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Reply on RC1

Ana M. Sabater et al.

Author comment on "Technical note: Validation of Aleppo pine transpiration rate measurements using the heat ratio method under laboratory conditions" by Ana M. Sabater et al., Hydrol. Earth Syst. Sci. Discuss., https://doi.org/10.5194/hess-2022-328-AC1, 2022

RC1: 'Comment on hess-2022-328', Anonymous Referee #1

The manuscript makes a substantial contribution to scientific progress because it attempts to validate an already published methodology to measure transpiration in Aleppo Pine and to make its extrapolation to forests easier. The manuscript is well-written and despite being a technical/methodological manuscript, it is easy to follow in all its sections. Moreover, it includes the study of the response to environmental variables (VPD and REW) which gives extra information of validation. It also recognizes the weakness of the study, which is the main problem of this novel manuscript from my point of view.

The validation of the method has been done only in three juvenile individuals, which is clearly not enough for the serious validation of any method. Besides, it has been done in juveniles, and not in adults, so intraspecific variability is expected to be higher than in adults. This would compromise the results, especially if we consider that the manuscript highlights the relevance of this study to scale transpiration in trees to a forest level, even when considering that the environmental conditions could fall within the same range as under field conditions. The length of the study does not compensate this low n, but it is true that it provides the opportunity to test the method under a wider range of environmental conditions. I suggest adding more n, or if this not possible, including another complementary study in adults with at least 6-10 adults. If this is not possible, I suggest rewriting some paragraphs or phrases so that the results match the facts. For example, excluding the word validation, recognizing and/or showing variability among juveniles....

Reply: *Dear referee,*

Thank you very much. We appreciate your comments and suggestions, which have been taken into account to improve the new version of the article.

As you mention, we have attempted to establish a test to improve and gain accuracy in the measurements involving the sap flow methodology, specifically the HRM method with Mediterranean trees of typically low transpiration rates. As you suggest, we have rewritten some paragraphs and contents to appropriately make our conclusions and results adequate to the initial preconceived experimental design. We agree that employing only three juvenile trees is a low replication design, which could not be further implemented for

structural/technical reasons (i.e. greenhouse space and design, limited facilities for transpiration controllers using more load cells, etc.). In addition, although trees presented functional variability, the results provide consistently enough evidence that the same procedure could be used in them all (see Sect. Appendix D for the tree variability analysis). We also measured several functional tree traits to analyze and take into account as much tree variability that we could include. In relation to your suggestions, now we explicitly state in the manuscript that trees were juveniles (lines of changes described in some comments below). Also, we have extensively used the terms "test" throughout the document instead of "validation" or "calibration", which require a more extensive replication methodology. Secondly, by adding Figure D2 and Table 2, we show that the transpiration of all the individuals was basically controlled by the interaction between VPD and REW despite their intraspecific variability. We also think that variability in juvenile individuals could not be weak point, but a strong point. This means that despite variability, the HRM is able to estimate high-quality transpiration rates accurately. Thirdly, we realise that using long experimental period, selecting a half-hourly basis, and the resulting load-cell high precision and resolution, confer the study remarkable strength. We also implemented low-speed fans inside the greenhouse for climatic homogenisation purpose, which allowed to maintain the three juvenile pines in their fixed positions for very stable measurements.

There are other reasons that support my suggestion of a major review.

There is no clear information of how much time plants were growing until the experiment started and under which environmental conditions they grew. We only know that plants were located in the experimental area 1 week before the experiment began (acclimation).

Reply: *Pine trees were acquired from a commercial nursery and were grown according to standard nursery protocols, which means moderate fertilisation and watering according to requirements. We have included a paragraph in the article that explains tree size (lines 136-144).*

Lines 136-144: "Pines were 3 m high, with a basal diameter of 5 cm on average. Pines displayed the same juvenile maturation status and presented a few reproductive structures (cones). Substrate was a commercial material made of a mixture of peat, compost and coconut fibre (1:1 v/v). It was not possible to obtain bigger trees due to limitations with managing such a size (i.e. transportation, space and head load limitations), but also because of container size. Container size was selected to avoid root growth limitations. This size limitation can negatively influence transpiration rates by limiting water absorption capacity during midday transpiration in bigger individuals."

We do not know the environmental conditions of the experimental area. Is it an open area? Is it a greenhouse? In the Abstract, the authors indicate that plants were under a wide range of abiotic conditions, but were they "natural" abiotic conditions or "controlled" ones? More information is needed. after reading App. C, it would seem that they were under controlled conditions, but the authors do not indicate the range/means of these environmental conditions.

