nature portfolio

Peer Review File

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

Corresponding Author: Professor José Ignacio Querejeta

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Attachments originally included by the reviewers as part of their assessment can be found at the end of this file.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
This is a wonderful manuscript and should eventually be published, but not before addressing the issues below.

[1] The introduction and justification for this manuscript missed two of the most important publications that laid the foundation for this worldwide leaf economic spectrum (WLES) functional approach, especially since that paper was on Mediterranean-climate vegetation. These are the classic pre-WLES publications by Hal Mooney and Lloyd Dunn:
• Mooney, H.A., and E.L. Dunn. 1970. Photosynthetic systems of Mediterranean-climate shrubs and trees of California and Chile. American Naturalist 104:447-453. Doi.org:/10.1086/282679
• Mooney, H.A., and E.L. Dunn. 1970. Convergent evolution of Mediterranean-climate evergreen sclerophyll shrubs. Evolution 24:292-303. Doi.org:/10.2307/1950744

[2] At no place in the manuscript or in the supplemental material could this reviewer find a list of the individual species sampled at each location. This consequence is several significant gaps critical to understanding and interpreting the data on which claims are made.
• Please provide a list of individual species at each site, at least as part of the supplemental materials.
• Please indicate for each species on these lists as to whether they are deciduous or evergreen.
• Please indicate for each species whether the plants are long-lived (more than 20 years) or short-lived.

[3] The authors could produce a stronger story if the manuscript addressed the issues of whether deciduous vs evergreen or long-lived vs short-lived characteristics played any significant or non-significant role in their analyses and data interpretations.

[4] There places in the manuscript where the manuscript is “loose” in the data interpretation or “over interprets the data”. For instance, consider
• Lines 246-248
• Lines 276-279
• Lines 357-359
• Lines 424-430

[5] Given the claim (lines 329-332), it would have been helpful if the manuscript actually provided or referenced a publication on plants from a Mediterranean climate and not constrained as gypsum soils. There is undoubtedly existing Mediterranean-climate vegetation data on this topic from Steve Davis and colleagues in the California chaparral.

[6] It would have been nice for the manuscript to acknowledge that the “metabolic set point” concept originated with Ehleringer and Cerling (1995, Tree Physiology 15:105-111)

Review submitted by Jim Ehleringer

Reviewer #2

(Remarks to the Author)
SUMMARY
Muñoz-Gálvez et al. looked at the diversity of water-use strategies among coexisting woody species in 10 Mediterranean ecosystems with different climates (62 species in all). The isotopic composition (soil, stem, and leaf) was analyzed to determine plant water uptake depth, water use efficiency. The findings suggest that ecohydrological niches are segregated based on water intake depth, with larger species accessing deeper soil water and exhibiting more conservative "water-saver" behaviors, whilst smaller species rely on shallow water and exhibit more "water-spender" behavior. Drought and heat stress in these ecosystems induce close coordination between above- and below-ground water-use features, leading to trade-offs that limit the diversity of viable water-use methods.
The idea is very interesting, the dataset highly valuable. The paper is well-written, albeit there is some brevity and lack that prevents issues from being completely addressed. I have some concerns about the methods and the analysis. The results section was not often clearly presented.
I have the following concerns about the introduction:
INTRODUCTION
1. (requires much bigger emphasis on study's relevance): Hypothesis and how they lead to unequivocal conclusions are not clear. Objectives are mostly exploratory – the scientific gap they address is not clear. Explain and underline more the relevance of the entire study, from topic (why is hydraulic an important trait?), and site selection (why do these sites represent the semiarid-wetter well?) to statistical analysis (why is trait interconnectivity and thus network analysis a useful approach to answer the research questions?). Underlining key strength: another key strength of this study is that it links stem to leaf hydraulic traits. This should be emphasized more in the introduction.
I also have some significant concerns regarding the analysis and method section
ANALYSIS
2. The analysis must go much beyond correlating a series of traits to produce robust, mechanistical, knowledge.
To enhance analysis consistency and integration, and allow for fair hydraulic trait comparison, include (a) a Principal Component Analysis to simultaneously test and visualise site differences (and tree/shrub differences). To show sites differences and trait relations in an integrated manner, perform a PCA. This is efficient and integrated data visualisation by simultaneously showing (1) sites are not very different, and (2) sites' position in relation to trait interplay. Currently most analyses are on a trait-by-trait basis.
and (b) (statistics- perform multivariate linear regression): this analysis falls rather short considering the available data, and is inconsistent with previous analyses. To see if there is an interaction effect between climate and hydraulic traits, differentiate between the sites in the analysis. Alternatively, include aridity index as a predictor variable in the regression to specify if climate plays a role if there is enough variation across sites. This is interesting and important as current literature has found a significant effect for vulnerability in arid but not humid regions. This makes your results all the more thought provoking. Therefore, the analysis, results, and discussion are lacking dramatically in this aspect of the study.
What is a trait network? How do you quantify the centrality of something on it? What are the alternative hypotheses that could similarly explain the pattern found? How do you negate them?
- Sensitivity analysis should be performed on the dataset as it includes many different groups (trees and small and tall shrubs). Ideally, those groups should be treated as factors in the analysis rather than globally joining the data.
- If I understood correct, part of the dataset is not original – that is, part of the data has already been published or measured in several years ago. Where traits measured in the same individuals always?
Another thing to clarify: I do have a problem with the used data, as the compiled data are collected across different years and sites but not consistently. Inconsistent data gathering across years and sites might complicate research and decision-making. These inconsistencies often lead to issues in data comparability, quality, and reliability, which can compromise the validity of any conclusions drawn. I was wondering how the authors dealt with that and how they came to the general conclusions with such inconsistency.
In addition, the authors are knowledgeable enough to know that the relationship between aboveground hydraulic traits and water status in woody species is inseparable. How differences in roots help to keep working (uptake water and mineral nutrients). I suggest that the authors consider this aspect in a little more detail in this text.

Reviewer #3

(Remarks to the Author)
Dear Authors,

Please find my comments in the attached pdf.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
The authors have addressed the comments of the reviewers. I recommend acceptance of this revised manuscript.

Jim Ehleringer

Reviewer #2

(Remarks to the Author)

Thank you for addressing all the previous suggestions/recommendations and including it with more details on the revised version. I have no more questions or concerns about the revised version. I totally understand the full data-sharing policy; however, I believe it is highly recommended to provide the complete code and the data used to ensure full transparency and reproducibility of the results. I recommend the manuscript publication when this revision is done.

Reviewer #3

(Remarks to the Author)
Review of the manuscript “Trait coordination and trade-offs constrain the diversity of water use strategies in 1 Mediterranean woody plants”.

