Dear Theresa Blume, thank you for spending time on this manuscript and for valuable feedback. We have, has requested, gone through it thoroughly and corrected for typos and unclarities. Below you will find point-by-point answers to your comments and a marked-up version of the manuscript. We look forward to your response.

L21-22: This is confusing. Do you mean that even when misalignment is small it can cause significant shifts/errors in sap flow estimation? Here it sounds like the misalignment errors are small but in contrast your corrections cause big shifts.

Yes, this is what we mean, that the difference in the output can be big although the misplacement is quite small. We have rephrased to make it read more clearly:

L22-23: "We conclude that even small geometrical misalignments in probe placement can create a significant error in sap flow estimations".

L25: Explain what this is.

We have explained this in the text (L149 and L161), and decided to not include this term in abstract, as it can cause confusion as pointed out by the editor.

L60: Did you contact the journal/authors (whoever has the copyright) for the right to reproduce these figures?

Yes, we have the permission from the authors and the journal (open access). We included 'with permission' in the figure legend.

L70: make clear what this is

We decided it would be best to introduce this term in the method section, and have therefore changed the phrase to (L69):

"Attention should be given to check the accuracy of the raw readings, in which the rest of the methodology is built on"

L96-L98: this is confusing/not clear. Do you mean: "As the wood differs between the upslope and downslope sides of the trees, we decided to consistently install all sensors into the upslope side."
I am not really following your statement about the water retention in the soil. Did you measure this? Is this relevant here? Another common criterion is to install the sensors

facing north to decrease solar radiation on the sensors. Were your sensors covered with radiation shields?

We understand that this wasn't clear. We have now emphasised that we are talking about the slope of the ground, and therefore it is relevant with the retention of water in the soil. It was not measured but used to set a criterion for all sensors when installed (L95-L97):

"To establish a criterion that unified the effect of the slope of the ground, with the upper part facilitating the retention of water in the soil, all the sensors were drilled into the uphill side of each tree trunk. All sensors were covered with radiation shields."

L125: Do you mean v1/v2? I do not think this is the common term for this

The v1/v2 ratio is an average ratio of multiple instantaneous ratios taken before the release of a heat pulse and between 60 and 100 seconds. It does not have a common term because it is not usually referred to as it is more common to refer to the averaged ratio (v1/v2) instead. However, the v1/v1 is not a precise term because averages are taken from however many heat ratios one chooses to use. In our case this is 41 (Fig. S1).

In the script (L125-133):

"Marshal (1958) parameterised the heat pulse velocity (V) in the HRM as a function of time (s) following a heat pulse, and the instantaneous ratio of the increase in temperature (°C) (from the temperature prior to the release of the thermal pulse) at the downstream (v_1) and upstream (v_2) from a line heater

$$= \frac{4Kt\ln\left(\frac{v_1}{v_2}\right) - (x_2^2 + y_2^2) + (x_1^2 + y_1^2)}{2t(x_1 - x_2)} \qquad [2]$$

where K is the thermal diffusivity ($cm^2\ s^{-1}$), t is the time passed from the release of thermal pulse in seconds, (x_1, y_1) and (x_2, y_2) are the relative positions (cm) of the thermocouples to the heater (considering x-axis along the xylem and y-axis in the perpendicular direction) (Fig. 2), and v_1, v_2 representing the temperature increases following the heat release".

L139: it is a bit confusing that your x and y axes are the opposite of the standard x = horizontal and y= vertical axes

Yes, we agree that this can seem confusing. We also included an x in figure 1 to make sure that we are talking about the vertical axis when referring to x. For figure 2 we wrote (L136-137):

"The x-axis represents the vertical distance from the heater, while the y-axis accounts for horizontal probe alignment from the heater".

L142: if sensors are installed vertically then y1=y2?

We have included a clarification in the sentence (L140):

"If probes are installed symmetrically, horizontally and vertically, in line with the heater, x = x1 = -x2 and y1 = y2=0."

L146: Make clear what this refers to. This is x1, right? Positive distance of heater to probe?

We have added the description for clarity (L145-L147):

"where ϕ is a priori, a constant only depending on the placement of the probes and on the thermal diffusivity of both the xylem and the material used in the sensors, and x, a positive magnitude representing the distance from the heater element for the two probes".

Combined explanations:

L150: Explain what this is, L159: why instantaneous? L180: Not clear. What do you mean here?

Clarified (L149):

"The $\frac{v_1}{v_2}$ is an average of multiple instantaneous heat pulse ratios (HPR), one for each second of which the temperature is registered, at 60 to 100 seconds after the heat pulse release.".

Combined explanations: L181: is this correct? L263, Figure 3C: why do we have negative slopes? Shouldn't they be positive?

The slope needs to be as close to 0 as possible, it does not mean it has to be positive. However, it can be argued that the threshold we sat of |slope – median (slope)| < 0.003 s^{-1} can vary depending on sensor performance. We clarified in the text as (L180):

"Deviations from an idealized slope of zero, positive or negative, means that the HPR is not being constant with time".

L183: Instantaneous ratios are not mentioned in Burgess et al.

Burgess et al. (2001) uses the $\frac{v_1}{v_2}$, but displays a figure with the relationship between this ratio and time in seconds. Each of those ratios is what we term instantaneous here, to go into detail of its slope and relative standard deviation.

L190-191: This is what you call HPR, or not?

Yes, we simplified accordingly (L192):

"the V1/V2 would be equal to 1 (v1 = v2 in equation 3)".

L203: or y1=y2

Yes! thank you for pointing this out.

Fig3A: but doesn't this figure show all the data, not just the night-time, wet conditions where we assume zero flow?

Yes, it is correct that the figure 3A includes all the data, but the baseline for the whole dataset should have been close to 1 and not somewhere between 1.4 and 1.5 as seen here.

L256: I don't understand this part. Please explain. why are these values eliminated?

Because the $\frac{v_1}{v_2}$ is an average of multiple instantaneous HPRs, the slope of each HPR will demonstrate how close they are to zero, which is what the slope should be if the sensors are perfectly symmetrical.

In the text it's explained accordingly (L183-186):

"The magnitude of the threshold chosen for the slope was taken from a modelled output of $\frac{v_1}{v_2}$ ratios performed by Burgess et al. (2001), were low sap flow velocities (5 cm h-1) combined with a small wound width (0.17 cm) were shown to display a slope of 0.001."

Your axis labels only show 0.01, we need more digits here!

Corrected, thank you.

L260: Is this a week were you assume that we have zero flow at night? Then state that.

No, it is not. I it is a zoomed in panel of a normal week of data. We included:
"Zoomed in panel of HPR data for one week of measurements to better see the diurnal pattern".

L265: You should show this in the plot - give the median and clearly indicate the areas on the plot with the erroneous data.

The read lines indicate the erroneous data. All data below or below these lines are filtered out.

L265: "Red line indicates threshold used for the quality control for this particular sensor. All data above or below these lines are filtered out as they did not correspond to HPR with |slope – median (slope)| < 0.003 s-1. Median slope for this sensor was -0.004"

Table1 and figure 6: Why not give the dates here? Or month and year?

