15 Oct 2024: Anonymous Referee #2, Report #1

Kowalski describes a model for oxygen transport from stomata that notes that Dalton's law implies that oxygen concentration within the stomatal cavity must be below ambient. The work I interesting but there are still a few points that need to be clarified and reconsidered.

I thank the anonymous referee for evaluating the manuscript.

A schematic could be helpful but perhaps even more so is the acknowledgement that plants also consume oxygen if their cells have mitochondria; oxidative respiration to drive cellular function has to come from somewhere. Obviously the respiratory needs of plants are rather small compared to most animals but worth mentioning for completeness as the results have implications for plant cellular function.

The referee suggests acknowledging that plant cells consume oxygen. I am afraid that this misses the point of the manuscript, which regards gaseous air spaces and not cell tissues. During active leaf functioning, the photosynthesising cells that line stomatal cavities must be well oxygenated, beyond their respiratory requirements, because they emit oxygen. However, their water vapour emissions exceed those of oxygen by orders of magnitude. Oxygen represents a sizeable fraction (about one-fifth) of ambient air but a far smaller fraction of leaf gas emissions, which are nearly pure water vapour and so dilute O_2 to force hypoxic conditions inside substomatal cavities. I have added this explicatory, underlined sentence to the beginning of Section 4, and hope that it helps the referee and future readers to focus on gases within substomatal cavities. Any discussion of cellular oxygen would distract and confuse the issue, and is best avoided.

On 38: delta_e here may not reflect the ambient VPD as commonly assumed if recent studies by Cernusak and colleagues regarding the non-saturation of the sub-stomatal space hold true. (There certainly is a surplus as noted on line 39).

The referee suggests that Δe might not reflect the VPD, if the sub-stomatal cavity is not completely saturated. I am afraid I disagree. Here, the verb "equal" was discarded in favour of "reflect", which has a less precise meaning. For example, in the image at right, the lake reflects the mountains and sky. It does so imperfectly since rocks are visible at the lake's bottom (it is not a mirror), but it does reflect. I think that the word "reflect" is correctly chosen here. This is also why the last sentence of section 3 (The Model) says "… 21% of the vapour pressure surplus of the substomatal cavity, or **about** 21% of the environmental VPD" (emphasis added here), allowing for possible under-saturation.

On 111 and elsewhere regarding the air jet, given that previous work is alluded to here rather than described is this related to the Bernoulli principle?

The referee asks whether the airflow is related to the Bernoulli principle. I do not believe that this is a relevant framework. The Bernoulli principle derives from the assumption of an inviscid fluid. Neglecting the effects of friction, it expresses energy conservation as a conversion between three types of energy: pressure-volume work, gravitational potential energy, and kinetic energy.

The tiny stomatal size and low velocity give the airflow exiting stomata a low Reynolds number. In such viscous flows, conversion of kinetic energy into molecular kinetic energy (by dissipation, raising the temperature) cannot be neglected in the equation of motion. This invalidates the Bernoulli derivation; in this viscous case, the Bernoulli principle is not an appropriate framework for estimating velocities from pressure differences.

However, I thank the reviewer for this comment because it has opened my eyes to the misleading effects of the terminology of “stomatal jets”. Although I published previously using this terminology (Kowalski, 2017), I have come to realise that it is unhelpful. The word “jet” has high-velocity connotations and may be misleading in this context. Air does not ***jet*** out of stomata; it ***oozes*** or ***seeps*** (which seem like better verbs for describing viscous motion). I have therefore revised the manuscript to correct this unhelpful terminology, and substituted “mass flow” for “jet” where appropriate.

5 Dec 2024: Anonymous Referee #3, Report #2

This report contains no comments that I can find, and a recommendation that the manuscript be accepted as is. Having served as editor for a different journal, I appreciate that such a review is of little help, neither to the editor nor to the author in revising for improvement. At least, however, it is an unambiguous opinion.

12 Dec 2024: Associate editor decision: Publish subject to minor revisions (review by editor)

I am now in receipt of two additional reviews who are generally favorable about the manuscript and its scope but discuss minor revisions that I feel will help clarify key points. Please consider these comments and please don't hesitate to reach out if questions arise. I am posting Reviewer #2's comments below as they may not be visible by the author in the Copernicus system as uploaded.

Again, I appreciate the editor's efforts in handling this manuscript. My replies to Reviewer #2 appear on previous pages at the beginning of this document. Since the comments below seem to come from a different scientist (they refer to reviewer 2), I will refer to them as having come from Reviewer X.

