

New Phytologist Supporting Information

Article title: Advancing the helox compensation method to estimate intercellular humidity in leaves of diverse species

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Table S1. Single-surface cuticular conductances (g_{cw}) used for each species.

species	cuticular conductance / [mol m ⁻² s ⁻¹]
<i>Gossypium hirsutum</i> L.	0.00292 ⁽¹⁾
<i>Helianthus annuus</i> L.	0.0016 ⁽¹⁾
<i>Heteromeles arbutifolia</i> M.Roem.	0.000955 ⁽²⁾
<i>Laurus nobilis</i> L.	0.00036 ⁽¹⁾
<i>Liriodendron tulipifera</i> L.	0.00409 ⁽³⁾
<i>Magnolia grandiflora</i> L.	0.00265 ⁽⁴⁾
<i>Oryza sativa</i> L.	0.00427 ⁽¹⁾
<i>Pyrrosia hastata</i> Ching	0.00073 ⁽⁵⁾

Sources: (1) Kerstiens (1996), (2) Pivovarov et al. (2018), (3) Burlett et al. (2025), (4) Hussain et al. (2024), (5) mean for six fern species by Trueba et al. (2026).

Table S2. List of mathematical parameters used in the SI, with symbols and units. PES, proximal evaporating site in 2-point model of leaf interior; DES, distal evaporating site.

parameter	symbol	units
transpiration rate in nitox	E	$\text{mol m}^{-2} \text{s}^{-1}$
transpiration rate in helox	E'	$\text{mol m}^{-2} \text{s}^{-1}$
ratio of transpiration rate in helox to nitox	ε	unitless
vapor flux from PES to DES	F_v	$\text{mol m}^{-2} \text{s}^{-1}$
leaf total conductance to water vapor	g_{tw}	$\text{mol m}^{-2} \text{s}^{-1}$
intercellular relative humidity	h	unitless
h corrected for systematic increase in E in helox	$h_{\text{corrected}}$	unitless
h at DES in nitox	h_d	unitless
h at DES in helox	h_d'	unitless
h at PES in nitox	h_p	unitless
ratio of g_{tw} in helox to nitox	η	unitless
gas constant	R	$\text{J mol}^{-1} \text{K}^{-1}$
liquid phase resistance from xylem to evaporating site	R_L	$\text{m}^2 \text{s MPa mol}^{-1}$
liquid phase resistance from xylem to DES	R_{LD}	$\text{m}^2 \text{s MPa mol}^{-1}$
liquid phase resistance from xylem to PES	R_{LP}	$\text{m}^2 \text{s MPa mol}^{-1}$
liquid phase equivalent of r_v	R_v	$\text{m}^2 \text{s MPa mol}^{-1}$
vapor phase resistance from PES to DES	r_v	$\text{m}^2 \text{s mol}^{-1}$
ratio of R_v to R_{LD}	ρ_L	unitless
ratio of R_{LP} to R_{LD}	ρ_v	unitless
temperature	T	K
molar volume of water	V_w	$\text{m}^3 \text{mol}^{-1}$
ambient water vapor mole fraction in nitox	w_a	mol mol^{-1}
ambient water vapor mole fraction in helox	w_a'	mol mol^{-1}
intercellular water vapor mole fraction	w_i	mol mol^{-1}
saturated water vapor mole fraction in nitox	w_s	mol mol^{-1}
saturated water vapor mole fraction in helox	w_s'	mol mol^{-1}
saturated water vapor mole fraction at DES in nitox	w_{sd}	mol mol^{-1}
saturated water vapor mole fraction at DES in helox	w_{sd}'	mol mol^{-1}
saturated water vapor mole fraction at PES in nitox	w_{sp}	mol mol^{-1}
saturated water vapor mole fraction at PES in helox	w_{sp}'	mol mol^{-1}
composite parameter (RT/V_w)	y	MPa
water potential of apoplastic water	ψ_{apo}	MPa
water potential at DES	ψ_d	MPa
water potential at PES	ψ_p	MPa
soil water potential	ψ_s	MPa
leaf xylem water potential	ψ_x	MPa

Table S3. Sets of simulations and associated parameter values.

Other parameter values were as follows unless otherwise noted: $E = 0.005 \text{ mol m}^{-2} \text{ s}^{-1}$, $T = 25^\circ\text{C}$, $w_a = 0.007 \text{ mol mol}^{-1}$, $\psi_x = -0.5 \text{ MPa}$, $T_{\text{proximal}} = T_{\text{distal}}$, $T_{\text{helox}} = T_{\text{nitox}}$.

simulation set	objective	parameters varied, and range of values
I	determine relevant ranges of unknown parameters	$R_{\text{LD}} \in [10^0, 10^5] \text{ m}^2 \text{ s MPa mol}^{-1}$ $\rho_L \in [10^{-3}, 10^3]$ $\rho_V \in [10^{-3}, 10^3]$
II	assess within-leaf vapor gradients and their impact on interpretation of results from one-point model of the leaf interior	$R_{\text{LD}} \in [10^3, 10^{4.25}] \text{ m}^2 \text{ s MPa mol}^{-1}$ $\rho_L \in [0.01, 1.0]$ $\rho_V \in [0.1, 1.0]$
III	assess effect of helox on within-leaf vapor gradients	
IV	assess impact of within-leaf temperature gradients on vapor gradients and experimental inference of intercellular humidity	<i>as simulation sets II and III, plus:</i> $T_{\text{proximal}} - T_{\text{distal}} \in [0, 0.3] \text{ K}$
V	assess impact of temperature shift upon switching from nitox to helox and experimental inference of intercellular humidity	<i>as simulation sets II and III, plus:</i> $T_{\text{helox}} - T_{\text{nitox}} \in [0, 0.5] \text{ K}$

