Dear referee, dear editor,
Thanks very much for your encouraging assessment of our manuscript and the extremely helpful suggestions for improvements. Given your positive feedback, we were very happy to use your comments to substantially improve the manuscript. In the below, the original reviewer comments are formatted in black normal font, whereas our responses are formatted in red. Since we re-structured large parts of the document in response to your comments, we do not provide a track-changes version, but instead point to the line numbers with the major changes in our responses below.

Referee #1 Evaluations:
Recommendation: Major changes needed

Referee #1 (Comments for the Author (Required)):

The authors present two new methods of using suction (induced by either evaporation or syringe) to move water through excised stem segments and measure the flow rates, pressures and conductivities. The basis for this study is that common methods of hydraulic measurement often push water through with positive pressures, which contrasts with how water moves in plants. They use these new methods with various modifications to simulate drought and other conditions and to induce water into or out of storage. I found this to be an interesting method with much potential for investigating dynamics of hydraulic transport. Overall in the writing I think there should be a reassessment of what the major findings were. The four goals listed in the Background and Aims are all worthy goals which the method addresses. But in the discussion and sometimes the abstract, the achievements being listed are that it can be used for leak detection (which as I note below is not very transparent), that it has educational potential (which is true but I view this as a side point), and that conductivity increases with flow rate (which I also note is not apparent). So a refocus on what this method shows or achieves that other methods do not would be beneficial.
Thank you for the suggestion. We have re-structured and re-focused the abstract, discussion, and conclusion accordingly. The point about increasing conductivity with flow rate was strengthened in the discussion, as explained below.

I also think the Background and Aims could be improved to provide a stronger basis for why the new methods are needed. Currently, the topic of each paragraph is as follows:
water CO_2 tradeoff and tension
cohesion
gravity and metastability
embolism and reduced conductivity
how vulnerability is measured
Sperry method
HPFM
Sperry and HPFM different from natural
Cavitron method
Cavitron details
Method shortcomings (no runaway cavitation, 2/3 above atmospheric, storage)
While the information in these paragraphs seems fine (except where noted below) and there is some logic to the organization of paragraphs, I felt like the writing could be structured better to make it apparent where this is going. The most obvious change would be to introduce Sperry method, HPFM and Cavitron all at once. As is, Sperry and HPFM are introduced and discussed and then Cavitron is brought up later. The overall message of the section should be something like "This is how water moves in planta. These are three common methods to move water in plant segments. These are the reasons why these methods differ from in planta and why that's a problem. This is

how the presented method will address those problems." It's also notable that this method was not used to measure vulnerability. So the paragraphs on vulnerability aren't necessarily needed.

Another concern (more details below) is whether the experiment where a bubble is introduced can be said to be simulating runaway air seeding. Given that the air simply passes through open vessels, it can't be said that air seeding is occurring (i.e. it's unlikely that air passed through any pit membranes).
Thank you for pointing out the lack of clarity, we addressed this under the comment for line 419 below.

Beyond this, I has a number of suggestions relating to clarity of the study.

7-8: For the general reader it would be useful to briefly state what the common methods are and why they are unnatural.

Thank you pointing this out, we changed it to “Common methods of measuring hydraulic conductivity control a pressure gradient to push water through plant samples, submitting them to conditions far away from those that are experienced in nature where flow is suction driven and determined by the leaf water demand.” L-7

16: Pulling water through a stem is not an entirely new approach. There's work by Kolb et al (1996 A method for measuring xylem hydraulic conductance and embolism in entire root and shoot systems) which does this. There is also work by Tyree using the Sperry method with the stem elevated above the reservoirs to create a sub atmospheric pressure.
RESPONSE: Thank you for pointing us to Kolb et al. 1996 and Tyree and Yang 1992. We now cite these papers in the intro and discussion. To induce an absolute pressure of 40 kPa, as shown in our Figure 6, the twig would have to be elevated 6 m above the water reservoirs, which is not feasible. However, the Kolb et al. method could indeed achieve similar pressures to our system. The work by Kolb et al. is now mentioned in the introduction at L-86 and in the discussion L-472:486.

31: Not all plants have vessels. "Conduits" should be used instead. But also, non-vascular plants should not be overlooked.
Agreed and changed.