We included the dates in the table description. Adding it directly to the header of the table would alter its format. Same for figure 6.

what do you mean by this?

L333-L336: "Because the HPR is an average of multiple instantaneous temperature ratios, it is difficult to ensure its accuracy only by visual inspection of the averaged output, without further statistical information. For each heat pulse measurement sequence, we suggest RSD and slope from linear regression of the instantaneous ratios versus time, to filter out random ratios and to ensure the quality of the data"

Combined explanations:

L337: What do you mean by this? erroneous? uncertain? Limited is too vague. L338: Not clear. This sentence is incomplete.

We have specified in the text accordingly:

"The HRM only measure values below 45 cm h-1, (Forster, 2017), due to a maximum ratio of v1/v2 = 20, for when it can be assumed accurately (Burgess et al., 2001; Marshall, 1958). Because our dataset showed no higher velocities than 8 cm h-1, it was not initially considered a limitation for the measurements.".

L347: S3 corrected to Fig. S3.

L384-L385: Deleted.

Please find the marked-up manuscript version below.

Technical note on long-term probe misalignment and proposed quality control using the heat pulse method for transpiration estimations

Elisabeth K. Larsen[1,3], Jose Luis Palau[1], Jose Antonio Valiente[1], Esteban Chirino[2] and Juan Bellot[3,4].

[1]Mediterranean Centre for Environmental Studies (Fundacion CEAM), Charles R. Darwin 14, Parque Tecnològico, 46980 Paterna, Valencia, Spain
[2]Faculty of Agricultural Sciences, Lay University Eloy Alfaro of Manabí, Ave. Circunvalación, Vía San Mateo, Manta 130802, Ecuador
[3]Department of Ecology, University of Alicante, Apdo. 99, E-03080 Alicante, Spain
[4]IMEM Ramón Margalef, Department of Ecology, Faculty of Sciences, University of Alicante, 03080 Alicante, Spain

Correspondence to Elisabeth K. Larsen (eli@ceam.es)

1 Abstract

Transpiration is a crucial component in the hydrological cycle and a key parameter in many disciplines like agriculture, forestry, ecology and hydrology. Sap flow measurements are one of the most widely used approaches~~methods~~ to estimate whole-plant transpiration in woody species due to its~~their~~~~its~~ applicability in different environments and in a variety of species, as well as having the capacity of continuous high temporal resolution measurements. Several techniques~~methods~~~~approaches~~ have been developed and tested under different climatic conditions and in different wood properties. However, the scientific literature also identifies considerable ~~several~~~~a number of error~~ sources of error when using sap flow measurements that need~~s~~ to be accounted for~~.~~: probe misalignment~~ of the probes~~, wounding ~~t~~~~o~~~~f the xylem~~, thermal diffusivity and stem water content. This study aims to integrate probe misalignment as a function of time to improve measurements during long-term ~~studies~~ field campaigns (> 3 months). The ~~h~~Heat ratio method (HRM) was chosen because it can assess low and reverse flows. ~~s~~Sensors were installed in four individuals of *Pinus halepensis* ~~during~~for 20 months. Pines were located~~,~~ in a coastal valley in South-Eastern Spain (39º57'45" N 1º8'31" W) ~~within~~characterized by a Mediterranean climate. We conclude that even small geometrical misalignments in the probe placement can create a significant error in sap flow estimations. ~~We conclude that even when geometrical misalignment errors are small, the introduced corrections can generate an important shift in sap flow estimations.~~ Additionally, we propose the need for ~~a~~ new ~~set of~~ statistical information to be recorded during the measurement period, which can be used as a quality control of the sensor output~~heat ratio readings obtained from the sensors~~. Relative standard deviation and slope against time of the averaged $\frac{v_1}{v_2}$~~heat pulse ratio~~, w~~er~~e~~as~~ used as quality indicators.~~, to~~ We conclude~~ing~~ that no general time limit can be decided for the longevity of the sensors but should rather be determined from individual performance over time.

2   Introduction

Plant transpiration is a key process in the hydrological cycle, generally being~~and in forest ecosystems it is often~~ the largest component of total evapotranspiration in forest ecosystems (Schlesinger & Jasechko et al., 2014). However, accurate estimations of transpiration are still difficult to obtain, making~~and~~ field assessments of transpiration estimations ~~are therefore~~ crucial in hydrological planning, as well as in forestry, ecophysiological research and climate forecasting. Sap flow measurements are one of the most widely used ~~techniques~~ approaches to estimate transpiration in woody species as it is readily automated for continuous readings and not limited to single leaf measurements (Forster, 2017; Peters et al., 2018; Flo et al., 2019). Although some sap is stored in the stem and leaves ~~will go to stem and leaf storage~~, the majority ~~it is estimated that~~ (99%~~)~~, is lost through transpiration.~~, and s~~ Sap flow measurements can therefore be used to directly estimate transpiration values (Forster, 2017).

There are a range of techniques used to measure sap flow, with method-families varying ~~There are a range of different approaches to sap flow measurements, and method families vary~~ between heat dissipation (HD) (Granier, 1987; Lu et al., 2004), stem heat balance (SHB) (Langensiepen et al., 2014), trunk segment heat balance (THB) (Smith and Allen, 1996), and heat pulse velocity (HPV) (Marshall, 1958). However, they are all based on tracing heat within the xylem (Burgess et al., 2001; Davis et al., 2012; Forster, 2017). Marshall (1958) developed a theoretical method to determine sap flow from thermal diffusion and dissipation theory of heat pulses in ~~heterogeneous material~~wood. His technique~~theory~~ relies on calculating the heat ratios measured in two parallel thermocouples aligned vertically and symmetrically with respect to a line heater along the direction of the xylem (Fig. 1). Burgess et al. (2001) developed an improved HPV technique, termed 'the heat ratio method' (HRM), based on the Marshall (1958) methodology.~~, which~~The HRM is sensitive to the direction of the sap flow, being able to ~~and is capable of measuring~~~~can to~~ measure low and reverse rates (Burgess et al., 2001). The HRM is therefore more appropriate to use in water-deficit environments were flow rates are low. Burgess et al. (2001) developed two steps of corrections for sap flow calculations by considering probe misalignment and wounding, caused by the implementation of the sensors (Fig. 1). By accounting for these sources of error and additionally estimating the stem moisture content and radial variability, the HRM has been evaluated as the HPV-method with the highest accuracy, although with a tendency of underestimating transpiration values (Forster, 2017), an error that is shown to increase with higher sap flow rates~~values~~ (Steppe et al., 2010).
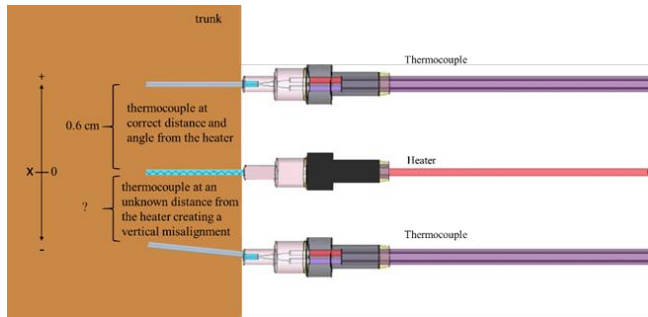
Figure 1. Schematic drawing of the three probes used for the heat ratio method: two thermocouple and one heater. One thermocouple demonstrates the correct placement, aligned vertically and symmetrically at 0.6 cm from the heater. The other thermocouple demonstrates the misalignment: when the probe is closer to the heater and not aligned symmetrically. The zero is marked at the height of the heater, indicating that the placement of the thermocouples are estimated in relation to the heater, at 0.6 cm and -0.6 cm distance, as indicated by the arrows. Sensors are modified from Davis et al. (2012) with permission.