Reply: *We already mentioned in Sect. Material and Methods that the experiment was performed in the Plant Experimental Unit, and in Sect. Appendix D that the three juvenile pines were placed inside a greenhouse module. We have added the information about the official website of the Plant Experimental Unit in the manuscript (lines 152-156). In appendix C, we have described explicitly the type of module (lines 496-497).*

The experimental conditions were partially controlled. Soil moisture was the variable that could be better controlled for a certain range (from 0.14 to 0.4 m^3 m^{-3}), together with climatic homogeneity with added ventilation (without generating perceptual currents). However, other variables were more exposed to external conditions, such as temperature and solar radiation, whose control is more complicated. We have added this information in lines 155-160.

We already described the environmental conditions in "Section 3.2. Effects of environmental conditions of the transpiration response". It is true that it is not clear enough in the manuscript. Thus to provide a better understanding, we have added a new table (Table 2) with the range and means, and a time series representation (Fig. C1), and have added more variables, such as global solar radiation.

Lines 149-152. "The greenhouses of the Experimental Unit are equipped with relative humidity and temperature control (see https://sstti.ua.es/es/infraestructuras-de-apoyo/unidad-de-experimentacion-vegetal.html, last accessed: 23 November 2022, Sect. Appendix C)."

Lines 152-156: "The VPD range went from 0.27 to 2.82 kPa. A wider range was not possible due to technical limitations. Soil moisture was better controlled by irrigation system within the wide 0.14 to 0.40 m^3 m^{-3} range. As pines could not be moved for head cell installation reasons, one big fan was installed to homogenise air the conditions and to avoid VPD gradients. Solar radiation followed the daily dynamics for the time of the year."

Lines 496-497: "The three juvenile pines were placed inside a module in a greenhouse (6.4x16x4.5 m, width x length x height)."

Table 2. Environmental condition during the experiment. Abbreviations: VPD: vapour pressure deficit, REW: relative extractable water.

	Minimum	**Mean ± SD**	**Maximum**
VPD (kPa)	0.27	0.78 ± 0.47	2.82
Global solar radiation (W m^{-2})	0.00	109.55 ± 188.02	1012.00

