

Anonymous Referee #2

We thank Referee #2 for reviewing our manuscript. We answer the comments below (in blue) and show how we will revise the paper based on this thoughtful input.

This study explores the role of soil and plant water status, evaporative demand and root distribution on the water use dynamics of two heavily-equipped, willow trees (*Salix viminalis*) installed in isolation within a buried lysimeter. Plant water deficit (noted ΔW , where $\Delta W=0$ means that maximum daily stem radius has not shrunk since the previous day, and that plant water status is “optimal”) is estimated from a micro-dendrometer at stem base. Soil water status is estimated from soil tensiometers at 5 soil depths, evaporative demand and tree transpiration is retrieved from lysimeter and sap flow data, and tree water origin is retrieved from water isotope ($^2\text{H}/^1\text{H}$ and $^{18}\text{O}/^{16}\text{O}$) tracing techniques and statistical mixing models, using three potential water sources: top (0- 50cm) and deep (50-150cm) bulk soil water, and mobile soil water (extracted at all depths using ceramic cups at 600hPa). The authors identify three distinct periods of plant water status (optimal, sub-optimal and transient) and find that, at least during periods when plant water status is sub-optimal ($\Delta W>0$), vertical variations in soil water matric potential, more than root distribution, explains the origin of tree water. Observing as well that soil water potential is the main explanatory variable of variations in ΔW , they conclude: “plant water status drives tree source water partitioning”.

First of all, I am not comfortable with the connection made between plant and soil water status. The two are linked of course but the results mostly show that plants take up soil water where it is available. The fact that the water uptake distribution may change between periods of contrasted plant water status is only because the soil water distribution also changes between these three periods.

We thank the Referee for raising this point. We would defend our approach by emphasizing that the connection between plant water status investigated with the use of dendrometers (and computed as tree water deficit) and soil water status has been previously explored in the literature in field conditions (Drew et al., 2011; Kocher et al., 2013; Oberhuber et al., 2015; Zweifel et al., 2005, 2016; Fan et al., 2019 and others). However, how this information can be used as a tool to understand plant water uptake has not been explored before. This is, in our opinion, one of the crucial point of this paper. We also want to recall that species differ markedly in the ways they adjust water balance (supply - loss) in response to environmental conditions (i.e. soil water availability). These distinct adjustments (i.e. via stomatal conductance, use of stored water – stem capacitance) reflect in different water status even when species coexist under same conditions, as shown in the literature. Therefore, plant water status has species-specific response and it is not just a function of soil conditions. If we define ‘dry’ or ‘wet’ conditions based on soil alone we are biased towards soil conditions, and we ignore species traits that drive water uptake and define extraction limits across changes in soil water potential (Sperry et al., 1998). Species-specific physiological responses are largely overlooked in ecohydrological investigations of plant water use. The use of metrics that allow

us to continually monitor physiological responses to water availability, such as tree water deficit provides opportunity to overcome limitations imposed by stable isotopes alone (i.e. uncertainty related to fractionation) and better understand species response to patterns in water availability.

Now, the idea that plants take up soil water where it is available is not too surprising, especially in trees where fine root length density is relatively high and well distributed across the soil horizons (Figure 7). Theories of soil water uptake by plants can explain this pattern (Cowan, 1965; Javaux et al. 2008). Even when fine root length density is not well distributed, root water uptake will depend mostly on the soil water status. This is because root water uptake increases with the soil-to-root water potential difference and decreases with the hydraulic resistance across the rhizosphere, the root endodermis and along the xylem network. In a drying soil, this network of hydraulic resistances is often dominated by the resistance through the rhizosphere, that depends on fine root density but mostly on soil hydraulic conductivity and thus soil water potential.

