Authors’ responses to the comments of reviewer#1, James Knighton

>> Major comment: the proposed methodology hasn't been validated with a parallel established method.

Direct validation with a parallel established method surely would have been desirable, but would have required more resources (technical equipment, sample analysis, time) than were available during the making of the presented study. However, we do think that the results of other studies that reported sap flow velocity profiles or breakthrough curves of isotopic labelling experiments are in good agreement with our findings.

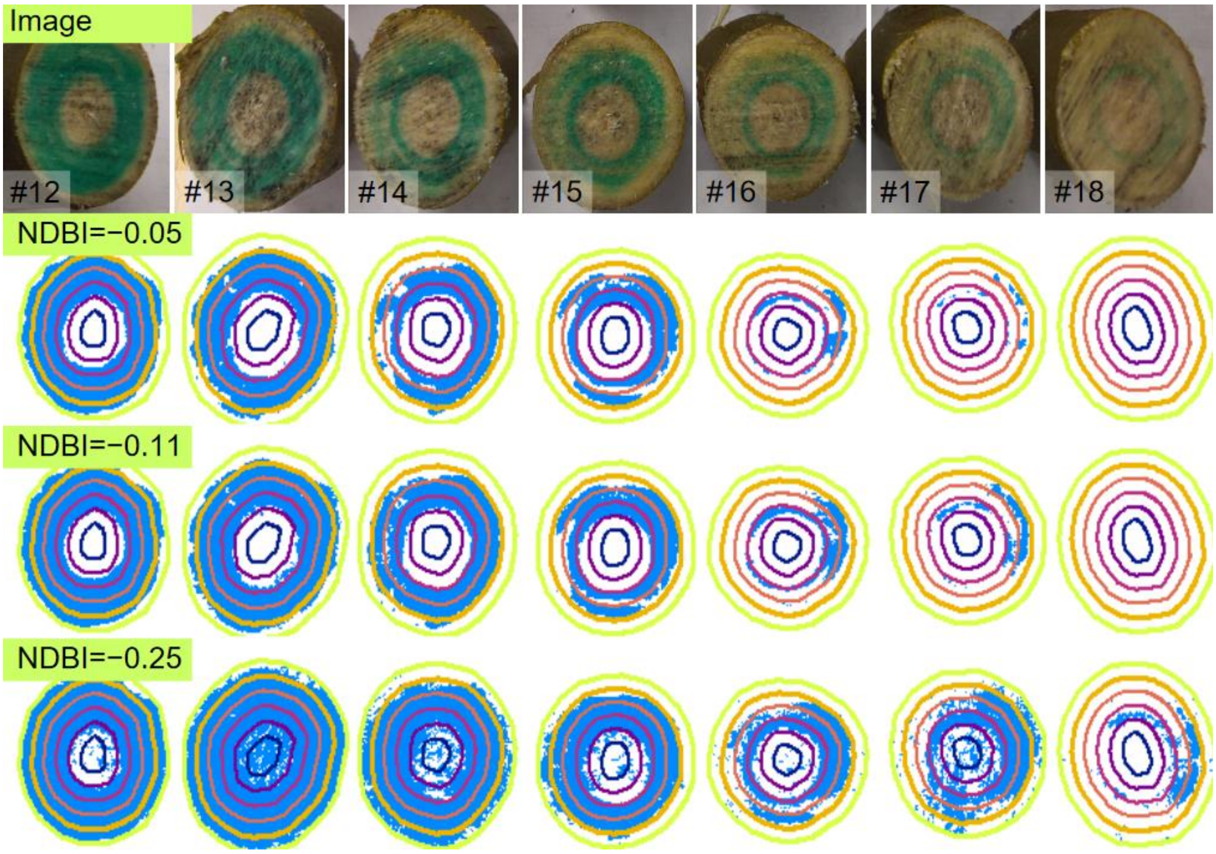
>> There is some discussion of how these results compare to radial variations in xylem water velocity measured with sapflux techniques; however, these prior measurements were made at different heights, depths into the xylem, and on different species.