Previous studies have suggested additional solutions for probe misalignment (Ren et al., 2017), for determining thermal diffusivity (Vandegehuchte and Steppe, 2012), and correcting for heterogeneous heat capacity in wood (Becker and Edwards, 1999). However, there is no recent recommendation for how long newly deployed sap flow sensors can be employed. Some studies have shown how sensor probes inserted into the xylem can dampen the signal due to blocking or destruction of vessels (Moore et al., 2010; Wiedemann et al., 2016). One way to account foravoid changes over time has been to reinstall sensors throughout the study period (Moore et al. 2010), interrupting continuous measurements in long-term datasets (Moore et al. 2010)., Furthermore, however there is little information to be found on the exact interval for which sensors should need be reinstalled this needs to happen (Vandegehuchte and Steppe, 2013). , and for continuous measurements this will interrupt the dataset (Moore et al. 2010). Therefore, we aim to find a quality indication that can ensure that the readings do n'not deteriorate over time, or if they do, that it would be detectable. Attention should be given to check the accuracy of the heat pulse ratio itselfraw readingsoutput data, in which the rest of the methodology is built on. In addition, to allow for sensors to be employed over longer periods, it i's necessary to develop a dynamic probe misalignment correction method due to observed changes in probe position over time. The adjustment proposed here, which is built on the calculations of Burgess et al. (2001), is necessary when monitoring transpiration continuously for more than 3 months due to an observed shift in probe placement from one season to another (Fig. 4, 6). Wood properties, the heterogeneity of the xylem, and plant tissue growth might further misplace the sensor after its implementation (Barrett et al., 1995). Burgess et al. (2001) corrected for linear probe misalignment *in situ*. , when an enforced zero sap flow was attained by severing root systems at the end of the experiment. This correction must be effectuated during zero flow. Actual probe placement is therefore suggested to be calculated one time, usually at the end of the experiment when the root system can be severed to enforce zero flow, to correct for the misalignment (Burgess et al., 2001). However, this solution is not suitable for long-term measurements, as the misplacement will change over time (Ren et al., 2017), or when intrusive methods are not an option. Thus, the objective of this research is firstly, to develop a statistical filtering method

to ensure the quality and consistency of the measurements over time, and secondly, to implement a modified version of the probe-placement calculation for sap flow series longer than 3 months.

This technical note is structured in two parts: the first one, dealing with the statistical analysis of long-term time series of averaged heat pulse ratios to ensure the quality of data and their stability over time; the second part, proposing an adaptation of the method developed by Burgess et al. (2001) to introduce a dynamic probe misalignment correction for the HRM. The aim is to obtain a more precise calculation of transpiration by parameterising the probe misalignment as a function of time to correct for the effect of tree growth.

3 Materials and method

3.1 Field site

This study was carried out in at an inland experimental plot located in the Turia river basin, Eastern Spain (39º57'45" N 1º8'31" W), in a Mediterranean climate with continental features. Average annual rainfall is 475 mm year $^{-1}$, average annual maximum and minimum temperature is 15.5 ºC and 4.4 ºC respectively. Sap flow sensors were installed in four pine trees (*Pinus halepensis* Mill.) according to the heat ratio method (HRM, Burgess et al., 2001). Each sap flow sensor consists of three needles; one heater and two thermocouples. We will refer to the thermocouples as probes, and when using the term "sensors" we refer to both probes and the heater. To establish a criterion that unified the effect of the slope of the ground, with the upper part facilitating the retention of water in the soil, all the sensors were drilled into the uphill side of each tree trunk. All sensors were covered with radiation shieldsTo a establish a criterion that unified the effect of the slope on all trees, with the upper part facilitating the retention of water in the soil, all the sensors were drilled into the uphill side of each tree trunk. Since *P. halepensis* has a higher sap velocity average near the cambium with the velocity steady declining nearer to the heartwood (Cohen et al., 2008), sensors were installed at 20 mm depth below the cambium to account for the average sap velocity rates, as estimated by Manrique-Alba (2017). A metal plate was used as a guide during installation to ensure 0.6 cm spacing between the drilleding holes. The selected pines had an average diameter of 24.5 cm at breast height. Continuous measurements were obtained from April 2017 to December 2018.

3.2 Environmental conditions

Air relative humidity (%) and air temperature (ºC) were registered every 30-minute (U23 Pro V2, Onset Computer Corporation, USA). Precipitation was registered using a rain gauge with 0.2 mm resolution (RGR-M, Onset Computer, USA). Three soil moisture probes were inserted at 20-25 cm depth to register soil water content, (SWC.) (S-SND.M005, Onset Computer Corporation, USA), using a datalogger for logging specifications (HOBO Micro Station, USA). To assume periods of zero- sap flow, relative extractable water (REW). was calculated using the method of Bréda et al. (1995):

$$REW = \frac{(\theta_t - \theta_{min})}{(\theta_{max} - \theta_{min})}$$

[1]

Where θ_t is the registered SWC, θ_{min} is the minimum SWC observed during the measurement period, and θ_{max} is the SWC at field capacity. Values surpassing one were converted into one, according to Granier et al. (2000).

3.3 Construction of the sensors

The thermocouples were made after Davis et al. (2012), with a type E junction of chromium and constantan. The wires of the thermocouples were soldered together at temperatures not surpassing 200°C, placed 20 mm inside a micropipette (10μL), and then into a needle (0.12 cm x 4 cm, Sterican, Braun). The heater was made from a constantan wire of 20 mm coiled around a 7 cm long aluminum wire and placed inside the same type of needle as the thermocouples. The wire-ends were then soldered on to an electrical cable. Another resistance of 4.95 ohms wasere then soldered onto to the cable to get a total electrical resistance of 14.95 ohms. The heater was connected through a solid-state relay to a 12 V battery, and deliveringed 7.0 W of power when emitting heat pulses. at the heat pulses. Both thermocouples outputs and heaters were connected to a CR800 datalogger for measurement recording (Campbell Scientific Inc., USA).