The authors did a good job in addressing my comments. I especially appreciate the added clarifications in the text and the expansion on the potential impact of ‘the height effect’. While I remain skeptical as to whether the height effect can be as easily excluded as suggested by the authors, I acknowledge that the authors did a excellent job in presenting the alternative hypothesis in light of their findings, to the benefit of the reader. For instance, the speculations on the effective time delay of 1-2 days seem rather optimistic, because such estimates are based on irrigated, ideal conditions at the peak of a plant’s sap flow performance. It would be interesting to see the exact integration used by the authors to upscale such sap flow measurements to their own system where drought stress causes water resource partitioning. However, I leave it to the discretion of the editor whether this would be to the merit of the reader.

Overall, I really enjoyed reading this study, and can support the publication of this manuscript.

REVIEWER COMMENTS

Reviewer #1 (Remarks to the Author):

This is a wonderful manuscript and should eventually be published, but not before addressing the issues below.

Response: We are thrilled and very thankful for the positive appraisal of our work. In the revised manuscript, we have carefully addressed all the important issues raised by the reviewer, as explained below.

[1] The introduction and justification for this manuscript missed two of the most important publications that laid the foundation for this worldwide leaf economic spectrum (WLES) functional approach, especially since that paper was on Mediterranean-climate vegetation. These are the classic pre-WLES publications by Hal Mooney and Lloyd Dunn:
• Mooney, H.A., and E.L. Dunn. 1970. Photosynthetic systems of Mediterranean-climate shrubs and trees of California and Chile. American Naturalist 104:447-453. Doi.org:/10.1086/282679
• Mooney, H.A., and E.L. Dunn. 1970. Convergent evolution of Mediterranean-climate evergreen sclerophyll shrubs. Evolution 24:292-303. Doi.org:/10.2307/1950744

Response: Thanks for the advice. These pioneering and highly relevant papers are now properly acknowledged and cited in the revised manuscript (Lines 733 -736).

[2] At no place in the manuscript or in the supplemental material could this reviewer find a list of the individual species sampled at each location. This consequence is several significant gaps critical to understanding and interpreting the data on which claims are made.
• Please provide a list of individual species at each site, at least as part of the supplemental materials.
• Please indicate for each species on these lists as to whether they are deciduous or evergreen.
• Please indicate for each species whether the plants are long-lived (more than 20 years) or short-lived.

Response: We are particularly grateful for this useful advice, as this essential information was unfortunately missing from the earlier version of the manuscript (our fault!). In the revised version, we have included two new tables in the Supplementary Material with all the requested information: Table S2 shows basic relevant information for each species (and the abbreviations used throughout the manuscript), including plant family, plant life form (small shrubs, large shrubs or trees), leaf habit (evergreen, winter deciduous or drought deciduous), approximate lifespan and maximum rooting depth according to the published literature; Table S18 shows the list of woody plant species present at each study site.

[3] The authors could produce a stronger story if the manuscript addressed the issues of

whether deciduous vs evergreen or long-lived vs short-lived characteristics played any significant or non-significant role in their analyses and data interpretations.

Response: Thanks for the insightful advice. In the revised manuscript, we have specifically addressed the impact of the species leaf habit (evergreen vs drought-deciduous) on their isotopic traits, and the main findings are highlighted in Figure S6 and briefly described and discussed in the main text (Lines 223 – 225, 272 – 275 and mentions in the discussion). Please note that winter-deciduous species were not included in this analysis because they were only present at the wettest and coldest site (Montejo, located at 1500 m elevation and representing the transition from Mediterranean-type climate to Temperate/Atlantic climate). We conducted an extensive literature search to compile all the available information on the lifespan of our target species. Unfortunately, given the lack of reliable information in the scientific literature regarding the lifespan of many of the species included in our study (especially for small shrub species of presumably short but uncertain lifespan), it was not possible to explicitly address the impact of this particular factor in the paper. However, we totally agree that this aspect could be highly relevant for the research topic at hand and of great interest for future research in this field.

[4] There are places in the manuscript where the manuscript is "loose" in the data interpretation or "over interprets the data". For instance, consider
• Lines 246-248
• Lines 276-279
• Lines 357-359
• Lines 424-430

Response: Considering your advice, in the revised manuscript we have carefully eliminated, toned-down or rephrased these statements to better clarify the arguments made, paying special attention to avoiding any "over-interpretation" of the data. In particular, we have carefully removed any mention previously made to plant variables or traits that were not actually measured in the present study (such as growth, photosynthesis, reproductive output, etc).

[5] Given the claim (lines 329-332), it would have been helpful if the manuscript actually provided or referenced a publication on plants from a Mediterranean climate and not constrained as gypsum soils. There is undoubtedly existing Mediterranean-climate vegetation data on this topic from Steve Davis and colleagues in the California chaparral.

Response: Thanks for the useful advice. We have added several relevant references reporting similar patterns in the California chaparral, Chile and other Mediterranean-type ecosystems around the world (Ackerly, 2004; Bachofen et al., 2024; Davis & Mooney, 1986; Jacobsen, Pratt, Davis, & Ewers, 2008).

[6] It would have been nice for the manuscript to acknowledge that the "metabolic set point" concept originated with Ehleringer and Cerling (1995, Tree Physiology 15:105-111)

Response: this important concept is first mentioned in L 91 of the revised manuscript, and the suggested reference has been properly acknowledged and cited.

Reviewer #2 (Remarks to the Author):

SUMMARY

Muñoz-Gálvez et al. looked at the diversity of water-use strategies among coexisting woody species in 10 Mediterranean ecosystems with different climates (62 species in all). The isotopic composition (soil, stem, and leaf) was analyzed to determine plant water uptake depth, water use efficiency. The findings suggest that ecohydrological niches are segregated based on water intake depth, with larger species accessing deeper soil water and exhibiting more conservative "water-saver" behaviors, whilst smaller species rely on shallow water and exhibit more "water-spender" behavior. Drought and heat stress in these ecosystems induce close coordination between above- and below-ground water-use features, leading to trade-offs that limit the diversity of viable water-use methods.

Response: We appreciate the faithful and accurate summary of the main findings of our study, thanks.

The idea is very interesting, the dataset highly valuable. The paper is well-written, albeit there is some brevity and lack that prevents issues from being completely addressed. I have some concerns about the methods and the analysis. The results section was not often clearly presented.

Response: We are very grateful for the appreciative and encouraging comments on our work, as well as for the insightful advice provided by the reviewer for improving the paper. All the relevant issues raised by the reviewer have been carefully addressed in the revised version of the manuscript, as explained below in more detail.

I have the following concerns about the introduction:

INTRODUCTION

1. (requires much bigger emphasis on study's relevance): Hypothesis and how they lead to unequivocal conclusions are not clear. Objectives are mostly exploratory – the scientific gap they address is not clear. Explain and underline more the relevance of the entire study, from topic (why is hydraulic an important trait?), and site selection (why do these sites represent the semiarid-wetter well?) to statistical analysis (why is trait interconnectivity and thus network analysis a useful approach to answer the research questions?). Underlining key strength: another key strength of this study is that it links stem to leaf hydraulic traits. This should be emphasized more in the introduction.