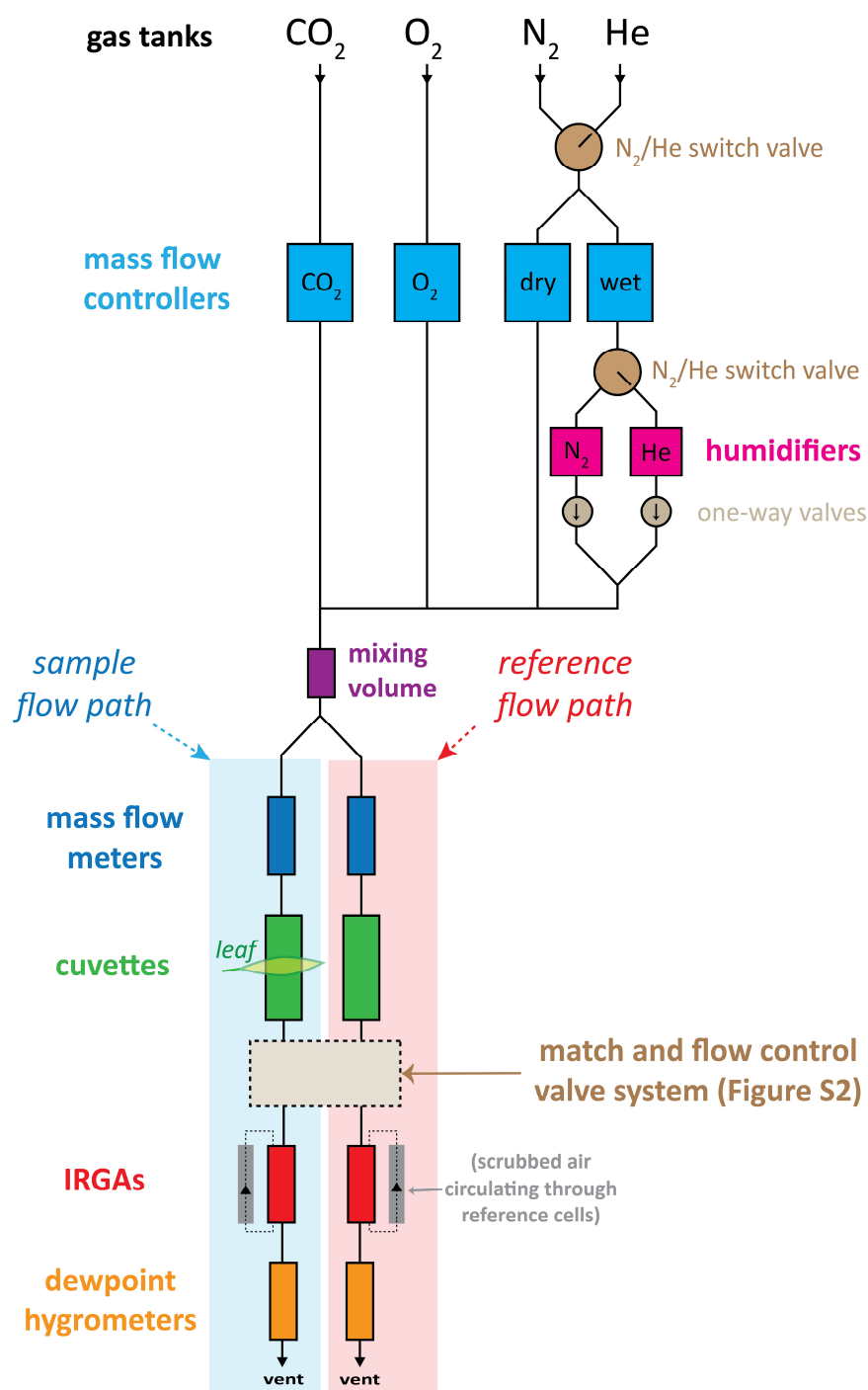


Figure S1. Diagram of the gas exchange system. Flow proceeds from top to bottom in this diagram. The match and flow control valve system is detailed in Figure S2.