We agree that observing that plants take up soil water where it is available may not be surprising. However, we show that this uptake is dynamic and can be observed by hydrometric measurements in the plant, other than stable isotopes alone. The plasticity of plant water uptake from different soil depths has been difficult to identify in field conditions (i.e. lack of knowledge of boundary conditions) and the mechanisms remains poorly understood. We use measurements that represents plant adjustment to fluxes and water availability over time to improve mechanistic understanding in shift in water uptake given limitations in stable isotopes (i.e. uncertainties regarding to fractionation, water extraction bias, labour intensive). Our data suggests that the shift in water uptake is related to plant water stress, which we would not be able to determine by simply looking at timeseries of soil moisture at different depths. The theories and models in the literature that supports compensation mechanisms in water uptake is in our discussion (4.1 – Lines 313-317, including Javaux et al., 2008). We have also explained in our manuscript the dependency in hydraulic resistance that increases in drying soils and dependence on water potential gradient between roots and soil (Line 348-364). However, soil hydraulic conductivity cannot explain patterns of plant water uptake alone. Previous studies that have investigated shifts in water uptake among different species using stable isotopes showed that not all species change the depth of uptake with changes in soil water availability (e.g. Ellsworth and Sternberg 2015; Volkmann et al., 2016; Antunes et al., 2018). Despite similar root distribution, the distinct source water partitioning during drought conditions among species has been suggested to be associated with species-specific hydraulic traits (Volkmann et al., 2016). Plants respond differently to changes in matric potential through the soil profile (that depends for example on species hydraulic safety margins and stem capacitance). The shift in uptake is not only a metric of soil conditions, but also of the plant's ability to decrease water potential while maintaining xylem within safety margins, and hydraulic conductivity. Thus, monitoring plant water status that captures species-specific adjustments to changes in water availability using isotopes may help us understand why we see distinct patterns of plant water

use. We will change the introduction and discussion in the revised manuscript to better reflect the current understanding in the literature and address the points raised by this comment.

Also the three periods identified by the authors are quite arbitrary. They could also correspond to periods of beginning of stem growth ($\Delta W=0$), growth ($\Delta W \geq 0$) and no growth ($\Delta W > 0$), or little rain but high water content ($\Delta W=0$), more intermittent rain and (deep) soil water deficit ($\Delta W \geq 0$) and no rain and higher (top) soil water deficit (until heavy rain comes) ($\Delta W > 0$). Ideally we would want to study the relationship between plant water sources and plant (or soil) water status on a more continuous basis, a bit like the relationship found between ΔW and sap flux (I guess mostly a result of an increase in both evaporative demand and functional sapwood area during periods of positive ΔW). Having only one sampling day for each period is a bit limiting to draw definite conclusions about how plant water uptake varies over the season.

We respectfully disagree that the three periods are arbitrary, as they are based on methods described in the literature to compute tree water deficit (ΔW). Tree water deficit (ΔW) over multiple days provides information on plant water status in response to environmental drivers. Despite the daily variability in stem shrinkage, the offset in stem radius in relation to its fully hydrated state can last longer if the plant is under water stress, as defined by Zweifel et al., 2016. Thus, multiple consecutive days of water deficit indicate periods of water stress (which we define as 'water deficit' $\Delta W > 0$; or what could be defined simply 'dry'). Conversely, 'no deficit' ($\Delta W=0$; or simply 'wet') periods are defined as periods when we did not observe any tree water deficit, meaning that tree water relations are balanced overnight and stem returns to fully saturated stages over many days. The intermediate state, where equal days of deficit and no deficit occur intermittently, is defined as the "intermittent" water deficit period.

