaf level could help deep rooted species, mainly large shrubs and trees in our study, to avoid hydraulic failure, canopy dieback, defoliation and mortality during long rainless periods (Fernández-de-Uña et al., 2023; Klein et al., 2013; Martin-StPaul et al., 2017; Yi et al., 2019).

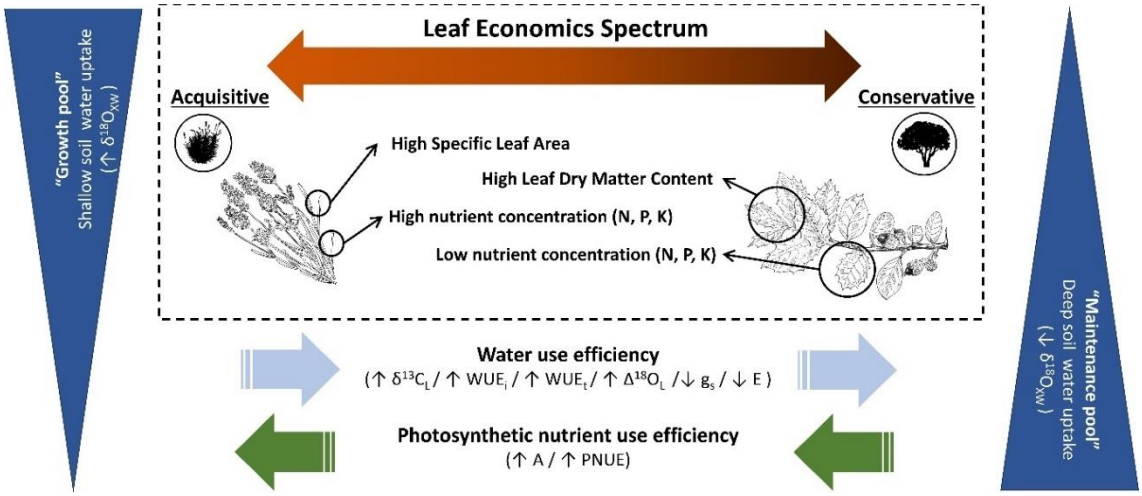

Figure 5. Conceptual model summarizing the significant coupling and functional coordination between carbon, water and nutrient use strategies across coexisting woody species in Mediterranean plant communities. On the left side of the figure are acquisitive species characterized by fast resource use, shorter growing season and shallow roots relying mainly on the nutrient-rich "growth water pool" available in upper soil layers. On the right side are conservative species characterized by slow resource use, longer growing season and deep roots capable of reaching the "maintenance soil water pool" stored in deeper subsoil/bedrock. Leaf carbon isotopic composition ($\delta^{13}C$) reflects time integrated water use efficiency; oxygen isotopic enrichment above source water ($\Delta^{18}O$) reflects time-integrated stomatal conductance; xylem water oxygen isotopic composition ($\delta^{18}O$) reflects soil water uptake depth. Stomatal conductance, g_s; transpiration rate, E; intrinsic water use efficiency, WUEi; instantaneous water use efficiency, WUE_t; net photosynthetic rate, A; photosynthetic nitrogen use efficiency, PNUE. All images included in this figure belongs to public image repositories.

In summary, our findings reveal two distinct and contrasting resource use strategies among Mediterranean woody plants. Larger plant species adopt a "slow" and conservative strategy with heavy investment in carbon-costly tissues (evergreen sclerophyll leaves and deep roots) to secure a stable water supply that allows sustaining moderate transpiration and photosynthetic activity during long rainless periods. Alternatively, "fast" and acquisitive species of smaller size prioritize investment in less carbon-costly tissues (shallow roots and leaves with high SLA, leaf nutrient concentrations and photosynthesis combined with looser stomatal regulation of plant water flux). This acquisitive strategy favours rapid resource uptake and plant growth after rain events, followed by leaf senescence and shedding during prolonged rainless periods to avoid water loss (De La Riva et al., 2016; Laughlin et al., 2023; Mooney and Dunn, 1970) (Figure 5). Interestingly, the relationships between species scores along the LES axis and their leaf level and belowground water use traits became stronger when

excluding Montejo, our wettest study site located at the transitional zone between Mediterranean and Eurosiberian temperate ecosystems. This suggests that the significant functional coordination and convergence among multiple resource use traits observed in our study might be an idiosyncratic feature of dryland floras (de la Riva et al., 2023). Low water availability entails high costs to plants in the Mediterranean drylands, so the diversity of resource-use trait combinations that are ecologically and physiologically feasible may be narrowly constrained. Economic and water use traits may thus necessarily converge in order to adapt to the harsh environmental conditions and irregular pulses of soil water and nutrient availability to plants that are typical of drylands (Carvajal et al., 2019; Yin et al., 2018). Thus, the inescapable trade-offs among plant structural and physiological traits together with severe environmental constraints imposed by frequent droughts may limit the number of feasible trait combinations in Mediterranean dryland plants (Asner et al., 2016; Díaz et al., 2016). In humid biomes, coordination and functional convergence among multiple resource use traits may not be required, as the absence of water limitations could favour the decoupling of carbon, nutrient and water use strategies, thereby leading to a higher diversity of feasible trait combinations (Beccari and Carmona, 2024; Blackman et al., 2016; Díaz et al., 2016; Joswig et al., 2022; Li et al., 2017, 2015; Ren et al., 2025; Wang and Wen, 2022). Nonetheless, considering the large variety of sites, climatic conditions, plant life forms and species encompassed in the present study, we suggest that the patterns of functional convergence among multiple resource use traits reported here could also be present in other non-Mediterranean dryland environments at global scale. The coexistence of species with sharply contrasting resource use strategies in dryland plant communities may enhance their drought-resistance, resilience and overall productivity through complementarity effects that attenuate interspecific competition for limiting soil water and nutrients.

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Supplementary Materials

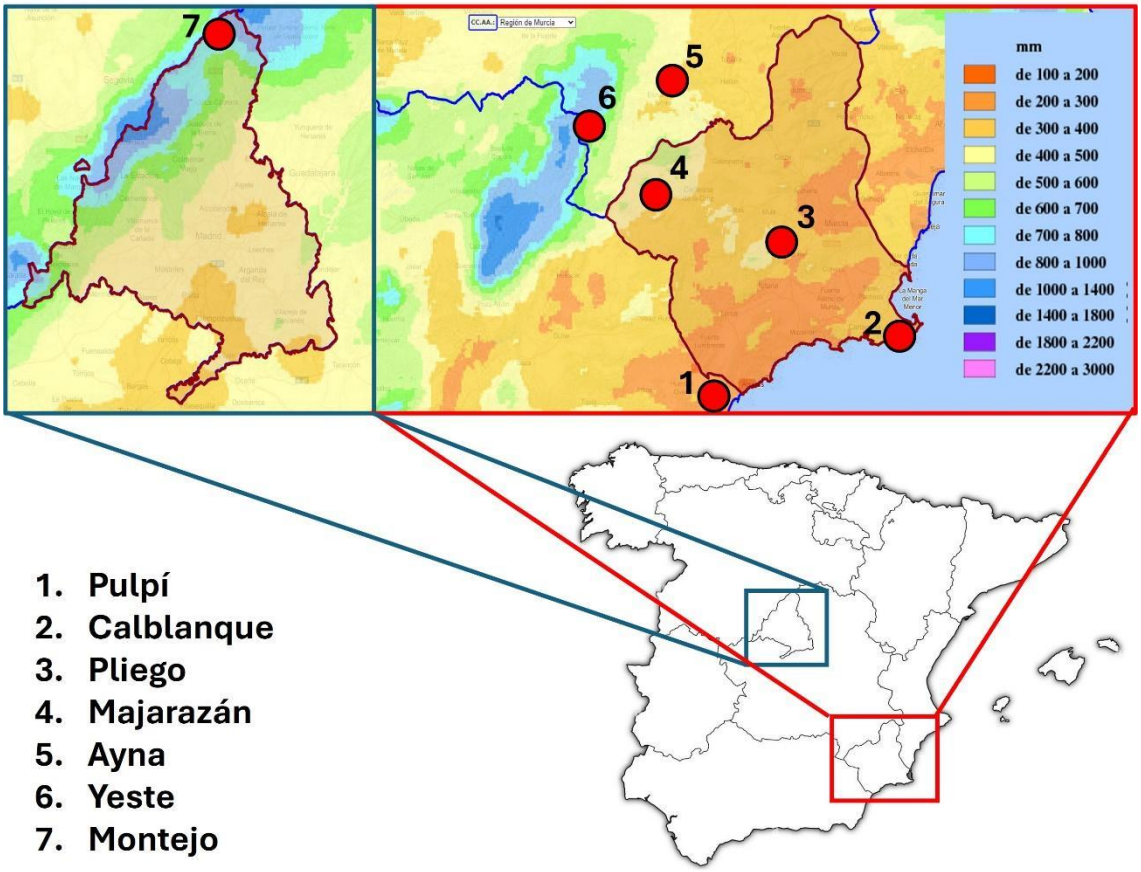
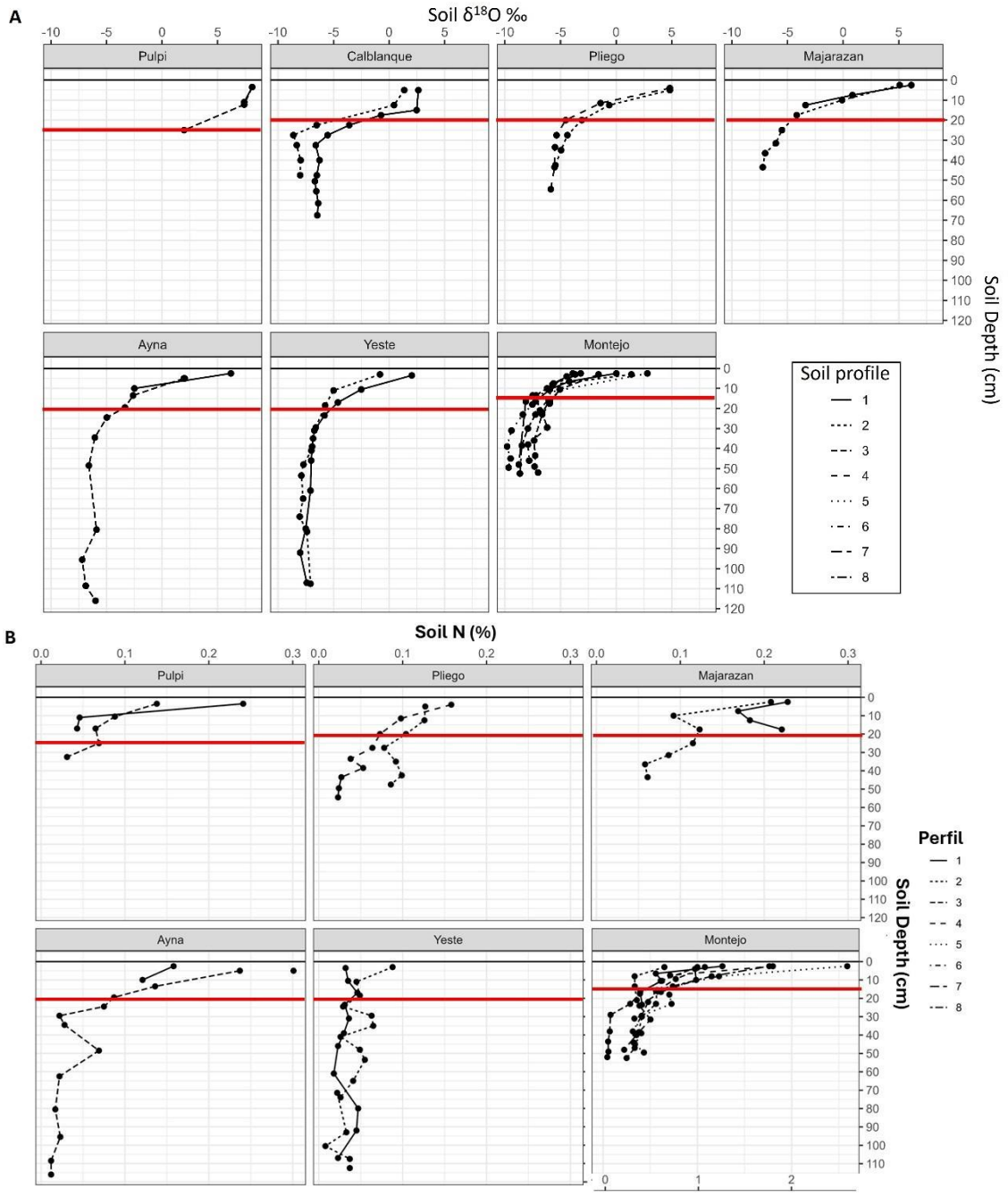


Figure S1. Map of the 7 study site locations along the Iberian Peninsula. Colour map represented mean annual precipitation (mm) (Ministerio de Medio Ambiente y Medio Rural y Marino. AEMET, 2011). Blue and red-framed inserts indicate the sub-Mediterranean and Mediterranean study region, respectively.


A
Soil δ18O ‰
Pulpi
Calblanque
Pliego
Majarazan
Ayna
Yeste
Montejo
Soil Depth (cm)
Soil profile
1
2
3
4
5
6
7
8
B
Soil N (%)
Pulpi
Pliego
Majarazan
Ayna
Yeste
Montejo
Soil Depth (cm)
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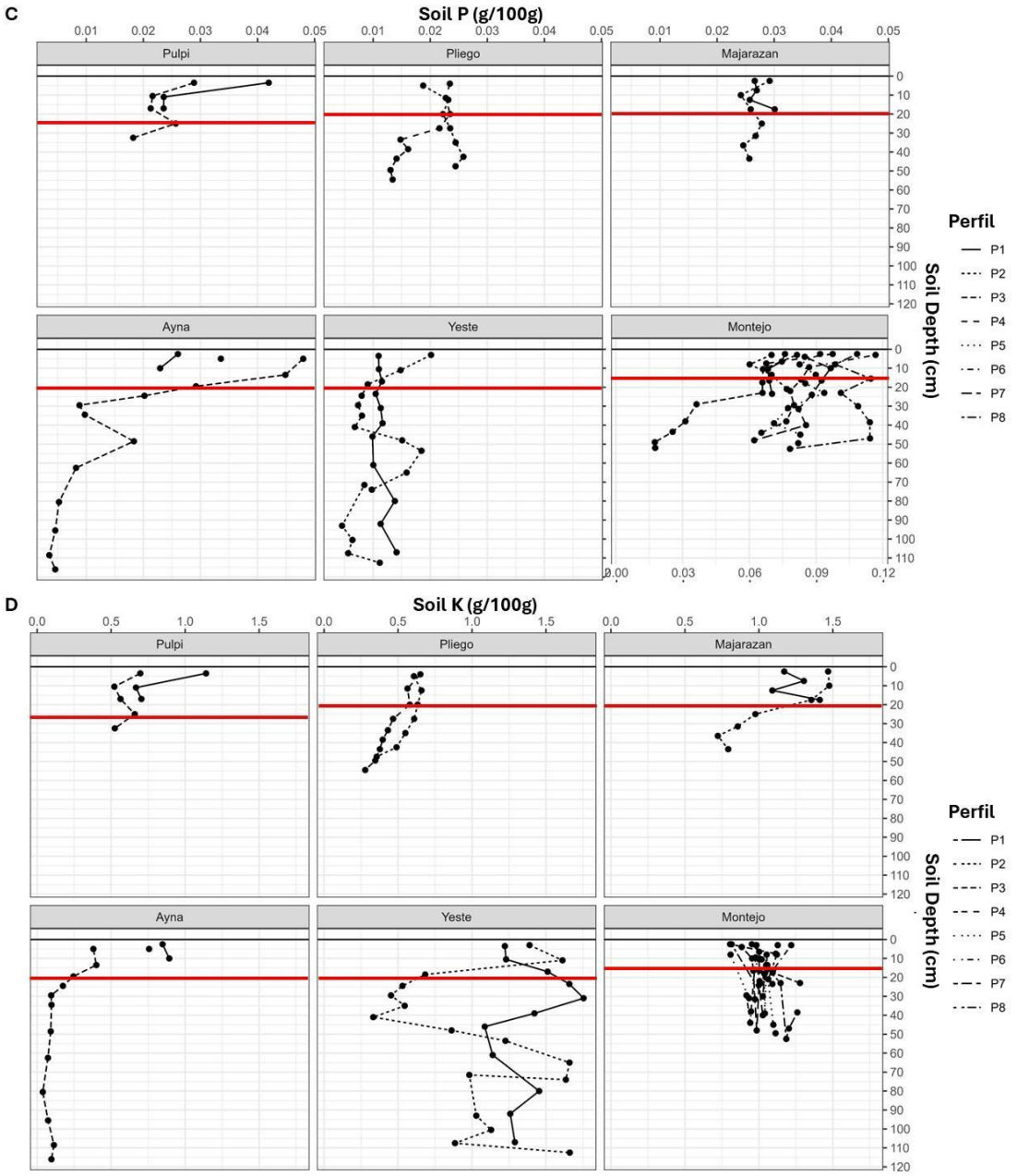

Figure S2. Steep gradients in gravimetric soil water $\delta^{18}O$ ‰ (A) and soil nitrogen (B), phosphorous (C) and potassium (D) concentration with soil depth (0 – 110 cm). Dots connected by lines depict the isotopic composition or soil water content of multiple soil profiles at each site. Red lines 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.