match and
flow control
valve system

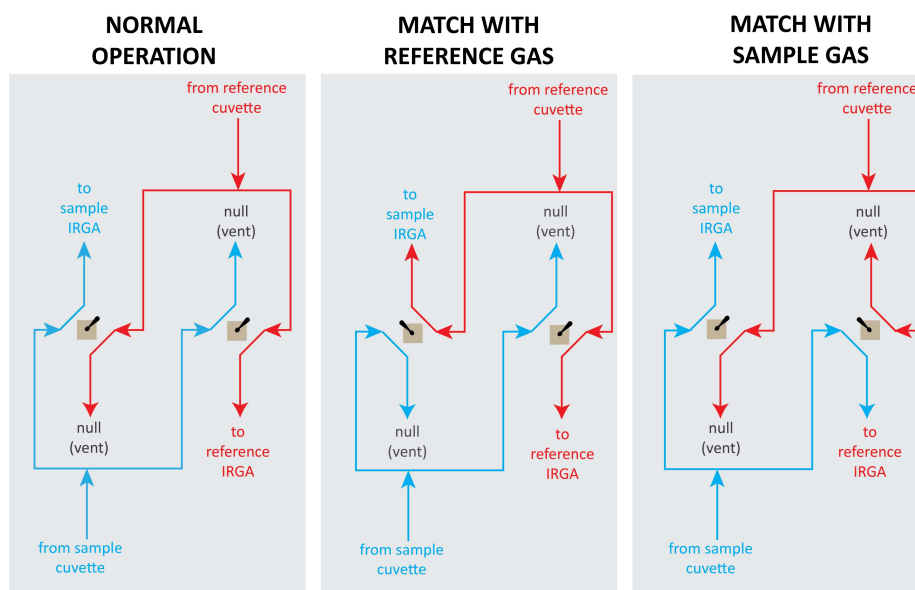
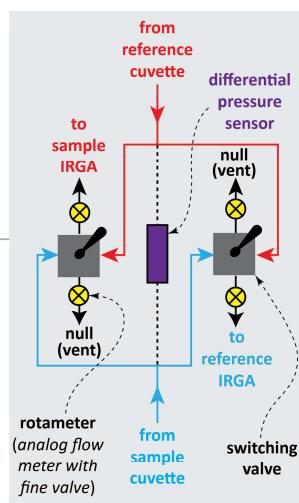


Figure S2. Diagram of the match and flow control system that lies between the cuvettes and the IRGAs in the overall system diagram shown in Figure S1. Switching valves enable matching of the analyzers using either sample or reference gas, and the combination of rotameters with fine needle valves and a differential pressure sensor enables balancing of flows and pressures in each of four gas streams (the primary and null streams for both reference and sample gas) to prevent changes in flow or pressure anywhere in the system when matching.

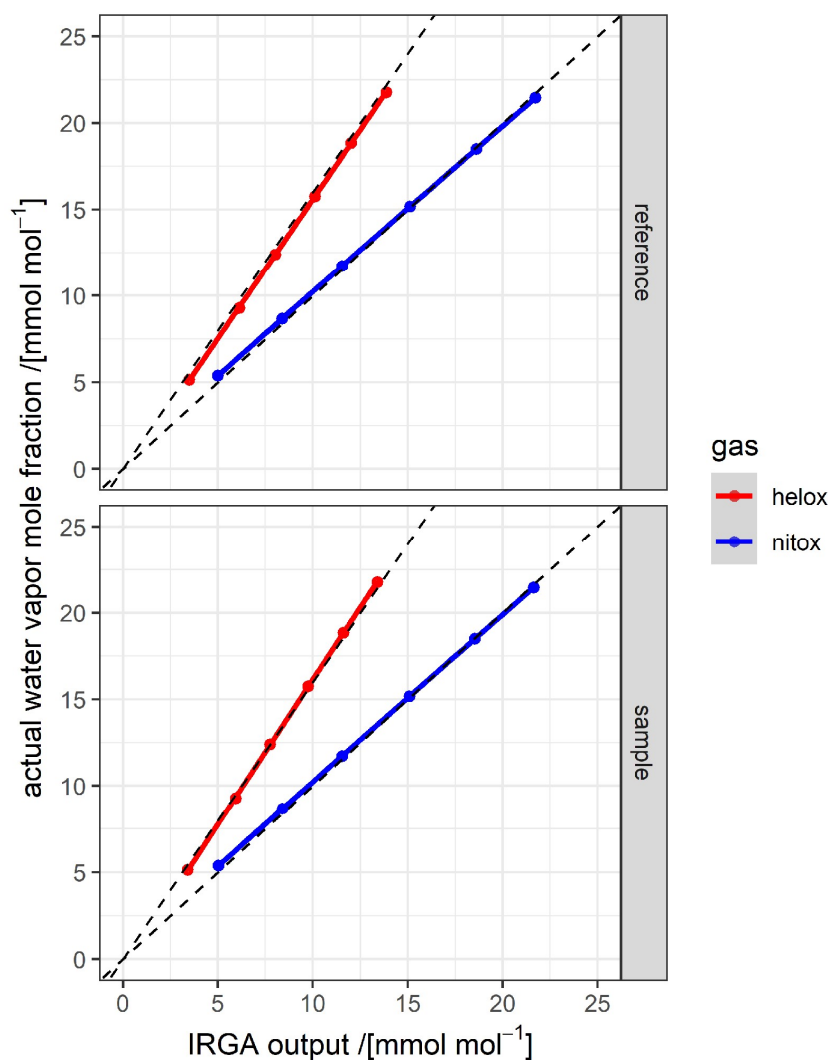


Figure S3. Infrared gas analyzer (IRGA) calibration; values on the horizontal axis are water vapor mole fractions reported by the reference IRGA (top) and sample IRGA (bottom) in nitox (blue) and helox (red), and values on the vertical axis are water vapor mole fractions reported by the chilled mirror dewpoint hygrometer. The colored lines are fitted second-order polynomials, and the dashed lines are lines passing through the origin, with slopes 1 (approximately aligned with the nitox data) or 1.6 (approximately aligned with the helox data).