3.4 Quality control of heat pulse ratios

Marshal (1958) parameterised the heat pulse velocity (*V*) in the HRM as a function of time (s) following a heat pulse, and the instantaneous ratio of the increase in temperature (ºC) (from the temperature prior to the release of the thermal pulse) at the downstream(v_1) and upstream(v_2), v_1 and v_2 respectively, from a line heater:

$$V = \frac{4Kt \ln\left(\frac{v_1}{v_2}\right) - (x_2^2 + y_2^2) + (x_1^2 + y_1^2)}{2t(x_1 - x_2)}$$

[2]

where *K* is the thermal diffusivity (cm^2 s^{-1}), *t* is the time passed from the release of thermal pulse in seconds, (x_1, y_1) and (x_2, y_2) are the relative positions (cm) of the thermocouples to the line heater (considering x-axis along the xylem and y-axis in the perpendicular direction) (Fig. 2), and v_1, v_2 representings the temperature increases following the heat release.

Con formato: Fuente: (Predeterminada) Times New Roman, Subíndice

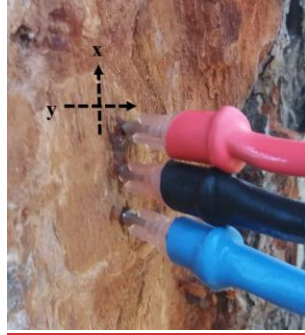Con formato: Fuente: (Predeterminada) Times New Roman, Subíndice

Figure 2. Sensor placements, from top: thermocouple, heater, thermocouple, in an Aleppo pine. The x-axis represents the vertical distance from the heater, while the y-axis accounts for horizontal probe alignment from the heater. The axes should cross where the heater is inserted but was moved for a clearer view of the coordinates.

If probes are installed symmetrically, horizontally and vertically, in line with the heater, $x = x_1 = -x_2$ and $y_1 = -y_2 = 0$, equation 2 simplifies into a function that is not dependent on time:

$$V = \phi \ln\left(\frac{v_1}{v_2}\right)$$

[3]

$$\phi = \frac{K}{x}$$

where ϕ is a priori, a constant only depending on the placement of the probes and on the thermal diffusivity of both the xylem and the material used in the sensors, and x, a positive magnitude representing the distance from the heater element for the two probes

The $\frac{v_1}{v_2}$ is an average of multiple instantaneous heat pulse ratios (HPR), one for each second the temperature was registered for, from 60 to 100 seconds after the heat pulse release. The "perfect symmetry" assumption renders that the heat pulse ratio (HPR) remains constant with time if heat pulse velocity (V), thermal diffusivity (K) and probe positions (in both, x and y directions) have negligible variations during the time following each heat pulse (Marshall, 1958). Burgess et al. (2001) further demonstrated how empirical results initially differed from the ideal approach described by equation 3 due to the blocking of xylem vessels and probe misplacement. However, the study concludes that the heat pulse ratio converges asymptotically at least 60 seconds after the heat pulse release and, for at least, 40 seconds more (until 100 seconds after the heat pulse release), when ratio should be measured because the stable conditions. Our study argues that a visual inspection of heat pulse velocities (V in equations 2 and 3) does not necessarily give enough information to decide if measured values are a good representation of the sap flow. The method does not consider that random ratios can arise, which due to the sensitivity of the method, are likely to occur in practice. On these premises, we have built a methodology to quality check sap flow measurements systematically by introducing a statistical analysis performed on the

instantaneous heat pulse ratio, acquired between 60 and 100 seconds after the heat pulse release. Hereafter, we will denote the averaged heat pulse ratio between 60 and 100 seconds as HPR. The quality check consisted of establishing threshold values for the slope of HPR against time and relative standard deviation (RSD), statistically defined as the standard deviation divided by the mean., and the slope of the HPR. The statistical information obtained would account for any deterioration of the measurement. Burgess et al. (2001) proposed two separately methods to correct for wounding and misalignment. The methods assume that errors arising from the wound inflicted by a sensor probe can be estimated using an empirical factor, whereas a misalignment of the probe needs to be calculated in situ. We propose a development of the misalignment correction method, while arguing that a statistical check of the HPR values would detect a deterioration of the signal caused by a worsening of the wound. This would lead to a smaller sample mean and hence a higher RSD and was The RSD was therefore chosen as a quality-check parameter along with the slope, which was a parameter proposed by Burgess et al. (2001).

3.4.1 Logging specifications

The proposed analysis to ensure the reliability of long time series of sap flow measurements require the storage of statistics that are not usually recorded (as it is considered unnecessary). The datalogger must have a minimum performance of the storage capacity (memory), and the processing speed of the algorithm implemented (especially in the routines related to the statistical calculations to be performed). In this study a CR800 (Campbell Scientific, USA) was used. A flow chart of sampling and data log (Fig. S1) was specifically designed, programmed and implemented in the datalogger to enable the calculationsus that are presented in the next sections of this paper.

We selected RSD (%) and Slope (s^{-1}) of the instantaneous heat pulse ratio versus time, calculated for each of the periods from 60 to 100 seconds to filter out random ratios (Fig. 3). Deviations from an idealized slope of zero, positive or negative, means that the HPR is not being constant with time. The slope should be as close to zero as possible, but any deviation, positive or negative, indicates that the HPR are not constant with time. All HPR with relative standard deviation > 5% and a |slope – median (slope)| < 0.003 s^{-1} were removed. The 5 % threshold was chosen to ensure that 95 % of the dataset could bewas considered in the data processing. The slope median was taken from all slope values obtained during the measurement period. The magnitude of the threshold chosen for the slope was taken from a modelled output of instantaneous ratios performed by Burgess et al. (2001), were low sap flow velocities (5 cm h^{-1}) combined with a small wound width (0.17 cm) were shown to display a slope of 0.001. Due to our low sap flow velocities (< 8 cm h^{-1}) and small probe diameter (0.12 cm), we expected the slope to be as close to the 0.001 levelas possible. The specific threshold of 0.003 was decided upon inspection of the measurements and can be modified according to needle size and magnitude of the sap velocity measured. According to Burgess et al. (2001), higher values of slope (0.01) can be expected with wound width and higher velocities.