56: How does one categorize it as being *the* primary focus rather than *a* primary focus?
Good point, we replaced “the” by “a”.

66: Indeed actual values are often not reported, but it's not clear if there is a follow up to this statement. If not, I suggest removing it.
Deleted as it did not fit

73: It should be something like "flows", not "drips".
Agreed and changed accordingly

90-91: You seem to be saying that with Sperry method and HPFM that flow is determined by conductance and "in contrast" the pressure difference is determined by flow rate in the natural system. But I don't think you can separate conductance, pressure difference and flow rate. Later (124) you state in the new method that flow rate is controlled and flow is a response. Without having read further, it's not clear why this matters. If I have a pump on the downstream side of the stem that draws 1 mg / s and the conductance is 1 mg / s kPa, then this will generate a 1 kPa pressure difference across the stem. But is this physically and meaningfully different from placing the downstream balance ~10 cm below the upstream reservoir to create a 1 kPa difference?

Thank you for pointing out our lack of clarity. We re-wrote the section and added points to to clearly summarize the drawbacks of current methods at L-109.

97: These are possibly better called "rotor cups" than "cuvettes".
RESPONSE: Thank you, changes applied.

111: According to your abstract, the new method cannot induce embolism so it seems it would also be unable to simulate runaway cavitation. So this should probably not be a point to make here.
RESPONSE: We only state in the abstract that the system cannot be run under negative pressure, but this does not necessarily mean that the method cannot simulate runaway cavitation. Even above boiling pressure, bubbles could expand and block vessels, leading to further reduction in pressure and expansion of more bubbles.

179-187: I guess I don't follow why you need to know the volume of the tube and T valve. Some justification and perhaps mathematical explanation would be helpful. Also, in using the ideal gas law, presumably you need temperature but you don't state how that was measured. Later (278) you state the lab temperature was around 21 C. Is this the temperature used for all calculations?
Thank you for pointing out the lack of clarity. The volume was needed as our initial volume for the ideal gas law calculations. This section has been re-written to state this more clearly, including the ideal gas equation, as well as the form we used to calculate the pressure in the syringe.
Assuming constant temperature during the half hour of calibration for both sensors, the temperature was not required for calculating the pressures. However, we added the information that the temperature in a beaker of water varied between 21 and 23 degrees over two weeks in the lab, and that 21 oC was used as reference in any calculations requiring temperature. L- 272:278

205: Is this mean vessel length or maximum length or...?
It was mean vessel length. The “maximum” in front of the following two instances of “vessel length” were removed for clarity.

Eqn 1: You have a negative sign which suggests Q or dP is negative but this is not apparent. "Pressure drop" can be positive or negative depending on your convention. Likewise the sign of Q depends on your convention. But a negative sign isn't typically used to calculate conductivity so consider if its necessary.
The negative sign was indeed for the pressure drop, as you mentioned, this is not apparent so the sign was left out.

227: Does A come from the stem diameter or the xylem diameter? Above you say "sample diameter" and "cross sectional area of flow"
The ‘A’ comes from the stem diameter, this has been added and the mention of “cross-sectional area of flow” was changed to “cross-sectional area of the whole twig”.

229: Earlier, you state that the flow meter is calibrated using the syringe. So they should be the same. But here you seem to be saying that the flow meter is more accurate. Please explain.
The flow meter measures the flow rate into the twig, while the syringe applies a set flow rate at the other end of the twig. As seen in the figure with storage, there can still be flow through the twig while the syringe has stopped pulling. The sentence was updated to mention this in parantheses:

"The flow rate in the syringe setup is measured by the syringe pump (as a set flow rate out of the twig), and the flow sensor (as an instantaneous flow rate into the twig)."

230: "values" appears twice
Thank you for noticing, the second "values" was removed.