Response: Thanks for the useful advice. In the revised manuscript, we have carefully reframed and rephrased the key research questions, current knowledge gaps, objectives and testable hypotheses, which we believe strengthens and clarifies their links to the main findings and conclusions of our study (by more explicitly linking the key results to the main testable hypotheses formulated in the Introduction). Following the reviewer's insightful advice, we have added a new hypothesis that explicitly refers to the link between stem and leaf hydraulic traits across species and functional types, to better

emphasize the relevance and novelty of this particular aspect of the study (hypothesis iii, lines 168 - 172). We have also provided a more clear and explicit justification for the selection of the 10 study sites with contrasting climates that were included in this research (Lines 149 - 156). Regarding the statistical analysis of the data, we provide further details below in response to specific comments addressing this aspect of the study.

I also have some significant concerns regarding the analysis and method section
ANALYSIS
2. The analysis must go much beyond correlating a series of traits to produce robust, mechanistical, knowledge. To enhance analysis consistency and integration, and allow for fair hydraulic trait comparison, include (a) a Principal Component Analysis to simultaneously test and visualise site differences (and tree/shrub differences). To show sites differences and trait relations in an integrated manner, perform a PCA. This is efficient and integrated data visualisation by simultaneously showing (1) sites are not very different, and (2) sites' position in relation to trait interplay. Currently most analyses are on a trait-by-trait basis, and (b) (statistics- perform multivariate linear regression): this analysis falls rather short considering the available data, and is inconsistent with previous analyses. To see if there is an interaction effect between climate and hydraulic traits, differentiate between the sites in the analysis. Alternatively, include aridity index as a predictor variable in the regression to specify if climate plays a role if there is enough variation across sites. This is interesting and important as current literature has found a significant effect for vulnerability in arid but not humid regions. This makes your results all the more thought provoking. Therefore, the analysis, results, and discussion are lacking dramatically in this aspect of the study.

Response: We are very grateful for this insightful advice, as we totally agree that adding the suggested multivariate analysis greatly helps to strengthen and clarify the interpretation of the data across sites and life forms. Following the reviewer's suggestion, we have performed a principal component analysis (PCA) that includes plant species height, and all the main isotopic traits measured in this study ($\delta^{18}O_L$, $\delta^{13}C_L$, $\delta 18O_{xw}$). Please note that we have chosen to include bulk $\delta^{18}O_L$ instead of $\Delta^{18}O_L$ data in the PCA because the $\delta^{18}O_{xw}$ is used to calculate $\Delta^{18}O_L$ and therefore they are mathematically correlated, which could eventually bias the interpretation of the results. In the revised version of the manuscript, we have included a new figure that highlights the results of the PCA analysis showing two twin panels, one of them highlighting the distribution of individuals according to their life form (Figure 5A) and a second one highlighting the distribution of individuals according to study site (Figure 5B). The main results of the PCA analysis are described and discussed in detail in lines 248-267 of the revised manuscript.

The overarching goal of our study was investigating the links and degree of coordination among water use traits across all species, life forms and study sites in Mediterranean woody vegetation. However, we agree that considering more explicitly the climatic differences among study sites and how they modulate plant traits and their coordination is interesting in itself and highly relevant for the interpretation and discussion of the patterns observed. Therefore, we extracted the individual plant scores

along the two main PCA axes and related these scores to the climatic variation among study sites regarding mean annual temperature, mean annual precipitation and aridity. We found that the second axis of the PCA was clearly related to climate-driven variation in the values of the water use traits included in the study, and this important aspect is now properly highlighted in the PCA figure. We have also conducted a new variance partitioning analysis for the main plant traits included in the PCA for a better understanding of the proportions of variance explained by site vs species form for each trait (Figure S10 and L 250 - 253). We also specify the proportion of variance absorbed by the site and life form in the first 2 PCA axis. (Table S13)

What is a trait network? How do you quantify the centrality of something on it? What are the alternative hypotheses that could similarly explain the pattern found? How do you negate them?

Response: Thanks for the useful advice. In Figure 9, we have added a new panel depicting a graphical representation of the trait network and trait interconnectivity observed in Mediterranean woody species, in which "Water uptake depth" (inferred from xylem water d18Oxw and deuterium-excess data) is highlighted as a key central trait of the network. We believe that this new panel depicting the trait network and its interconnectivity provides a more integrative and holistic overview of the major patterns encountered in our study, so we are particularly grateful for this insightful suggestion.

Alternative explanations for the patterns encountered and reported in this article have been carefully addressed and discussed in detail in a new paragraph in the Discussion section (L 385 - 410), in response to the suggestions and critical comments provided by Reviewer 3.

Sensitivity analysis should be performed on the dataset as it includes many different groups (trees and small and tall shrubs). Ideally, those groups should be treated as factors in the analysis rather than globally joining the data.

Response: Thanks for this useful advice. As proposed by the reviewer, we have now included both "study site" and "plant life form" as crossed random factors in all the mixed models performed in this article. After re-analysing the dataset according to the reviewer's indication, we find the same patterns and significant relationships between traits that we reported in the earlier version of the manuscript.

If I understood correct, part of the dataset is not original – that is, part of the data has already been published or measured in several years ago. Where traits measured in the same individuals always? Another thing to clarify: I do have a problem with the used data, as the compiled data are collected across different years and sites but not consistently. Inconsistent data gathering across years and sites might complicate research and decision-making. These inconsistencies often lead to issues in data comparability, quality, and reliability, which can compromise the validity of any conclusions drawn. I was wondering how the authors dealt with that and how they came to the general conclusions with such inconsistency.

Response: Thanks for offering us the opportunity to better clarify this important aspect of the study. Please note that none of the data included in this article have been published before, so the whole dataset is completely novel and original. The core dataset encompasses 6 study sites located along a climatic aridity gradient in the Iberian Peninsula that were sampled in two distinct years (2019 and 2022, excepting Montejo which was sampled in 2021 and 2022). This core dataset was supplemented with data from four Mediterranean plant communities that were sampled some years before (2008 and 2017; see table S15 for exact year of field sampling at each study site), to increase the number of plant species and range of climatic conditions encompassed by the study, thereby broadening the scope of the research. However, please note that all the field sampling campaigns were conducted by the same team of researchers, following exactly the same protocols and procedures in all cases. The samples collected in the field were thereafter processed and prepared in the same laboratory at CEBAS-CSIC by the same team and always using the same laboratory equipment and protocols. Moreover, all stable isotope measurements for all the samples included in the present study were conducted at the Center for Stable Isotope Biogeochemistry, University of California Berkeley (led by Prof. Todd E. Dawson).

In the first two sections of the Results dealing with water source partitioning among coexisting species, ecohydrological niche segregation and correlation between stem water and leaf isotopic composition across species, we used only data collected in a single year per site (10 sites). In the last section of the Results where we assessed the temporal stability of isotopic water-use traits between years with contrasting rainfall patterns, we used data collected in two distinct years at the 6 core study sites where field sampling was conducted twice. This comparison was done using mean species values per site and year, given that the individuals sampled for each species were different from one year to another. For leaf: sapwood area ratio data, measurements were conducted only for the Montejo (2021) and Yeste (2023) sites. To control for the potential influence of the "site" factor on trait coordination, we performed mixed models setting site and life form as crossed random effects, so we could address trait coordination after accounting for the climatic and environmental differences between sites.