REW

Pine 1	0.20	0.31 ± 0.20	1
Pine 2	0.22	0.32 ± 0.26	1
Pine 3	0.28	0.41 ± 0.25	1

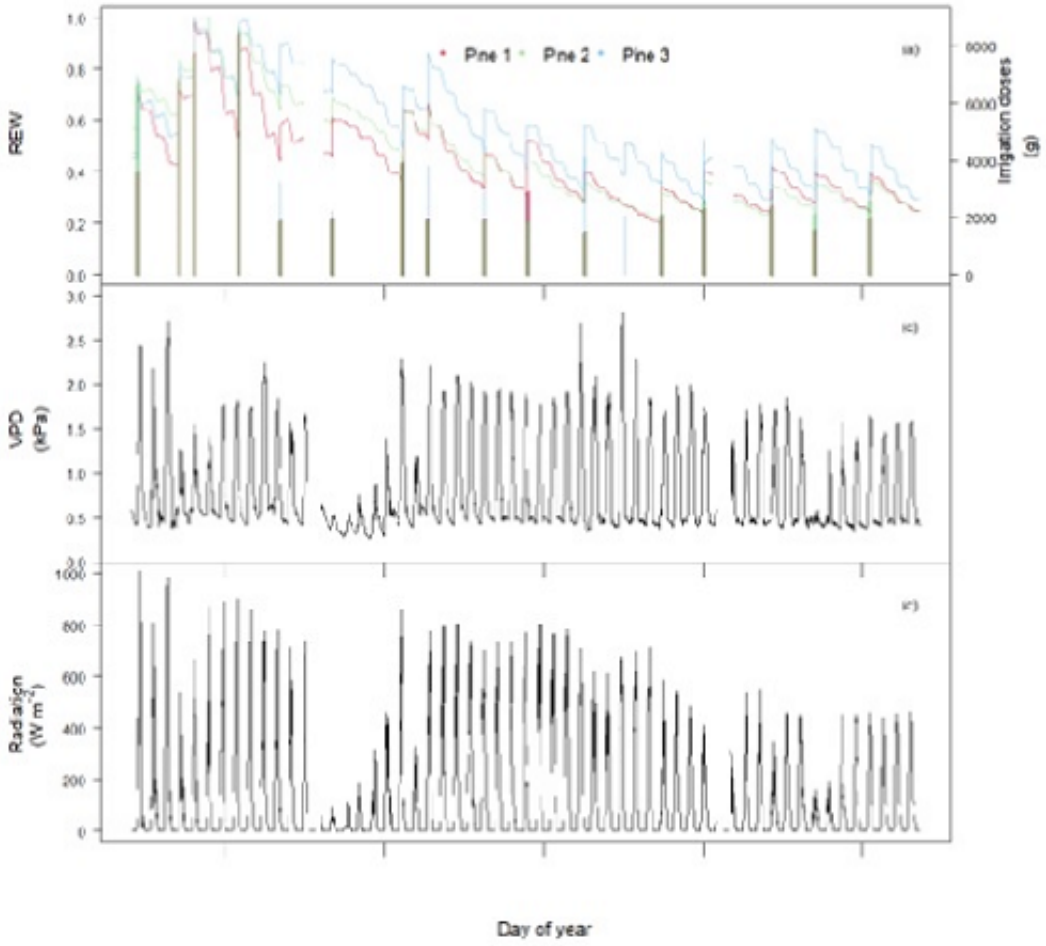

Figure C1. Representation of the transpiration and environmental conditions throughout the study period. Abbreviations: REW: relative extractable water; VPD: vapour pressure deficit.

Could the highest absolute error of lead cella in pine 3 be due to it being another model compared to pines 1 and 2? This point should be justified.

Reply: *Yes it is true that the load cell in pine 3 was another model showing a bigger error (Table 1), but all three load cells had been previously validated. We were sure that they were accurate enough to measure transpiration rates in Aleppo pine.*

Other minor comments:

In the Abstract, the authors generalize the results at the species level. However, this cannot be done because we are talking about juveniles, and only about three juveniles. This should be put into context.

Reply: *We agree. We already clarified that trees were juveniles in the title (Technical note: Testing transpiration rates of juvenile Aleppo pine trees using the heat ratio method under laboratory conditions), Abstract (lines 25-27, 36-39), Introduction (lines 118-121), and Material and methods (lines 134-136 and 137-138). We have also included a specific paragraph in the discussion (lines 369-386). In Sect. Appendix A and Appendix C, we put into context the methodological aspect of the probes to be adapted to juvenile pine individuals (lines 424-425 and 496-497). We do not replace them in all cases to make the reading more fluent.*

Lines 25-27: "This study simultaneously recorded the transpiration rate on juvenile pine trees by the sap flow HRM technique (T_{HRM}) and water losses were measured by load cells (T_{OBS})."

Lines 36-39: "Our results also support the use of probe misalignment correction to estimate transpiration in juvenile Aleppo pines, mainly when high transpiration values are recorded."

Lines 118-121: "This study aimed to: i) test the Aleppo juvenile pine transpiration rates measured by the HRM technique compared to load cells as a direct method taken as the "gold standard" to measure plant transpiration rates under a variety of laboratory environmental conditions"

Lines 134-136: "Intraspecific variation appeared in juvenile pines, but the transpiration of all the individuals was controlled by the interaction between VPD and REW (Figure D2, Table D2)"

Lines 137-138: "Pines displayed the same juvenile maturation status and presented a few reproductive structures (cones)."

Lines 370-386: "On the technical issues of this study, plant material contained only three pines in the juvenile life stage, which can be considered a poor replication design. However, for structural/technical reasons (i.e., greenhouse space and design, facility to control transpiration by load cells), it was not possible to implement higher replication and older trees and, consequently, a bigger size. According to recent review works about calibration experiments, these limitations fall in line with other studies. Not only are the

cost of plant material, time and equipment challenges, but so are transport logistics and performing calibration with increasing tree size (Dix and Aubrey, 2021). The use of big containers was possible, and no root limitations were found in containers due to the size-tree age combination. On the contrary, big trees could pose root limitations for absorbing water and, therefore, an anomalous transpiration response with different water availabilities that are unrelated to the sap flow method. In addition, previous sap flow calibration studies have been done in juvenile trees, and found no effects caused by the followed method (Sun et al., 2012). Although some studies have reported intraspecific variability with individuals' status (Aranda et al., 2012; Delzon and Loustau, 2004), the variability herein observed could not be a weak point, but a strong one for testing the method and misalignment correction under different conditions."

Lines 424-425: "Probes should be adapted to sap wood depth of juvenile pine individuals"

Lines 496-497: "The three juvenile pines were placed inside a module in a greenhouse (6.4x16x4.5 m, width x length x height)."

Any reference to hypothesis i? How do the authors know?