235: How large was this gas bubble? I assume it would need to be either conduit size or tubing size (but not in between) to be drawn into the xylem.
The bubble was tubing sized. The size was added when describing the addition of the bubble. "The gas bubble was the width of the tubing and 1.5 times the width in length, and was added through a valve below the lower pressure sensor and rose to the twig while flow, pressure, and hydraulic conductivity were being measured. " L-296

305-312: It would be useful to see the time series of this experiment, at least as a supplement.
The timeseries for the three trials has been added to the supplementary information along with a diagram and explanation. We also moved parts of the introduction and discussion related to these into the SI as it felt out of place but is still important to have documented,

Fig 2: Please label the components as you've done in Fig 1. Also, in Fig 1 the valves have a red rectangle that more or less seems to indicate it is a valve. But here the red is missing which suggests it is an open junction. These junctions are not labeled so it's not clear if they are indeed valves or junctions. If they are valves, please indicate. And for all valves, consider symbology that indicates the valve position during measurement (e.g. the T symbol rotated accordingly)
This is a very good point. The positions have been added just as in Fig. 1.

Fig 4: You have a general downward trend of K over time. Could this be a gradual clogging of the xylem?
341: You don't mention that K and downstream P decline during this time, which seems like a curious result.
Addressing both points above: Thank you for pointing this out, we are actually working on a follow up paper where we look into this in more detail. To clarify, we added this note at the end of the stress simulation experiment in the results: "Conductivity in Segment 'f-g' decreased steadily from 0.26 to 0.22 kg m^{-1} MPa^{-1} s^{-1} . The overall decrease in conductivity throughout the experiment could be seen in Fig. 4-6." L-415
We also added a paragraph in the discussion where we put the decline in hydraulic conductivity in context with similar declines reported previously in the literature.

356: It's a little confusing that sometimes you refer to a range of letters (e.g. a-b) and sometimes just letters. But ofter for the latter you are describing what happened between two points. It would be clearer to refer to ranges more often. For example, you state "Increase in water demand was simulated at 'e'" and then go one to say K had a "steady decline". The setup change occurred at e but the observations occurred between e and f.
The use of the letters was indeed inconsistent, we have updated the whole section, labeling the ranges as Segments to increase the clarity.

363: I note that you generally use base SI units (kg, Pa, m), giving values like K=0.00026 kg m^{-1} Pa^{-1} s^{-1}. I think units like g or mg and kPa or MPa and cm or mm can make the values more relatable (e.g. 0.26 g mm-1 s-1 kPa-1 instead of 0.000026 kg m^{-1} Pa^{-1} s^{-1}) but I will accept if the goal is the use base SI units only. However, you use uL (instead of L) consistently for volume and you use kPa and minutes at times, which suggests using all base SI units is not the goal, so consider using units that makes values more relatable.

We changed the conductivity units throughout to kg m−1 MPa−1 s−1, in the text as well as the graphs, as this is commonly found in papers such as the ones mentioned when introducing the hydraulic conductivity units. This makes the values more relatable to literature values.

389: It's not clear how the flow rate of the leak was calculated or on what basis it was assumed to be constant. The presence of the leak is unfortunate as it muddies up the result. It would have been nice to repeat the experiment without the bubble.
We saw this bubble formation in the first experiment (Fig. 3), and then again when repeating the experiment to focus on storage changes (Fig. 4). There is no good reason to believe it was constant other than the fact that the assumption of a constant leak equivalent to the difference in syringe and flow meter measurements at steady state lead to complete recovery of the storage depletion when flow ceased at the end of the experiment. We clarified this in the text: “The difference between the steady state flow rate measured by the flow meter at ‘d’, and the flow rate imposed by the syringe pump suggests that a leak occurred in the system, as a steady-state difference cannot be explained by changing storage. In addition, we observed a bubble in the syringe, confirming the presence of an air leak. The leak was assumed to have formed at ‘c’ due to a slight kink in the pressure, which would likely occur when air entry is initiated. In the absence of more detailed information, the air entry rate was assumed constant in Segment ‘c-d’ and to be equal to the flow rate difference between the steady state flow at ‘d’ and the syringe flow. Using the flow rate based on the assumed air entry rate, storage would have decreased by a maximum of 300 μL at ‘d’ and the end storage at ‘e’ would have been 7 μL larger than the initial twig water storage at ‘a’.”… L-438:449

419: I wouldn't characterize this observation as air seeding. It's interesting to see that air passed through the stem but this is not surprising. Given that the segment was only 2 cm long, there are likely many open vessels, allowing air to pass through without encountering vessel endwalls, which the induced pressures would likely not allow air seeding.
Thank you for pointing us to the misleading use of the term “air seeding”, it has been changed throughout the paper. A sentence was also added in the background and aims to clarify our use of the term: “Here we refer to air seeding as any process that introduces air into the xylem (Tyree and Zimmermann, 2002), such as air entry through pit membranes or wounds.”. L-52

436: What is meant by "This simple experiment" is not clear in the this context. Is it the whole study or the measurement on a long twig, which this statement follows?
Agreed that it is unclear which experiment is referred to, the start of the sentence was changed to: “The experiment with a long and short twig”

439: is "mean" meant?
Thank you for pointing this out, it is the maximum vessel length that determines if there are continuous vessels in a twig sample. “mean” has been changed to “maximum”.