In addition, the authors are knowledgeable enough to know that the relationship between aboveground hydraulic traits and water status in woody species is inseparable. How differences in roots help to keep working (uptake water and mineral nutrients). I suggest that the authors consider this aspect in a little more detail in this text.

Response: Thanks for the advice. We think that the nutritional aspects mentioned by the reviewer really fall beyond the scope of the present study, which has a strong focus on plant water use strategies. The present manuscript already includes a very large database encompassing multiple plant water-use traits, so here we have prioritized investigating their multiple complex interrelations in depth. Moreover, expanding the present database to include nutritional aspects would result in an exceedingly complex and long manuscript that would exceed by far the article length limits recommended by Nature Communications. However, we intend to address and investigate the interplay between

water and nutrient uptake more in depth in a separate manuscript that specifically focuses on nutritional aspects, and which is currently in preparation.

Reviewer 3

Review of the manuscript by Muñoz-Gálvez and co-authors: "Trait coordination and trade-offs constrain the diversity of water use strategies in Mediterranean woody plants".

Short description: Muñoz-Gálvez and co-authors use an incredible dataset of soil and stem water isotopic signatures to study the water use uptake strategies of lignified shrubs and trees in multiple plots in the Mediterranean region. The authors suggest that within Mediterranean plant communities, larger woody species extract water from deeper soil layers coupled to a much more conservative water use strategy than observed for shrubs. The latter exhibit a much more wasteful strategy with the extraction of water from shallower soil layers. The authors link such distinct strategies to a difference in coupling between above and below ground water use traits, leading to the establishment of ecohydrological niche segregation.

Appreciation and main comment: I appreciate the ambitious nature, and the incredible dataset collected for this study. Sampling 771 individuals belonging to 10 different Mediterranean ecosystems is indeed a truly admirable feat and could be a great addition to the scientific community interested in the use of stable water isotopes. However, this specific scientific community has seen a strong increase in studies scrutinizing the use and interpretation of stable water isotopes in the last years (Schwendenmann et al., 2010; Gaines et al., 2016; Evaristo et al., 2019; Barbeta et al., 2020; von Freyberg et al., 2020; De Deurwaerder et al., 2020; Knighton et al., 2020; Magh et al., 2020; Marshall et al., 2020; Seeger & Weiler, 2021; Mennekes et al., 2021; Bernhard et al., 2024, and others). It is therefore surprising that this voice of concern is not mentioned in the manuscript. It is particularly concerning as these studies uphold an alternative, but equally elegant explanation of the presented observations: defined as 'the height effect', i.e., a difference in lag-time due to differences in transportation length along the stem.

Response: Thank you very much for the appreciative comments regarding the scope and relevance of the dataset and ambitious nature of our work. Thanks also for the knowledgeable and thoughtful comments, suggestions and valuable references offered to improve and strengthen the interpretation of the data. We really appreciate the reviewer's suggestion to more explicitly consider and discuss an elegant alternative explanation for the patterns observed in our study. We provide a detailed response to the concerns expressed by the reviewer in our response to the specific comments below.

The height effect could be an alternative, if not more likely explanation of the observations, and lack of conclusive proof or at least a critical discussion undermines this study. This study setup compares shrubs with trees for which branch selection did not account for sampling height differences and hence hydraulic pathway length. Shrubs are much shorter and seem to have a much faster hydraulic conductivity as alluded to by the authors' own observations (i.e., shrubs have a more 'wasteful' strategy). Its short hydraulic pathway length and high hydraulic conductivity is conducive to a fast bridging and hence little time-lag between water uptake and that signature to reach the sampling

heights (i.e., hours/days). In contrast, the signature in trees must bridge a much longer hydraulic pathway length and are subject to lower conductivity ('more conservative strategy', low sap flow rates). Strong time delays between water uptake and the signature reaching the height of sampling can be substantial (i.e., multiple days/weeks). This 'height effect' phenomena has been described in detail in various recent studies (i.e., Schwendenmann et al., 2010; Gaines et al., 2016; Evaristo et al., 2019; De Deurwaerder et al., 2020; Magh et al., 2020; von Freyberg et al., 2020; Mennekes et al., 2021; Seeger & Weiler, 2021; Bernhard et al., 2024). Consequently, the signature observed in the shrubs likely reflect a shallow water uptake strategy at the time of measuring. However, this is not necessarily true for trees, which may be subject to long time-lags. It is possible that at the time of sampling, trees are taking up water from shallow soil layers, yet the signature measured by the authors at the branch level simply reflects the water uptake strategy several days/weeks preceding the sampling. Since at that time, the soil gradient may not yet have been established, the observed signatures would show a depleted signature which would erroneously be linked to deeper root water uptake. To me, this seems an equally likely scenario, which unfortunately undermines the entire study. Similarly, the authors should consider that the observations could be impacted by differences in exchange rates and storage capacity of xylem, phloem, and storage water (i.e., Knighton et al., 2020; Bernhard et al., 2024). I would encourage the authors to, at the minimum, discuss these likely scenarios in the discussion of the manuscript, as omission could present a misleading story.

Response: In the revised manuscript, we have included a whole new paragraph in the Discussion section that specifically addresses the key issues and concerns expressed by the reviewer regarding data interpretation (L 385 - 410). In this new paragraph, we explain in detail why this alternative interpretation of the data proposed by the reviewer seems unlikely and far less plausible than our own original interpretation. We are fully aware that the considerable hydraulic pathway length for water transport in large trees can lead to substantial time delays (several days or weeks) between soil water uptake by roots and the isotopic signature of this xylem sap reaching the branches sampled for isotope measurement (Bernhard et al., 2024; Evaristo et al., 2019; Schwendenmann, Dierick, Köhler, & Hölscher, 2010; von Freyberg, Allen, Grossiord, & Dawson, 2020). We are also aware that this so-called "height effect" can 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, Cuntz, Beyer, Dubbert, & Kuehnhammer, 2020; Mennekes, Rinderer, Seeger, & Orlowski, 2021). However, please note that the occurrence of such long-time lags between soil water uptake by roots and internal water transport to the sites of twig sampling can be safely ruled out in our study, for the following reasons. First, the average height of the tree individuals sampled in our study was only 8.5 m (ranging from 3 to 20 m), and, which considerably limits the length of the hydraulic pathway for water transport from roots to sampled branches. Second, the high xylem sap velocity typically encountered in trees during the spring growing season under Mediterranean conditions (characterized by high ambient temperature and VPD) favours rapid internal water transport in trees, thereby leading to rather short or even negligible time lags between root water uptake and transport to lower canopy branches. For example, 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 of less than 1-2 days from roots to canopy branches in our Aleppo pine and holm oak trees, which were by far the most abundant and widespread tree species across study sites.

