Reviewer #1

The paper was significantly revised and improved. There are a few technical comments to the chapter 6, only.

Line 150

I guess the term " Θ - the risk associated with stomatal functioning" can be replaced by the term used in Sperry et al 2017 - transpirational cost function.

[Response]

In Sperry et al. (2017), we called it cost. However, since the term is more a shadow cost that does not show up in real instantaneous carbon gain (as pointed out in Buckley (2017) <u>https://doi.org/10.1104/pp.16.01772</u>), we started to use the term risk as used in Venturas et al. (2018).

Line 154

The term "the leaf xylem end water pressure" needs some explanation.

[Response]

We clarified this in lines 150-151 (clean revision, hereafter) that " P_{canopy} is the water pressure at the end of leaf xylem" (also in our new Table 1).

Line 156

"transpiration rate is maximum"

May be - expressions 'all stomata are completely open' or 'maximum stomatal conductance' - are more correct?

[Response]

Stomatal conductance may not be able to fully open during drought stress. For example, if maximal stomatal conductance is 0.3 mol m⁻² s⁻¹, xylem may not even cavitate at well watered condition. In another case, xylem may lose all its conductivity even when stomata are not fully open during a drought. Therefore, we are using the physical limitation to xylem to describe kcrit. We clarified this in lines 151-152 that "k_{crit} is the k_{canopy} when transpiration rate reaches the maximum transport capacity of the xylem".

Reviewer #2

32: ...focuses... ... is not encouraged...[Response]Thanks. We have made the change in the revision.

37-38: It also includes diffusion among mesophyll cells in the liquid phase, and diffusion through the mesophyll in the vapor phase. Vapor-phase transport affects water potentials of all tissues downstream from the leaf xylem, so it must be considered part of "water transport" even though we may prefer to think of "water transport" as ending at the sites of evaporation. Water transport in plants may also include liquid-phase bulk flow through the apoplastic spaces in the mesophyll, and either diffusion or effusion through plasmodesmata, although the importance of those latter two pathways is largely speculative.

[Response]

Thanks for correcting this. We revised the sentence to "Water movement **in xylem conduits** includes mass flow through xylem conduits and diffusion between xylem conduits and capacitance tissues" to focus on xylem water movement only (lines 37-38, clean revision, hereafter). We also added a new paragraph to highlight that the water potential gradient should be used for diffusion among living cells and that water pressure difference should be used for mass flow in the apoplastic spaces: "We note that water transport in plants also include mass flow within the apoplastic spaces (e.g., in roots and leaves; Aloni et al. (1998)) and through plasmodesmata (e.g., between bundle sheath and phloem; Schulz (2015)), liquid water diffusion among living cells, and gaseous vapor phase diffusion among water-air interfaces (e.g., vapor diffusion within the stomatal chamber; Buckley (2015); Buckley et al. (2017)). As recommended, it is more accurate to use potential for diffusion and pressure to mass flow" as in lines 66-70.

39: please define the symbols in the text here, or better, make a table listing all symbols, units, etc., and then refer to the table at line 39.

[Response]

Thanks for the suggestions, and we added a new Table 1 (pasted below).