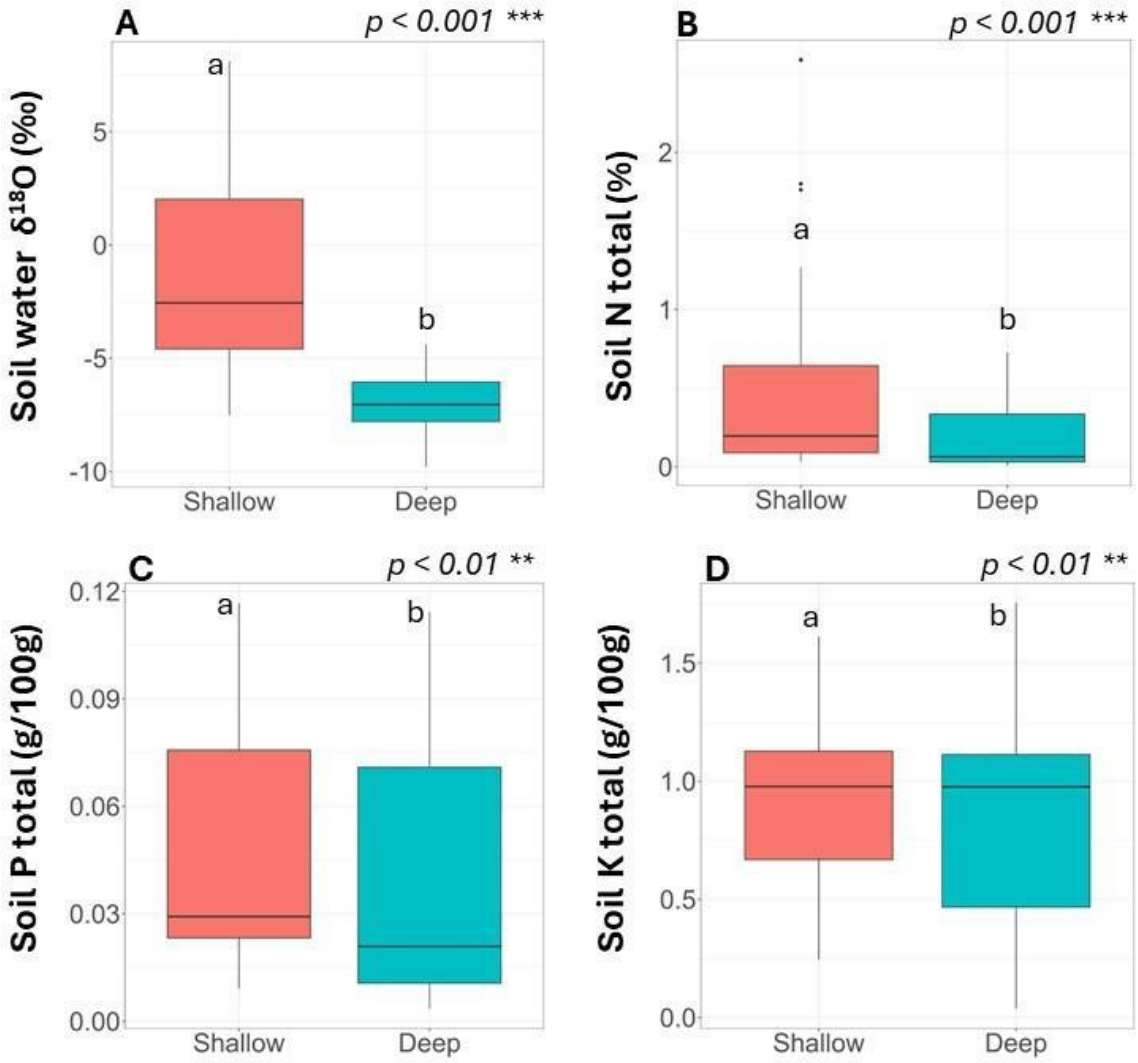

Figure S3. Boxplot depicting soil water $\delta^{18}O$ ‰ (A) and soil nitrogen (B), phosphorous (C) and potassium (D) concentration by soil layer across study sites. Boxplots represent median (line) values, while lower upper hinges correspond to the 25th and 75th percentiles and whiskers depicts 1.5*IRQ (interquartile ranges); outlier values are also shown. P values depict significant differences between soil layers.

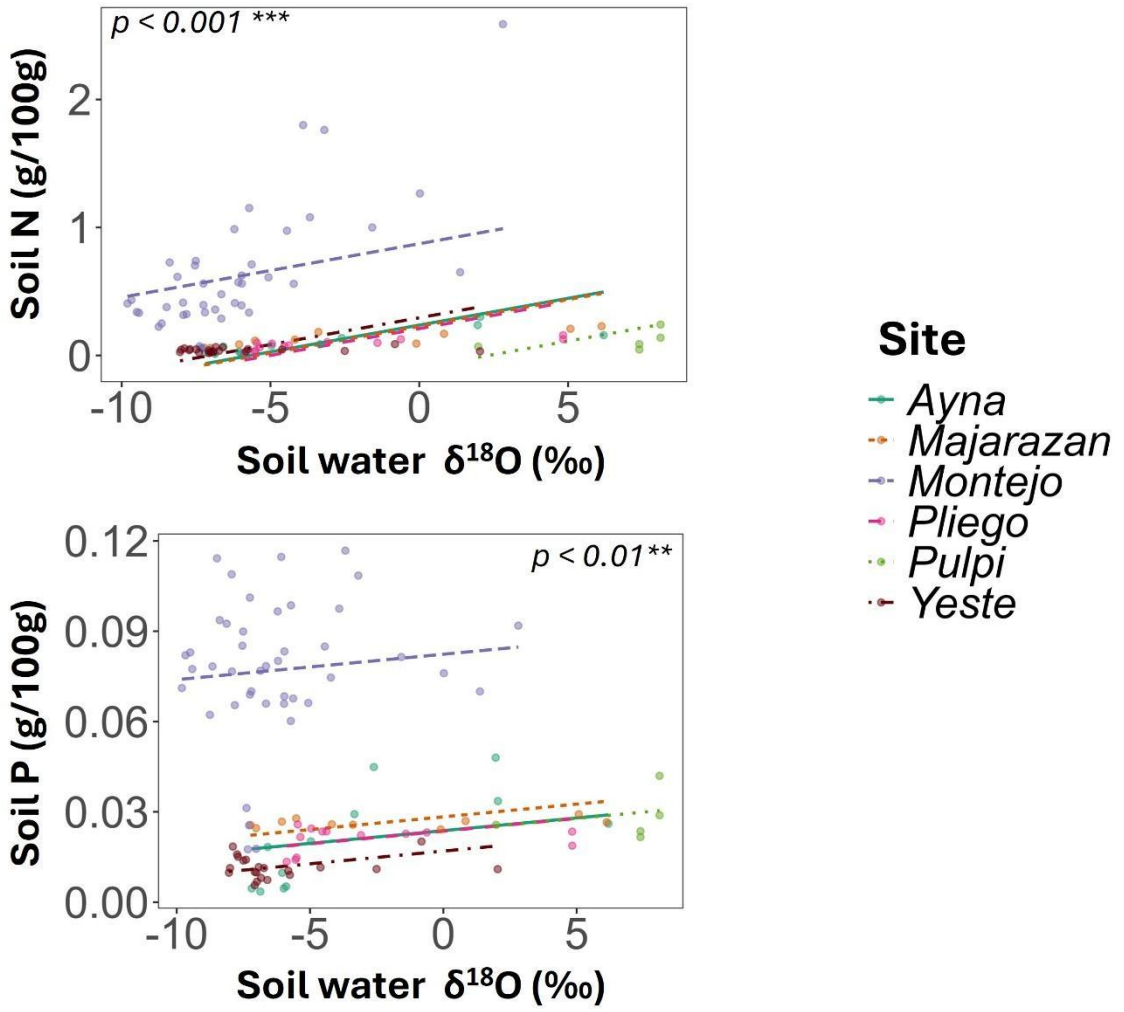

Figure S4. Relationship between soil nutrient concentration (N and P) and soil water $\delta^{18}O$ ‰. Models are linear mixed regressions with site as a random effect. Coloured dots represent individual soil samples at each site and depicted are individual within site relationships. P-values within each panel indicate the significance of the corresponding linear mixed regression model.

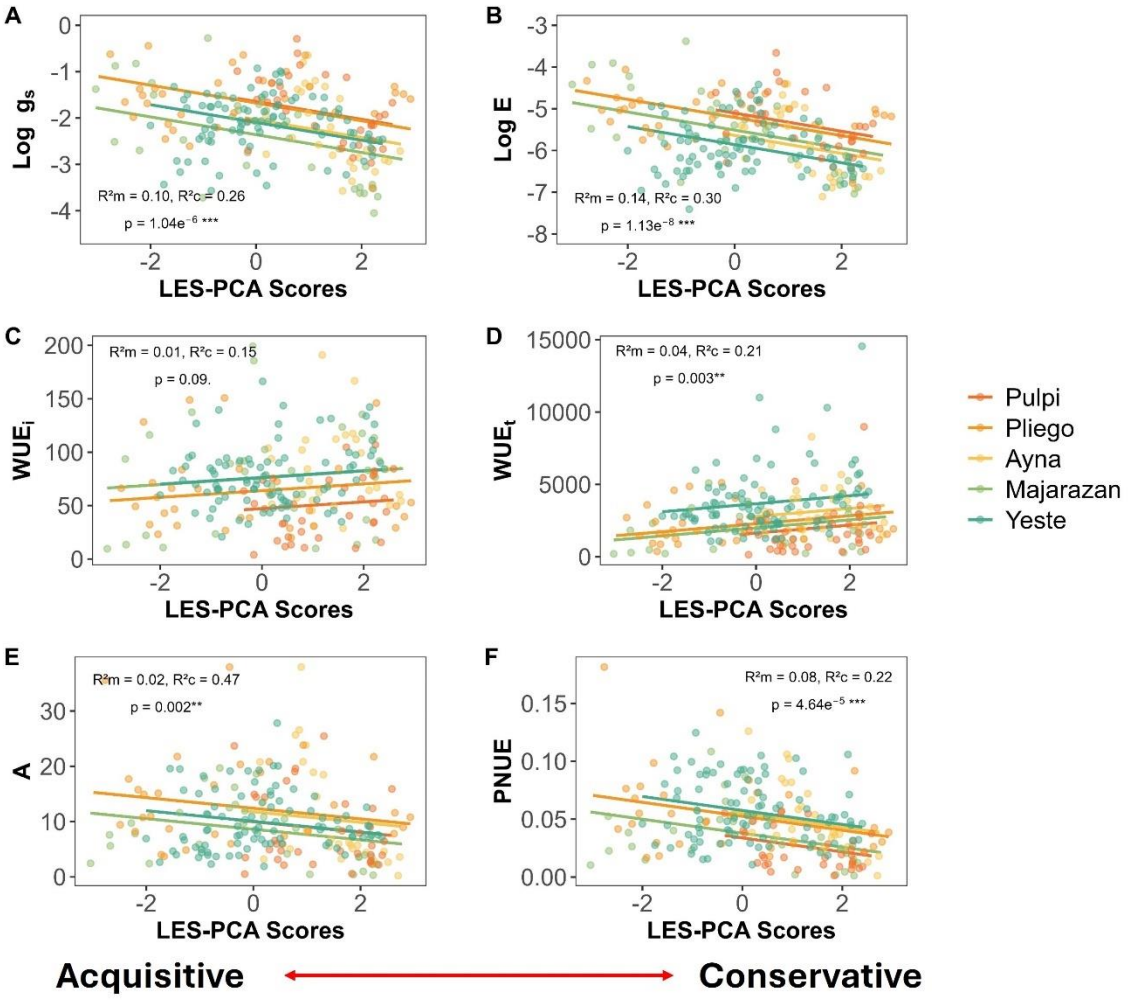

Figure S5. Within-site relationships between water use traits obtained by leaf gas exchange measurements (g_s, E , WUE_i, WUE_t, A and PNUE) and LES-PCA axis scores from general PCA across coexisting woody plant species excluding the more humid Sub-Mediterranean site (Montejo). Positive values in LES-PA_{axis} scores represents conservative leaf economy traits and negative values indicate acquisitive leaf economy traits.. Coloured dots represent individual plants for each site. Marginal (R^2m) and conditional R^2 (R^2c) indicates 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. For clarity, instead of the global regression line across sites, individual regression lines per site are shown in each panel to represent the different intercepts for each site . Stomatal conductance, g_s (mol m^{-2} s^{-1}); transpiration rate, E (µmol mol^{-1}); intrinsic water use efficiency, WUE_i (µmol CO_2 mol H_2O); instantaneous water use efficiency, WUE_t (µmol CO_2 mmol H_2O); net photosynthetic rate, A (µmol m^{-2} s^{-1}); photosynthetic nitrogen use efficiency, PNUE (µmol CO_2 g N^{-1} s^{-1}).

Table S1. Location (latitude, longitude), 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), topsoil (0-20 cm) water content (%) and topsoil (0-20 cm) nutrient composition (organic carbon, N, P, K, g kg^{-1}). Data not available for the Calblanque site.

Site	Coordinates	Altitude	MAT	MAP	PET	Aridity	Topsoil Organic carbon	Topsoil N	Topsoil P	Topsoil K
Calblanque	37.6040, -0.7471	10	18.5	278	1380.30	0.80				
Pulpi	37.411726, - 1.583253	195	17.6	272	1323.28	0.79	1.13 ± 0.29	0.09 ± 0.02	0.03 ± 0.002	0.69 ± 0.07
Pliego	38.061736, - 1.487976	473	14.8	414	1328.71	0.69	1.43 ± 0.25	0.11 ± 0.01	0.02 ± 0.001	0.62 ± 0.02
Ayna	38.543699, - 1.956891	900	13.7	432	1296.31	0.67	3.30 ± 0.71	0.17 ± 0.03	0.03 ± 0.004	0.59 ± 0.11
Majarazan	38.17702, - 2.098562	1235	12.7	466	1238.18	0.62	3.68 ± 0.24	0.17 ± 0.02	0.03 ± 0.001	1.32 ± 0.06
Yeste	38.336746, - 2.432745	1151	12.1	496	1205.84	0.59	1.25 ± 0.30	0.05 ± 0.01	0.02 ± 0.002	1.27 ± 0.13
Montejo	41.111487, - 3.501143	1450	8.6	726	951.51	0.24	13.72 ± 1.81	0.96 ± 0.12	0.08 ± 0.004	1.00 ± 0.02

Table S2. 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*
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*
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*

Table S3. Complete list of 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 (de la Riva et al., 2018; Navarro et al., 2009) (E: evergreen; WD: winter-deciduous; DD: summer drought-deciduous or semideciduous), according to the scientific literature and completed by expert knowledge when possible.

Species	Family	Abb	Life Form	Leaf habit
Adenocarpus hispanicus	Fabaceae	Ahis	LS	E
Anthyllis cytisoides	Fabaceae	Acyt	SS	DD
Artemisia barrelieri	Asteraceae	Abar	SS	DD
Ballota hirsuta	Lamiaceae	Bhir	SS	DD
Chamaerops humilis	Arecaceae	Chum	LS	E
Cistus albidus	Cistaceae	Calb	SS	DD
Cistus clusii	Cistaceae	Cclu	SS	DD
Cistus ladanifer	Cistaceae	Clad	SS	DD
Cistus monspeliensis	Cistaceae	Cmon	SS	DD
Coronilla juncea	Fabaceae	Cjun	SS	DD
Crataegus monogyna	Rosaceae	Cmono	T	WD
Cytisus purgans	Fabaceae	Cpur	SS	WD
Cytisus scoparius	Fabaceae	Csco	LS	DD
Daphne gnidium	Thymelaeaceae	Dgni	SS	E
Dorychnium pentaphyllum	Fabaceae	Dpen	SS	DD
Erica arborea	Ericaceae	Earb	LS	E
Fagus sylvatica	Fagaceae	Fsyl	T	WD
Fumana ericoides	Cistaceae	Feri	SS	DD
Genista florida	Fabaceae	Gflo	LS	DD
Genista Scorpius	Fabaceae	Gsco	SS	DD
Hedera helix	Araliaceae	Hhel	LS	E
Helianthemum syriacum	Cistaceae	Hsyr	SS	DD
Helichrysum stoechas	Asteraceae	Hsto	SS	DD
Ilex aquifolium	Aquifoliaceae	Iaqui	T	E
Juniperus oxycedrus	Cupressaceae	Joxy	LS	E
Juniperus phoenicea	Cupressaceae	Jpho	LS	E
Launaea arborescens	Asteraceae	Larb	SS	DD
Lavandula stoechas	Lamiaceae	Lsto	SS	DD

Lycium intricatum	Solanaceae	Lint	LS	DD
Maytenus senegalensis	Celastraceae	Msen	LS	DD
Olea europaea	Oleaceae	Oeur	LS	E
Osyris quadripartita	Santalaceae	Oqua	LS	E
Paronychia suffruticosa	Caryophyllaceae	Psuf	SS	E
Periploca angustifolia	Apocynaceae	Pang	LS	E
Phagnalon rupestre	Asteraceae	Prup	SS	DD
Phillyrea angustifolia	Oleaceae	Pan	LS	E
Pinus halepensis	Pinaceae	Phal	T	E
Pinus pinaster	Pinaceae	Ppin	T	E
Pistacia lentiscus	Anacardiaceae	Plen	LS	E
Prunus avium	Rosaceae	Pavi	T	WD
Quercus coccifera	Fagaceae	Qcoc	LS	E
Quercus faginea	Fagaceae	Qfag	T	WD
Quercus petraea	Fagaceae	Qpet	T	WD
Quercus pyrenaica	Fagaceae	Qpyr	T	WD
Quercus rotundifolia	Fagaceae	Qrot	T	E
Rhamnus lycioides	Rhamnaceae	Rlyc	LS	DD
Rosa canina	Rosaceae	Rcan	LS	WD
Rosmarinus officinalis	Lamiaceae	Roff	SS	DD
Rubus ulmifolius	Rosaceae	Rulm	SS	E
Sorbus aria	Rosaceae	Sari	T	WD
Sorbus aucuparia	Rosaceae	Sauc	T	WD
Staehelina dubia	Asteraceae	Sdub	SS	E
Teucrium capitatum	Lamiaceae	Tcap	SS	DD
Thymelaea hirsuta	Thymelaeaceae	Thir	SS	DD
Thymus hyemalis	Lamiaceae	Thye	SS	DD
Thymus membranaceus	Lamiaceae	Tmem	SS	DD
Thymus vulgaris	Lamiaceae	Tvul	SS	DD

Table S4. Loadings on axis 1 and 2 for all variables included in the PCA.