Meinzer et al., (2006) used heat pulses and deuterated water (D_2O) as tracers to characterize whole-tree water transport and storage properties, encountering that xylem sap velocities inferred from heat dissipation measurements were only about 20% of those estimated from transport of the D_2O tracer. They measured deuterium tracer velocities in xylem sap ranging from 390 to 540 cm per day in young Douglas fir trees (*Pseudotsuga menziesii*) growing in the US Pacific Northwest. Moreover, they argued that these values should be considered as conservative estimates of maximum sap velocity because of the potential for tracer loss via lateral diffusion, so they suggested that the true absolute maximum sap velocities were likely even higher than that in their conifer trees (F. C. Meinzer et al., 2006). Likewise, Gaines et al., (2016) measured xylem sap velocities of 230 to 900 cm per day (average 500-600 cm per day) in two oak species growing under temperate forest conditions in Pennsylvania (*Quercus prina* and *Q. rubra*). These authors found no evidence of any relationship between sap velocity and xylem specific conductivity, which varied by nearly an order of magnitude among their target tree species. They argued that the magnitude of the soil-to-leaf driving force for water transport (i.e. the difference between soil matric potential and mid-day leaf water potential, which is largely driven by ambient temperature and VPD) may be the key determinant of sap velocity across all tree species. This would contribute to explain the remarkably high xylem sap velocity values that have been measured in tropical angiosperm trees growing in seasonally dry tropical forest ecosystems (up to 25 m per day; see Meinzer et al., 2006 and references therein). Seeger & 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 distance between roots and the sites of sampling, as was always the case in our own study. Most importantly, distinct ecohydrological niche segregation between trees and co-occurring shrubs was further supported in our study by the highly significant correlation encountered between the species maximum rooting depth and their xylem water isotopic composition across plant life forms (Fig S8). We are particularly grateful to the reviewer for suggesting this additional analysis of the data, which we believe lends further support for our own original interpretation of the data.

On the other hand, we acknowledge that interspecific differences in xylem sapwood hydraulic capacitance and exchange of water between tree internal storage compartments and the transpiration stream could have potentially affected the isotopic composition of xylem water and cannot be completely ruled out in our study (Bernhard et al., 2024; Fabiani, Penna, Barbeta, & Klaus, 2022; Knighton et al., 2020). However, please note that the relative contribution of internally stored water to the tree transpiration stream is thought to be rather small (around 5-10%), although it generally increases markedly with tree size and main trunk volume (F. C. Meinzer et al., 2006; F. C. Meinzer, James, &

Goldstein, 2004; see Figures 13.1 and 13.2 in Scholz, Phillips, Bucci, Meinzer, & Goldstein, 2011). Therefore, tree storage water would be expected to play a rather minor role in our Mediterranean trees of rather modest size and diameter at breast height, especially during the peak of the spring growing season when tree transpiration rates are highest under Mediterranean climate conditions.

Preferentially, the authors should also supplement their study with additional data/insight supporting the hydraulic niche segregations, such as actual rooting profiles and maximal rooting depths, if such data is available.

Response: As suggested by the referee we have carried out an extensive literature search to compile all the published information on the maximum rooting depth of the target species and managed to find information for 48 of the 63 species included in the study (Table S2). As rooting depth data was obtained per species, we then calculated mean $\delta 18O_{xw}$ isotopic composition per species at each site and performed a mixed model to assess the relationship between maximum rooting depth and this isotopic proxy of water uptake depth, including site as random factor. We encountered a highly significant negative correlation between maximum rooting depth and xylem water oxygen isotopic composition across species and sites, indicating that tree species with a deeper rooting system consistently exhibited more negative $\delta 18O_{xw}$ isotopic composition, thereby supporting water uptake from deeper soil layers (Figure S8). Once again, we would like to express our sincere gratitude to the reviewer for suggesting this insightful and smart additional analysis that lends further support for our own interpretation of the data.

Other comments

[Results - section 1 and 2; R181-270]: (1) Vertical soil water source segregation and partitioning among coexisting woody species is widespread in Mediterranean plant communities. And (2) Coordination between belowground water uptake depth and aboveground leaf-level water use traits: the role of phylogeny.

The height effect complicates the interpretation of both sections, which could have an alternative explanation than the ones presented here.

Response: Please see above our response to the previous reviewer's comments on the "height effect".

[Results – section 3; R271] Isotopic water-use traits provide reliable proxies for species water use strategies in 271 Mediterranean plant communities.

This analysis relies on the comparison of data collected in two different years, which according to the authors show contrasting rainfall patterns. However, I argue that this might not be the case and would explain why they observe a 'stable' trend. It is true that the years show a larger precipitation amount during the winter period (Jan-March), but what is the relevance of this difference to measurements performed at the end of April/May? With an analysis based on isotopic signatures, this should be shown that the isotopic and soil moisture content profiles were strongly different by the rainfall events in both years, of which no proof is presented. If these isotope gradients are similar, and the current temperature and water conditions in the soil are the same, why would you

expect anything different? For this reason, I am not convinced that this interpretation is supported by the data.

Response: Thanks for the comment and for offering us the opportunity to better clarify this important aspect of the study, which was not sufficiently well explained in the earlier version of the manuscript. Regarding the influence of rainfall events during late winter-early spring on plant isotopic measurements performed in mid-late spring, it is important to note that year 2022 was one of the wettest of the historical record at all the study sites. In particular, the rainfall amounts received during March (late winter-early spring transition) were unprecedented in the climatic data series and exceeded by far all the previous historical rainfall records for this month across all the sites. This climatic exceptionality of year 2022 had a strong influence on the recharge of the soil profile and water availability especially in deeper layers during the following spring months with less abundant rainfall. According to a recent global meta-analysis study, about 30% of Mediterranean woody plant transpiration relies on recent precipitation stored in upper layers, so water uptake from deeper soil and bedrock moisture sources has an increasingly important role as the dry season progresses and the shallow soil dries out in seasonally dry climates (Bachofen et al., 2024; Miguez-Macho & Fan, 2021).

We agree with the reviewer that a more detailed analysis of the isotopic signals in rainwater and soil water profiles during the wetter year (2022) would have allowed a more rigorous comparison with the patterns encountered in the earlier, drier year (2019). However, we believe that the dramatic difference in precipitation regimes between 2019 (dry or average across sites) and 2022 (exceptionally wet period across sites) during the winter-spring months justifies the comparison of plant water use traits between the two years, and strongly supports our interpretation of the data. Moreover, please note that there was a marked offset in plant isotopic values between study years (see Table 1 below). This marked offset resulted in consistently lower $\delta^{13}C_L$ values in 2022 relative to 2019 across species and sites, as shown by a slope lower than 1 in the mixed model (slope = 0.78, Figure 7; cross-species averages = -28.23 and -28.77 in 2019 and 2022 respectively). 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 pattern was particularly strong and apparent for plants growing at the most arid sites (Pulpí, Pliego), and much weaker for plants growing at the most humid site (Montejo) (Table 1). This interpretation is further supported by the much lower leaf $\delta^{18}O_L$ values encountered in the exceptionally rainy year of 2022 at all the 5 pure Mediterranean sites (Table 1), 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). Furthermore, $\delta 18O_{xw}$ values were also less enriched in 2022 compared to 2019 across all sites, as shown by a slope lower than 1 in the mixed model (slope = 0.58, Figure 7) and more negative $\delta 18O_{xw}$ values in 2022 (cross-species averages= -4.82 and -5.43 in 2019 and 2022 respectively). This pattern suggests that much greater rainfall amounts and soil water availability combined with more humid ambient conditions (higher relative humidity, lower VPD) likely led to weaker evaporative isotopic enrichment of the source water used by plants in 2022 (wetter year) across sites, compared to the earlier, drier year

(2019). Lastly, leaf $\Delta^{18}O_L$ values did not show a consistent trend between years across sites (Table 1), likely due to the large differences in $\delta 18O_{xw}$ between sites and years.