3.5 Correction for long-term probe misplacement

Under the assumption of "perfect symmetry", in periods when $V = 0$ cm h^{-1}, ~~the~~ $\frac{v_1}{v_2}$~~the ratio of the increase in temperature at the downstream and upstream from a line heater~~ would be equal to 1 ($v_1 = v_2$ in equation 3). However, this is not always the case with field measurements due to misalignment of the probe, damage to xylem vessels when inserting the probes, and the different thermal properties of wood and needle. ~~(Fig. 3A,~~ Burges et al.~~,~~ (2001) proposed ~~. The same study showed how probe placement can be estimated *in situ* with the HRM. Further, it proposed~~ a methodology applied to in situ HRM measurements to account for this type of ~~identifyfor~~ probe misalignment, ~~which~~ build~~ings~~ on the assumption that errors arising from inaccurate probe spacing can be treated one-dimensionally. This approach assume~~s~~ that the total effect of probe misalignment (in both axes directions), observed in the ratio of the increase in temperature, can be parameterised calculating an "effective" probe misalignment in only one direction (x direction, parallel to the xylem). Thus, without the assumption of "perfect symmetry", in periods when $V = 0$ cm h^{-1}, equation 2 takes a more simplified form:

$$4Kt \ln\left(\frac{v_1}{v_2}\right) = (x_2^2 + y_2^2) - (x_1^2 + y_1^2)$$

[4]

Which becomes equation (5) if $y_1 = -y_2$ or $y_1 = y_2$.

$$4Kt \ln\left(\frac{v_1}{v_2}\right) = x_2^2 - x_1^2$$

[5]

As it is unknown if the misalignment is in x_1 or in x_2, the calculation is repeated twice in the present study, assuming first that x_1 is correct when ~~calculating actual~~estimating x_2 placement, and vice versa. Actually~~Usually~~ both x_1 and x_2 may be considered~~are~~ slightly misaligned, but in this approximation, ~~which means that~~ each of the two correction step~~s~~ will assume ~~an error,~~ that the other probe is correctly placed. However~~Then,~~ ~~taking~~ ~~t~~the average of the obtained misalignment~~s~~ calculated for~~rom~~ each of the probe~~s~~ will be considered as the resulting ~~, both~~ misalignment~~s sought~~ ~~will be accounted for~~ (Burgess et al., 2001). Each probe placement is calculated as:

$$x_2 = \sqrt{\left(4kt \ln\left(\frac{v_1}{v_2}\right) + x_1^2\right)}$$

[6]

Therefore, ~~T~~two different heat pulse velocities, V_1 and V_2, are~~then~~ derived (using equation 2 but with~~the~~ assumptions $y_1 = y_2$ ~~assumption~~ or ~~-~~$y_1 = -y_2$) for the x_1 and x_2 obtained; and the final V provided as their average.

Zero flow conditions can either be imposed artificially by severing the root or stem (Burgess et al., 2001), or found when there is no biophysical driving force (Forster, 2007). These conditions are accomplished ~~That is,~~ when atmospheric vapour pressure deficit (VPD) is close to zero, ~~the~~ soil is saturated~~,~~ usually after substantial

precipitation, and~~–~~ measurements~~values~~ are taken at predawn (Forster, 2017). Zero flow conditions were assumed at night (22:00-03:00h solar time) during days when ~~relative extractable water,~~ REW > 0.75, and vapor pressure deficit (VPD) w~~as~~as close to zero (Fig. S2). Multiple readings were used to produce an average of each event. These conditions were limited to five occasions during ~~our~~ the study period. Saturated soil is a necessary criterion due to the possibility of reverse flow at night-time (Forster, 2014). If not considered, low HPR values representing reverse flows can be interpreted as zero flow. During long field operations, Tthe non-intrusive approach of zero flow allows the parameterisation of misalignment as a function of time (equation 7), if several calculations of x_1 and x_2 (equation 6) are performed throughout the duration of the campaign. By applying equation 6 at varies times throughout the measurement period, it is possible to calculate a linear regression using the estimation of Burgess et al. (2001) for the obtained misalignment~~s~~ for each of the ~~probes~~thermocouples:

$$
\begin{aligned}
x_1 &= m_1\, d + n_1 \\
-x_2 &= m_2\, d + n_2
\end{aligned} \qquad [7]
$$

Where d is the time along the measuring campaign in days, x_1 and -x_2 are the ~~relative~~ positions of the ~~thermocouples~~ probes relative to the ~~to the line~~ heater element in cm, ~~d is the time along the measuring campaign in days,~~ m_1 and m_2 are the slopes ~~of the regressions for thermocouple 1 and 2,~~ and n_1 n_2 are the interception coefficients of the linear regressions for probes 1 and 2, respectively. The negative sign in front of x_2 in equation 7 is to clarify that this probe needs a negative root square solution.

By introducing equation 7 in equation 2 and allowing the simplification ~~y₁ = y₂~~assuming or $y_1 = -y_2$, two equations of the corrected heat pulse velocities are obtained as a function of the time during~~in~~ the measuring field campaign:

$$
\begin{aligned}
V_1 &= \frac{4Kt\, ln\left(\frac{v_1}{v_2}\right) + (m_1\, d + n_1)^2 - 0.6^2}{2t(m_1 d + n_1 + 0.6)} \\[8pt]
V_2 &= \frac{4Kt\, ln\left(\frac{v_1}{v_2}\right) + 0.6^2 - (m_2\, d + n_2)^2}{2t(0.6 + m_2 d + n_2)}
\end{aligned} \qquad [8]
$$

In accordance to what Burgess et al. (2001) proposes, our approach averages the two ~~estimates~~ values obtained from equation 8 to ~~obtain~~ estimate a corrected heat pulse velocity.

4 Results

4.1 Heat pulse ratios

The HPR obtained during the measurement period displayed a clear positive shift away from the theoretical ideal where the HPR would equal to one at zero flow (Fig. 3A). This gives an indication of the necessity of corrections, that being due to wound inflicted by the probe, misalignment, misestimation of thermal diffusivity-