Traits	Loadings	
	Axis 1	*Axis 2*
LDMC	0.403	-0.627
SLA	-0.493	0.018
N	-0.449	-0.457
K	-0.455	0.365
P	-0.432	-0.514

Table S5. Model summary table for linear models between PCA-LES scores and water use traits including the six Mediterranean sites (Calblanque, Pulpí, Pliego, Ayna, Majarazán, Yeste) and with the sub-mediterranean site (Mediterranean sites + Montejo). Model estimates and standard error (SE) are shown for each linear model. R-squared indicates the proportion of variance explained by the model (R^2), and p-value indicates the significance of each linear model.

		Mediterranean sites			
Response	**Predictor**	**Estimate**	**SE**	**R^2**	**p-value**
PCA-LES scores	**$\delta^{13}C_L$**	0.488	0.031	0.399	<0.001***
	$\Delta^{18}O_L$	0.065	0.017	0.042	<0.001***
	$\delta^{18}O_{XW}$	-0.154	0.045	0.033	<0.001***
	g_s	-0.533	0.115	0.092	<0.001***
	E	-0.604	0.112	0.119	<0.001***
	A	-0.039	0.014	0.037	<0.01**
	WUE_i	0.003	0.003	0.005	0.287
	WUE_t	0.001	0.0004	0.021	<0.05*
	PNUE	-15.040	3.020	0.106	<0.001***

Chapter 3

Water and leaf economic strategies shape functional trait variation in woody plant Mediterranean communities along an aridity gradient

Chapter 3: Water and leaf economic strategies shape functional trait variation in woody plant Mediterranean communities along an aridity gradient

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Introduction

Arid areas cover more than 40% of the planet's surface and are expected to increase because of climate change (Huang et al. 2016; Yao et al. 2020). Increased aridity leads to structural changes in plant communities worldwide, ranging from morphological and physiological adaptations such as reduced leaf size, changes in stomatal control to reduce water loss, or decreased photosynthetic capacity, to replacements in the dominant species and functional types in communities at different levels of aridity (Berdugo et al. 2020). But aridity effects are scale-dependent as plant responses could vary between molecular, individual, community and even spatial and time scales (Evans, Hu, and Michaletz 2025). Similar species could appear under different climatic conditions, while similar climatic conditions could support different types of species (Bruelheide et al. 2018; Muñoz-Galvez et al. 2025). In this regard, along with aridity level, there are many other factors at the local scale, such as soil conditions, the availability of ecological niches, and biotic interactions between species that also modulate the type of plant communities that can develop under certain environmental conditions (Ordoñez et al. 2009; Marks and Lechowicz 2006; Cornwell et al. 2018). Therefore, conducting studies that combine approaches focusing on different scales can enable us to better understand the dynamics of plant communities in adapting to ongoing climate change involving aridification.

In Mediterranean and dry ecosystems, where water is the main limiting resource, water use strategies play a crucial role in the development and survival of plant communities. Water use strategies have been found to be closely related to the type of aboveground

plant growth, including plant height, leaf morphology and physiology (Muñoz-Galvez et al. 2025). According to the fast-slow plant resource use theory (Reich 2014), being acquisitive or conservative (fast or slow) in the acquisition and use of any kind of resource requires this species to be acquisitive or conservative in the acquisition and use of other resources too. Coordination between resource (e.g. water and nutrients) acquisitive or conservative strategies across coexisting species has been found at local scales (Garnier et al. 2025; Illuminati et al. 2022; Prieto et al. 2018), and for multiple plant organs (roots, stems, and leaves) at both specie and community levels (de la Riva et al. 2016; Pérez-Ramos et al. 2012), but whether this coordination varies across plant communities along environmental gradients is less explored. The Leaf Economic Spectrum (LES) is a widely accepted framework to study the balance between plant resource acquisition (leaf nutrient concentrations and carbon assimilation) against leaf construction costs (indicated by specific leaf area, SLA; and leaf dry matter content, LDMC) at leaf level, that has also been extended to whole plant level (Ian J. Wright et al. 2004; Díaz et al. 2016; de la Riva et al. 2016). While the LES is a useful theoretical framework for evaluating carbon and nutrient use strategies, the combined study of carbon and oxygen stable isotopes composition in leaves emerges as a powerful tool for assessing plant water use strategies (Siegwolf et al. 2023). The carbon isotopic composition of leaves ($\delta^{13}C_L$) has proven to be a reliable proxy for time-integrated intrinsic water use efficiency at leaf level (Dawson et al., 2002; Moreno-Gutiérrez et al., 2012; F. Muñoz-Gálvez et al., 2025) that also informs about how leaf structure influences CO_2 and water diffusion from the sub-stomatal cavity through the leaf mesophyll to the chloroplasts (de la Riva, Prieto, and Villar 2019; Vitousek, Field, and Matson 1990; Hultine and Marshall 2000), net photosynthetic rates and stomatal conductance (Prentice et al. 2014; Farquhar, Ehleringer, and Hubick 1989). The oxygen isotopic composition of leaves ($\delta^{18}O_L$), or preferably the oxygen isotopic composition enrichment above source water ($\Delta^{18}O_L$) (Barbour 2007) provides a reliable time-integrated proxy of cumulative stomatal conductance and transpiration over the growing season (Moreno-Gutiérrez et al. 2012) in studies across climatic gradients. $\Delta^{18}O_L$ offers a measure of stomatal conductance and evaporative effects at leaf level that is independent from the isotopic composition of the source water used by plants. Source water isotopic composition varies markedly along geographical and environmental gradients as rainwater ^{18}O becomes more depleted as temperature decreases at higher latitudes and altitudes (Barbour 2007; Bowen and Revenaugh 2003; Gat 1996), and also with depth as due to evaporation of water near the surface it became more enriched than water in deeper soil layers (Allison, Barnes, and Hughes 1983)

In Mediterranean ecosystems characterized by prolonged rainless periods, nutrients and water are not always available for plants at the same time or within the same soil depth interval (Querejeta, Ren, and Prieto 2021), so the combined assessment of water and nutrient use strategies together allows to cover and encompass the complexity of adaptations to drought in Mediterranean plant communities in a more integrative and holistic manner. Soil nutrient and water limitation are the main drivers of functional structure in Mediterranean plant communities (Pérez-Ramos et al. 2012; Bernard-Verdier et al. 2012; Gross et al. 2013; Nunes et al. 2017). Functional changes between communities may be promoted by intraspecific variations, but when it comes to large environmental gradients, the turnover of species with contrasting resource use strategies has a greater impact (Garnier et al. 2019; Pérez-Ramos et al. 2012). Functional response to aridity has shown to move towards a higher abundance of conservative resource use strategies in drier environments, such as smaller and thicker leaves with lower nutrient concentrations, longer lifespans and higher water use efficiency (Gomarasca et al. 2023; Garnier et al. 2019; Kazakou et al. 2007; Liu et al. 2024; Cornwell et al. 2018). Stomatal conductance also tends to decrease with aridity in order to maintain plant physiological water balance (Lavergne et al. 2020). However, functional changes in response to drought are largely determined by the functional type of each plant species (Navas et al. 2010; de Oliveira et al. 2020), as different life forms (small shrubs, large shrubs or trees) often display contrasting acquisitive or conservative water and nutrient use strategies (Muñoz-Galvez et al. 2025). Therefore, sharply contrasting rooting depths, hydraulic traits and leaf structure among different plant life forms could modulate stomatal regulation differently in response to soil water stress (Lavergne et al. 2020), thereby enabling strategies adapted to the resources available to each type of species. Many of these studies have been based on community weighted means (Pérez-Ramos et al. 2012; Garnier et al. 2019; Navas et al. 2010; Kazakou et al. 2007), which presents some limitations when exploring the diversity of strategies within communities, as it disregards intraspecific trait variability (Carmona et al. 2016). Therefore, in addition to community weighted means, a more detailed study at the community level incorporating intraspecific trait variability can help us interpret the complementarity of strategies that may occur across different aridity levels.

Studying functional trait coordination at the individual level reveals the diversity of adaptive strategies to aridity in these ecosystems, but a community perspective allows us to understand which trait combinations are favoured in each community when faced with different levels of aridity. Using the trait probability density framework (TPD) (Carmona et al. 2016), a function representing the distribution of probabilities of

observing each possible trait value in a given ecological unit, provides a method to define the functional trait space for each plant community by integrating the probabilistic nature of trait distribution with the concept of multidimensional hypervolumes (Blonder et al. 2014; Hutchinson 1957). This approach also enables to evaluate the mechanisms driving spatial differences in the functional structure of plant communities by calculating the degree of overlap between different TPD functions (Beccari et al. 2024; Carmona et al. 2016), and allows to assess how different components of functional diversity, such as functional richness (FRic), the amount of functional space occupied by the organisms in each ecological unit (Carmona et al. 2019), vary along environmental gradients. In this regard, previous studies have reported that aridity acts as a strong environmental filter constraining functional trait spaces, particularly traits within the LES framework, thus limiting possible trait combinations (de la Riva et al. 2018; Rodríguez-Alarcón, Tamme, and Carmona 2022; Medeiros et al. 2025; Sfair, Rosado, and Tabarelli 2016). However, to our knowledge no studies to date have simultaneously studied water and LES resource use strategies, which could offer new insights on possible drought adaptation strategies in Mediterranean plant communities along environmental gradients.

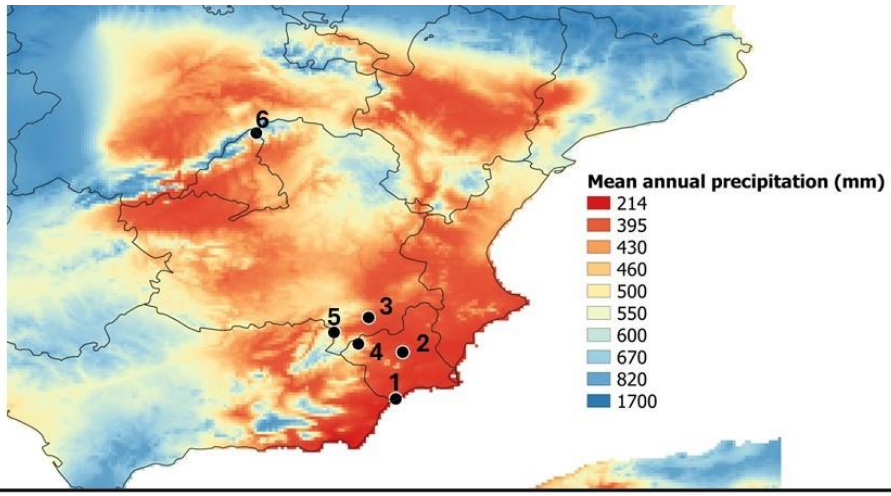
In this study, we explored the effect of aridity on plant water and nutrient use strategies of Mediterranean plant communities along a steep climatic gradient in the Iberian Peninsula. To this end we measured community weighted mean values for five leaf traits of the leaf economics spectrum (SLA, LDMC and N, P, K leaf concentrations per mass and area, Wright et al. 2005) and two leaf water use traits indicative of stomatal conductance and water use efficiency ($\Delta^{18}O_L$ and $\delta^{13}C_L$, respectively) in a total of 370 individuals from 42 native plant species in six plant communities with contrasting aridity. We then calculated the functional leaf trait space for each plant community based on their combination of plant resource use and leaf water use traits and explored the effect of aridity on each individual trait and on multiple trait assembly among communities. Based on previous studies, we hypothesized (I) that aridity has a strong effect on LES and leaf water use traits; (II) that there is a tight coordination between plant water use strategies and leaf economics traits at community level along aridity gradients; (III) that plant life form represents a main driver of functional trait variation across the aridity gradient and iv) that aridity shapes the functional trait space across plant communities along aridity gradients. We expected i) a greater dominance of species with more conservative water and resource use strategies towards the arid end of the gradient as a response to more severe water and nutrient limitations, and a shift towards more acquisitive strategies as aridity decreases, that ii) plant water and LES community weighted mean traits will be coordinated along an acquisitive-conservative spectrum,

that iii) communities towards the more arid end of the gradient will be dominated by more conservative life forms like trees and large shrubs (Muñoz et al. 2025) and (IV) that more climatically distant communities should also occupy more distant volumes in leaf functional trait space, indicating that aridity plays a key role in shaping the dominant trait values within each plant community.

Methods

Study site and sampling design

For the present study we selected six Mediterranean shrublands, open woodlands and closed-canopy forest communities with contrasting climatic conditions. The sites were distributed from the arid southeastern part of the Iberian Peninsula to the more humid Central Range Mountains along a 600 km linear transect (Figure 1). We characterized the climate conditions at each site using the Terraclimate database (Abatzoglou et al. 2018) where we extracted and calculated mean temperature (MAT, ºC), annual precipitation (MAP, mm) and potential evapotranspiration (PET, mm) values for the 1958-2021 period. Aridity was then calculated as A = 1 – MAP/PET. Mean annual precipitation and mean annual temperatures ranged from 272 mm and 17.6ºC at the warmest and most arid site near the Mediterranean coast, to 726 mm and 8.6ºC at the coolest and wettest site. Mean altitude above sea level of the study sites ranged from 195 m near the Mediterranean coast to 1450 m at the Central Ranges site. Soils are predominantly sedimentary calcareous with a mixture of marls, sandstones and clays depending on the site. Schist formations interspersed with calcareous lithologies can also be found at the northernmost and wettest site (Montejo) (Rodríguez-Fernández et al. 2015).



Site	Coordinates	Altitude (m.a.s.l)	MAT (°C)	MAP (mm)	PET (mm)	Aridity (1-P/PET)
Pulpi[1]	37.3601, -1.7331	195	17.6	272	1323	0.79
Pliego[2]	37.9546, -1.5013	473	14.8	414	1328	0.69
Ayna[3]	38.5224, -2.0874	900	13.7	432	1296	0.67
Majarazan[4]	38.0997, -2.0709	1235	12.7	466	1238	0.62
Yeste[5]	38.3899, -2.4575	1151	12.1	496	1206	0.59
Montejo[6]	41.1114, -3.5011	1450	8.6	726	951	0.24

Figure 1. Map of mean annual precipitation (mm) using Terraclimate database (period 1958-2021) (Abatzoglou et al. 2018) in the study area. Points indicate each study site, which coordinates, altitude, MAT, MAP, PET and aridity values were showed in the table below.

In each site, 10 parallel 25 m long transects were randomly selected with a minimum distance of 5 m between transects. Species composition (identity) and abundance (% cover) were recorded for each woody species intercepted by the transect line (Table S1). Species abundance was calculated as the species projected length over the transect in metres and species relative abundances were calculated as the species abundances divided by the total across species length (note that total abundance across species may be greater than 25 m due to vertical overlap between overstory and understory vegetation layers). We also calculated the relative abundance of each life form at each site by summarizing the relative abundance of all species within each life form at each site. Leaf traits were measured in species that together encompassed > 90% of the total plant cover within each community. At each study site, we sampled 6 replicate individuals of each species, which resulted in a total of 370 individuals collected from 42 woody species. The species were categorized into three plant functional groups (life forms) according to their vegetative aboveground height using an adaptation from Raunkaier's life form classification (Tumber-Dávila et al. 2022; Raunkiær 1934): small shrubs (SS, <1m), large shrubs (LS, 1-3 m) and trees (T, > 3 m) (Table S1). The field sampling campaign was carried out during the year 2022, starting in April at the warmest and driest

sites (Pulpí and Pliego) and ending in July at the wettest and coldest site (Montejo), with the aim of matching the peak of physiological and phenological activity of the vegetation at each location. The sampling date was carefully chosen at each study site to ensure at least a 2- week period with no rainfall prior to the sampling date. The rationale for this was to ensure the development of a steep isotopic gradient of soil water with depth along the edaphic profile, which is formed in response to topsoil water evaporation and isotopic fractionation during prolonged rainless periods (Allison, Barnes, and Hughes 1983).

Leaf nutrients and morphological traits.

To characterize plant resource use strategies, we measured several plant functional traits related to the Leaf Economic Spectrum. We sampled sun-exposed, fully expanded and healthy-looking leaves from each individual plant. These leaves were immediately placed in sealed bags containing a piece of wet paper in the field to ensure proper hydration, transported to the lab and stored in the fridge at 4ºC for leaf material processing within 24-48 hours. To characterize leaf morphology, we measured specific leaf area (SLA, cm^2 g^{-1}) and leaf dry matter content (LDMC, mg g^{-1}) using 4-6 leaves per individual, or up to 20-40 leaves for species with very small leaves (e.g. *Thymus vulgaris*). We first hydrated leaves overnight at 4º C in the dark and weighted them (saturated weight, SW, g). After weighing, a picture of the leaves was taken with a Nikon 90D (Tokyo, Japan) and the resulting image was processed with Photoshop (v22.5, Tokyo, Japan) to determine leaf area (LA, cm2). Leaves were then oven dried at 60ºC for 48 hr and weighted again to obtain their dry weight (DW, g). Specific leaf area (SLA, cm^2 g^{-1}) was calculated as SLA = (LA / DW) x 1000 and leaf dry matter content (LDMC, mg g^{-1}) was calculated as LDMC = ((DW / SW) x 1000).