Overall, and considering all of the above, we still believe that it is quite remarkable noteworthy that within-site species rankings in isotopic traits were maintained so well across sites between years with such contrasting climatic conditions, despite the marked offsets between years, which in our view lends very strong support to the claims made in L 307 - 323 of the Results and Discussion sections.

Table 1. Mean ± standard error per site for all isotopic traits in 2019 and 2022 (2021-2022 in Montejo). Total mean ± standard error also showed 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

[Discussion; 307] At this point, I have reservations with the interpretation of the observations, and whether the presented data can conclusively support the claims made by the authors. This discussion section lacks a more critical assessment or reflection on potential alternative hypotheses (see main comment).

Response: The suggested alternative explanation has been carefully considered and extensively discussed in a whole new paragraph in the revised manuscript (L 385 – 410), as explained above in response to previous comments.

[Figures]

Please be mindful that some of the figure labels (i.e. Fig S3) are presented in Spanish, which could present a language barrier for your audience

Response: Thanks for noticing this mistake, in the revised manuscript we have translated all the figure labels to English.

Bibliography

Ackerly, D. D. (2004). Functional Strategies of Chaparral Shrubs in Relation to Seasonal Water Deficit and Disturbance. *Ecological Monographs*, *74*(1), 25–44.

Bachofen, C., Tumber-Dávila, S. J., Mackay, D. S., McDowell, N. G., Carminati, A., Klein, T., … Grossiord, C. (2024). Tree water uptake patterns across the globe. *New Phytologist*. https://doi.org/10.1111/NPH.19762

Bernhard, F., Floriancic, M. G., Treydte, K., Gessler, A., Kirchner, J. W., & Meusburger, K. (2024). Tree- and stand-scale variability of xylem water stable isotope signatures in mature beech, oak and spruce. *Ecohydrology*, *17*(2), 1–20. https://doi.org/10.1002/eco.2614

Cohen, Y., Cohen, S., Cantuarias-Aviles, T., & Schiller, G. (2008). Variations in the radial gradient of sap velocity in trunks of forest and fruit trees. *Plant and Soil*, *305*(1–2), 49–59. https://doi.org/10.1007/s11104-007-9351-0

Davis, S. D., & Mooney, H. A. (1986). Water use patterns of four co-occurring chaparral shrubs. *Oecologia*, *70*(2), 172–177. https://doi.org/10.1007/BF00379236

de Deurwaerder, H. P. T., Visser, M. D., Detto, M., Boeckx, P., Meunier, F., Kuehnhammer, K., … Verbeeck, H. (2020). Causes and consequences of pronounced variation in the isotope composition of plant xylem water. *Biogeosciences*, *17*(19), 4853–4870. https://doi.org/10.5194/bg-17-4853-2020

Evaristo, J., Kim, M., van Haren, J., Pangle, L. A., Harman, C. J., Troch, P. A., & McDonnell, J. J. (2019). Characterizing the Fluxes and Age Distribution of Soil Water, Plant Water, and Deep Percolation in a Model Tropical Ecosystem. *Water Resources Research*, *55*(4), 3307–3327. https://doi.org/10.1029/2018WR023265

Fabiani, G., Penna, D., Barbeta, A., & Klaus, J. (2022). Sapwood and heartwood are not isolated compartments: Consequences for isotope ecohydrology. *Ecohydrology*, *15*(8). https://doi.org/10.1002/eco.2478

Gaines, K. P., Meinzer, F. C., Duffy, C. J., Thomas, E. M., & Eissenstat, D. M. (2016). Rapid tree water transport and residence times in a Pennsylvania catchment. *Ecohydrology*, *9*(8), 1554–1565. https://doi.org/10.1002/eco.1747

Jacobsen, A. L., Pratt, R. B., Davis, S. D., & Ewers, F. W. (2008). Comparative community physiology: Nonconvergence in water relations among three semi-arid shrub communities. *New Phytologist*, *180*(1), 100–113. https://doi.org/10.1111/j.1469-8137.2008.02554.x

Knighton, J., Kuppel, S., Smith, A., Soulsby, C., Sprenger, M., & Tetzlaff, D. (2020). Using isotopes to incorporate tree water storage and mixing dynamics into a distributed ecohydrologic modelling framework. *Ecohydrology*, *13*(3). https://doi.org/10.1002/eco.2201

Magh, R. K., Eiferle, C., Burzlaff, T., Dannenmann, M., Rennenberg, H., & Dubbert, M. (2020). Competition for water rather than facilitation in mixed beech-fir forests after drying-wetting cycle. *Journal of Hydrology*, *587*(April), 124944.

https://doi.org/10.1016/j.jhydrol.2020.124944

Marshall, J. D., Cuntz, M., Beyer, M., Dubbert, M., & Kuehnhammer, K. (2020). Borehole Equilibration: Testing a New Method to Monitor the Isotopic Composition of Tree Xylem Water in situ. *Frontiers in Plant Science*, *11*(April), 1–14. https://doi.org/10.3389/fpls.2020.00358

Meinzer, F. C., Brooks, J. R., Domec, J. C., Gartner, B. L., Warren, J. M., Woodruff, D. R., … Shaw, D. C. (2006). Dynamics of water transport and storage in conifers studied with deuterium and heat tracing techniques. *Plant, Cell and Environment*, *29*(1), 105–114. https://doi.org/10.1111/j.1365-3040.2005.01404.x

Meinzer, F. C., James, S. A., & Goldstein, G. (2004). Dynamics of transpiration, sap flow and use of stored water in tropical forest canopy trees. *Tree Physiology*, *24*(8), 901–909. https://doi.org/10.1093/treephys/24.8.901

Mennekes, D., Rinderer, M., Seeger, S., & Orlowski, N. (2021). Ecohydrological travel times derived from in situ stable water isotope measurements in trees during a semi-controlled pot experiment. *Hydrology and Earth System Sciences*, *25*(8), 4513–4530. https://doi.org/10.5194/hess-25-4513-2021

Miguez-Macho, G., & Fan, Y. (2021). Spatiotemporal origin of soil water taken up by vegetation. *Nature*, *598*(7882), 624–628. https://doi.org/10.1038/s41586-021-03958-6

Scholz, F. G., Phillips, N. G., Bucci, S. J., Meinzer, F. C., & Goldstein, G. (2011). Hydraulic Capacitance: Biophysics and Functional Significance of Internal Water Sources in Relation to Tree Size. In F. Meinzer, B. Lachenbruch, & T. Dawson (Eds.), *Size- and Age-Related Changes in Tree Structure and Function.* Dordrecht: Tree Physiology, vol 4. Springer.