Reply: *We agree with this comment and have added two references at the end of the sentence: Burgess et al., 2001 and Burgess and Dawson, 2008 (lines 123-124).*

Lines 123-124: "i) the thermal response of the HRM method (Burgess et al., 2001; Burgess and Dawson, 2008) will allow high accuracy at low sap flow rates, but the high sap flow rates measured by this technique will globally underestimate transpiration rates."

Do the three pines present the same maturity status?

Reply: *All the individuals were in the same juvenile maturation state, which presented poorly developed reproductive structures (cones). We have added this information in lines 137-138.*

Lines 137-138: "Pines displayed the same juvenile maturation status and presented a few reproductive structures (cones)."

Line 158. Considering trees...they are juveniles, not adult trees....the same happens in some manuscript sections. This might be confusing.

Reply: *We agree. As mentioned in other comments, we already clarified that trees were juveniles in the title (Technical note: Testing transpiration rates of juvenile Aleppo pine trees using the heat ratio method under laboratory conditions), Abstract (lines 25-27, 36-39), Introduction (lines 118-121), and Material and methods (lines 134-136 and 137-138). We have also included a specific paragraph in the discussion (lines 369-386). In Sect. Appendix A and Appendix C, we put into context the methodological aspect of the probes to be adapted to juvenile pine individuals (lines 424-425 and 496-497). We do not replace them in all cases to make the reading more fluent.*

Tables 1, 2 and 3 could be combined to better compare the results.

Reply: *We agree. We have combined Tables 2 and 3 in the new version to better compare the results. However, Table 1 has been left as a separate table because it presents the methodology description.*

Table 3. Summary statistics of the linear model of (a) the transpiration rate measured by the HRM probes ($T_{HRM,}$ kg h^{-1}) according to the transpiration rate measured by load cells ($T_{OBS,}$ kg h^{-1}); (b) the transpiration rates measured by the HRM probes corrected by the probe misalignment correction that Larsen et al. (2020) proposed ($T_{HRM\ MIS,}$ kg h^{-1}) according to T_{OBS}.

	Pine 1	Pine 2	Pine 3
(a) T_{HRM}			
Intercept	-0.030 ± 0.002	-0.026 ± 0.002	0.020 ± 0.002
T_{OBS}	0.853 ± 0.016	0.785 ± 0.014	0.475 ± 0.017
R^2	0.88	0.86	0.50
p-value	< 0.05	< 0.05	< 0.05
(b) $T_{HRM\ MIS}$			
Intercept	-0.002 ± 0.002	-0.012 ± 0.001	0.023 ± 0.002
T_{OBS}	0.871 ± 0.017	0.790 ± 0.014	0.476 ± 0.017

R^2	0.88	0.87	0.50
p-value	< 0.05	< 0.05	< 0.05

Did the authors move plants during the experiment to avoid the effect of being near the fog system generation?

Reply: *No, we did not consider moving pines while running the experiments to avoid any disturbance with inserting the HRM probes or the bias measured by load cells. For this reason, throughout the experimental period, all three juvenile pines were in the same position and on their respective load cell. Furthermore, fog system generation did not affect pine trees because it always operated in combination with ceiling fans to promote good homogenisation conditions (lines 154-156 and 507-508). They were used only for a very limited period during the experiment.*

Lines 154-156: "As pines could not be moved for head cell installation reasons, one big fan was installed to homogenise air the conditions and to avoid VPD gradients"

Lines 507-508: "A big fan was installed to homogenise air conditions and to avoid VPD gradients".

I suggest including in the results the response of each pine to VPD and REW to check any variation in the response to these variables depending on the studied pine. I expect the response to VPD to be high among pines due to not only the intraspecific variation in juveniles, but also to the position in the experimentation area.

Reply: *We have changed Figure 2 and Table 5 to the following Fig.D2 and Table D2. Now, Figure D2 and Table D2 are found in Appendix D following Referee 2's recommendations.*

Fans promoted good homogenisation of air conditions (lines 154-156 and 507-508). Thus VPD should be the same for all the trees and intraspecific variation may be caused by trees' juvenile status. Even though there was intraspecific variation in juvenile pines, the transpiration of all the individuals was controlled by the interaction between VPD and REW. We have added this consideration in lines 369-393.