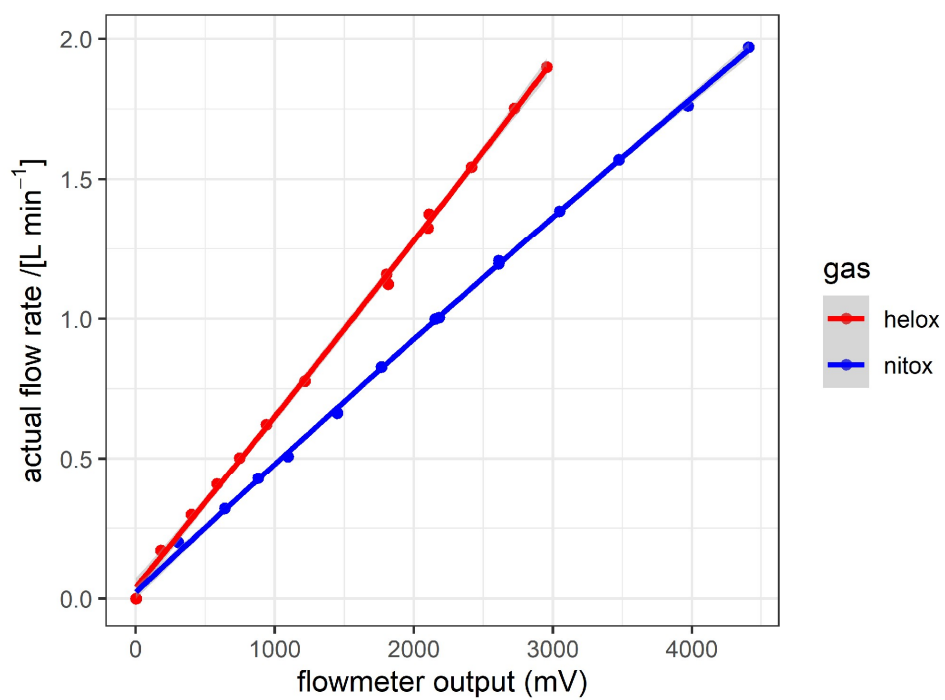


Figure S4. Calibration of leaf cuvette flow meter in nitox (blue) and helox (red). The lines are fitted second-order polynomials (nitox: $r^2_{\text{adj}} = 0.9993$, $p < 2.2 \cdot 10^{-16}$; helox: $r^2_{\text{adj}} = 0.9987$, $p < 2.2 \cdot 10^{-16}$).

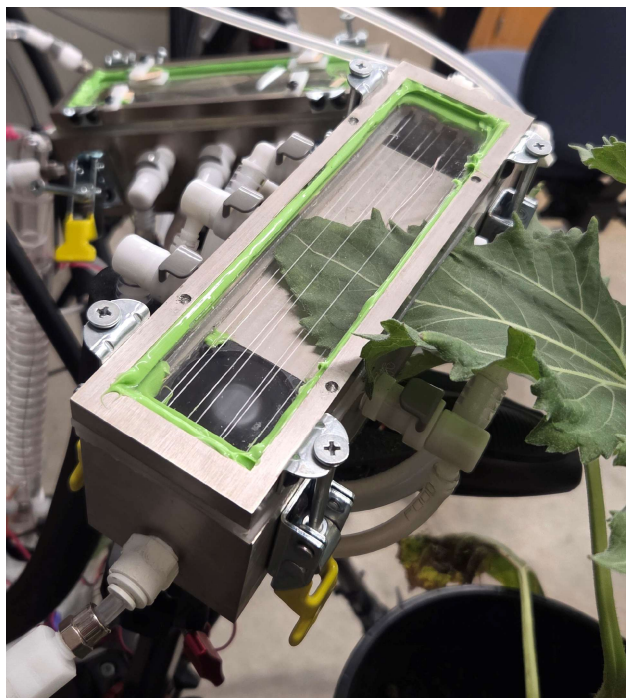


Figure S5. Photo of the sample leaf cuvette with a leaf of *Helianthus annuus* in the cuvette. The gas inlet is in the foreground, on the short side of the cuvette; the fittings on the long sides of the cuvette control water flow from the temperature-controlled recirculating bath. The green material on the rim of the lid is dental putty, used to seal the window; it did not protrude into the area occupied by the leaf. The reference cuvette is visible in the background. The light source is not shown; it was removed to enable this photo.

Main block of chamber
(baseplate not shown,
lid shown below)