The remaining leaves were dried at 60ºC for 48h and finely ground using a ball mill (MM200, Retsch, Germany) to measure leaf nutrient contents (C, N, P, K; mg g^{-1}). C and N concentrations were measured using an elemental analyser (Thermo- Finnigan EA1112, Milan, Italy); while leaf K and P were determined using inductively coupled plasma optical emission spectrometry (ICP-OES, Thermo Elemental Iris Intrepid II XDL, Franklin, MA, USA) after a microwave-assisted digestion with HNO_2:H_2O_2 (4:1, v:v) at the Ionomics Facility at CEBAS-CSIC (Spain). We also calculated leaf nutrients (N, P, K) per area (mg cm^{-2}) using this formula: $Nut_{area} = Nut_{mass}$ x SLA.

Xylem water and leaf isotopic measurements

For each, individual we sampled one basal 5 cm fully lignified stem from a terminal branch to avoid potential issues related with related to xylem isotopic enrichment due to backflow from transpiring leaves or green photosynthetic stems (Schwinning 2008). The

stems were debarked to isolate the xylem, placed in 5 ml glass vials sealed with parafilm to prevent water loss and stored at -20ºC until water extraction. We used a cryogenic vacuum distillation line at 85ºC and 10 militorr vacuum pressure for at least 2 hours to extract all the water contained in the stems (Ehleringer and Osmond 1989; Diao et al. 2022). Water extraction efficiency was above 97% for all samples (Ceperley et al. 2024). All water extractions and isotopic analyses were performed at the Stable Isotope Research Center (SIRC/WSL, Birmensdorf, Switzerland). The $\delta^{18}O$ of water samples ($\delta^{18}O_{XW}$) were measured with a high temperature conversion elemental analyser coupled to a DeltaPlus XP isotope ratio mass spectrometer (TC/EA-IRMS; Finnigan MAT, Bremen, Germany). Oxygen ($\delta^{18}O_{XW}$) isotopic composition is expressed in ‰ notation relative to the standard V-SMOW (Vienna standard Mean Ocean SMOW (Vienna standard Mean Ocean Water). Long-term external precision values for water $\delta^{18}O_{XW}$ determinations was ± 0.12‰.

To measure dual C and O isotopic composition ($\delta^{13}C_L$ and $\delta^{18}O_L$) we weighted finely ground dry leaf material (4 mg for $\delta^{13}C_L$ and 0.3 mg for $\delta^{18}O_L$) and encapsulated it into tin or silver capsules for $\delta^{13}C_L$ and $\delta^{18}O_L$ isotopic analysis, respectively. Leaf $\delta^{13}C$ was measured by continuous flow (CF) dual isotope analysis using a CHNOS Elemental Analyzer interfaced to an IsoPrime100 mass spectrometer and leaf $\delta^{18}O_L$ was determined in continuous flow (CF) using an Elementar PYRO Cube interfaced to a Thermo Delta V mass spectrometer at the Center for Stable Isotopes Biogeochemistry, University of California-Berkeley (USA). Both $\delta^{13}C_L$ and $\delta^{18}O_L$ are expressed in delta notation (‰) relative to the Vienna Pee Dee Belemnite standard (V-PDB) and Vienna Standard Mean Ocean Water (VSMOW), respectively. Long-term external precision values for leaf $\delta^{13}C_L$ and $\delta^{18}O_L$ determinations were ± 0.1‰ and ± 0.2‰, respectively. To isolate the leaf-level evaporative signal from the source water isotopic signal(Barbour 2007) imprinted on leaves, we calculated the oxygen isotopic enrichment above source water of leaf dry matter ($\Delta^{18}O$) as: $\Delta^{18}O = \delta^{18}O_L - \delta^{18}O_{XW}$, where $\delta^{18}O_{XW}$ is the oxygen isotopic composition of xylem water.

Statistical analysis

Each plant community were divided into three plots, grouping 3 proximate 25 metres long transects to calculate relative species abundance for each plot. We then calculated the community weighted mean for all main traits measured in each plot (LDMC, SLA, N_{mass}, P_{mass}, K_{mass} , $\delta^{13}C_L$ and $\Delta^{18}O$) and explored the relationship between each individual CWM trait value and AI using linear models. To explore the overall change in the functional structure of our plant communities we calculated the relative contributions of species replacement (species turnover, i.e. the sum of contributions of species

occurrence and species abundance) and intraspecific trait variability (ITV) in the main traits measured, using the method developed by Lepš et al., (2011). To assess if the relationship between leaf water use traits and aridity was different for each life form (trees, large shrubs and small shrubs) we performed linear models between $\delta^{13}C_L$, $\Delta^{18}O$ and AI separately for each life form.

We identified the main axes of functional trait variation across the target plant species and communities by performing a principal component analysis (PCA) with leaf morphological (SLA and LDMC), nutrient (leaf N, P and K concentrations) and isotopic ($\delta^{13}C_L$ and $\Delta^{18}O$) traits. Using the *funspace* package (Carmona, Pavanetto, and Puglielli 2024) we built the functional space across sites, which showed the density of covarying trait combinations, and explored the variation in the density of plant trait combinations across sites and life forms. Then we fitted a GAM model using aridity as a response variable and the functional trait space axis as a two-dimensional smoother predictor to test how the combination of traits varied in relation to this environmental variable across sites and life forms (Carmona, Pavanetto, and Puglielli 2024).

We estimated the probabilistic distribution of the species within the functional trait space within each site by performing multivariate kernel density estimations with the trait probability density (TPD) and ks packages (Carmona et al. 2019, 2016). To scale from individual trait values to community trait probability distribution, a kernel density estimator was associated with each individual of each species (TPD_I), then we summed all the TPD_I values of a species to calculate the trait probability distribution of that species in each location (TPD_s). Finally we combined the TPD_s of the species present in each site, rescaling according to the relative abundance of the species in each community, obtaining a function that integrates the probability density function of the whole community (TPD_c) (Carmona et al. 2016). We estimated the functional dissimilarity between pairs of plant communities (functional β-diversity) calculated as 1-overlap between their respective TPD_c (Carmona et al. 2019). TPD-based dissimilarities considered the boundaries and the differences in how densely occupied the functional space at each site weighted by the relative abundance of each species at each site is. We have also calculated one of the components of functional β-diversity, functional turnover, which indicates the extent to which differences between two plant communities arise because they occupy different functional spaces. Functional turnover is complementary to nestedness, which reflects how differently two sites occupy the functional space they share (Carmona et al. 2016; Beccari et al. 2024). High functional turnover values indicate that each site is occupying a different part of the functional space, while for sites that share the same part of the functional space turnover values

will be close to 0. We assessed climatic dissimilarity between sites by calculating Euclidean distances of Aridity between sites. We then assessed the correlation between functional (β-diversity) and both climatic dissimilarity and functional turnover between plant communities by performing Mantel tests using the Pearson correlation coefficient and the maximum number of permutations possible based on our matrices (719)(Oksanen et al. 2013). Finally, we used major axis (MA) regression models to elucidate the trends between β-diversity, climatic dissimilarity and functional turnover. These models were performed using the "lmodel2" function of the lmodel2 R package (Legendre 2024).

We also calculated the functional richness (FRic) of each plant community as the amount of functional space occupied by the 0.99 quantile of each TPD. As functional richness of each plant community is positively related to the number of species that compose it, we calculated Standardize Effect Sizes (SES) using null models to express functional richness independently from species richness ($FRic_{SES}$) (Carmona et al. 2021). We compared the observed functional richness for each community with that calculated from a random assignment of species for each site. We estimated 500 null values of functional richness for each community and use them to calculate a SES of functional richness for each community by using the following formula: $FRic_{SES} = (Fric_{obs} – \text{mean } Fric_{null}) / Sd\ FRic_{null}$. Values lower than 0 for a given plant community indicate that the observed functional richness is lower than expected for a random set of species and thus its functional space is contracting while positive values indicates an overdispersion of functional traits (Carmona et al. 2021; de la Riva et al. 2023).

Results

Aridity effects on plant functional trait variation.

We found significant relationships between classical CWM LES traits, such as leaf morphology (SLA and LDMC) and nutrient concentrations (N_{mass}, P_{mass} and K_{mass}), and aridity. More conservative CWM trait values (lower SLA and N_{mass}, P_{mass} and K_{mass} and higher LDMC) were found towards the most arid end of the gradient (Figure 2A,B,C,D and E). Nutrients per area had opposite trends to nutrients per mass, with significant positive relationships between P_{area} and K_{area} with aridity, while the relationship was only marginal for N_{area} (Figure 2F,G,H).

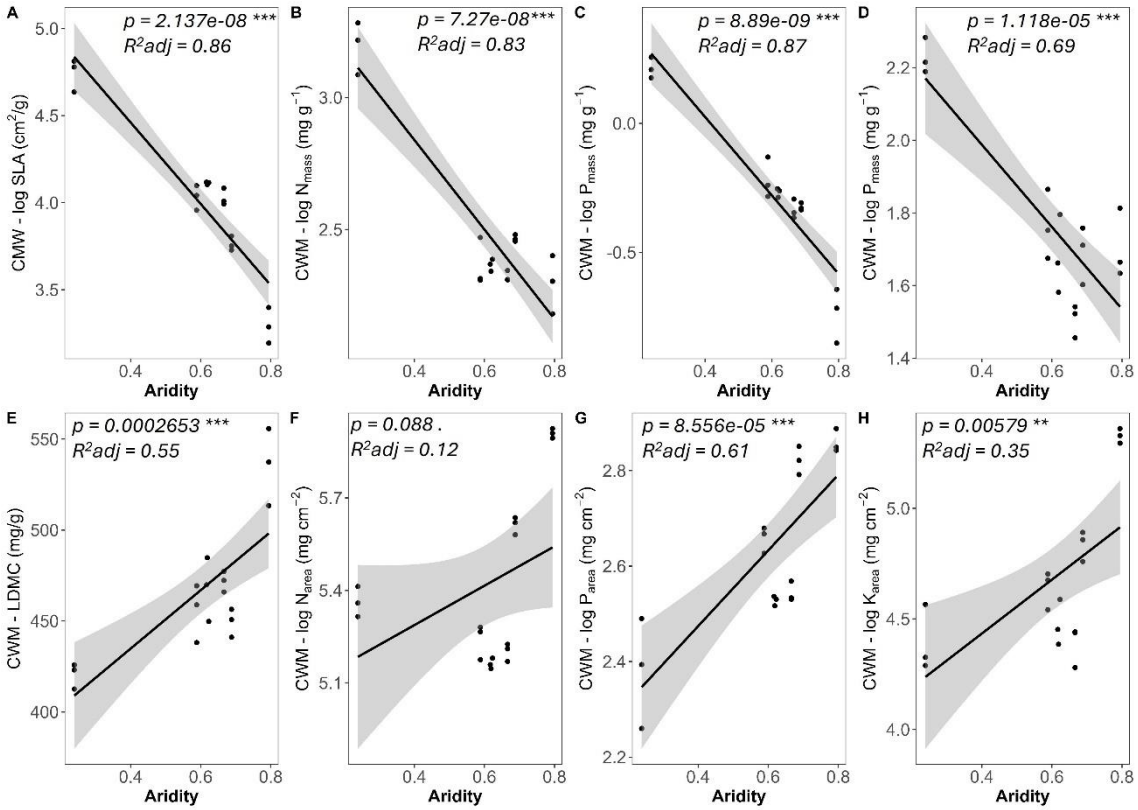


Figure 2. Relationships between community weighted mean leaf morphological (CWM-log_SLA and CWM-LDMC), mass and area based nutrient status (CWM -N_{mass}, -P_{mass}, -K_{mass} and CWM-N_{area},-P_{area}, -K_{area}) and aridity (1- P/PET). Relationships are based on three plots per community at the six sites along the aridity gradient. The adjusted R^2 (R^2adj) and p values of each linear regression model are also shown.

Leaf water use traits also varied with aridity at community level with increasing CWM water use efficiency (higher $\delta^{13}C_L$) and increasing CWM stomatal conductance (lower $\Delta^{18}O_L$) with increasing aridity (Figure 3). Species-level $\delta^{13}C_L$was consistently and positively related with aridity within trees and large shrubs, but not within small shrubs, although there is no significant difference between their slopes (F = 2.40, p-value = 0.09). Whereas the negative relationship between $\Delta^{18}O_L$ and aridity was consistent and significant within all three life forms (trees, large shrubs and small shrubs, Figure 4), with small shrubs showing higher slope than trees and large shrubs slopes (F = 5.64, p-value = 0.003). The greater proportion of the functional variance for the main leaf resource (SLA, LDMC, N, P, K) and water use traits ($\delta^{13}C_L$ and $\Delta^{18}O$) was mainly explained by species turnover between sites rather than by intraspecific trait variability or their covariation (Figure 5), thus highlighting the large shifts in plant species composition and community structure along the aridity gradient.

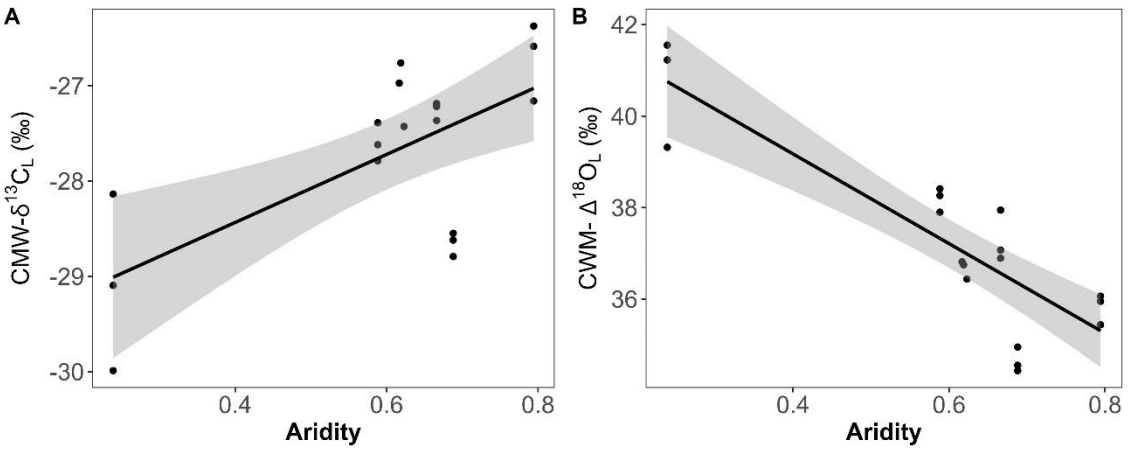


Figure 3. Relationships between community leaf intrinsic water use efficiency (CWM-$\delta^{13}C_L$), stomatal conductance (inversely related to CWM- $\Delta^{18}O_L$) and Aridity (1- P/PET), using three plots per community along the aridity gradient. The adjusted R^2 (R^2adj) and p values of each linear regression model are also shown.

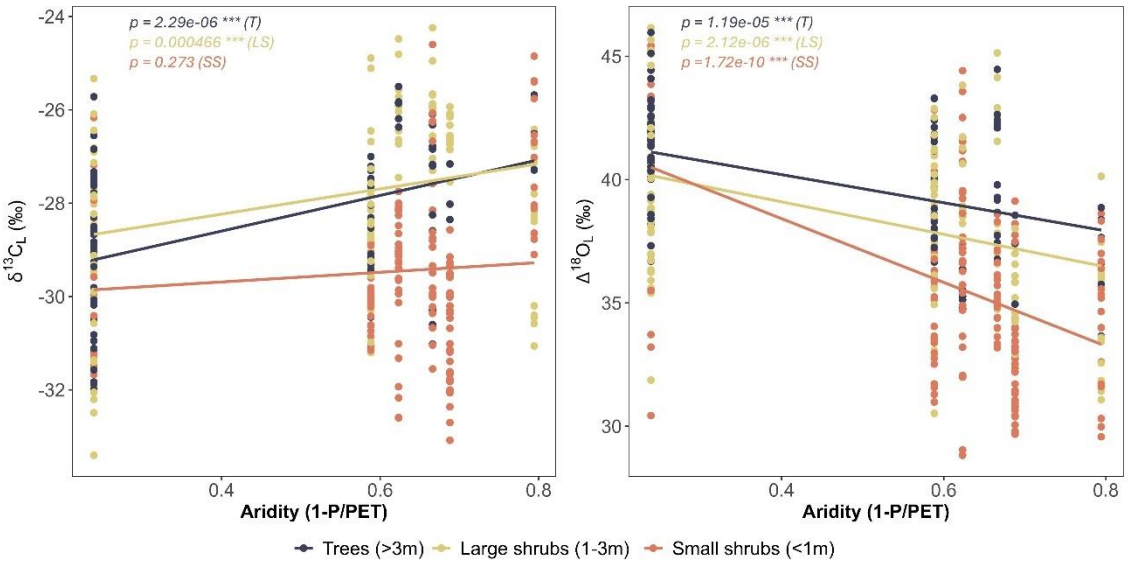


Figure 4. Relationships between leaf intrinsic water use efficiency ($\delta^{13}C_L$), stomatal conductance (inversely related to $\Delta^{18}O_L$) and Aridity (1- P/PET) for each life form (trees in blue, large shrubs in beige and small shrubs in orange). P values the linear model for each life form are shown.