I read the revised manuscript by Kowalski and their response to reviewers. Kowalski addresses their scientific concerns. I think they could engage more with the reviewers' comments about tone and understanding, as the reviewers' comments likely reflect the broader sentiment of potential readers. However, I tend to defer to the author to let them communicate how they feel is best. I do not have a a strong background a fundamental physics, but instead have learned along the way as I became interested in plant ecophysiology. Hence, I found the manuscript interesting, very clear, and I learned from it. My gut reaction is that if non-diffusive transport was important, wouldn't there be a large body of observations that were inconsistent with existing models? I recognize this isn't very sound logic on my part, but the paper would be more convincing if this could be demonstrated. Alternatively, a quantitative demonstration that jets can explain the decoupling between A and E at high temperatures would be more convincing. This decoupling is an interesting observation, but seems like it could be readily explained by other factors such as deactivation of enzymes either by regulation or denaturation, for example. I also wish that Kowalski engaged with reviewer 2's comment about a clearer physical description of what is going on with these jets. To extend that, I would like to know what sort of experiments or measurements should be done to work out the significance of O2 jets. It's not clear to me if the physics is so well validated that we can trust the model or if there are hidden assumptions or oversimplifications that are being neglected. I do not necessarily think all of these points need to be addressed in the revision, but as a practicing ecophysiologist, addressing these points would make the argument more convincing.

To clarify the subjects of my replies, I have highlighted five sections above of the comments from Reviewer X:

1. <u>Tone and understanding</u>: I have consulted with a highly regarded colleague, and received and heeded some advice on how to improve the tone of my writing. The manuscript is now rewritten without changing the message, and I hope that the editor and reviewers (if consulted) will agree that the tone is improved. (Experience indicates that I am a poor judge of this.) I also hope that both the sentence added in response to the comment by Anonymous Referee #2 above, and also the revised terminology (avoiding the term "jets" where possible), will improve understanding.

2. Large body of observations inconsistent with existing model: This is precisely the point of Section 4's final paragraph. There is indeed a body of observations that the existing model does not explain, namely the decoupling of transpiration and photosynthesis that has been observed widely at very high leaf *T*. The manuscript revised on 26 September cited five articles in this regard, all quite recent; two new citations from 2024 have now been added. Adhering to the diffusion-only model of leaf gas exchanges, plant ecologists have sought purely physiological explanations for such decoupling. I believe that they should take into account how it can result from gas transport processes, and hope that this is one of the clear messages of the manuscript.
3. Quantitative demonstration: Whether or not it was easy to understand, the original manuscript was in fact quite quantitative regarding decoupling. Referring to line numbers in the old revised manuscript (submitted on 26 September), it was explained how:
    a. 121-125: carbon dioxide concentrations are reduced as a consequence of Δe (the surplus of water vapour pressure inside substomatal cavities), supressing photosynthesis and
    b. 131-135: for same the gradient in water vapour concentration (or VPD), elevated non-diffusive transport (at high q) enhances water vapour egress and so enhances transpiration.

   The newly revised manuscript attempts to make this more explicit to the reader, referring back to suppression of CO_2 at the end of the paragraph regarding enhanced transpiration, and just prior to discussing water-use efficiency and decoupling. I considered going into more detail, with additional quantitative examples of decoupling and its magnitude, but feel that this would take away from the concision of the paper.
4. Clearer physical description: Having read (and replied to) the comment by Anonymous Referee #2, Report #1 regarding the Bernoulli principle, supported by these suggestions from Referee X, I realise that I chose my words unartfully (hardly surprising) when publishing the terminology of "stomatal jets" (Kowalski, 2017). The word "jet" has high-velocity connotations and is inappropriate in this context. Air does not jet out of stomata, but rather oozes (which seems a better verb for describing viscous motion). I have therefore revised the manuscript to avoid the use of this unhelpful terminology.
5. What sort of experiments should be done: A new Section 5 ("Prospects for Unveiling Stomatal Fluid Mechanics") has been added prior to the paper's conclusions.

13 Dec 2024: Co-editor-in-chief decision: Publish subject to minor revisions (review by editor)

Thank you for uploading responses to the referees. I feel that in principle the manuscript is publishable but I would appreciate if you note in the text the importance of further studies into the fluid mechanics of the stomatal / atmosphere interface, and to consider the comments by the additional reviewer posted underneath my brief letter from Dec. 12. I think that we all agree that we need to revisit popular assumptions regarding stomatal exchange, and to do so a bit more guidance to the community can make your work be of even greater value to the community.

As noted above, I have added a new Section 5 suggesting further studies into the fluid mechanics of stomatal gas exchanges, and made several other modifications as consequences of the comments from Reviewer X.