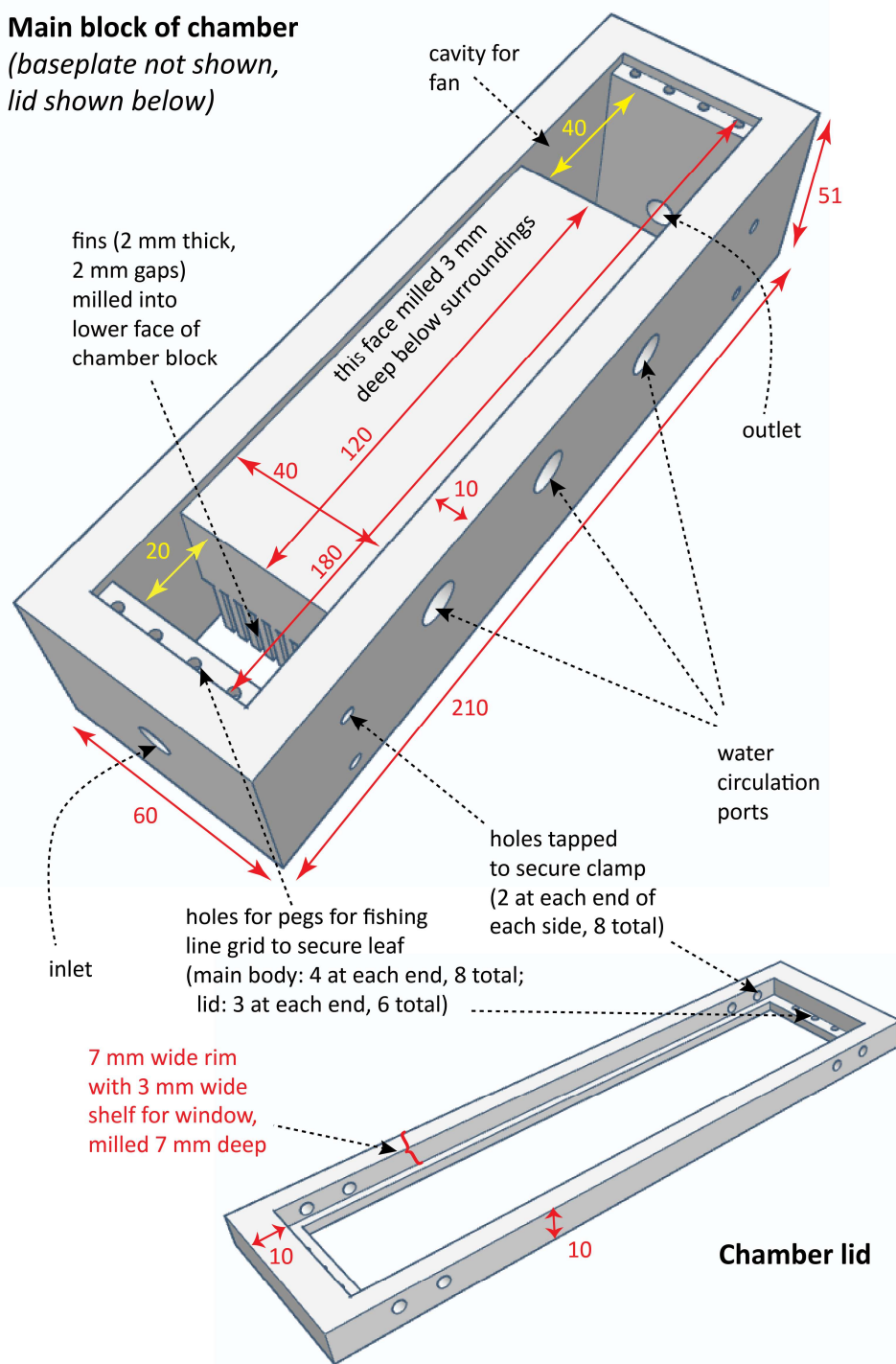


Figure S6. Drawings of the leaf cuvette design. All dimensions are given in millimeters. Not shown are (1) a mesh of fishing lines used to hold the leaf in a fixed lateral plane between the gaskets; (2) the gaskets themselves, which are each 3 mm thick and mounted to the upper rim of the chamber body and the lower rim of the chamber lid; (3) the glass window in the chamber lid; and (4) the baseplate that seals the bottom of the chamber body.