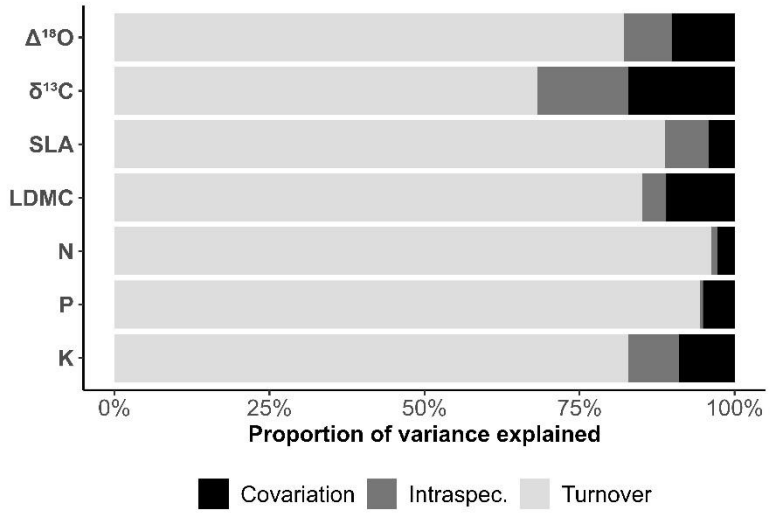


Figure 5. Decomposition of the total variance of the main leaf functional traits studied: leaf oxygen isotopic enrichment above source water ($\Delta^{18}O$), leaf carbon isotopic composition ($\delta^{13}C$), specific leaf area (SLA), leaf dry matter content (LDMC), leaf nitrogen, phosphorous and potassium concentration per mass (N, P, K). Each column reflects the percentage of variance explained by species turnover across sites (light grey), intraspecific trait variability (grey) and covariation between the two (black) (Lepš et al. 2011).

Analysis of plant functional trait spaces along the aridity gradient.

The first two axes of the principal component analysis of the functional trait space accounted for 74% of the total leaf trait variability (Figure 6A). The first axis alone explained 51.5% of leaf trait variation with all Leaf Economics Spectrum traits together with $\delta^{13}C_L$ loading heavily on this axis (Figure 6A and Table S2). Traits associated with a resource-acquisitive strategy (high SLA and nutrient concentrations) had negative loads on this axis while traits related to a resource-conservative strategy (high LDMC and $\delta^{13}C_L$) had positive loads (Figure 6A). The second axis explained an additional 22.5% of leaf trait variation and represented mainly the variation in $\Delta^{18}O_L$, thus representing variability in stomatal conductance. Water saver strategies at leaf level (high $\Delta^{18}O_L$, i.e. lower time-integrated stomatal conductance) correlated positively with PCA axis 2, while water spender strategies (low $\Delta^{18}O_L$, i.e. higher time-integrated stomatal conductance) correlated negatively with this axis (Figure 6A). Interestingly, leaf $\delta^{13}C_L$ also loaded heavily on this second PCA axis with same sign as $\Delta^{18}O_L$, thereby indicating higher intrinsic water use efficiency (WUE_i) at leaf level in species exhibiting tighter stomatal regulation of plant water flux (i.e. water saver species with high $\Delta^{18}O_L$). Two distinct density centroids appeared within the functional trait space, one was linked to more conservative LES traits (higher loads on PCA1) and lower stomatal conductance and

higher WUE_i (lower loads on PCA2), mainly associated to large shrubs and trees (Figure 6A). The other centroid was linked to acquisitive LES traits, and higher stomatal conductance and lower WUE_i that corresponded mainly to small shrubs (Figure 6A). Results from GAM showed a significant effect of aridity on the functional trait space (R^2_{adj} = 0.80 and p-value of smoothing terms <0.001***) (Figure 6B). More arid sites hosted a combination of species with more conservative LES traits (higher LDMC) and with lower $\delta^{13}C_L$ (lower WUE_i) and $\Delta^{18}O_L$ (higher stomatal conductance), while more humid sites hosted species with acquisitive LES traits (high SLA and nutrient concentrations), higher $\delta^{13}C_L$ (higher WUE_i) and high $\Delta^{18}O$ (lower stomatal conductance) (Figure 6B). The functional changes of plant communities along the aridity gradient can be observed by examining the functional spaces at each of the sites (Figure 7). The functional trait space shifted gradually from the conservative end of the LES axis (PCA1) and higher stomatal conductance (PCA2) at the drier sites (Pulpi and Pliego), towards the more acquisitive end of the LES axis (PCA1) and lower stomatal conductance (PCA2) at the wettest site of the gradient (Montejo) with Majarazán and Yeste in between (Figure 7).

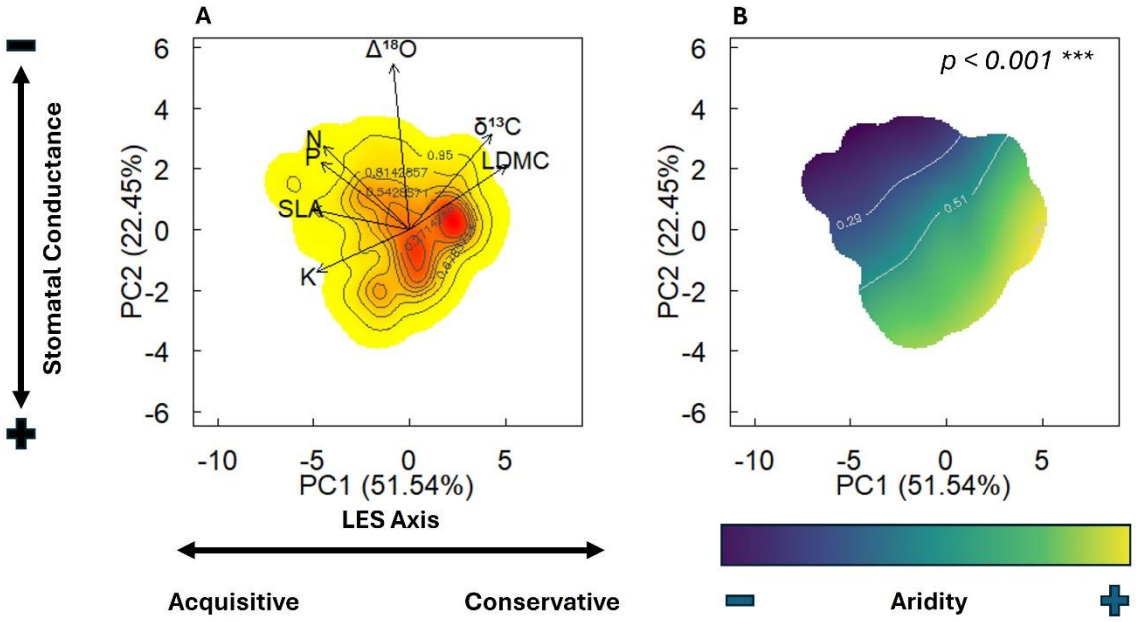


Figure 6. A) Probabilistic distribution of individuals in the functional trait space defined by a PCA including leaf water and resource use traits: leaf oxygen isotopic enrichment above source water ($\Delta^{18}O$), leaf carbon isotopic composition ($\delta^{13}C$), specific leaf area (SLA), leaf dry matter content (LDMC), leaf nitrogen, phosphorous and potassium concentration per mass (N, P, K). Colours indicate the probabilistic distribution of trait combinations in the functional trait space (red = high probability; yellow = low probability). Contour lines indicate quantiles of the probability distribution. The variance explained by each axis of the PCA are also shown. B) Plow showing GAM predicted values for aridity (1-P/PET) within the same functional space (yellow = high aridity; blue = low aridity). Contour lines indicate the quantiles of GAM predictions expressed in the same unit as

the response variable. P-value indicate significance effect of smoothing factors (PCA axis) on aridity. A and B were built using funspace () and funspaceGAM() functions (Carmona, Pavanetto, and Puglielli 2024), respectively.

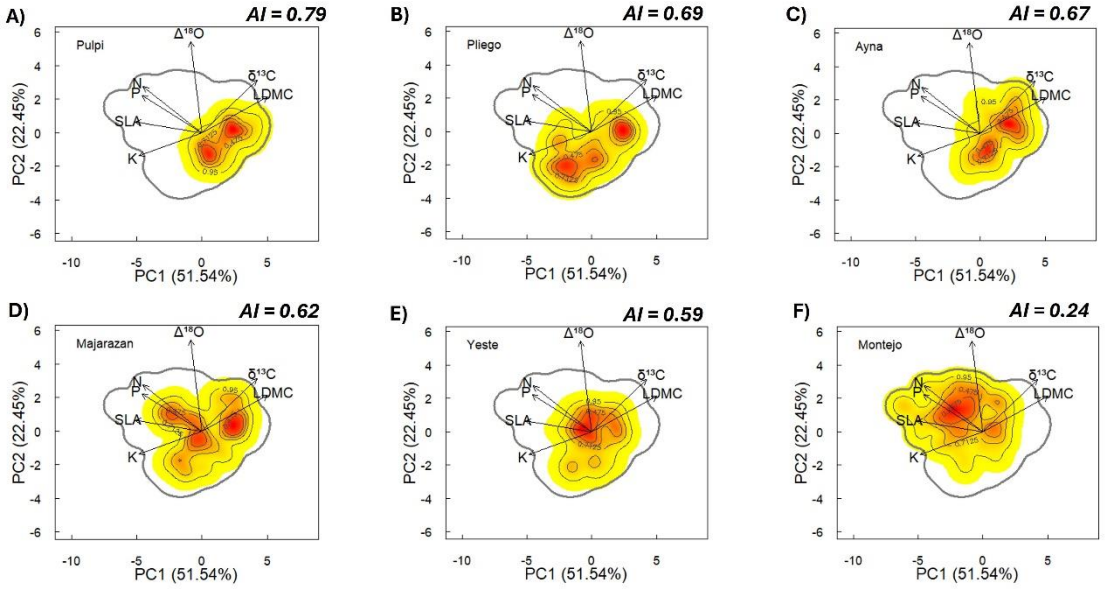


Figure 7. Probabilistic distribution of individuals at each study site in the functional trait space defined by a PCA including leaf water and resource use traits: leaf oxygen isotopic enrichment above source water ($\Delta^{18}O$), leaf carbon isotopic composition ($\delta^{13}C$), specific leaf area (SLA), leaf dry matter content (LDMC), leaf nitrogen, phosphorous and potassium concentration per mass (N, P, K).. Colours indicate the probabilistic distribution of trait combinations in the functional trait space (red = high probability; yellow = low probability). Contour lines indicate quantiles of the probability distribution. The variance explained by each axis of the PCA are also shown. Aridity (AI) values are shown per each site. All figures were built using the *funspace* function (Carmona, Pavanetto, and Puglielli 2024).

Analysis of community structure along the aridity gradient.

The relative abundance of each life form differed markedly among sites (Figure 8), with communities dominated by a higher proportion of small shrubs such as *Thymus vulgaris, Cistus clusii* or *Fumana ericoides* at the driest and warmest sites (Pulpi and Pliego, reaching 52% and 42%, respectively), while the relative abundance of small shrubs at the coldest and most humid site of the gradient (Montejo) decreased to as low as 7%. Conversely, the proportion of large shrubs and trees increased steeply towards the more humid sites of the gradient, changing from open *Pinus sp.* forests with interspersed large shrubs in more arid Pulpí, Majarazán or Ayna to mixed pine-oak evergreen (*Quercus rotundifolia*) forests in Yeste, to closed canopy deciduous forests dominated by *Fagus sylvatica, Quercus pyrenaica and Quercus petraea* in the most humid site (Montejo).

According to their position and density in the functional trait space, small shrubs were characterized by a water spender strategy and more acquisitive LES traits than large shrubs and trees, which presented water saver strategies and more conservative LES traits (Figure S1 A, B, C). As aridity increased, the LES axis (PC1) explained a much larger proportion of leaf trait variation than did the stomatal conductance axis (PC2) for both trees (R^2_{adj} = 0.69 and R^2_{adj} = 0.13 for LES and water axes, respectively) and large shrubs (R^2_{adj} = 0.44 and R^2_{adj} = 0.13 for LES and water axes, respectively). In contrast, in small shrubs a larger proportion of leaf trait variation along the aridity gradient was explained by the stomatal conductance PCA axis (PC2, R^2_{adj} = 0.07 and 0.17 for LES and water axes, respectively).

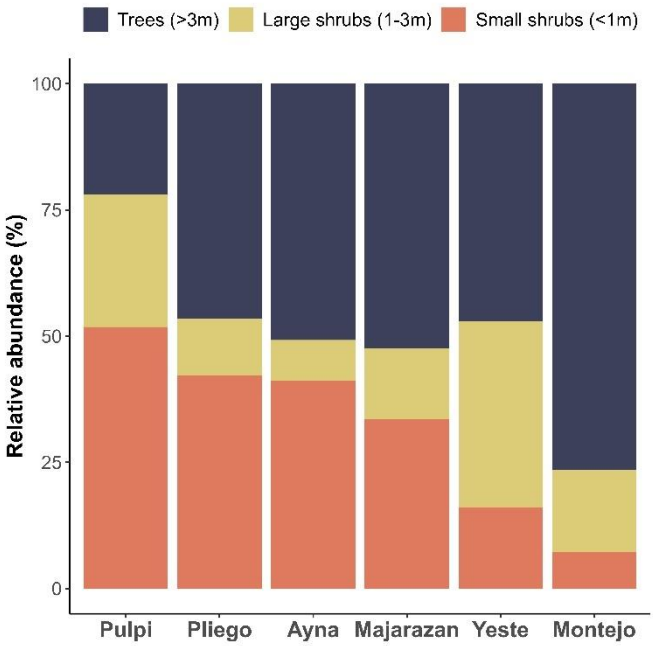


Figure 8. Relative abundance (%) of each life form (small shrubs (<1m), large shrubs (1-3 m), trees (> 3m)) at each site. Sites are ordered from higher (left) to lower (right) aridity values.

Differences between plant communities along the gradient

We found a significant positive correlation between functional β-diversity and climatic dissimilarity using Mantel test (Figure 9A), showing that communities located at more climatically dissimilar sites along the aridity gradient were also more different functionally. Functional β-diversity values ranged from 0.65, between the two communities in the mid-range of the aridity gradient (Pliego and Majarazan), to 0.92 between the two communities located at the opposite extremes of the aridity gradient (Pulpi and Montejo).

We encountered very different functional turnover between pairs of sites, from very low between functionally similar communities (0.03, Majarazan and Ayna) to very high between functionally different communities (0.85, Pulpi and Montejo). Mantel tests showed a significant positive relationship between functional β-diversity and functional turnover (Figure 9B), indicating a large differentiation in the functional spaces occupied by communities at the opposite extremes of the aridity gradient and lower differentiation in plant communities exposed to more similar climatic conditions. Functional richness decreased significantly with increasing aridity () (Figure 9 C). Standardized effect sizes (SES) for functional richness were lower than expected by random in communities located at more arid locations (-1.64 and -1.09 in Pulpi and Ayna, respectively), showing a contraction of the functional space and therefore a higher functional redundancy with increasing aridity. On the other hand, SES were higher than expected by random in plant communities growing at less arid sites (3.78 and 13.51 in Majarazan and Montejo respectively), indicating an overdispersion of functional traits in these sites and therefore an expansion of the functional trait space, especially in Montejo (Table S3).

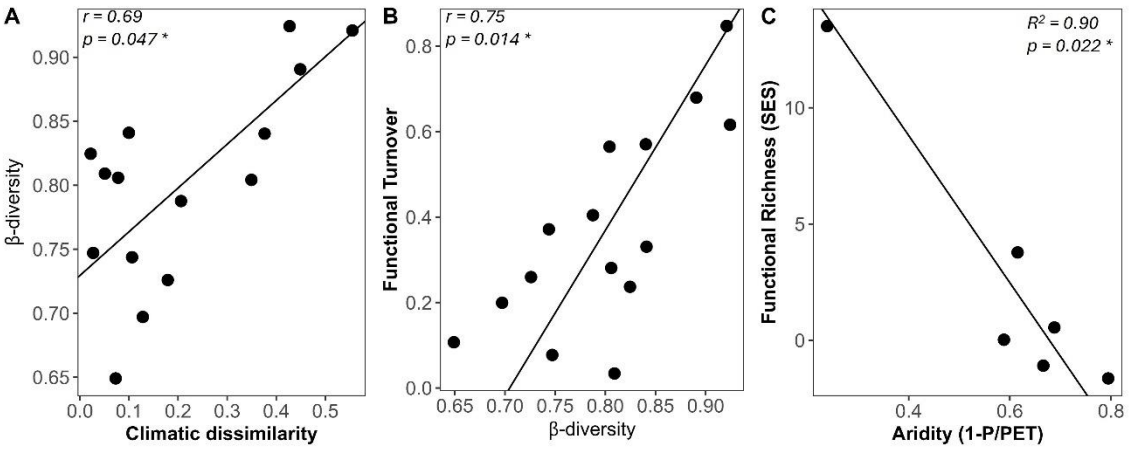


Figure 9. Relationships between (A) climatic dissimilarity and functional β-diversity and (B) between functional β-diversity and functional turnover for each pair of sites. The Mantel tests coefficient of correlation (r) and significance (p) are shown for the relationship between the two data matrices. C) Relationship between functional richness SES ($FRic_{SES}$) and aridity (1-P/PET) for each site. In all plots, the major- axis regression line is shown in black (A: $R^2 = 0.47$, p = 0.002; B: $R^2 = 0.56$, p = 0.001; C: $R^2 = 0.90$, p = 0.022, also in the figure).