or stem water content. However, the HPR data by itself does not give an indication of the quality of each measurement, nor if it deteriorates over time. Therefore, the quality of the measurements was indicated by calculating the RSD ~~, the relative standard deviation divided by the sample mean,~~ and the slope associated with each HPR (Fig. 3B and 3C). All HPR with RSD higher than 5% were eliminated. The data points eliminated corresponded to a 1 % of the total dataset. Because the HRM is built on the theoretical assertion that the temperature ratio ~~in each of~~ ~~the~~between the thermocouples is steady with time, specifically between 60 and 100 seconds after the release of a heat pulse, the slope of the HPR should be close to zero. All HPR with |slope – median (slope)| < 0.003 s^{-1} were eliminated, which corresponded to a 12 % of the original dataset (Fig. 3C).
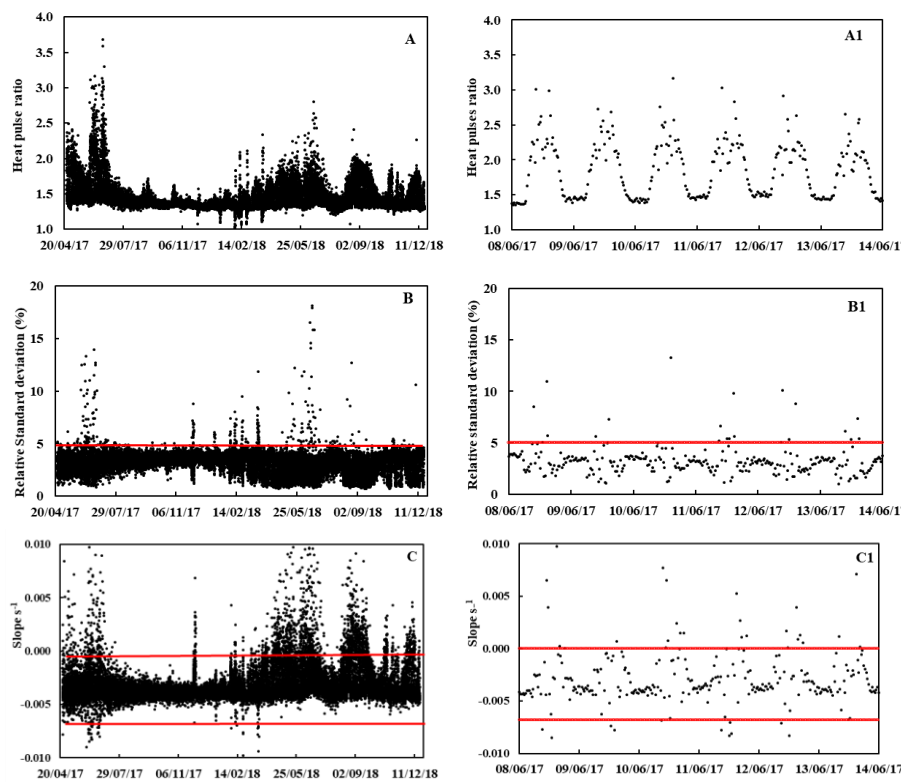
Figure 3. (A) Heat pulse ratios (HPR) throughout the measurement period at 30-minute intervals in tree number 1. Each HPR is an average of 41 instantaneous ratios corresponding to the temperature difference in two thermocouples at 0.6 cm up- and downstream from a heater ~~probe~~ element at 0.2 cm depth. (A1) Zoomed in panel of HPR data for one week of measurements to better see the diurnal pattern. (B) Relative standard deviation (%) for each HPR in tree number 1 for the whole measurement period. Red line indicates threshold used for the quality control where all HPR relative standard deviation > 5% were removed from the data analysis. (B1) Zoomed in panel of RSD data for one week. (C) Slope (s^{-1}) for each HPR in tree number 1 for the whole measurement period. Red line indicates threshold used for the quality control for this particular sensor. All data above or below these lines are filtered out as they did not correspond to HPR with |slope – median (slope)| < 0.003 s^{-1}. Median slope for this sensor was -0.004 s^{-1}. ~~Red line indicates threshold used for the quality~~

(C1) Zoomed in panel of slope data for one week of measurements.

4.2 Heat pulse velocities

A linear regression was obtained from misalignment calculations for each sensor performed during zero-flow conditions. Zero-flow conditions were assumed at night (22:00-03:00h solar time) during days when ~~relative extractable water,~~ REW > 0.75, and vapor pressure deficit (VPD) was close to zero (Fig. S2). Multiple readings were used to produce an average of each event. These conditions were limited to five occasions during ~~our~~ the study period. The outputs indicate a clear shift of placement in each of the sensors over time, here denoted as x_1 and x_2 in each tree (Fig. 4). The eight probes, two per tree, all deviated from the ideal of 0.6 cm. Probe x_2 in pine number 1 had the highest inaccuracy with the initial value close to 0.3 cm. On average, the probes showed a shift of 0.04 cm in placement after twenty months of measurement (Fig. 4). The equation obtained from the linear regression was then implemented in equation 8, and corrections of the heat pulse velocity data was done using an average of V_1 *and* V_2 (Fig. 5). Outputs were compared with one-time misalignment corrections, which w~~ere~~as calculated in the beginning of the measurement period to demonstrate the evolution of the probe misalignment. The one-time correction demonstrated a steady decline in accuracy over time (Fig. 5). The difference between the two correction methods showed significance after three months of employment (table 1).
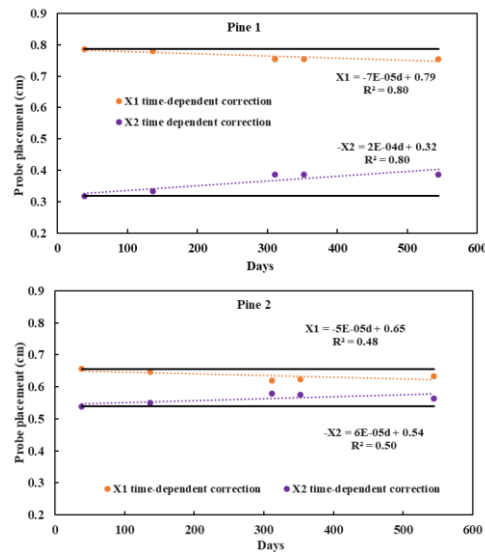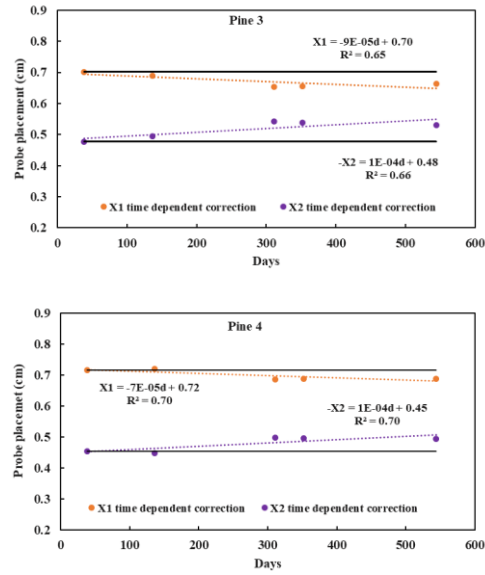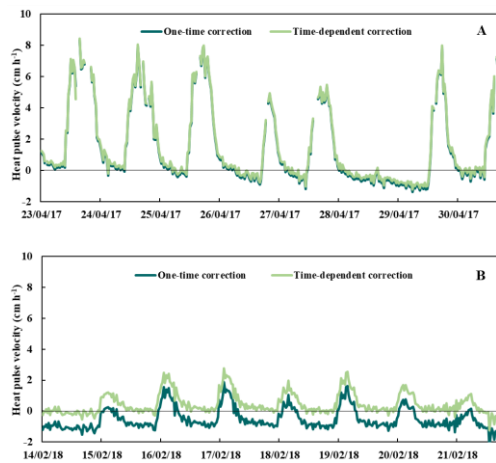
Figure 4. Placement of the probes are shown as distance from the heater (cm). Probe placement was calculated once at the beginning of the measurement period (solid lines) for the whole study period and compared to probe placement calculated varies times (solid circles with dotted lines) throughout the study period. Each point represents the probe position calculated during its respective zero-flow event

Heat pulse velocity (cm h^{-1})

One-time correction   Time-dependent correction   C

10
8
6
4
2
0
-2

12/12/18   13/12/18   14/12/18   15/12/18   16/12/18   17/12/18   18/12/18   19/12/18
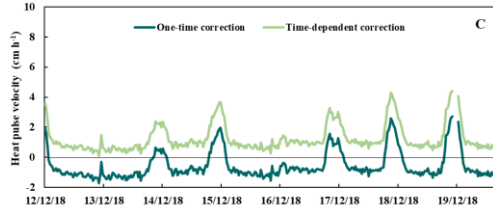
Figure 5. Heat pulse velocities during one week of measurements in the beginning (A), halfway (B) and at the end (C) of the measurement period. Dark green lines represent velocities with probe misalignment corrected for once at the beginning of the experiment. Light green lines represent velocities with the time- dependent probe misalignment corrections.