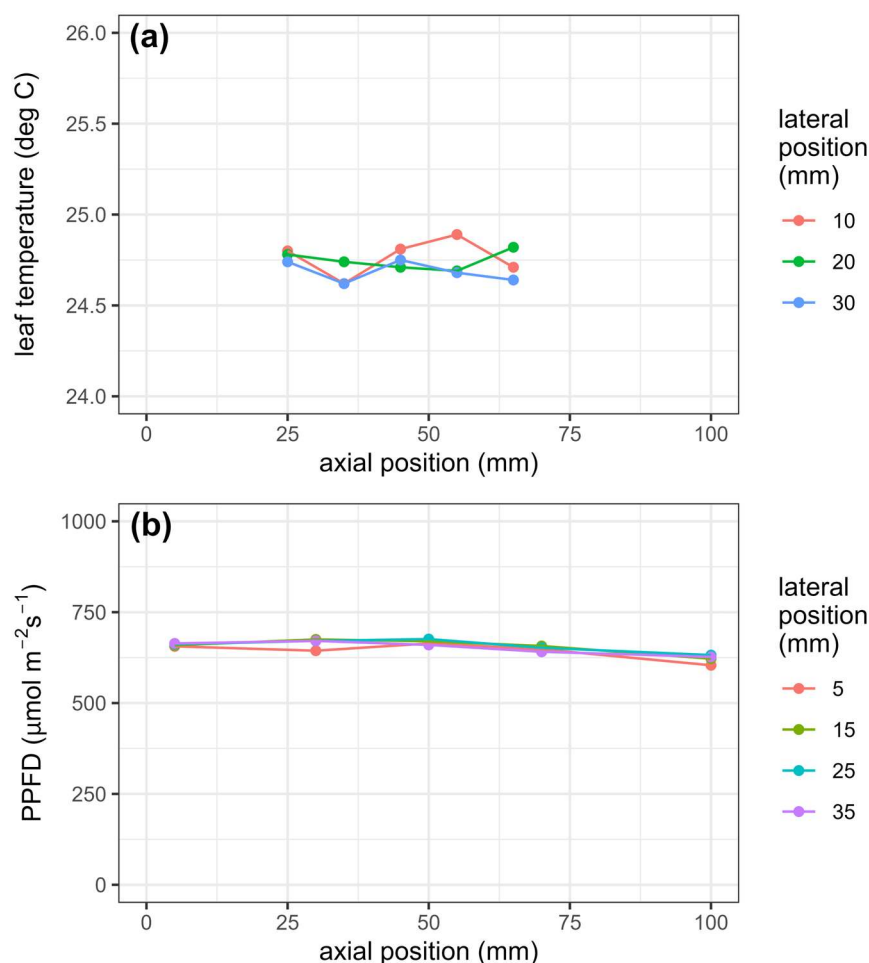


Figure S7. Variation in (a) leaf temperature and (b) photosynthetic photon flux density (PPFD) at the leaf surface. For (a), the leaf thermocouple was moved axially in 10-mm steps across most of the axial width (where axial refers to the long axis of the cuvette, which is also the direction of airflow) of a *Helianthus annuus* leaf in the gas exchange cuvette, then repositioned laterally (i.e., along the narrow axis of the cuvette), and the procedure was repeated. The leaf was illuminated at $900 \mu\text{mol m}^{-2} \text{s}^{-1}$, and had a steady transpiration rate and stomatal conductance of $4.2 \text{ mmol m}^{-2} \text{s}^{-1}$ and $0.32 \text{ mol m}^{-2} \text{s}^{-1}$, respectively, with Δw of 8 mmol mol^{-1} . The highest and lowest temperatures measured were 24.89 and 24.62 $^{\circ}\text{C}$, respectively, the mean was 24.73 $^{\circ}\text{C}$, and the standard deviation was 0.078 $^{\circ}\text{C}$. For (b), the light source was located at a distance of approximately 12 cm from the leaf surface and the cuvette lid was held in place but the cuvette body was removed, a LI-190R quantum sensor (LI-COR, Lincoln, NE) was placed beneath the cuvette lid, at the height that a leaf would normally occupy), and the sensor was moved to each of 20 points across the portion of the leaf cuvette that typically contained a leaf. The highest and lowest values measured were $676 \mu\text{mol m}^{-2} \text{s}^{-1}$ and $604 \mu\text{mol m}^{-2} \text{s}^{-1}$, respectively, the mean was $652 \mu\text{mol m}^{-2} \text{s}^{-1}$, and the standard deviation was $19 \mu\text{mol m}^{-2} \text{s}^{-1}$.