Symbol	Description	Unit
DP	Driving pressure $(P_{x1} - P_{x2} + \rho g h_1 - \rho g h_2)$	MPa
$P ext{ or } p$	Water pressure	MPa
$P_{\rm c}$	Cell turgor pressure	MPa
$P_{\rm x}$	Xylem water pressure	MPa
Ψ or ψ	Water potential	MPa
$\Psi_{\rm s}$	Osmotic potential from dissolved solute	MPa
Ψ_{sc}	$\Psi_{\rm s}$ of living cells	MPa
$\Psi_{\rm sx}$	$\Psi_{\rm s}$ of xylem sap	MPa
$A_{\rm L}$	Leaf area the xylem supports	m^2
$A_{\rm S}$	Sapwood area	m^2
k	Hydraulic conductance	$\rm mol\; MPa^{-1}\; s^{-1}$
k_{branch}	Hydraulic conductance of the branch	$\rm mol\; MPa^{-1}\; s^{-1}$
$k_{\rm L}$	Leaf area specific hydraulic conductance	$\rm mol\; MPa^{-1}\; m^{-2}\; s^{-1}$
K	Hydraulic conductivity	$\rm mol \ m \ MPa^{-1} \ s^{-1}$
$K_{\rm L}$	Leaf area specific hydraulic conductivity	$\rm mol\; MPa^{-1}\; m^{-1}\; s^{-1}$
K_{S}	Sapwood area specific hydraulic conductivity	$\rm mol\; MPa^{-1}\; m^{-1}\; s^{-1}$
Q	Flow rate through the xylem segment	$ m mol~s^{-1}$
A_{max}	Maximum achievable photosynthetic rate	$\mu mol \; m^{-2} \; s^{-1}$
E	Transpiration rate of the whole plant	$mol s^{-1}$
$E_{\rm crit}$	Maximum E beyond which the plant desiccate	$mol \ s^{-1}$
k_{canopy}	Marginal hydraulic conductance of the canopy $(\mathrm{d}E/\mathrm{d}P_{\mathrm{canopy}})$	$mol MPa^{-1} s^{-1}$
$k_{\text{canopy,ref}}$	k_{canopy} when transpiration rate is 0	$mol MPa^{-1} s^{-1}$
k_{plant}	Whole plant hydraulic conductance	$\rm mol\; MPa^{-1}\; s^{-1}$
P_{canopy}	Water pressure at the end of leaf xylem	MPa
$\Psi_{\rm soil}$	Soil water potential	MPa
Θ	Risk associated with stomatal opening	$\mu mol \; m^{-2} \; s^{-1}$
VC	Vulnerability curve	-
a, b	Logistic function parameters	-, MPa ⁻¹
B, C	Weibull function parameters	MPa, -
k_{\max}	Maximum hydraulic conductance	$\rm mol\; MPa^{-1}\; s^{-1}$
$k_{\rm max,25}$	Maximum hydraulic conductance at 25 $^{\circ}C$	$\rm mol\; MPa^{-1}\; s^{-1}$
m, n	Power function parameters	MPa ⁻ⁿ , -
P_{50}	Xylem water pressure where xylem loses 50% conductance	MPa
η, η_{25}	Viscosity of water (at 25 °C)	Pas
γ , γ_{25}	Surface tension of water (at 25 °C)	${\rm N~m^{-1}}$

39: at the end of this sentence, it may help to explain for readers why you're saying this about the ions: namely, that osmotic potential does not contribute to the driving forces for long-distance transport in the xylem, because (1) the reflection coefficient in the xylem is zero, and (2) the distances involved are too long for diffusion and thus chemical potential to be an important contributor.

[Response]

Reason (1) for the reflection coefficient would require a too detailed (and distracting) explanation to most readers, so we preferred not to include it in the revision. However, see our revised text "Dissolved ions only play a role in liquid water density but do not contribute to the driving pressure for long-distance transport in the xylem, because the distance involved is too long for diffusion and thus chemical potential to be an important contributor." as in lines 39-41.

41: The symbol "psi_m" for osmotic potential is likely to generate confusion. I suggest you use "psi_s" or reverse the sign convention and use greek "pi" for osmotic pressure. psi_m looks like matric potential or mesophyll water potential to me.

[Response]

We changed the symbol from Psi_m to Psi_s (text and figure).



line 43: There is actually a general name for Px + rho*gh, which is the "head" (or hydraulic head).

[Response]

We thought about using water head that has a similar definition in our last revision, but they differ in their units. We removed the sentence and revised the sentence to "Therefore, water potential is imperceptibly used in place of mass flow driving pressure (i.e., DP) because of the gravity term in it." as in lines 44-45.

48-49: This example illustrates *how* delta psi might not equal delta P, but it doesn't explain *why* delta P is the correct driving force in this context instead of delta psi. That explanation is

the one I gave in my first review, about the relevance of different modes of transport (bulk flow vs diffusion) at different spatial scales. Since the purpose of this ms is to clarify these issues and prevent confusion and error, I really think it's necessary to explain this point explicitly.

[Response]

We added the sentence "Besides the fact that the values of DP and water potential difference do not always equal, the primary reason for not misuse them is that water potential describes the tendency for water to move between adjacent phases (where water molecules will diffuse), whereas pressure is more relevant to bulk water movement" as in lines 50-53 to clarify that the reason to not use potential in place of pressure is that they are responsible for diffusion and bulk flow, respectively.

56: The criteria for the delta values to be equal are a bit more strict than this – it also assumes the osmotic potential in the xylem is zero. It's typically pretty small, as you noted earlier, but not zero. And there are circumstances where it's not negligible.

[Response]

We made the criteria more strict in the revision: "(e.g., when there is no height change or external air pressure, and osmotic potential in the xylem is zero)" as in lines 61-62.

61: I used the word "sophistic" in my review in a different context; I wouldn't say the use of leaf water potential is a "sophistic mistake". You could say a "mistake of interpretation" or just mistake.