440-441: I think it's generally accepted that pit membranes prevent gas transport rather than maintain flow.
Thank you for directing this to us, the sentence has been removed.

459: There's a 15 kPa difference between the two pressures you cite. The source of the difference is not apparent.
The difference is that the first pressure is subtracted from atmospheric pressure to obtain the second value. The text has been rearranged to be clearer on this point. We also changed the text in the summary to reflect that 49.5 kPa is the experimental minimum we attained and not the theoretical limit.

461: You haven't previously mentioned this issue of the distance and gravity. It's a good point but it's not fitting as a "summary" point.
This is a good point, we removed the mention of it in the re-structuring of the discussion.

473: It's not clear at what time in Fig 3 you are referencing here.
We agree that this was not clear and have added the time period we are referencing to in parentheses.

513: In Fig 4, there's not an obvious effect of flow rate affecting conductivity. Rather, there is a gradual decline in conductivity as the experiment progresses punctuated by transient spikes when pressure is changed. If these transient spikes are what you refer to as flow rate affecting conductivity, then I disagree. transient spikes should be expected when pressure is changed due to water uptake or release. To convincingly show that flow affects conductivity, it would be necessary for K to stabilize at each given pressure - or at least be able to account for the gradual decline in conductivity.
Thank you for pointing out that our point is not so obvious here. Indeed, the changes in K due to a change in flow rate are small, and partly obscured by the continuously declining trend in K. However, they are consistent across both methods, and more closely related to flow rate than to pressure. In Fig. 4 a, step increases in K are associated with step increases in both pressure and flow rate, whereas in Fig. 4 b, step increases in K are associated with step increases in pressure but DECREASES in pressure. The reverse is seen for step declines in flow rate, which are clearly associated with step declines in K in all but one case (at 1.5 hrs, where no clear step change in K is visible). We modified the discussion to:
"Surprisingly, our experiment showed that an increase in flow rate increased the conductivity of the sample, both at constant pressure difference (Sperry method) and constant flow rate (Syringe method). Conversely, step decreases in flow rate resulted in step reductions in conductivity in three out of four cases in Fig. 4. Since flow rates are positively correlated with pressure in the Sperry method (increase in pressure drives flow), but negatively correlated with pressure in the Syringe method (increase in suction drives flow), the combination of both methods enables the conclusion that the sample's conductivity indeed depends on the flow rate, not the liquid pressure. The positive correlation between flow rate and hydraulic conductivity was also found in Fig. 5, at the transitions marked as d, e, and f. More targeted experiments on different species could shed light into potential mechanistic reasons for this behaviour." L-580:592

521: Indeed you detected a leak but as I note above, the leak is not obvious in the raw data so I question whether this should be a selling point.
The difference in flow rates at steady state (no more change in flow rate or pressure), can only be explained by a leak in our opinion. See our response to L-389, and clarification in the text.

552: There is no partial calculation in Fig S3. If providing a calculation, please provide a full calculation.
Thank you for picking this up! We are not sure what happened here, but we removed this sentence and described Fig. S3 further above in the section.

Discussion: You begin the discussion with a recap of the experimental setup and a comparison about which of your new methods is better and introduce the prospect of one method for education. But none of these topics address what the key findings were and why the new methods are a necessary contribution. It would be nice to start the discussion by focussing on what the new method tells us that other methods cannot tell us.
Thank you for pointing out the lack of clarity in the discussion, we have restructured it to start with a paragraph describing the presented methods against current methods, how it is a combination of

concepts and the advantages we present. We then continue into dissecting the different methods' advantages, drawbacks, preliminary findings and potential for new insights.