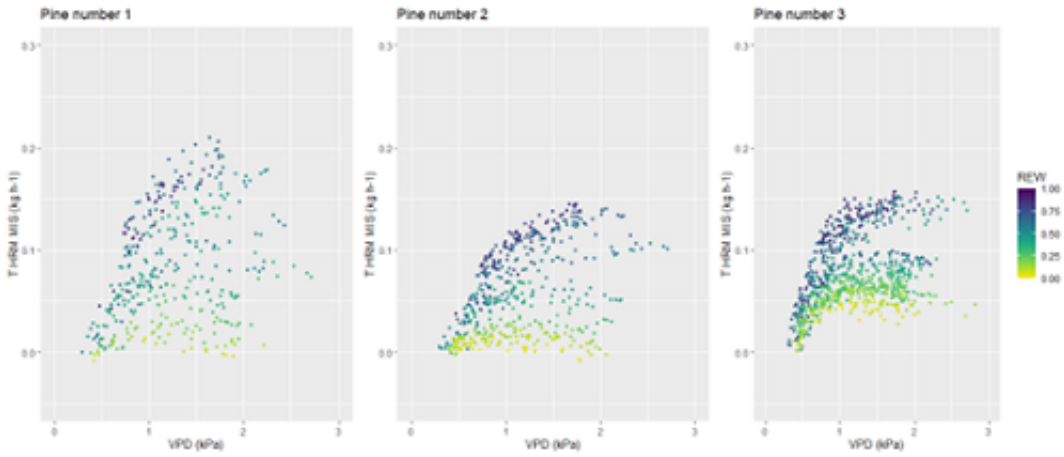

Figure D2. Representation of the response of the transpiration rate measured by the heat ratio method probes and corrected by Larsen's misalignment probes corrections ($T_{HRM\ MIS}$) to the environmental variables interaction (VPDxREW, vapour pressure deficit x relative extractable water) in the three juvenile Aleppo pines. REW is represented as a gradient scale colour, where purple depicts wet conditions and yellow dry conditions. The figure is a graphical explanation of the models in Table D2.

Table D2. Summary statistics of the linear mixed-effects models of transpiration measured by heat ratio method probes and corrected by Larsen's misalignment probes corrections ($T_{HRM\ MIS}$) according to the environmental variables. Pine individuals are fitted as a random factor. Abbreviations: VPD: vapour pressure deficit, REW: relative extractable water. A cross indicates the VPDxREW interaction. An asterisk (*) depicts a p-value lower than 0.05.

$T_{HRM\ MIS}$	Pine number 1	Pine number 2	Pine number 3
Intercept	0.025 ± 0.012 *	0.016 ± 0.004 *	0.032 ± 0.004 *
VPD	-0.027 ± 0.009 *	-0.019 ± 0.003 *	0.001 ± 0.003
REW	0.001 ± 0.025	0.014 ± 0.007	0.008 ± 0.008
VPDxREW	0.165 ± 0.020 *	0.103 ± 0.006 *	0.075 ± 0.006 *

R^2	0.66	0.86	0.66

Lines 154-156: "As pines could not be moved for head cell installation reasons, one big fan was installed to homogenise air the conditions and to avoid VPD gradients"

Lines 507-508: "A big fan was installed to homogenise air conditions and to avoid VPD gradients".

Lines 370-393: "On the technical issues of this study, plant material contained only three pines in the juvenile life stage, which can be considered a poor replication design. However, for structural/technical reasons (i.e., greenhouse space and design, facility to control transpiration by load cells), it was not possible to implement higher replication and older trees and, consequently, a bigger size. According to recent review works about calibration experiments, these limitations fall in line with other studies. Not only are the cost of plant material, time and equipment challenges, but so are transport logistics and performing calibration with increasing tree size (Dix and Aubrey, 2021). The use of big containers was possible, and no root limitations were found in containers due to the size-tree age combination. On the contrary, big trees could pose root limitations for absorbing water and, therefore, an anomalous transpiration response with different water availabilities that are unrelated to the sap flow method. In addition, previous sap flow calibration studies have been done in juvenile trees, and found no effects caused by the followed method (Sun et al., 2012). Although some studies have reported intraspecific variability with individuals' status (Aranda et al., 2012; Delzon and Loustau, 2004), the variability herein observed could not be a weak point, but a strong one for testing the method and misalignment correction under different conditions. From our point of view, the confidence in our results for its use as a test in the Aleppo pine transpiration estimations by the HRM method is reinforced by: (i) transpiration was measured by head cells ("gold standard") with accurate precision; (ii) the long experimental period (57 days); (iii) the resolution of the transpiration rates methodology on a half-hourly basis; (iv) estimating individual-specific functional traits; (v) the range of environmental conditions. In fact the range of environmental conditions (VPD and REW) used in this paper is consistent with the range observed in fieldwork studies."

Please also note the supplement to this comment:
https://hess.copernicus.org/preprints/hess-2022-328/hess-2022-328-AC1-supplement.pdf