Schwendenmann, L., Dierick, D., Köhler, M., & Hölscher, D. (2010). Can deuterium tracing be used for reliably estimating water use of tropical trees and bamboo? *Tree Physiology*, *30*(7), 886–900. https://doi.org/10.1093/treephys/tpq045

Seeger, S., & Weiler, M. (2021). Temporal dynamics of tree xylem water isotopes: In situ monitoring and modeling. *Biogeosciences*, *18*(15), 4603–4627. https://doi.org/10.5194/bg-18-4603-2021

von Freyberg, J., Allen, S. T., Grossiord, C., & Dawson, T. E. (2020). Plant and root-zone water isotopes are difficult to measure, explain, and predict: Some practical recommendations for determining plant water sources. *Methods in Ecology and Evolution*, *11*(11), 1352–1367. https://doi.org/10.1111/2041-210X.13461

Response to Reviewer's comments

Reviewer #1 (Remarks to the Author):

The authors have addressed the comments of the reviewers. I recommend acceptance of this revised manuscript.

Jim Ehleringer

Response: We are greatly honoured and thrilled to learn that Prof. Ehleringer recommends publication of our manuscript in Nature Communications. We are also very thankful for the insightful and useful advice he provided to improve the earlier version of our manuscript.

Reviewer #2 (Remarks to the Author):

Thank you for addressing all the previous suggestions/recommendations and including it with more details on the revised version. I have no more questions or concerns about the revised version. I totally understand the full data-sharing policy; however, I believe it is highly recommended to provide the complete code and the data used to ensure full transparency and reproducibility of the results. I recommend the manuscript publication when this revision is done.

Response: We are pleased that we have managed to improve the document with the changes made based on your recommendations. Regarding the open access publication of the full dataset used for this manuscript, please note that we have uploaded a short version of it in an open repository, and we are open to share the full version with other researchers upon reasonable request. At this stage, we are still working in the preparation of additional manuscripts using this same dataset (plus additional data on leaf economic traits), so the open publication of the complete database could potentially compromise the novelty of this research. Once we have completed the publication of the pending manuscripts, we will publish the complete database so that other researchers can make use of it. Finally, all the R programming codes, statistical functions and their structure are referenced and explained in the methods section. Since we have not developed any new codes or functions during this work, we consider that the explanation given is adequate to replicate the methodology in other works.

Reviewer #3 (Remarks to the Author):

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

The authors did a good job in addressing my comments. I especially appreciate the added clarifications in the text and the expansion on the potential impact of 'the height effect'. While I remain skeptical as to whether the height effect can be as easily excluded as suggested by the authors, I acknowledge that the authors did an excellent job in presenting the alternative hypothesis in light of their findings, to the benefit of the

reader. For instance, the speculations on the effective time delay of 1-2 days seem rather optimistic, because such estimates are based on irrigated, ideal conditions at the peak of a plant's sap flow performance. It would be interesting to see the exact integration used by the authors to upscale such sap flow measurements to their own system where drought stress causes water resource partitioning. However, I leave it to the discretion of the editor whether this would be to the merit of the reader.

Overall, I really enjoyed reading this study, and can support the publication of this manuscript.

Response: Thank you very much for the appreciative comments regarding the changes included in the revised manuscript and for your explicit support for publication. We acknowledge that some uncertainties may remain regarding the effect of height on the isotopic composition of xylem water in tree species, and we believe that our paper could certainly provide a good starting point for future work to continue exploring this intriguing question more specifically. However, we believe that the references and examples from the literature that we use to support our interpretation of the data and to discuss the potential influence of the height effect are perfectly valid and relevant, as they reflect very similar environmental conditions to the ones encountered in our own study. In particular, Cohen *et* al. (2008) measured xylem sap flow velocities of at least 20 to 30 cm per hour in two iconic Mediterranean tree species that were widespread across our study sites (*Pinus halepensis* and *Quercus rotundifolia*), and these xylem sap flow measurements were conducted in the field under very similar, non-irrigated natural conditions in Israel. In our study, stem samples were collected from branches at a maximum height of 5 metres above ground level, or even less in the two tree species mentioned (3-4 m height). However, even when considering 5 metres height and the sap flow velocities measured by Cohen *et* al (2008) as a reference, xylem water could potentially move from ground level to the sampling point within 16-25 hours. In our study, stem samples were always collected at the peak of growing season at each study site, when the soil water content is still relatively high (especially in subsurface soil layers), which should favour a much faster sap flow velocity than during the subsequent dry summer in Mediterranean-type ecosystems. Nonetheless, we agree with the reviewer that the height effect and its potential influence on the xylem water isotopic composition of upper canopy branches in Mediterranean woody vegetation remains an intriguing question that should be specifically addressed in future studies.

Round 1, Reviewer 3 Attachment:
Review of the manuscript by Muñoz-Gálvez and co-authors: "**Trait coordination and trade-offs constrain the diversity of water use strategies in** 1 **Mediterranean woody plants**".

Short description: Muñoz-Gálvez and co-authors use an incredible dataset of soil and stem water isotopic signatures to study the water use uptake strategies of lignified shrubs and trees in multiple plots in the Mediterranean region. The authors suggest that within Mediterranean plant communities, larger woody species extract water from deeper soil layers coupled to a much more conservative water use strategy than observed for shrubs. The latter exhibit a much more wasteful strategy with the extraction of water from shallower soil layers. The authors link such distinct strategies to a difference in coupling between above and below ground water use traits, leading to the establishment of ecohydrological niche segregation.

Appreciation and main comment: I appreciate the ambitious nature, and the incredible dataset collected for this study. Sampling 771 individuals belonging to 10 different Mediterranean ecosystems is indeed a truly admirable feat and could be a great addition to the scientific community interested in the use of stable water isotopes. However, this specific scientific community has seen a strong increase in studies scrutinizing the use and interpretation of stable water isotopes in the last years (Schwendenmann *et al.*, 2010; Gaines *et al.*, 2016; Evaristo *et al.*, 2019; Barbeta *et al.*, 2020; von Freyberg *et al.*, 2020; De Deurwaerder *et al.*, 2020; Knighton *et al.*, 2020; Magh *et al.*, 2020; Marshall *et al.*, 2020; Seeger & Weiler, 2021; Mennekes *et al.*, 2021; Bernhard *et al.*, 2024, and others). It is therefore surprising that this voice of concern is not mentioned in the manuscript. It is particularly concerning as these studies uphold an alternative, but equally elegant explanation of the presented observations: defined as 'the height effect', i.e., a difference in lag-time due to differences in transportation length along the stem.