We agree that plant hydraulics and growth are not disconnected processes, and there is evidence in the literature that shows trees will not grow under periods of water stress because of the tight relationship between xylem water potential and cambial cell expansion (Boyer and Silk, 2004; Steppe et al., 2006; Korner et al., 2015). The method we used to define tree water deficit also supports this evidence. However, we do not think that other factors can explain the observed ΔW patterns. The possible alternative suggested by the Referee that ΔW dynamics could be related to "*beginning of stem growth ($\Delta W=0$), growth ($\Delta W \geq 0$) and no growth ($\Delta W > 0$)*" is inaccurate. Despite the fact that growth (GRO, as defined in the manuscript) is tightly linked to the definition of ΔW , this interpretation does not explain the observed shrinkage in stem diameter in relation to stem fully saturated stage (water-related shrinkage) during 'no growth'. Second, growth patterns alone do not explain the variability observed in the intermittent period, as we observed stem diameter swelling related to changes in water content. As per the alternative "*little rain but high water content ($\Delta W=0$), more intermittent rain and (deep) soil water deficit ($\Delta W \geq 0$) and no rain and higher (top) soil water deficit (until heavy rain comes) ($\Delta W > 0$)*", our soil tension data shows that there is not a simple and unequivocal relationship between moisture and plant water status. Alternative definitions do not have a clear physiological interpretation regarding water stress as the method applied here.

We do acknowledge lack of clarity in our methodology and discussion. Thus, we will incorporate the points discussed here in our revised manuscript. We will also increase the number of sampling days observed per period as required by both referees.

Finally, I do not understand how the different potential water sources are treated. The authors consider only three potential water sources: “mobile” (i.e. “extractable” at a suction of 600hPa) soil water at all depths, bulk “shallow” (0-50cm) soil water and bulk “deep” (50-200cm) soil water. But the bulk water includes the mobile water then. How can the authors argue: “shallow and deep [water samplings] represented water pools that were held under tensions below 600hPa”?

We agree with the reviewer, the current definition of sources is limiting. We had selected sources based on the sampling methodology and statistical difference among end members that were identified using non-parametric Kruskal-Wallis test (not reported). However, during wet periods the mobile and bulk water isotopic signatures did not differ statistically. To address this issue, we will focus on bulk soil water samples and will focus on shallow vs deep sources. Time series visualization of isotopic composition of xylem and sources and preliminary Bayesian mixing model analysis show that the use of bulk water as end-members (shallow and deep) continues to indicate the larger use of water from deep layers during the period of water deficit. We also observed some variability in the partitioning among the different days within the same period which will further be discussed. We will include the isotopic composition of the soil profile in relation to xylem water to provide more evidence and understanding of the patterns observed.

In conclusion, I find the experimental work carefully designed and of overall very good quality but the amount of sampling campaign for water isotope analysis is a bit limiting, the interpretation of the results is a bit problematic and the overall conclusions are mostly confirmatory.

We thank Referee #2 for the positive evaluation of our experimental work. We agree that the current manuscript is somewhat limited by the small sample size. We will revise our results based on all the available raw data (rather than the summary findings as presented in the original draft). We will conduct new analyses based on the updated definition of water sources and address Referee #1 concerns about potential overlap in signatures of the two different water pools. Here again, we will let the data and analysis speak—and not be so definitive in our statements about what exactly is going on—but rather, discuss how different processes may be occurring based on these different perspectives discussed above and uncertainties of our data set. We will therefore tone down the definitiveness of our findings and explore openly other possible explanations, and do our best to defend which one we think is consistent with other findings. However, we do maintain our opinion that our results and conclusions are not confirmatory. Our study goes beyond what has been reported or investigated previously in the ecohydrology (plant water source investigations) literature. We use knowledge of plant water status (current physiological response to environmental conditions) simultaneously with stable

isotopes to investigate patterns of plant water use. There is a gap in the current mechanistic understanding of what drives the partitioning of sources. We show that incorporating measurements that have a physiological understanding of plant response to environmental conditions at time of uptake could fill part of this gap. Despite the broad application of stable isotopic data to estimate root water uptake strategies, several aspects of this methodology are limiting (i.e. uncertainty with fractionation processes, low sample size, labor intensive). Thus, a combination of approaches is crucial. We will also do a better job at clarifying how our work advances the logical progression in ecohydrological separation literature—and therefore, is an important and useful contribution in moving this body of knowledge forward. Measures of plant water status that uses relative water content (i.e. tree water deficit) provides an integrative approach on the understanding of plant response to drought and incorporates temporal response to changes in environmental conditions (Martinez-Vilalta et al., 2019).

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