We are well aware that our selected specimen was far from being very representative. But this study was not supposed to deliver final representative values for more relevant tree species, but rather to introduce a methodology that might be suited to investigate sap flow velocity distributions for a wide range of trees of different species and ages. In the revised version of the manuscript we will make sure to highlight that the obtained values should not be generalized.

>> I would also guess that this underestimate is what causes the authors to calculate that a substantial proportion of outer xylem tissue does not conduct water (Fig. 6c-d). It may be the case, but this type of finding would impact the conclusions of many prior studies and should therefore be carefully validated.

We think that is a misunderstanding: The unstained areas in the outer xylem of cut surface #15 (as shown in Fig. 2) do not imply that any part of the outer xylem is not conducting water. They merely imply that some water flowing in the outer xylem was not fast enough to reach cut surface #15 within the time of the experiment. In fact, our results in Fig. 5b-c show that all transport velocities within the outer xylem were greater than 0. What our dye tracer experiment could show, is that the water in the outermost xylem is not moving the fasted. As already mentioned in the discussion of the manuscript, similar findings were reported by studies based on sap flux measurements on different species (see Luttschwager2007, Cermak2008 and Gebauer2008).

The immobile water fractions shown in Fig. 6c-d are actually a combination of two kinds of "immobile" water: first: the 10% of cell bound water that is assumed throughout the entire xylem and second: the “free” water in the inner xylem that simply is not moving. Our proposed dye tracer method would certainly not be suited to quantify the fraction of cell bound water. Even when 100% of a xylem section would appear stained from a dye tracer, we still would have to expect that a certain fraction the water (i.e. the cell bound water) is actually not moving at all. However, the work of Fabiani2022 clearly suggests that there is some exchange between the water in the transpiration stream and less mobile xylem water. We will make sure to include this into the discussion.



Extra-Figure 1: Original images of the last seven cut surfaces on top of three rows that used different NDBI thresholds to detect blue stained areas.

>> This is a concern to me particularly because the proposed methodology required subjective visual tuning of the NDBI classification of dye versus no-dye in each cross section. This visual tuning seems to have resulted in an underestimate of the dye-stained area (Fig. 2)

After your hint to the obvious underestimation in Fig. 2 we reran our scripts and actually found that an NDBI threshold of -0.11 produces better results. However even with that threshold we did not manage to properly detect the visibly blue stained areas in cut surface #18. We have created Extra-Figure 1 that shows the last seven cut surfaces together with their digitized versions for three different NDBI thresholds: -0.05 (as in the original manuscript) -0.11 (optimum) and -0.25 (obvious overshoot). Interestingly, an NDBI threshold of -0.25 seems to be suited to detect the faintly blue stained areas of cut surface #18, but on other images this threshold leads to clear misclassifications with too many false positives. We do agree that the automated detection of blue stained area fractions has potential for improvement. We already discussed many of the possible improvements that could be made to obtain better images and we will add your suggestion of reference swatches to the discussion.

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>> The ideal resolution to this comment would be to provide a validation or comparison of the technique. I would imagine that this experiment could accommodate sap-flux sensors installed along the sample and/or xylem water isotopic sampling (if labeled and dyed water were used at the boundary condition). I recognize that this would be a substantial amount of work. At the very least, I strongly caution against using the results of this preliminary experiment to guide field sampling and model development and think the results and conclusions could be tempered a bit more than they already have been.

Within the revised version of the manuscript we will make sure to clarify that the results of this study should not directly be the basis for decisions regarding field sampling or model development. The presented study could be a starting point to devise more refined experiments in order to investigate the distribution of flow velocities within the xylem.