The height effect could be an alternative, if not more likely explanation of the observations, and lack of conclusive proof or at least a critical discussion undermines this study. This study setup compares shrubs with trees for which branch selection did not account for sampling height differences and hence hydraulic pathway length. Shrubs are much shorter and seem to have a much faster hydraulic conductivity as alluded to by the authors' own observations (i.e., shrubs have a more 'wasteful' strategy). Its short hydraulic pathway length and high hydraulic conductivity is conducive to a fast bridging and hence little time-lag between water uptake and that signature to reach the sampling heights (i.e., hours/days). In contrast, the signature in trees must bridge a much longer hydraulic pathway length and are subject to lower conductivity ('more conservative strategy', low sap flow rates). Strong time delays between water uptake and the signature reaching the height of sampling can be substantial (i.e., multiple days/weeks). This 'height effect' phenomena has been described in detail in various recent studies (i.e., Schwendenmann *et al.*, 2010; Gaines *et al.*, 2016; Evaristo *et al.*, 2019; De Deurwaerder *et al.*, 2020; Magh *et al.*, 2020; von Freyberg *et al.*, 2020; Mennekes *et al.*, 2021; Seeger & Weiler, 2021; Bernhard *et al.*, 2024). Consequently, the signature observed in the shrubs likely reflect a shallow water uptake strategy at the time of measuring. However, this is not necessarily true for trees, which may be subject to long time-lags. It is possible that at the time of sampling, trees are taking up water from shallow soil layers, yet the signature measured by the authors at the branch level simply reflects the water

uptake strategy several days/weeks preceding the sampling. Since at that time, the soil gradient may not yet have been established, the observed signatures would show a depleted signature which would erroneously be linked to deeper root water uptake.

To me, this seems an equally likely scenario, which unfortunately undermines the entire study. Similarly, the authors should consider that the observations could be impacted by differences in exchange rates and storage capacity of xylem, phloem, and storage water (i.e., Knighton *et al.*, 2020; Bernhard *et al.*, 2024). I would encourage the authors to, at the minimum, discuss these likely scenarios in the discussion of the manuscript, as omission could present a misleading story. Preferentially, the authors should also supplement their study with additional data/insight supporting the hydraulic niche segregations, such as actual rooting profiles and maximal rooting depths, if such data is available.

Other comments

[Results - section 1 and 2; R181-270]: (1) *Vertical soil water source segregation and partitioning among coexisting woody species is widespread in Mediterranean plant communities.* And (2) *Coordination between belowground water uptake depth and aboveground leaf-level water use traits: the role of phylogeny.*

> The height effect complicates the interpretation of both sections, which could have an alternative explanation than the ones presented here.

[Results – section 3; R271] *Isotopic water-use traits provide reliable proxies for species water use strategies in* 271 *Mediterranean plant communities.*

> This analysis relies on the comparison of data collected in two different years, which according to the authors show contrasting rainfall patterns. However, I argue that this might not be the case and would explain why they observe a 'stable' trend. It is true that the years show a larger precipitation amount during the winter period (Jan-March), but what is the relevance of this difference to measurements performed at the end of April/May? With an analysis based on isotopic signatures, this should be shown that the isotopic and soil moisture content profiles were strongly different by the rainfall events in both years, of which no proof is presented. If these isotope gradients are similar, and the current temperature and water conditions in the soil are the same, why would you expect anything different? For this reason, I am not convinced that this interpretation is supported by the data.

[Discussion; 307]

> At this point, I have reservations with the interpretation of the observations, and whether the presented data can conclusively support the claims made by the authors. This discussion section lacks a more critical assessment or reflection on potential alternative hypotheses (see main comment).

[Figures]

Please be mindful that some of the figure labels (i.e. Fig S3) are presented in Spanish, which could present a language barrier for your audience.

References

Barbeta A, Gimeno TE, Clavé L, Fréjaville B, Jones SP, Delvigne C, Wingate L, Ogée J. **2020**. An explanation for the isotopic offset between soil and stem water in a temperate tree species. *New Phytologist* **227**: 766–779.

Bernhard F, Floriancic MG, Treydte K, Gessler A, Kirchner JW, Meusburger K. **2024**. Tree- and stand-scale variability of xylem water stable isotope signatures in mature beech, oak and spruce. *Ecohydrology* **17**: 1–20.

De Deurwaerder HPT, Visser MD, Detto M, Boeckx P, Meunier F, Kuehnhammer K, Magh RK, Marshall JD, Wang L, Zhao L, *et al.* 2020. Causes and consequences of pronounced variation in the isotope composition of plant xylem water. *Biogeosciences* **17**: 4853–4870.

Evaristo J, Kim M, van Haren J, Pangle LA, Harman CJ, Troch PA, McDonnell JJ. **2019**. Characterizing the Fluxes and Age Distribution of Soil Water, Plant Water, and Deep Percolation in a Model Tropical Ecosystem. *Water Resources Research* **55**: 3307–3327.

on Freyberg J, Allen ST, Grossiord C, Dawson TE. **2020**. Plant and root-zone water isotopes are difficult to measure, explain, and predict: Some practical recommendations for determining plant water sources. *Methods in Ecology and Evolution* **11**: 1352–1367.

Gaines KP, Meinzer FC, Duffy CJ, Thomas EM, Eissenstat DM. **2016**. Rapid tree water transport and residence times in a Pennsylvania catchment. *Ecohydrology* **9**: 1554–1565.

Knighton J, Kuppel S, Smith A, Soulsby C, Sprenger M, Tetzlaff D. **2020**. Using isotopes to incorporate tree water storage and mixing dynamics into a distributed ecohydrologic modelling framework. *Ecohydrology* **13**: e2201.

Magh R-K, Eiferle C, Burzlaff T, Dannenmann M, Rennenberg H, Dubbert M. **2020**. Competition for water rather than facilitation in mixed beech-fir forests after drying-wetting cycle. *Journal of Hydrology* **587**: 124944.

Marshall JD, Cuntz M, Beyer M, Dubbert M, Kuehnhammer K. **2020**. Borehole equilibration: testing a new method to monitor the isotopic composition of tree xylem water in situ. *Frontiers in Plant Science* **11**: 358.

Mennekes D, Rinderer M, Seeger S, Orlowski N. **2021**. Ecohydrological travel times derived from in situ stable water isotope measurements in trees during a semi–controlled pot experiment. *Hydrology and Earth System Sciences Discussions* **2021**: 1–34.

Schwendenmann L, Dierick D, Köhler M, Hölscher D. **2010**. Can deuterium tracing be used for reliably estimating water use of tropical trees and bamboo? *Tree Physiology* **30**: 886–900.

Seeger S, Weiler M. **2021**. Temporal dynamics of tree xylem water isotopes: In-situ monitoring and modelling. *Biogeosciences Discussions* **2021**: 1–41.