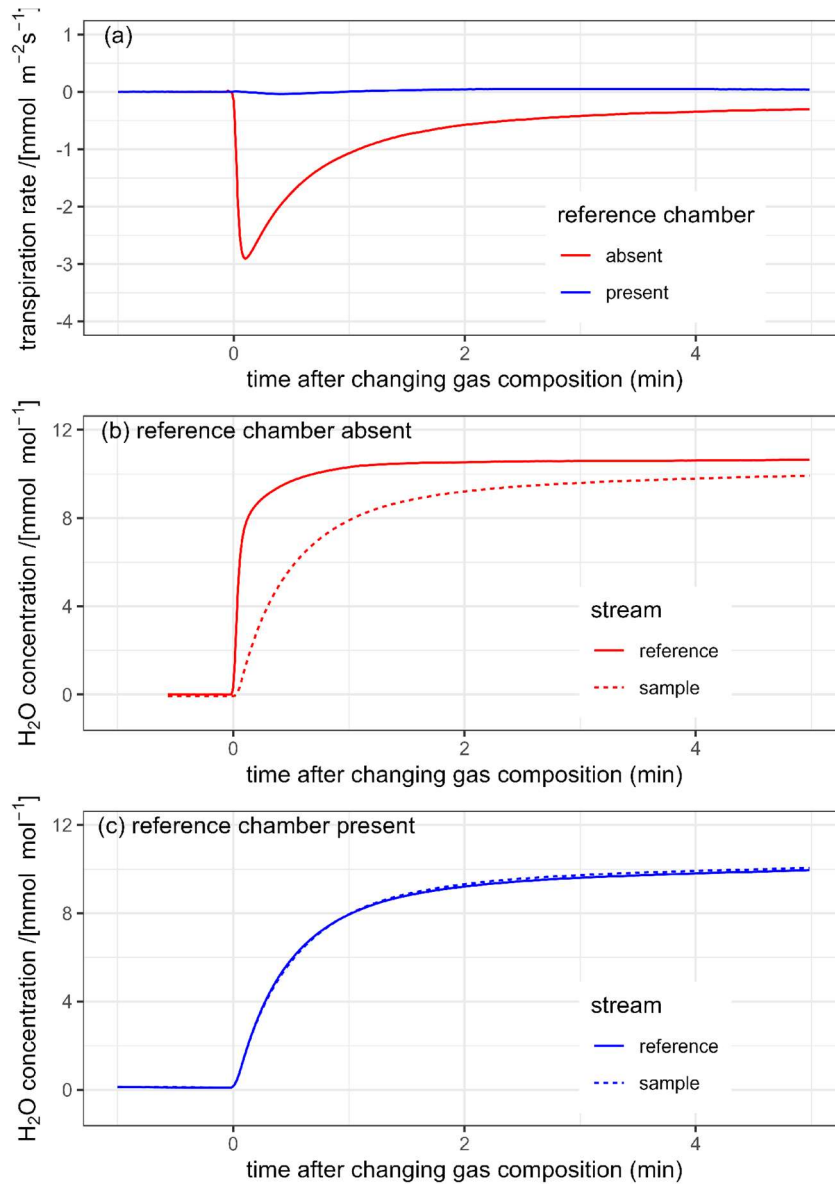


Figure S8. (a) Even with no leaf in the chamber, calculated transpiration rate diverges far from zero for a time after the reference gas vapor composition is changed (red line in (a)). However, including an empty 'reference chamber' in the reference stream (blue line in (a)) largely eliminates the artefact. (b) The artefact occurs because the compositional change propagates through the reference stream (solid lines in b,c) faster than through the sample stream (dashed lines), due to the presence of the leaf chamber in the latter but not the former. (c) This effect is eliminated when an empty chamber is present in the reference stream.

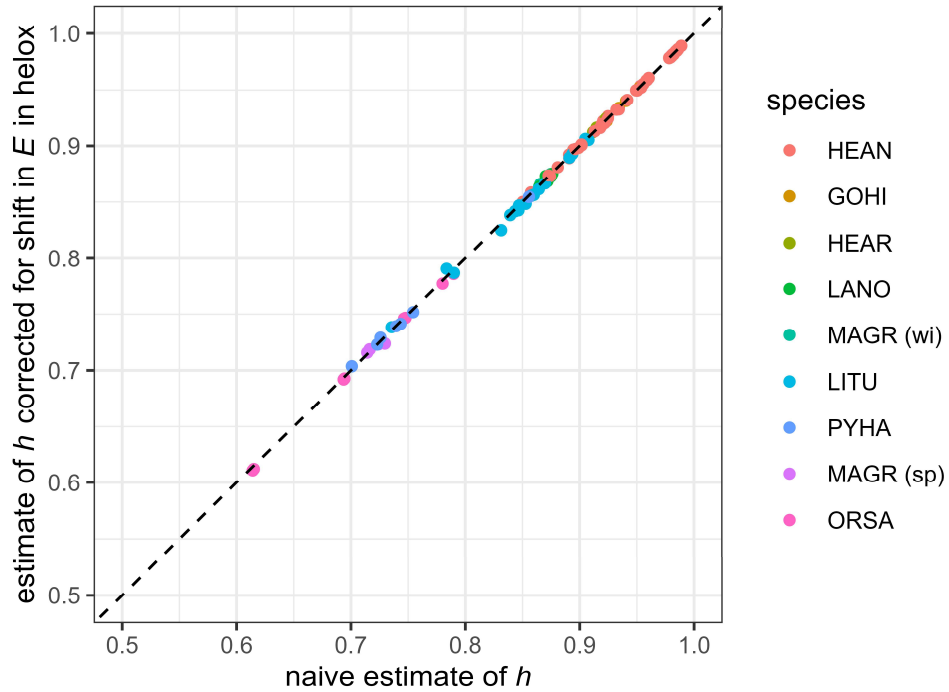


Figure S9. Applying an approximate correction for the effect of a small change in E that occurs upon switching to helox (Eqn S9) has negligible impact on the estimate of intercellular relative humidity (h). The "naive estimate" refers to the quantity $\hat{h}$ in Eqn S9. The dashed line is a 1:1 line.

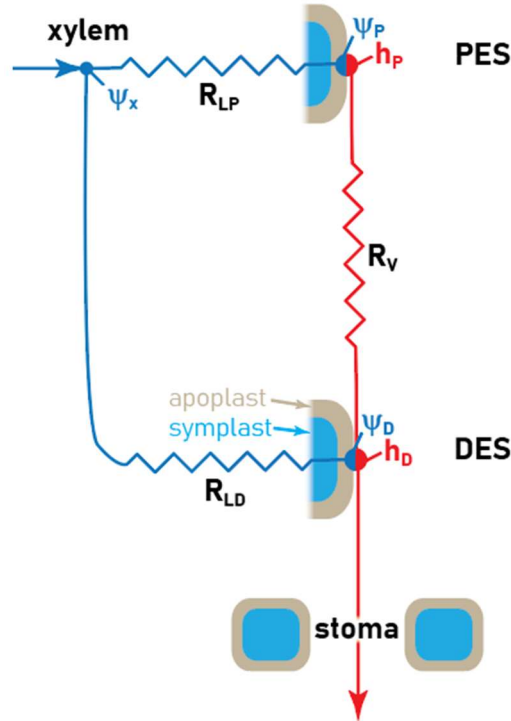


Figure S10. Diagram of two-point model of the leaf interior. We imagine the leaf interior to comprise two evaporating sites (the proximal evaporating site [PES] and the distal evaporating site [DES]), which are each fed by liquid-phase resistances to water flow from the xylem of R_{LP} and R_{LD} , respectively, and which are separated by an airspace resistance of R_V (which is expressed with units of a liquid-phase resistance for commensurability with R_{LP} and R_{LD} ; see Eqn S21). The transpiration stream is partitioned through the PES and DES according to the total resistance of each pathway; distal to the DES, all water moves in the vapor phase, through stomata to the external atmosphere. ψ_x , xylem water potential; ψ_P and ψ_D , liquid phase water potentials at the PES and DES, respectively; h_P and h_D , intercellular relative humidities of air adjacent to the PES and DES, respectively.