[Response]

We revised the sentences to "A commonly seen mistake is the use of leaf water potential to describe measurements from the pressure chamber method (Scholander et al., 1964; Boyer, 1967), which gives a decent estimate of xylem water pressure. People often refer to the measurement as leaf water potential as (a) xylem conduit water has very low solute content, (b) gravity term is often negligible compared to the very negative leaf xylem water pressure, and (c) if the water has reached equilibrium internally prior to the pressure chamber measurement, xylem water potential should equal that in the mesophyll" as in lines 71-75.

Also, on 61, the first sentence needs to be linked to the second to make sense. Perhaps "A commonly seen mistake is the use of leaf water potential to describe measurements from the pressure chamber method (Scholander...), which gives a decent estimate of the xylem water potential. People often refer to this measurement as leaf water potential because..." [Response]

See our response above.

63: should add to this list: (c) provided the leaf has been allowed to equilibrate internally prior to the pressure chamber measurement, the water potential in the xylem should equal that in the mesophyll.

[Response]

See our response above.

65: "For example, when the whole plant is under equilibrium, leaf water potential should be equal everywhere, but the measured leaf xylem pressure would differ for leaves at different height." The first part of this sentence is incorrect (if you interpret water potential as a measurable) or inadvertently misleading (if you interpret it to include the un-measurable "gravitational component"). *Measured* leaf water potential (e.g. by psychrometry or the Shardakov method) would not be equal everywhere; assuming no solutes in the xylem, leaf water potential would necessarily equal xylem water potential everywhere, which in turn would equal xylem pressure (if no solutes). Now, that's not true if you include the 'gravitational component', but including that term is always confusing because *it cannot be measured* - any method of measuring WP involves chemically equilibrating the leaf's water with some other pool of water whose potential is either known (as in Shardakov) or can be measured (as in the vapor in the psychrometer chamber), and that act of chemical equilibration requires the two pools of water to be close enough that the gravitational component can't differ measurably. Something that cannot be measured in practice and only exists in a theoretical definition is only going to generate confusion... unless you tackle the confusion head-on. I realize this issue is confusing for readers, but again, if this ms is to serve its intended purpose, it should tackle these difficult issues openly!

[Response]

The definition of water potential is the sum of water pressure, osmotic potential, and gravity component. However, the measurements do not include the gravity component, and a "true" water potential has to be computed. The main idea of the sentence is that we should refer to the pressure chamber measurements as estimates of xylem pressure. As the idea has been conveyed already, we removed the sentence to avoid confusion.

We clarified this in the revision: "It is recommended to refer to the measurement as leaf/xylem water pressure or balance pressure in the future, rather than leaf/xylem water potential that is not directly measurable." as in lines 77-79.

73: I think the proper term here is "extensive" properties, not bulk properties. They are extensive because their numerical values depend on the extent (size) of the system. e.g., flow rate will be greater for 10 xylem conduits taken together than for just one conduit, whereas flux (flow per cross sectional area) would be the same in both cases, and would be an "intensive" quantity. [Response]

Thanks for the suggestion. We revised the text to "Hydraulic conductance (flow rate divided by driving pressure) is an extensive property (depends on the extent/size of the system), whereas hydraulic conductivity is an intensive property that is supposed to represent different xylem anatomy" as in lines 82-84.

74: It's true that xylem structure is often highly non-uniform, but you could say the same thing about electrical properties of most realistic things in nature. The electrical vs hydraulic distinction doesn't really clarify here. What matters is that "conductivity" is meant to describe an intensive property.

[Response]

See our response above.

76: Using "E" for flow rate here could confuse readers, because it so often means "transpiration rate", which in turn is conventionally expressed on a leaf area basis – so k would then look like a conductivity because E would look like a flux.

[Response]

Thanks for pointing it out. I have the tendency to define E as flow rate, and E_{leaf} as transpiration rate per leaf area. To not confuse readers, we used Q for the hydraulic conductance equations, and E as the whole plant transpiration in the paper (Table 1).

77: I'm not sure what you mean by "and area not accounted for by k".

[Response]

We clarified this in the revision: "(a) hydraulic conductance (namely k) is the ratio between flow rate through the segment (Q) and driving pressure ($\Delta P - \rho g \Delta h$) (an extensive parameter depending on segment length and cross-section area), (b) hydraulic conductivity (namely K) is the ratio between flow rate and driving pressure gradient (an extensive parameter depending on segment cross-section area)" as in lines 84-87.