Discussion

Our study encompassing 42 species growing at 6 Mediterranean plant communities highlights how both the leaf economics axis (PC1) and the stomatal conductance axis (PC2) jointly shape community functional spaces along a Mediterranean aridity gradient (Figure 6A). The functional space occupied by the 6 target plant communities is largely determined by an axis that reflects variation in the leaf economic spectrum (Ian J. Wright et al. 2004; Díaz et al. 2016), expressing a continuum from acquisitive species in the use of carbon and nutrients (high SLA and nutrient concentration) to conservative species (high LDMC and low nutrient concentration). We also found that CWM LES axis traits were strongly influenced by aridity (Figure 2), with conservative LES traits being dominant in the drier sites of the gradient, while plant communities at wetter sites were dominated by acquisitive LES traits at community level. The predominance of more sclerophyllous leaves (lower SLA and higher LDMC) in drier environments is linked to more tightly packed cells with fewer inter-cellular air spaces that enhance leaf wilting resistance and minimize foliar water loss (José M. Costa-Saura et al. 2017; José María Costa-Saura et al. 2016), thus preventing cell damage during drought events (De Micco and Aronne 2012; Carrascosa et al. 2023). These traits of sclerophyllous leaves are also compatible with the presence of additional layers of photosynthetically active cells that allow the accumulation of a greater amount of photosynthetic proteins per unit leaf area (Niinemets 2001). Although, at the community level, there was a reduction in foliar nutrient concentrations per mass along the aridity gradient, nutrient concentrations per unit area increased with aridity due to an increase in leaf mass per area (LMA, inverse of SLA), especially for P_{area} and K_{area}, but less so for N_{area} that only increased marginally (Figure 2). These trends are strongly supported by theory, which show that nutrient concentrations per area increase, even as nutrient concentrations per mass decrease, due to increases in leaf mass area (LMA) with increasing climatic aridity (Ian J. Wright et al. 2005; Wright, Reich, and Westoby 2003). Leaf nutrients such as N or P are key elements for the construction of proteins involved in the photosynthetic machinery, including Rubisco, which mediate CO_2 fixation inside the leaf. Additionally, these nutrients are also an integral part of nucleic acids and bioenergetic molecules like ATP (Ian J. Wright et al. 2004). This linkage between resource conservative strategies within the LES and aridity has been documented at both global (Liu et al. 2024; Cornwell et al. 2018) and regional scales (de Tomás Marín et al. 2023; Wei, Luo, and Wu 2016; Cornwell and Ackerly 2009). However, very few studies have simultaneously integrated water with other resource use strategies with variable outcomes (J. Wang and Wen 2022b, 2022a; Illuminati et al. 2022). This work represents one of the first attempts to encompass and

integrate water, carbon and nutrient use strategies to advance our understanding of plant adaptations to variable environmental conditions in drylands (Figure 10).

Regarding water use traits, our results show that, contrary to expectations, water use efficiency and stomatal conductance exhibit partially diverging trends at the community level along the aridity gradient, with both higher water use efficiency (CWM-$\delta^{13}C_L$) and higher stomatal conductance (CWM-$\Delta^{18}O_L$) observed in the most arid areas (Figure 3). Notably, $\delta^{13}C_L$ is strongly associated with LES traits (PC1) in the functional trait space along the aridity gradient (Figure 6A). $\delta^{13}C_L$ reflects the trade-off between photosynthesis and stomatal conductance (Siegwolf et al. 2023), and these leaf gas exchange traits are further influenced by leaf morphology and its effects on the diffusion of water and CO_2 through the leaf tissue to the carboxylation sites in the chloroplasts (Hultine and Marshall 2000; Vitousek, Field, and Matson 1990; Li et al. 2017; Niinemets 2001). Moreover, $\delta^{13}C_L$ is strongly and positively associated with both leaf photosynthetic capacity expressed on an area basis and with stomatal regulation stringency, both of which increase steeply with N cotent per area (N_{area})(I. J. Wright, Reich, and Westoby 2001; Paillassa et al. 2020; Querejeta et al. 2022; Wright, Reich, and Westoby 2003; Westerband et al. 2023). Conversely, the steep decrease in $\Delta^{18}O_L$ with aridity appears to be largely driven by climatic factors, as rising temperatures and VPD with aridity increases the atmosphere evaporative demand. thereby enhancing time-integrated stomatal conductance and cumulative transpiration (Grossiord et al. 2020; Diao et al. 2024). The increase in transpiration under high temperatures may be facilitated by reduced water viscosity and increased cuticula and membrane permeability (Sadok, Lopez, and Smith 2021), which facilitates water movement through the leaf. This phenomenon has been observed even in species exhibiting conservative LES traits and regardless of plant water access (Aparecido et al. 2020; Marchin et al. 2023). Enhanced transpiration also favours evaporative leaf cooling that prevents or delays heat-induced damage and inactivation of the photosynthetic machinery, thereby sustaining carbon assimilation for longer periods (Peguero-Pina et al. 2020; Lawson et al. 2014; Ren, Tian, and Querejeta 2025; Diao et al. 2024; Urban et al. 2017)., but at risk of increasing mortality when surpassing hydraulic safety thresholds (Marchin et al. 2022).

This increase in time-integrated stomatal conductance and cumulative transpiration under warmer environmental conditions along the aridity gradient would be expected to result in lower intrinsic water use efficiency (lower $\delta^{13}C_L$), as reported in other studies at the local level or in those that have controlled for environmental variation (Illuminati et al., 2022; Muñoz-Gálvez et al., 2025; Prieto et al., 2018). However, the parallel increase in leaf nutrient contents per unit area mediated by a decrease in SLA (increased LMA)

towards the arid end of the aridity gradient, substantially enhances carboxylation and photosynthesis (Sardans and Peñuelas 2015; S. Wang et al. 2025; I. J. Wright, Reich, and Westoby 2001). This enhancement offsets or even outweighs the opposing influence of increased stomatal conductance on $\delta^{13}C_L$, thereby explaining the observed increases in WUEi with aridity (Cornwell and Ackerly 2009; Prentice et al. 2014; Wright, Reich, and Westoby 2003; Ian J. Wright et al. 2005). Leaf sclerophylly in arid communities (lower SLA and higher LDMC and LMA) enables the stacking of a higher number of photosynthetically active cell layers per unit leaf area, which increases the accumulation of photosynthetic proteins per area, and allows maintaining photosynthesis for longer periods under drought stress (Niinemets 2001; De Micco and Aronne 2012). This allows a stronger CO_2 drawdown during photosynthesis and a more favourable trade-off between carbon assimilation and water loss resulting in higher time-integrated WUE_i, and thus higher $\delta^{13}C_L$ (Ian J. Wright et al. 2005; Maire et al. 2015; Wright, Reich, and Westoby 2003). However, we found that small shrubs, which exhibit more acquisitive LES traits and a more water spender strategy than large shrubs and trees (Figure S1), are unable to balance water loss and carbon assimilation showing no significant changes in their water use efficiency ($\delta^{13}C_L$) with aridity (Figure 4A). In contrast, large shrub and tree species in arid zones are able to use the water they transpire through the stomata more efficiently, maintaining much higher leaf-level water use efficiencies (as inferred from higher $\delta^{13}C_L$) than would be predicted given their simultaneously high stomatal conductance and cumulative transpiration (inferred from low $\Delta^{18}O_L$), both of which are driven by high ambient temperature and vapor pressure deficit (VPD).

The proportion of each life form within plant communities changed markedly along the aridity gradient (Figure 8), with small shrubs becoming more abundant at the most arid sites, while the proportion of large shrubs and trees increases towards the more humid end. Increased aridity thus favours the selection of plants with low stature and highly acquisitive resource-use strategies (i.e. high SLA and nutrient concentrations) and a water spender use strategy (i.e. high stomatal conductance and low WUE_i, Figure S1). These small shrubs struggle to balance water losses during prolonged drought periods typical of the Mediterranean region, frequently adopting a summer-deciduous leaf habit that enables them to shed their leaves during drought periods and sprout again when conditions improve (de Oliveira et al. 2020; Nunes et al. 2017; Blanco-Sánchez et al. 2022; Gross et al. 2013). Small shrubs mainly depend on the nutrient-rich, but ephemeral, shallow water pool stored in the topsoil (Muñoz-Galvez et al. 2025; Ryel et al. 2008), which promotes the adoption of an acquisitive C and nutrient use strategy along with a fast use of water to take full advantage of short periods after rainfall events

when conditions are more favourable for photosynthesis (Ryel et al. 2008). On the other hand, we found that larger shrubs and trees in the most arid end of the gradient, which access deeper and more stable soil water pools in the subsoil or bedrock (Muñoz-Galvez et al. 2025; Ryel et al. 2008) displayed a more conservative resource use (low SLA and nutrient concentrations) and water use strategy (low stomatal conductance and high water use efficiency) that allowed them to tolerate the long drought periods characteristic of these Mediterranean arid sites (Figure 4 B, C). Access to deeper water sources allows large shrubs and trees to maintain perennial leaves and sustain moderate level of photosynthetic activity during extended dry periods, when small shrubs have often lost most of their foliage, thereby reducing competition for resources.

Under this strong environmental stress, the coexistence of species with different strategies reduces competition and favours a more efficient use of the limiting soil resources, including water. Trees and large shrubs, although often less abundant in more arid communities, may facilitate the establishment of other species beneath their canopies through shading effects, moderation of climate extremes in the understory and the redistribution of water and nutrients from deeper soil layers to dry topsoil through hydraulic redistribution by roots (Prieto, Armas, and Pugnaire 2012; Caldwell and Richards 1998), potentially expanding water and resource niches and supporting seedling establishment in arid areas. Such increase in water availability can maintain larger perennial species, which benefit from more stable water supply over time (Nunes et al. 2017; Berdugo et al. 2020). A different pattern emerges at the coldest and most humid extreme of the gradient, where water limitation is less relevant and winter-deciduous tree species with acquisitive LES traits (high SLA and nutrient concentrations per mass) but a lower stomatal conductance such as *Fagus sylvatica, Quercus pyrenaica or Quercus petraea* dominate. In this multilayered forests, temperature and light availability become more limiting than water. Here, winter-deciduous trees show acquisitive LES traits aimed at maximizing light and nutrient capture, photosynthesis and resource assimilation during shorter growing seasons (Reich 2014; Pérez-Ramos et al. 2013). Under these colder and wetter environmental conditions, shorter shrubs with smaller and thicker leaves (*Lavandula stoechas, Cytisus purgans or Juniperus communis*) can only persist and thrive in open areas and forest gaps with sufficient light availability (Gross et al. 2013). Taken together, these findings suggest that the coexistence of plant life forms with sharply contrasting resource and water use strategies is sustained by spatial partitioning of resources and water within sites and across gradients of aridity, thereby promoting complementarity and increasing overall resource use efficiency at the community level.

Our results suggest that variation in functional trait ranges and species composition leads to plant communities occupying different functional spaces along the aridity gradient (Figure 9B). Communities experiencing similar climatic conditions show geater functional similarity in both resource-use (LES) and water use strategies (Figure 8A). This pattern is consistent with findings in Carmona et al., (2021) for above and belowground plant morphological traits, but, our study is one of the firsts to integrate leaf water, carbon and nutrient use dimensions at the community level. Functional dissimilarity among plant communities across the aridity gradient can be attributed to variation in environmental conditions and constraints environmental conditions and constraints, such as water and nutrient availability, temperature and light, which are often the main drivers of plant trait variation at large spatial scales (Cornwell and Ackerly 2009; Ian J. Wright et al. 2017). The combination of local abiotic conditions and site-specific biotic interactions (niche partitioning, competition, facilitation) can also drive the community assembly and functional dissimilarity of these communities (Kraft et al. 2015; Bruelheide et al. 2018). We also found that functional richness, encompassing carbon, nutrients and water use dimensions, decreased towards the most arid sites (Figure 8C). This shift was characterized by trait overdispersion (greater within-community functional richness) towards the wettest end of the aridity gradient, to functional trait at the arid extreme, indicating a contraction of the feasible functional space. This contraction is likely favoured by the dominance of fast-growing small shrubs, which rapidly deplete topsoil nutrients and water, thus hampering the establishment of larger, longer-lived species with more conservative water and resource use strategies (Gross et al. 2013; Valencia et al. 2015). In contrast, the functional space expands towards the most humid sites as increased water availability allows large shrubs and trees to establish, act as nurse species and promote microhabitat and niche heterogeneity via facilitative mechanisms (Maestre et al. 2009). At the coldest and most humid environment (Montejo), intense competition for limiting resources such as light may promote niche partitioning and trait divergence among coexisting species, thus maintaining high functional diversity (Kraft et al. 2015; Medeiros et al. 2025). Interestingly, recent studies also suggest that drylands can exhibit relatively high functional trait diversity relative to more humid biomes, allowing the codominance of functionally contrasting life forms (Gross et al. 2017, 2024). The observed contraction of the functional space with increasing aridity in Mediterranean plant communities adds a new dimension by demonstrating that aridity may restrict the pool of physiologically viable combinations of water, carbon and nutrient use strategies in drylands, particularly when accounting for specific water use traits.

In this study, we demonstrate that Mediterranean plant communities adopt different resource and water use strategies in response to varying levels of climatic aridity. These functional shifts are reflected at the community level by changes in the dominant life forms along the aridity gradient, with small shrubs dominating plant communities at the driest sites and trees and large shrubs dominating in more humid areas (Figure 10). Our research adds a novel dimension to earlier studies showing that increased aridity favours conservative traits along the leaf economics spectrum (LES). Specifically, we demonstrate that increasing aridity leads to an unavoidable increase in stomatal conductance and cumulative water loss across all plant life forms, driven by warmer temperatures and VPD, which is offset and outweighed by parallel increases in leaf-level intrinsic water use efficiency. This efficiency gain is driven by enhanced leaf sclerophylly and higher nutrient concentrations per area under more arid conditions. These findings highlight the importance of regional-scale studies, as global environmental gradients can mask population-specific responses constrained to a narrower part of the gradient (Evans, Hu, and Michaletz 2025). Thus, by explicitly incorporating a water-use dimension into the functional trait space, we provide a mechanistic framework to understand the shifts in resource (C and nutrient) and water use strategies along aridity gradients, as well as for predicting the responses of Mediterranean plant communities to increasingly arid climate scenarios under ongoing climate change.

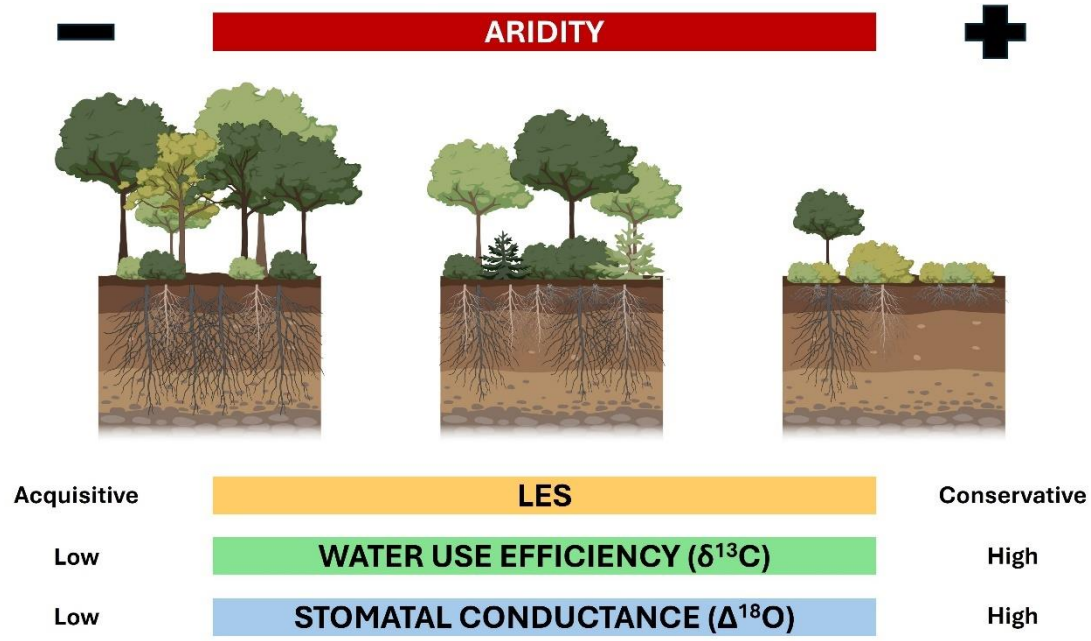


Figure 10. Conceptual model summarising changes in community structure and functional traits along the aridity gradient. From left to right, we show how communities in more humid areas have a higher proportion of trees. These communities have more acquisitive traits in the LES (higher SLA and nutrient concentration per mass), lower intrinsic water use efficiency ($\delta^{13}C_L$) and lower stomatal conductance values ($\Delta^{18}O_L$) than communities in more arid areas due to lower atmospheric demand in these sites. Towards the arid end of the gradient, the proportion of shrubs, especially small shrubs, increases relative to trees. Communities in more arid areas show more conservative values in the LES (higher LDMC and lower nutrient concentration per mass), greater intrinsic water use efficiency ($\delta^{13}C_L$), and higher stomatal conductance ($\Delta^{18}O_L$), forced by higher evaporative demand from the atmosphere and higher temperatures. Image made using BioRender.