4.3 Sap flow

Heat pulse velocities (cm h^{-1}) were converted into sap flow (cm^3 cm^{-2} h^{-1}) according to Burgess et al. (2001). Our data demonstrated that by not correcting for changes in probe misalignment under continuous measurement for more than 3 months, the errors corresponded to an averaged difference of 0.29 (cm^3 cm^{-2} h^{-1}) in sap flow per quartile for the four trees. At the end of the 20-months period, this corresponded to an averaged difference of 0.53($\pm$0.23) cm^3 cm^{-2} h^{-1} (Table 1, Fig. 6). In terms of transpiration values, this corresponded to a mean difference of 0.7 L tree $^{-1}$ day^{-1} (sapwood area = 170 cm^2), which would correspond to a difference of 542 L ha $^{-1}$ day $^{-1}$ (775 tree ha $^{-1}$) assuming 8 hours of daylight. It is relevant to note that our conversion did not consider the differences in stem moisture content, which can also affect the output values (Vandegehuchte and Steppe, 2013). The outputs obtained should be considered as relative differences as the one-time correction was applied in the beginning of the experiment.

Table 1. Seasonal averages of sap velocity for 4 different pines. All sap flow values are expressed in cm^3 cm^{-2} h^{-1}. Sap flow corrected for with time-dependent misalignment calculations are compared with sap flow corrected for once in the beginning of the measurement period. Averages were taken from daily values. Abbreviations Sp, Su, Fa, and Wi indicates Spring (March-May), Summer (June-August), Fall (September-November) and Winter (December-February) respectively, each with the corresponding year.

| Pine	Correction method	Sp-17	Su-17	Fa-17	Wi-17	Sp-18	Su-18	Fa-18
1	One-time correction	0.98±1.2	0.23±0.8	-0.31±0.2	-0.37±0.5	0.12±0.7	0.05±0.5	-0.16±0.5
	Time-dependent correction	1.08±1.2	0.44±0.7	0.04±0.2	0.10±0.5	0.64±0.7	0.70±0.6	0.69±0.5
2	One-time correction	1.33±2.1	0.49±1.2	-0.28±0.4	-0.25±0.5	0.06±0.6	0.04±0.5	-0.05±1.8
	Time-dependent correction	1.40±2.1	0.66±1.2	-0.02±0.4	0.09±0.5	0.36±0.7	0.42±0.5	0.45±2.0
3	One-time correction	0.82±1.7	0.73±1.1	-0.02±0.5	-0.24±0.5	0.57±0.6	0.75±0.5	0.53±0.60
	Time-dependent correction	0.84±1.7	0.79±1.1	0.09±0.5	-0.09±0.5	0.76±0.6	0.99±0.5	0.80±0.63
4	One-time correction	0.79±1.1	0.56±0.7	0.09±0.4	-0.05±0.4	0.29±0.6	0.18±0.5	0.05±0.5
	Time-dependent correction	0.82±1.1	0.66±0.7	0.29±0.4	0.23±0.4	0.61±0.6	0.56±0.5	0.55±0.5
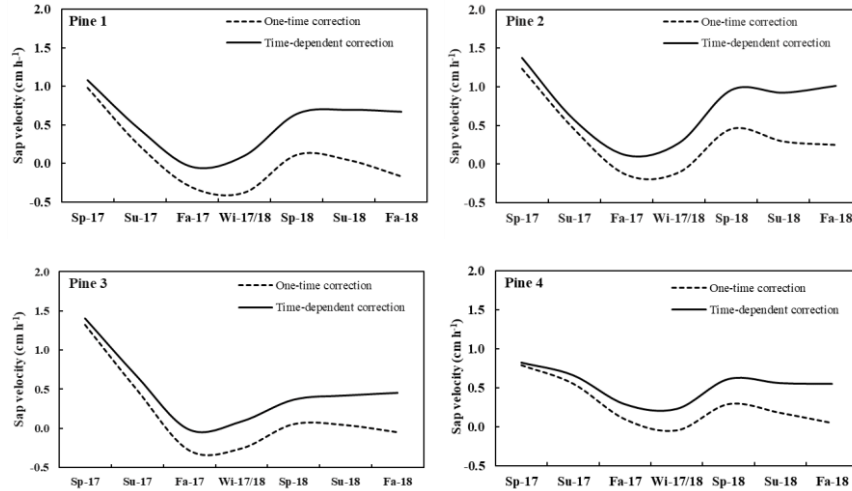
Figure 6. Seasonal averages of sap flow (cm^3 cm^{-2} h^{-1}) calculated using the two misalignment corrections throughout the 20-month measurement period. Dotted lines represent probe misalignment corrected for once. Solid lines represent sap flow corrected for with the time-dependent probe misalignment method. Abbreviations Sp, Su, Fa and Wi indicates Spring (March-May), Summer (June-August), Fall (September-November) and Winter (December-February) respectively, each with the corresponding year.

4.4 Transpiration

To demonstrate the difference in terms of transpiration, sap flow values (cm^3 cm^{-2} h^{-1}) without misalignment correction (Tr), with one-time correction (Tr_{one}), and with time-dependent correction (Tr_{time}) were converted into transpiration values (L $tree^{-1}$ h^{-1}) and compared against each other during a period of three weeks. Data from three of the pines (1, 3 and 4) were taken towards the end of the measurement period, to demonstrate the greatest differences (Fig. 7). However, the sensor placement in pine number two demonstrated a misplacement closer to target (0.6 cm) towards the end of the measurement period (Fig. 4), and another measurement period was therefore chosen for this tree, for a better demonstration of the differences between the estimations. Note that the transpiration estimations without misalignment correction still went through the wound correction step as shown in Burgess et al. (2001). Pine number 1 displayed the biggest differences between the Tr and Tr_{time}, with an average daily difference of 1.5±0.002 (L $tree^{-1}$) during the three weeks, and a difference in daily average difference of 0.3±0.003 (L $tree^{-1}$) between Tr_{one} and the Tr_{time}. Pine number 3 and 4 showed similar differences between the methods, with a daily average of 0.1±0.001 and 0.2±0.002 (L $tree^{-1}$) respectively, between Tr and T_{time}, and bBoth had a difference in n daily average difference of 0.1±0.001 (L $tree^{-1}$) between Tr_{one} and Tr_{time} (Fig. 7).
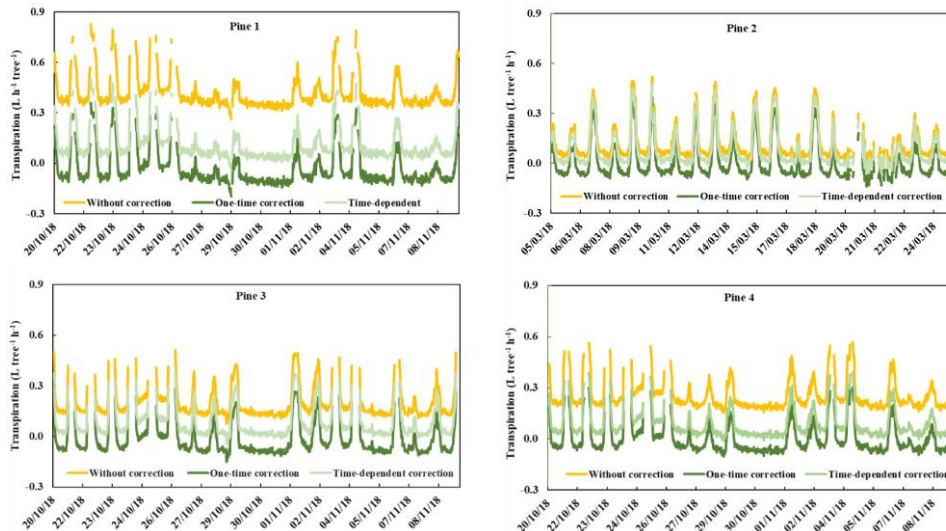
Figure 7. Three weeks of transpiration rates (L tree^{-1} h^{-1}). 'Without correction' represents rates without misalignment-corrections, 'One-time correction' represents rates corrected for once in the beginning of the measurement period, and 'Time-dependent correction' represents rates being corrected for at varies days throughout the measurement period.