Regarding your suggestion for validation with other approaches, we would argue that our findings fit very well to already published distributed sap flow measurements (Luttschwager2007, Cermak2008 and Gebauer2008), even though those measurements may have been made at different species and at different spatial dimensions. The dye tracer approach may even be an option for smaller plant compartments, that could hardly be investigated by distributed sap flow measurements. The idea of comparing a stable water isotopic label with our dye tracer was already mentioned in the discussion part of the manuscript (lines 263-265), but this approach would not be suited to evaluate the spatial resolution that can be achieved with the dye tracer approach. At least for tracing the water flux through soil, a recent study by Llorens2022 showed that isotopic labeling and brilliant blue labelling do lead to pretty similar results.

>> Section 1: I appreciate the focus on process-based modeling of root to xylem conduit time lags. I would also point out that research using storage selection (SAS) functions have been presented as a parallel way of dealing with this problem. These models don't need to explicitly consider where water spent time (i.e., inside the plant versus in the soil). SAS functions can numerically represent complex age-distributions of water in xylem. I understand that this isn't the primary focus of the paper, but this comes to mind as I read the argument the authors are making. Here are several examples…

We are aware of SAS functions, but within our introduction we tried to trace the path from traditional approaches towards more process based approaches that enable a more realistic representation of xylem water transport. From that perspective we would prefer to keep the introduction short and not include additional references towards a purely statistical/parametric approach (i.e. SAS functions) that plays no role for the further content of the study.

>> Line 49: The process-based modeling papers described by the authors all shared a similar challenge in that they were attempting to simultaneously simulate the both the soil water balance (the root boundary condition) and xylem water isotopic observations. Some of the model error in representing xylem water was likely error that cascaded down from an imperfect representation of the soil water, and not only an imperfect representation of xylem water transport. Smith et al (2022) demonstrated this directly. This might be worth mentioning.

At least for the study of Seeger2021 the soil water used for the computations did not come from a model, but from interpolated in-situ measurements of soil moisture and soil water isotopes. Therefore, I do not think that this point applies here. The observed discrepancies in that work clearly were caused by a lack of a proper representation of water storage and transport within the xylem.

Line 55: This transition in text is kind of abrupt. The text goes from advances in modeling the soil-plant system to measurement techniques. Would this paragraph make more sense moved down to be the first paragraph of Section 1.2?

We agree that the transition is abrupt. Instead of moving it to the first paragraph of section 1.2, we would place them into a new subsection about tracers (dyes and isotopes) that will open section 1 of the revised manuscript.

>> Line 75: A definition of "mobile" would be useful here. Is "mobile" water extracted under a certain pressure? Many researchers remove heartwood from tree core samples prior to isotopic analysis to sample only the "mobile" water; however, recent studies have suggested that heartwood is "mobile" to a certain degree. I think the field at large needs a better definition for "mobile." For example, see Fabiani et al (2022).

As already mentioned above, we are actually dealing with two kinds of immobile water (cell bound water and free water that is not moving). We will make sure to clearly define what we are talking about at this point.

>> Line 87: Possibly no one has done this with stable isotopic techniques or dye tracers, but there are many studies where the radial variations in xylem velocity have been observed (Ford et al 2004). Maybe I'm not understanding what the authors are suggesting, but this seems a like a new method of measuring something that has been measured many times before.

The point is that sap flux velocities and water transport velocities within the xylem are not identical - in our case the water transport is on average three times (and at a maximum five times) faster than the mean sap flux velocity. Furthermore, sap flux sensors do give one value per depth class (usually not finer than 5 mm) while the dye tracer approach allows for a much higher spatial resolution. Additionally, the dye tracer approach returns whole velocity distributions for each depth class. In order to achieve the same with sap flux sensors, unreasonable amounts of sensors would be needed.

>>Line 107: How long did this dye breakthrough require?

42 minutes. This information is given right at the beginning of the results section (line 183).