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Supplementary Materials

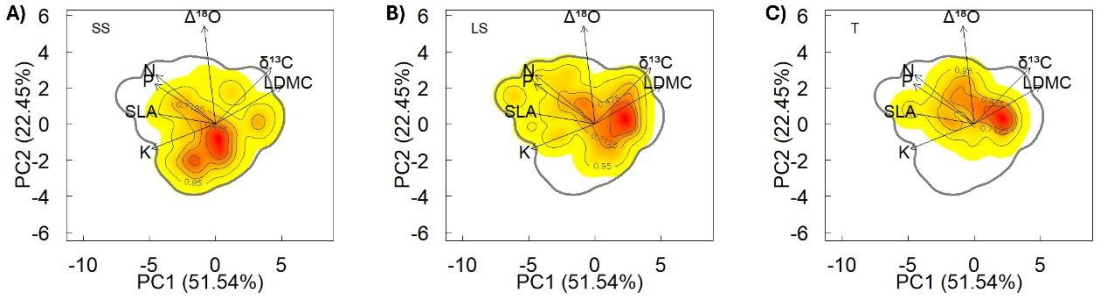

Figure S1. Probabilistic distribution of individuals from each life form (SS = small shrubs, LS = large shrubs and T = trees) in the functional trait space defined by a PCA with the main leaf trait studied: leaf oxygen isotopic enrichment above source water ($\Delta^{18}O$), leaf carbon isotopic composition ($\delta^{13}C$), specific leaf area (SLA), leaf dry matter content (LDMC), leaf nitrogen, phosphorous and potassium concentration per mass (N, P, K). Colours indicate the probabilistic distribution of trait combinations in the functional trait space (red = high probability; yellow = low probability). Contour lines indicate quantiles of the probability distribution. The variance explained by each axis of the PCA are also shown. This figures were built using funspace () function (Carmona, Pavanetto, and Puglielli 2024).

Table S1. Complete list of species, family and life form (Small shrub, SS, <1 m; Large shrub, LS, 1-3 m; Trees, T, > 3 m) composition at each study area. Species are ordered according to their relative abundance (%) at each community.

Site	Species	Family	Life form	Relative Abundance (%)
Pulpi	*Stipa tenacissima*	Poaceae	SS	42.2
	Pinus halepensis	Pinaceae	T	19.8
	Rahmnus lycioides	Rhamnaceae	LS	18.3
	Pistacia lentiscus	Anacardiaceae	LS	3.1
	Rosmarinus officinalis	Lamiaceae	SS	3.0
	Olea europaea	Oleaceae	LS	2.3
	Cistus clusii	Cistaceae	SS	1.4
Pliego	*Pinus halepensis*	Pinaceae	T	43.8
	Rosmarinus officinalis	Lamiaceae	SS	28.8
	Juniperus oxycedrus	Cupressaceae	LS	10.6
	Thymus membranaceus	Lamiaceae	SS	3.3
	Teucrium capitatum	Lamiaceae	SS	2.2
	Fumana ericoides	Cistaceae	SS	1.7
	Dorychnium pentaphyllum	Fabaceae	SS	1.6
	Helichrysum stoechas	Asteraceae	SS	1.5
	Cistus clusii	Cistaceae	SS	0.6
Ayna	*Pinus halepensis*	Pinaceae	T	45.7
	Rosmarinus officinalis	Lamiaceae	SS	25.3
	Stipa tenacissima	Poaeceae	SS	12.5
	Juniperus oxycedrus	Cupressaceae	LS	5.3
	Quercus rotundifolia	Fagaceae	T	3.2
	Juniperus phoenicea	Cupressaceae	LS	2.4
	Thymus vulgaris	Lamiaceae	SS	1.6
	Cistus clusii	Cistaceae	SS	0.2
Majarazan	*Pinus halepensis*	Pinaceae	T	51.2
	Rosmarinus officinalis	Lamiaceae	SS	16.1
	Staehelina dubia	Asteraceae	SS	10.8
	Juniperus oxycedrus	Cupressaceae	LS	8.5
	Juniperus phoenicia	Cupressaceae	LS	5.2
	Genista scorpius	Fabaceae	SS	4.1
	Thymus vulgaris	Lamiaceae	SS	1.3
	Daphne gnidium	Thymelaeaceae	SS	0.5
Yeste	*Pinus pinaster*	Pinaceae	T	33.1
	Juniperus oxycedrus	Cupressaceae	LS	26.2

	Dorychnium pentaphyllum	Fabaceae	SS	6.8
	Cistus ladanifer	Cistaceae	SS	5.3
	Quercus faginea	Fagaceae	T	4.7
	Pinus halepensis	Pinaceae	T	4.5
	Quercus rotundifolia	Fagaceae	T	4.5
	Erica arborea	Ericaceae	LS	4.3
	Rubus ulmifolius	Rosaceae	SS	2.7
	Rosa canina	Rosaceae	LS	2.3
	Rosmarinus officinalis	Lamiaceae	SS	1.6
	Cistus monspeliensis	Cistaceae	SS	1.3
	Phyllyrea angustifolia	Oleaceae	LS	0.9
	Lavandula stoechas	Cupressaceae	LS	0.8
	Daphne gnidium	Thymelaeaceae	SS	0.2
Montejo	*Quercus pyrenaica*	Fagaceae	T	22.0
	Quercus petraea	Fagaceae	T	17.9
	Fagus sylvatica	Fagaceae	T	17.7
	Ilex aquifolium	Aquifoliaceae	T	10.5
	Lavandula stoechas	Lamiaceae	SS	6.1
	Adenocarpus hispanicus	Fabaceae	SS	5.1
	Rosa canina	Rosaceae	LS	5.0
	Juniperus communis	Cupressaceae	LS	3.6
	Sorbus aria	Rosaceae	T	2.5
	Sorbus aucuparia	Rosaceae	T	2.3
	Crataegus monogyna	Rosaceae	T	2.0
	Prunus avium	Rosaceae	T	1.4
	Cytisus purgans	Fabaceae	SS	1.0
	Hedera helix	Araliaceae	LS	1.0
	Genista florida	Fabaceae	LS	0.6
	Erica arborea	Ericaceae	LS	0.5
	Rubus ulmifolius	Rosaceae	SS	0.3
	Cytisus scoparius	Fabaceae	LS	0.2

Table S2. Loadings on the first and second axes (PC1 and PC2, respectively) for all traits included in the functional space principal component analysis (PCA).

Trait	PC1	PC2
SLA	-0.836	0.108
LDMC	0.823	0.347
Leaf N	-0.737	0.448
Leaf P	-0.752	0.361
Leaf K	-0.789	-0.225
$\delta^{13}C_L$	0.692	0.513
$\Delta^{18}O$	-0.142	0.891

Table S3. Summary of the observed functional richness ($Fric_{obs}$) in each plant community along with the mean functional richness obtained in the null model by randomizating species ($Fric_{null}$) and its standard deviation (Sd $FRic_{null}$). Also shown are the standardize effect size values for functional richness (SES) calculated as: $FRic_{SES} = (Fric_{obs} – Fric_{null}) / Sd\ FRic_{null}$.

Site	$FRic_{obs}$	$FRic_{null}$	Sd $FRic_{null}$	$FRic_{SES}$
Pulpi	12.89	17.66	2.91	- 1.63
Pliego	21.19	19.74	2.63	0.55
Ayna	15.83	18.79	2.72	-1.09
Majarazan	28.90	18.68	2.70	3.78
Yeste	23.48	23.43	1.92	0.02
Montejo	45.03	24.79	1.50	13.51

General Discussion

General discussion

The present study represents one of the largest field-based research efforts undertaken so far to improve current understanding of plant functional diversity and strategies to cope with water scarcity along the soil-plant-atmosphere continuum in the Mediterranean region, as it spans multiple native plant communities exposed to contrasting environmental conditions. We found significant coordination between carbon, nutrient and water use strategies along an acquisitive-conservative resource-use spectrum ranging from the uptake of water and nutrients by roots to gas exchange at leaf level. On the one hand, we identified species of large size, both above- and belowground, which allows them to access deeper soil/bedrock water sources that are more stable over time. These large species typically invest in evergreen and more sclerophyllous leaves capable of tighter stomatal regulation and higher water use efficiency at leaf level. On the other hand, we found species of smaller size that rely mainly on temporally fluctuating shallow soil water sources, which typically invest on fast-growing thin leaves of shorter lifespan that operate with higher stomatal conductance and lower water use efficiency. We also found that climatic aridity exerts a marked effect on water use strategies at the plant community level, as it shapes the dominant life forms and leaf habits of the different species living in these native Mediterranean plant communities. In fact, we have observed that time-integrated stomatal conductance and transpiration (inferred from leaf oxygen isotopic composition, $\Delta^{18}O$) tend to increase under warmer ambient temperatures and increasing aridity despite a parallel increase in intrinsic water use efficiency (inferred from leaf carbon isotopic composition) and more conservative leaf economic traits (higher LDMC and lower SLA). This is possible because species that inhabit the warmest and most arid areas manage to increase their photosynthetic capacity by producing thicker leaves (lower SLA/higher LMA), thereby increasing the amount of nutrients and the number of photosynthetically active layers per unit leaf area. However, not all species can achieve this, as small shrubs are unable to compensate for higher stomatal conductance with enhanced water use efficiency under warmer and drier environmental conditions, leading many of these species to adopt drought-avoidance strategies such as drought deciduousness. Aridity also acts as a selective force in these Mediterranean plant communities, with drought-avoidance strategies being the most dominant in the warmer and most arid communities. These results highlight the wide diversity of resource use strategies that can be found in Mediterranean plant communities, which promotes complementarity among coexisting species and the efficient use of limiting resources (water and nutrients) in the face of adverse climatic conditions.

Stable isotopes as integrative functional traits for water use strategies in dryland ecosystems.

The use of carbon and oxygen stable isotopes as reliable proxies for plant leaf gas exchange and physiological status has been well described over the past decades (Barbour 2007; Cernusak et al. 2013; Dawson et al. 2002; Flanagan, Ehleringer, and Pataki 2005; Dawson and Siegwolf 2007). A myriad of studies in plant science and ecology have successfully used this approach for the past 40 years as evidenced by the extensive scientific literature reviewed for this thesis. Our results indicate that the isotopic composition of carbon ($\delta^{13}C$) and oxygen ($\delta^{18}O$) in leaves (particularly oxygen isotopic enrichment above source water, $\Delta^{18}O$) are good predictors of time-integrated water use efficiency and stomatal conductance, respectively, as supported by the reasonably good match and agreement with direct leaf gas exchange measurements conducted in the field (Chapter 1, Figure 8). We have also observed that these measurements, especially leaf $\delta^{13}C$, but also $\Delta^{18}O$, are reasonably stable through time across species, as demonstrated when comparing the data obtained in two years with sharply contrasting climatic conditions (Chapter 1, Figure 7). However, the complexity of the signals they reflect can complicate the interpretation of plant isotopic composition, especially when analysed under non-controlled environmental conditions, such as in our field study, and this important question remains a topic of ongoing debate among researchers (Lin, Barbour, and Song 2022; Guerrieri et al. 2022). The major point of discussion derives from the fact that the oxygen isotope composition of plants is strongly influenced not only by the leaf-level evaporative isotopic enrichment caused by the opening or closing of stomata but is also highly sensitive to environmental factors and the isotopic signal of plant water sources (Barbour 2007). These include large geographical differences in temperature or vapour pressure deficit, the isotopic composition of rainwater, and evaporative enrichment of source water in the soil before root water uptake. In order to be able to attribute variation in leaf $\delta^{18}O$ only to the stomatal conductance signal of interest, all the climatic and soil factors mentioned above should be kept constant, which can only be achieved under laboratory conditions or in common garden experiments (Roden and Siegwolf 2012). However, when conducting field studies that cover large geographical and environmental gradients, as is the case in this work, it is necessary to control as best as possible for these sources of variation in plant oxygen isotopic composition. One option for this may be to limit the range of variation of this trait whenever possible. For example, by calculating leaf $\Delta^{18}O$ ($\Delta^{18}O = \delta^{18}O_L - \delta^{18}O_{XW}$) we can control for spatial and temporal variations in the isotopic composition of the source water used by plants at each site, which is one of the largest sources of variation in leaf $\delta^{18}O$, (Barbour 2007). However, xylem water collection and extraction is very labour

demanding and time consuming, so this detailed information is neither always available or feasible, nor does this approach help control for the variation in other environmental factors, like temperature or VPD, between sites.

The dual C/O isotope – gas exchange conceptual model offers a mechanistic approach to interpretating changes in water use efficiency and stomatal conductance in response to environmental variables based on a dual isotope response ($\delta^{13}C$ and $\delta^{18}O$) (Siegwolf et al. 2023; Scheidegger et al. 2000). $\delta^{13}C$ is a complex signal that integrates the influences of both photosynthetic carbon assimilation (A) and stomatal conductance (g_s) in the balance between supply and demand of CO_2 in leaves. However, the relative contribution of each of these two key physiological processes to the isotopic composition of the carbon that ends up forming plant tissues is often difficult to separate and disentangle. The simultaneous measurement of $\delta^{18}O$, which is a proxy for evaporative leaf water flux that is influenced by g_s but not A, within the same individual leaves can help improve the interpretation of $\delta^{13}C$ variation and disentangle the relative influences of A and g_s on plant isotopic composition. Thus, the combined measurement of carbon and oxygen isotopes in leaves can help researchers interpret plant responses to variation in multiple of environmental factors (temperature, relative humidity, precipitation amount, etc).

Alternatively, other researchers are proposing novel methods and approaches to further improve current understanding of the relationship between carbon gain and water loss in plants, such as the Integrated Metabolic Strategy (IMS) index (Goud et al. 2019), which is a synthetic measure that describes the ratio between leaf carbon isotope composition ($\delta^{13}C$) and oxygen isotope composition above source water ($\Delta^{18}O$). Higher IMS values indicate a higher metabolic carbon set point per unit of increasing evaporative water loss conditions in leaves and thus reveal a more favourable trade-off between carbon assimilation and water loss. The large number of studies using stable isotopes in plant ecophysiology, as well as the ongoing effort to refine and improve our interpretations, highlights the great interest generated by their use as integrative indicators of biological processes in vegetation. Stable isotopes allow obtaining integrated measurements over time, and this methodology enables large field sampling campaigns to be carried out in less time than with other more conventional methods (e.g. leaf gas exchange measurements or root excavation). This work contributes to expanding their potential applications and generating extensive field-based databases that enable more ambitious and larger-scale field studies. Our findings are also useful to feed models that improve our predictions of the behaviour of Mediterranean vegetation in the face of future climate scenarios brought about by global warming and regional climate aridification.

Differences in soil water uptake depth drive ecohydrological niche segregation in Mediterranean plant communities

Using these isotopic tools, we were able to document patterns of ecohydrological niche segregation among coexisting plant species that would have been otherwise impossible to detect with traditional methods. We found distinct ecohydrological niche segregation among coexisting species within all the Mediterranean plant communities studied (Chapter 1, Figure 1), regardless of their environmental conditions, with small shrubs using a larger proportion of shallow soil water than large shrubs and trees (50%, 36% and 24%, respectively). Ecohydrological niche segregation among co-occurring plant life forms and species is a widespread phenomenon that extends to a wide variety of ecosystems beyond drylands (Silvertown, Araya, and Gowing 2015). The drivers of hydrological niche segregation can be the appearance of vertical soil moisture gradients during rainless periods, differences in water-acquisition strategies among species (rooting depth, different phenology, etc.), seasonal differences in the temporal availability of water along the soil profile or their combination. However, the extent to which ecohydrological niche segregation affects the relative success of different strategies in native plant communities is still poorly understood. The seasonality of rainfall in the Mediterranean has a marked effect on water uptake strategies and their success at the community level (Ackerly 2004; Mooney and Dunn 1970). Globally, Mediterranean forests and shrublands have one of the greatest water extraction depths of all biomes, more than humid forests that host larger species (Bachofen et al. 2024). Mediterranean-type ecosystems are characterised by infrequent but very heavy rainfall, which can effectively recharge deep soil, bedrock and aquifers. However, moisture persists for only a short time in the topsoil during rainless periods due to high evaporation rates, thereby promoting the formation of deeper root systems (Schwinning, Starr, and Ehleringer 2005). The fact that they have deeper roots does not mean that these species necessarily use a large proportion of deep-water sources. This is so because many native Mediterranean species have dimorphic root systems that allow them to shift and alternate between the use of shallow or deep water sources, depending on seasonal variations in soil moisture availability along the soil/bedrock profile (Filella and Peñuelas 2003).

The ability of native plants to shift between the different water sources available throughout the year is one of the factors that most determines the leaf habit, growth pattern and phenology of each co-occurring species in dry ecosystems. In our study, we found that there is a strong correlation between water extraction depth by roots, stomatal regulation stringency and water use efficiency across and within these Mediterranean

plant communities (Chapter 1, Figure 4). Shallow-rooted species that use a higher proportion of topsoil water generally exhibit higher stomatal conductance and lower water use efficiency, while the opposite is true for deep-rooted species that use a larger proportion of deep-water sources. In arid ecosystems, the presence of deep roots allows a certain degree of stomatal conductance and transpiration to be maintained even during prolonged rainless periods, thereby sustaining photosynthetic activity for longer during dry spells (Bachofen et al. 2024; Laughlin et al. 2023). However, species lacking deep roots need to shorten or accelerate their life cycles to take full advantage of the transient pulses of water that become available after occasional rainfall. The success of each of these strategies at the community level depends largely on the severity of the climatic aridity to which each plant community is exposed. In this study, we found that smaller shrub species with shallower roots and faster water use and consumption are more abundant at the driest end of our aridity gradient, while the proportion of large shrubs and trees with slower and more conservative water use strategies increases towards the wetter end (Chapter 3, Figure 8). Although it may seem counterintuitive, it is important to note that, once certain aridity thresholds are exceeded, conservative strategies cease to be efficient in ensuring plant survival and growth, as the severity of droughts causes even deep water pools to be depleted, making it impossible to maintain plant activity (Berdugo et al. 2020). Thus, smaller plant species with shorter life cycles, shallower roots and more opportunistic water-spender strategies tend to dominate in the most arid environments (Blanco-Sánchez et al. 2022; Carvajal et al. 2019; Laughlin et al. 2023).