5 Discussion

5.1 Filtering heat pulse ratios

Because the HPR is an average of instantaneous temperature difference ratios, it is difficult to ensure its accuracy only by visual inspection of the averaged output, and without further statistical information. For each heat pulse measurement sequence, wWe suggest RSD and slope from linear regression of the instantaneous ratios versus time to filter out random ratios, and to ensure the quality of the data. When this procedure is considered for long filed campaigns, Aa clear tendency may be observedwas seen of higher RSD and slope values during periods of higher flow rates when compared to the HPR data (Fig. 3A, 3B and 3C), indicating more noise and less trustworthy data during those episodeshigher flow rates. The HRM only measure values below 45 cm h-1, (Forster, 2017), due to a maximum ratio of $\frac{v_1}{v_2}$ = 20, for when it can be assumed accurately (Burgess et al., 2001; Marshall, 1958). Because our dataset showed no higher velocities than 8 cm h^{-1}, it was not initially considered a limitation for the measurements. The HRM is known for being limited at higher rates (> 45 cm h^{-1}, Forster, 2017), but our dataset showed no sap velocity values higher than 8 cm h^{-1}, and therefore was not initially considered as a limitation.

During twenty months of field measurements, the thermocouples showed no visible sign of deterioration with time. However, it is still important to note that this does not consider the possible diminishing amplitude of the ratios over time, which can also lead to underestimations of actual flow (Barett et al., 1995; Green et al., 2003;

Forster, 2017). Therefore, this should be observed separately, comparing sap flow ratios using data obtained under similar climatic conditions (Moore et al., 2010). Due to the variation in rainfall between the two years, SWC differed significantly on similar calendar dates ~~with the exception of~~apart from six days in June (9 – 15). When comparing the relationship of sap flow versus VPD on these days, there was an increase in the slope; 2.3 for 2017 and 2.9 in 2018. This, we attributed to an overall weaker correlation between VPD and sap flow in 2017; $R^2 = 0.4$ in 2017 versus $R^2 = 0.6$ in 2018, due to higher values of VPD the first year (~~S~~Fig. S3). In conclusion, it was clear that the HPR readings had not decreased in the second year, when compared to the first year, under similar environmental conditions.

5.2 Long term variation in probe placement

When applying the HRM for longer than a few weeks it is relevant to quantify how ϕ in equation (3) and the misalignment term in equation (5) evolve during the measuring period. The predicted variation of x_1 and x_2 is due to the growth of the tree and, on the other hand, periodical variations ~~of~~in K due to annual and seasonal variations of the physiological properties of the tree (Green et al., 2003; Vandegehuchte and Steppe, 2012; Ren et al., 2017). In the HRM, the probe misalignment calculations can be corrected using the methodology proposed here, considering that as an average, each sensor displayed a 0.04 cm displacement within the tree after twenty months of measurements. The correction would also be more rigorous with ~~more~~added ~~assumed~~ zero flow events, which would be easier to obtain in humid environments with more periods of saturated soil.

The theory behind the HPV method were tested on conifers (Marshall, 1958), whereas Burgess et al. (2001) more generally refers to woody species when working with HRM. The time-dependent correction method could be useful with any species already tested with the HRM, where misalignment of probes can create a source of error and sensors are installed over a longer period. Specifically, where the movement of the wood might cause further displacement of the sensor.

By going back to the original assumption of "perfect symmetry", we investigated the original premises the method is built on. Even though Burgess et al. (2001) elaborated a correction method for sensor misalignment we saw that changes in sensor misplacement was detectable after each season. Therefore, multiple corrections should be carried out throughout the measurement period. The proposed modified method coincides with the one-time correction method (Burgess et al., 2001) for short time periods (< 3 months) but differs progressively over time. We found that this shift in placement is significant already after 3 months, and therefore dynamically misplacement calculations should be carried out or sensors should be reinstalled at this frequency. However, as pointed out by Moore et al. (2010), re-installing the sensors might create a shift in the data due to spatial variation within the tree. Leaving the same sensors in the tree throughout the study period avoids this problem and enables the study to focus on the intrinsic factors affecting the sap flow rates.

2. Conclusion

In conclusion, we found that high quality measurements with sap flow sensors can be ensured over longer periods (>3 months), if the HPR is assessed using the proposed filtering method, and the probe misalignment variability over time corrected for. In this study, we observed data over a 20-month period in *Pinus halepensis*, and saw no sign of deterioration in the second year compared to the first, when observing the values obtained for slope and relative standard deviation. However, when observing the alignment of each probe, there was a clear shift from the beginning to the end of the measurement period. This indicate that measurements can be obtained during a second season without the need of re-installing sap flow sensors, if the proposed time-dependent misalignment correction is incorporated in the data processing. This would increase the accuracy of point measurements, and consequently transpiration estimations. The different errors related to upscaling are beyond the scope of this paper, but significant differences were observed when comparing sap flow estimations with no correction, one-time correction, and time-dependent correction for probe misalignment. ~~To avoid sensor reinstallation, this should therefore be considered.~~

Author contributions

JLP and ECH conceptualized the study. EKL made the sensors with the supervision of JB. EKL implemented the sensors and had the responsibility of the data processing and field site. JLP, EKL and JAV all worked on the data analysis. JAV wrote the script for the datalogger and had the technical responsibility. EKL and JLP prepared the manuscript with inputs from all the co-authors. JLP and JAV designed the flow chart to be programmed in any data logger system.

The authors declare that they have no conflict of interest

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