>> Figure 6c-e: This is implying that some shallow xylem tissue existed that was not transporting water. It seems more likely that this was because the NDBI algorithm was missing areas where dye was showing up weakly. You can see that this is likely the case on the left-hand side of Figure 2a and 2c. There is a clear blue-green coloring along the left of the cross section that was not identified by the algorithm and (I'm assuming) coded as "immobile." The authors also point out that the blue dye leaked from the last cross section (mobile) but did not stain this cross section (not identified as mobile). I would guess that the reported "immobile" water percentages are more related to methodological limitations than evidence that immobile water is being sampled in short cores.

I hope that part was already answered above. Short repetition: Undyed areas further down the flow path length merely imply that the water transport was not fast enough to reach this point within the time of the experiment – it does not mean that the water is not flowing. The immobile water that can be expected to be sampled as part of short cores is cell bound water, which might be released if an aggressive extraction method like CVD is used.

>> Line 225: I'm not sure if it would work with this the methodology, but we often apply mineral oil to increase visibility when counting rings on tree cores. It makes the wood grain much more distinct. This might be an inexpensive way to quickly improve image quality.

>>Line 230: You could also include a reference tile or swatch next to each stem cross section for image post-processing to a visual standard.

Thank you for these suggestions, we will include these suggestions to the discussion of the method.

>> Line 284: The wording here is a bit awkward: "a lot more, very common trees."

This will be rephrased in the revised manuscript.

>> Line 295 - 296: This recommendation seems like an overreach given that this methodology hasn't been validated (see major comments).

Actually, for mature trees the deep core case will rarely ever apply. But we will rewrite that part.

>> Line 304: "wide range of velocities" maybe isn't the most accurate wording. As discussed in section 4.2 this study shouldn't be taken as a measurement of velocity because of the unusual boundary conditions. Maybe instead: "a large variance in the radial distribution of velocities" which avoids talking about the actual velocities.

This will be rephrased in the revised manuscript.

>> Line 305: Minor comment here, but Knighton et al (2020) investigated both piston flow and a fully-mixed reservoir, which was a little more complicated than piston flow.

We have mentioned the fully-mixed reservoir of Knighton2020 in section 1.1 of the introduction. At that point, we would argue that a from a physical perspective the fully-mixed reservoir seems less plausible than a piston flow representation. Therefore, we do not think that the manuscript would gain anything by mentioning the fully-mixed reservoir at that point.

>> Line 305: There is also a possible difference in the temporal scales between that modeling experiment and this study presented in the manuscript. I'm assuming this study occurred over a time period of less than one working day (<8 hours). If a boundary condition is introduced and held constant for a sufficient period of time (e.g., rainfall onto soils followed by a period of no rain for a week) the radial distribution of xylem velocities will stop mattering after the slowest path has reached the sampling height. The variation in flowpath velocities would only be a strong consideration if the water boundary condition at the roots was rapidly changing relative to the transit time between roots to the sampling point, which might not always be a real-world concern.

We agree that the effect introduced by a distribution of transport velocities may be negligible whenever the considered systems can be considered to be close enough to steady state conditions. They also do not play a role when the ratio of transport distance to velocity is small enough. However, as shown by Seeger2021, a temporal lag between RWU and xylem water measurement as well as non-negligible blurring introduced by different transport velocities do occur at real world uptake conditions (even though artificial labelling can enhance the visibility of such effects). Even if a less complex representation might not do any harm in most cases, there will definitely be occasions where the inclusion of transport velocity distributions can help to achieve a more realistic relationship between the observed signatures in RWU and xylem water.

>> Line 316: Something wrong with this reference "?Dubbert"

Error in the bibtex file was fixed.

References

Lüttschwager, D., and Rainer, R.:
"Radial distribution of sap flux density in trunks of a mature beech stand." *Annals of forest science* 64.4 (2007): 431-438.

Čermák, J., Nadezhdina, N., Meiresonne, L., and Ceulemans, R.:
"Scots Pine Root Distribution Derived from Radial Sap Flow Patterns in Stems of Large Leaning Trees" Plant and Soil, 305, 61–75, https://doi.org/10.1007/s11104-007-9433-z, 2008.