Evidence for a unified acquisitive-conservative spectrum encompassing carbon, nutrient and water use

Beyond documenting the diversity of plant water use strategies within plant communities, a central question emerges: are belowground and aboveground water-use strategies coordinated with carbon- and nutrient-use strategies (LES)? This study represents a major effort to jointly explore the different dimensions involved in resource use (water, nutrients, carbon) by native plants in Mediterranean ecosystems. In this regard, we have found evidence of significant coordination between water, nutrient and carbon use strategies across plant species and study sites (Chapter 2, Figure 3,4). We found that smaller plant species with a water-spender strategy (shallower water uptake depth, higher stomatal conductance and lower water use efficiency) presented also acquisitive leaf economic traits (low carbon investment in leaves, high leaf nutrient concentrations) and a higher nutrient use efficiency during photosynthetic carbon assimilation. Conversely, an opposite pattern was found in larger species with a water-saver strategy (deeper water uptake depth, lower stomatal conductance and higher water use

efficiency) that typically showed conservative leaf economic traits along the LES (i.e. high carbon investment in leaves, low leaf nutrient concentrations) and low nutrient use efficiency during photosynthesis. This is in line with the findings of earlier work that has defined the existence of an acquisitive-conservative (or fast-slow) axis in the use of carbon and nutrients at the leaf level, as posed by the leaf economic spectrum (LES) conceptual framework at the global scale (Reich 2014; Díaz et al. 2016; Wright et al. 2004). However, the potential coordination of plant water use strategies with the LES in terms of water use efficiency and water uptake depth has received considerably less research attention and remains poorly explored. Coordination between water extraction depth and carbon, nutrient and water use strategies aboveground might be stronger under dry Mediterranean conditions, which are stressful for plants (Mueller, Kray, and Blumenthal 2024). Conversely, when environmental stress is less severe, the decoupling of water, carbon and nutrient use strategies may allow for a wider array of diverse resource use strategy combinations to adapt to different local environments (Li et al. 2015; Wang and Wen 2022). To the best of our knowledge, only a few studies have addressed this important issue (Prieto et al. 2018; Illuminati et al. 2022; Garnier et al. 2025), and have done so with fewer species and life forms and only at local spatial scales. So, the present study represents a step forward in advancing understanding of the ecohydrological and ecophysiological functioning of plant communities in Mediterranean and other arid and semiarid ecosystems. To the best of our knowledge, our experimental approach is the first to combine the LES conceptual framework (Wright et al., 2004) with the dual C and O isotope-gas exchange model (Scheidegger et al., 2000; Siegwolf et al., 2003) and the isotopic determination of soil water uptake depth (Dawson 2022) across multiple plant communities with contrasting environmental conditions. This novel approach advances current understanding of the diversity of plant resource-use strategies within and across Mediterranean plant communities, and their response to the changing environment.

As a result of significant coordination between water, nutrient and carbon use strategies in Mediterranean woody plants, some key trade-offs emerge that reduce the number of possible trait combinations that can be found in the studied native plant communities. These trade-offs generally respond to balances between the demand for different resources (water, nutrients) and their relative availability in the ecosystem. One of these trade-offs relates to safety versus efficiency in transporting water through the plant, in which the height of the plants plays a very important role (Manzoni et al. 2013; Nardini et al. 2014). On the one hand, large trees can access deeper water sources that are more stable over time, but this also makes them much more exposed to hydraulic failures

caused by the drop in plant internal water potential with increasing height. This leads them to develop a series of adaptations to transport water more safely and efficiently through the xylem, enabling them to better withstand long periods of drought (Fernández-de-Uña et al. 2023). On the other hand, smaller species do not have to cope with this drop in water potential with height, and thus can develop larger conduits in the xylem, which allow them to transport water more quickly and efficiently during periods when it is available. However, this makes them more vulnerable to cavitation and hydraulic failure during droughts (Maherali, Pockman, and Jackson 2004; Gleason et al. 2016). Another key constraint is the trade-off between SLA and leaf lifespan (LLS), which determines both the potential rate of photosynthetic carbon return per unit leaf mass investment and the duration of the return (Westoby et al. 2002). A higher SLA with lower LLS is related to greater photosynthetic capacity and allows for greater flexibility and faster response to spatial and temporal changes in soil resources availability, enabling acquisitive plant species to take advantage of water and nutrient pulses more quickly after rainfall. Meanwhile, lower SLA (i.e. higher LMA) and higher LLS may offer more advantages in the longer term under stressful environmental conditions, as species adopting this more conservative strategy can accumulate a greater number of leaves that increase the plant's total photosynthetic surface area. Even though photosynthetic capacity per unit leaf area is lower in these conservative species, they can still achieve similar primary productivity over longer periods (Wright et al. 2004; Reich 2014). Finally, the relationship between stomatal regulation stringency and water use efficiency, which depends on both stomatal conductance and photosynthetic capacity, is another of the most important trade-offs determining plant resource use strategies and growth in Mediterranean environments (Moreno-Gutiérrez et al. 2012). When we analyse the general relationship between these two key water use traits within and across sites, we find that higher stomatal conductance allows for higher photosynthesis and greater productivity over short favourable periods, but does so at the unavoidable cost of lower water use efficiency at leaf level. In short, these relationships reveal the existence of a single main axis of resource acquisition-conservation variation in plant traits that constrains the diversity of water, nutrients and carbon use strategies combinations that are physiologically feasible for plants in Mediterranean ecosystems.

From species to communities: scaling up functional diversity across environmental gradients.

These patterns of variation in water-use strategies and in trait coordination across species have profound implications for how functional diversity is structured at the community level and how it responds to environmental gradients. Plant functional traits determine how species respond to their environment, allowing us to scale individual responses up to the community level (Gross et al. 2009). As evidenced by our results, plant traits covaried along axes of specialization reflecting functional trade-offs, such as resource acquisition-conservation, that ultimately affect species' reproduction, growth and survival. The Mass Ratio Hypothesis that posits that the functioning of ecosystems is determined by the trait values of the most dominant species (Grime 1998). Therefore, the use of trait community weighted means (CWM) provides information about the dominant traits in an ecological unit that can be used to summarise changes in mean trait values across plant communities in response to environmental selection for any given functional traits (Carmona et al. 2016; Ricotta and Moretti 2011). However, ecosystem responses to environmental changes go beyond those of dominant traits, as the position of species in a multidimensional trait space can reveal much greater functional diversity and the existence of distinct ecological niches within plant communities as a result of biotic and abiotic interactions (Maire et al. 2012). Biotic interactions may cause species to coexists either because they are functionally different (niche differentiation) or because they are functionally similar (weak competitive exclusion due to habitat filtering) (De Bello et al. 2012). Species abundance and distribution within plant communities can be affected by both habitat filtering, promoting dominant species, and niche differentiation, acting as stabilizing force on rare species, which may act differently on distinct groups of functional traits (Gross et al. 2013). Increasing niche differentiation increases the number of feasible trait combinations, which can enhance species richness and coexistence within communities. Conversely, an increase in environmental severity enhances the habitat filtering effect, promoting the exclusion of rare species (Maire et al. 2012). Our results suggest that, as environmental stress increases, the functional richness of Mediterranean plant communities decreases (Chapter 3, Figure 9), so the number of feasible trait combinations is reduced, yet we could still find a wide diversity of carbon, nutrient and water use strategies at the warmest, driest and most arid end of our climatic gradient. (Gross et al. 2024) Thus, the combined effects of habitat filtering and niche differentiation in response to the changing environment promote the complementarity of water, carbon and nutrient use strategies among coexisting plant species, thereby maximising the efficient use of the resources available in each community.

Beyond the wide functional diversity found within plant communities at each site, our results indicate that increased aridity also leads to variation in the functional traits we measured across sites (Chapter 3, Figure 7). In the most arid areas, we found more conservative leaf economic traits (more sclerophyllous leaves) combined with higher water use efficiency at leaf level. However, contrary to expectations, we also found that time-integrated stomatal conductance increases with increasing aridity towards the drier end of the gradient (Chapter 3, Figure 3). Water flux in leaves is strongly influenced by the environmental conditions of each location, so the higher temperatures and greater atmospheric water demand found in the most arid locations inevitably forces leaves to transpire more water despite decreasing soil water availability (Diao et al. 2024; Urban et al. 2017; Marchin et al. 2023, 2016; Aparecido et al. 2020; Posch et al. 2024). This also serves as a strategy to enhance evaporative leaf cooling through increased transpiration and to thereby extend the periods during which the enzymes involved in photosynthesis remain active through heat-stress alleviation (Lawson et al. 2014; Peguero-Pina et al. 2020). However, this increase in stomatal conductance has costs and risks that become more pronounced as water stress increases, thus causing sequential changes in the functioning and composition of plant communities with increasing aridity (Berdugo et al. 2020).

In response to increasing drought stress, there is a progressive reduction in vegetation productivity mediated by an increase in leaf sclerophylly (thicker leaves with lower maximum photosynthesis rates) (Carrascosa et al. 2023; Cornwell and Ackerly 2009; Pérez-Ramos et al. 2020; Ramos-Muñoz et al. 2024). As aridity intensifies, vegetation can shift from grassland to shrublands with deeper root systems that are less sensitive to seasonal droughts (Gross et al. 2013; Maestre et al. 2009). As aridity intensifies even further, an ecological breakdown occurs that limits the existence of drought tolerant species, which are replaced by drought-deciduous species better adapted to take advantage of short and unpredictable rain pulses (Berdugo et al. 2020). Thus, once a certain aridity threshold is exceeded and access to deep water does not compensate for the increasing water losses caused by enhanced transpiration, the dominant strategies in woody plant communities shifts towards more acquisitive strategies. Acquisitive species with water-spender strategies are capable of optimizing the use of brief pulses of water, while surviving the harshest drought periods by shedding their leaves (Carvajal et al. 2019; Blanco-Sánchez et al. 2022). According to IPCC RCP 8.5 predictions for 2100, 22% of the planet's terrestrial surface is expected to be affected by increased aridity, and 3.7% of the surface, including the most arid areas of our gradient, could exceed the critical thresholds of ecosystem breakdown (Berdugo et al. 2020). Therefore,

the research carried out in this doctoral thesis is of great importance, as understanding the wide diversity of mechanisms that operate in Mediterranean plant communities to acquire, use and conserve water and other nutrients will allow us to design more climate-smart vegetation conservation, management and restoration plans under the ongoing climate aridification scenario across the Mediterranean region. In this sense, promoting a higher functional and species diversity in Mediterranean ecosystems is crucial to preserve ecosystems that are more resilient and better adapted to climate change.

Limitations and future directions

The main focus of this work has been to attempt and describe the wide diversity of species and resource use strategies present in the woody plant communities studied, thereby advancing current understanding of the complexity of coexisting resource use strategies and their potential complementarity in Mediterranean ecosystems. However, this broad objective has entailed some limitations that are worth mentioning and that also may stimulate future lines of work. The target plant communities were sampled only once during the year, coinciding with the peak of the plant's maximum physiological activity, in order to maximise the number of species we could sample with the resources at our disposal. Because of this, we still have room to learn more about how these communities behave throughout the year, during the coldest and wettest months, but also during the most extreme drought periods in summer. For this purpose, we suggest that stable isotopes of C and O in leaves might not be as useful at reflecting differences in leaf gas exchange at finer temporal scale during unfavourable periods, given that C and O incorporation into plant tissues primarily occurs during periods of high plant physiological activity and growth. The use of leaf gas exchange and plant water potential measurements in repeated measurements throughout the year could provide a finer temporal resolution than isotopes in this respect. In contrast, xylem water isotopes do allow us to detect differences in soil water extraction depth throughout the year, as long as we find an isotopic enrichment profile in the soil that allows us to differentiate the isotopic composition of the different water sources available in the soil profile. Furthermore, although the aim of this study has been to provide a holistic picture of how different species use resources in dry ecosystems by integrating water, carbon and nutrient use strategies, other relevant aspects for understanding plant responses to drought, such as hydraulic capacity (water transport capacity, resistance to embolism, etc.), should also be considered in future research (Choat et al. 2012; Nardini, Battistuzzo, and Savi 2013; McDowell et al. 2022; Nardini et al. 2016). Finally, disentangling the influence of different climatic variables on the plant isotopic values observed will need further research. For example, disentangling the relative influence of

temperature vs. vapour pressure deficit (together and independently) on leaf $\delta^{18}O$ and $\Delta^{18}O$ values along the aridity gradient, or the weight of each climatic variable on the variability of each isotopic trait (including also $\delta^{13}C$). In this regard, one of our goals for the future is to use the extensive database we have generated, together with other available databases (Kattge et al. 2020), to feed models that allow us to predict the impact of climate change on plant water use strategies and their impact on different plant communities.

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General Conclusions

General Conclusions

1. We found a wide variety of above- and belowground water use strategies among coexisting plant species at all the study sites (stomatal conductance, $\Delta^{18}O_L$; intrinsic water use efficiency, $\delta^{13}C_L$; water uptake depth, $\delta^{18}O_{XW}$, $\delta^{2}H_{XW}$, d-excess), which was largely mediated by the size of the different species that make up these ecosystems (small shrubs, large shrubs and trees). This large functional diversity allows distinct ecohydrological niche segregation among co-occurring species at all the sites studied, regardless of the degree of aridity to which they are exposed.
2. Within sites, aboveground ($\delta^{13}C_L$, $\Delta^{18}O_L$) and belowground water use strategies ($\delta^{18}O_{XW}$, $\delta^{2}H_{XW}$, d-excess) are coordinated between them and with leaf hydraulic traits (leaf area to sapwood area ratio) along a physiological spectrum ranging from a profligate water-spender strategy to conservative water-saver strategy. Water spender species, which generally show smaller size, present shallower root water uptake, higher stomatal conductance, lower water use efficiency and sparser leaves along shoots; while the opposite traits are found in bigger water saver species (larger shrubs and trees).
3. The trade-offs arising from coordination between above- and belowground water use strategies constrain the diversity of whole-plant water-use strategies present in upland Mediterranean ecosystems.
4. Carbon and oxygen stable isotopes in leaves, as well as oxygen and deuterium isotopes in plant xylem water, are good indicators of whole-plant water use strategies. Moreover, leaf $\delta^{13}C_L$ and $\Delta^{18}O_L$ show reasonably good correlations with gas exchange measurements (intrinsic water use efficiency and stomatal conductance, respectively). Within study sites, plant species rankings and differences in isotopic traits are maintained and well preserved between years with contrasting climatic conditions (i.e. wet vs dry years).
5. Leaf economic traits (SLA, LDMC, and N, P, K concentrations) converge along a single Leaf Economics Spectrum axis across species in Mediterranean vegetation. There is a high diversity of leaf economic traits among coexisting species within each study site along the aridity gradient, encompassing an spectrum from acquisitive (high nutrient concentration and SLA) to conservative (low nutrient concentration and high LDMC) carbon and nutrient use strategies.
6. Leaf economic traits and water use traits at leaf level are tightly coordinated across species. Acquisitive species along the LES also exhibit a water-spender

strategy at leaf level (higher stomatal conductance and transpiration and low intrinsic and instantaneous water use efficiency) combined with higher photosynthesis rates (A); while their coexisting conservative species along the LES exhibit a water-saver strategy and lower photosynthesis rates.

7. Water uptake depth is also strongly related to leaf economic traits. Acquisitive species along the LES use a higher proportion of shallow soil water that is rich in nutrients but unstable over time, while conservative species along the LES use a greater proportion of deep soil/bedrock water that is more stable over time but poorer in nutrients.
8. Acquisitive species along the LES with shallow water uptake pattern tend to prioritise nitrogen use efficiency over water use efficiency at leaf level, while more conservative species with deeper water uptake pattern instead prioritise water use efficiency over nitrogen use efficiency.
9. The convergence of water, carbon and nutrient use strategies along a single "fast-slow" spectrum is more pronounced in strictly Mediterranean sites (i.e. when the wetter sub-Mediterranean site Montejo is excluded from the analyses), where climatic aridity and water shortage constrain carbon and nutrient acquisition more strongly.
10. Aridity has a strong effect on LES and water use traits at leaf-level. There is a higher dominance of conservative leaf economic traits (lower nutrient concentrations per mass, lower SLA and higher LDMC) coupled with higher intrinsic water use efficiency in drier plant communities.
11. Contrary to what we expected, intrinsic water use efficiency and stomatal regulation stringency diverge along the aridity gradient, despite tight their tight coupling across coexisting species within sites. Although stomatal conductance inevitably increases with aridity due to rising temperatures and atmospheric water demand, especially in small shrubs, species in the most arid sites still manage to maintain higher intrinsic water use efficiency thanks to increased nutrient contents and photosynthetic capacity per unit leaf area.
12. The functional space occupied by different plant communities varies along the aridity gradient, mainly due to species turnover, with those communities that are more climatically separated being also more functionally distinct.
13. Although plant functional richness and the diversity of resource-use traits and their combinations tend to decrease with aridity, all the study sites and plant communities along the gradient still exhibited sharply contrasting water and nutrient use strategies among coexisting species.

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