Figure S11. Predicted intercellular humidities for a wide range of within-leaf resistances. Long-dashed and short-dashed lines: simulations of relative humidity at the proximal (h_p) and distal (h_d) evaporating sites, respectively, from a two-point model of the leaf interior; solid lines: value of intercellular humidity (h) that would be inferred from experimental data using Eqn 5 in the main text, which is implicitly based on a model that treats the leaf interior as a single point. In many cases, h_d is very similar to h and is thus obscured by the solid lines.

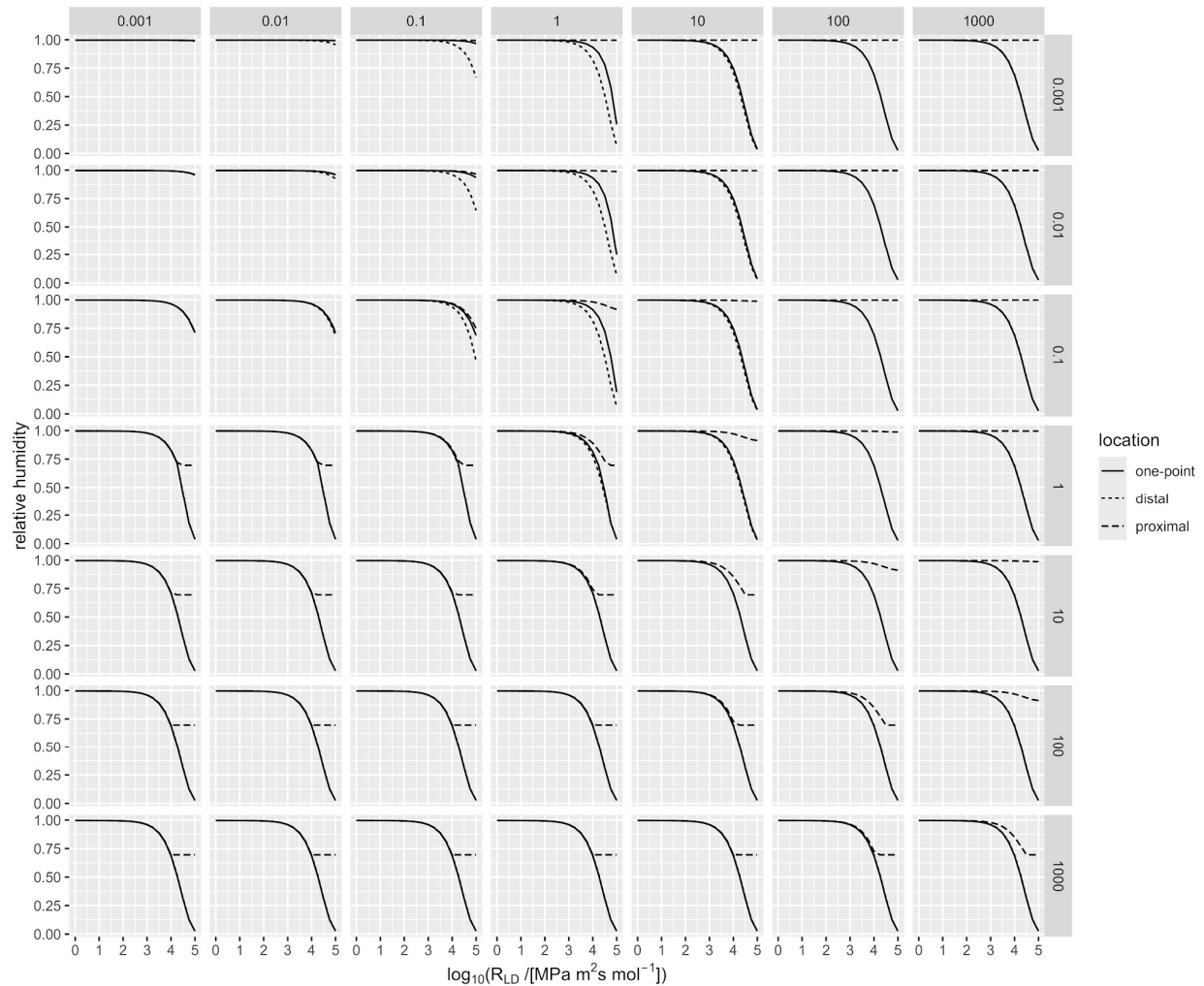


Figure S12. Predicted intercellular humidities. Long-dashed and short-dashed lines: simulations of relative humidity at the proximal (h_p) and distal (h_d) evaporating sites, respectively, from a two-point model of the leaf interior; solid lines: value of intercellular humidity (h) that would be inferred from experimental data using Eqn 5 in the main text, which is implicitly based on a model that treats the leaf interior as a single point. ρ_v is shown in the grey bars at top; ρ_a is shown in the grey bars at right.

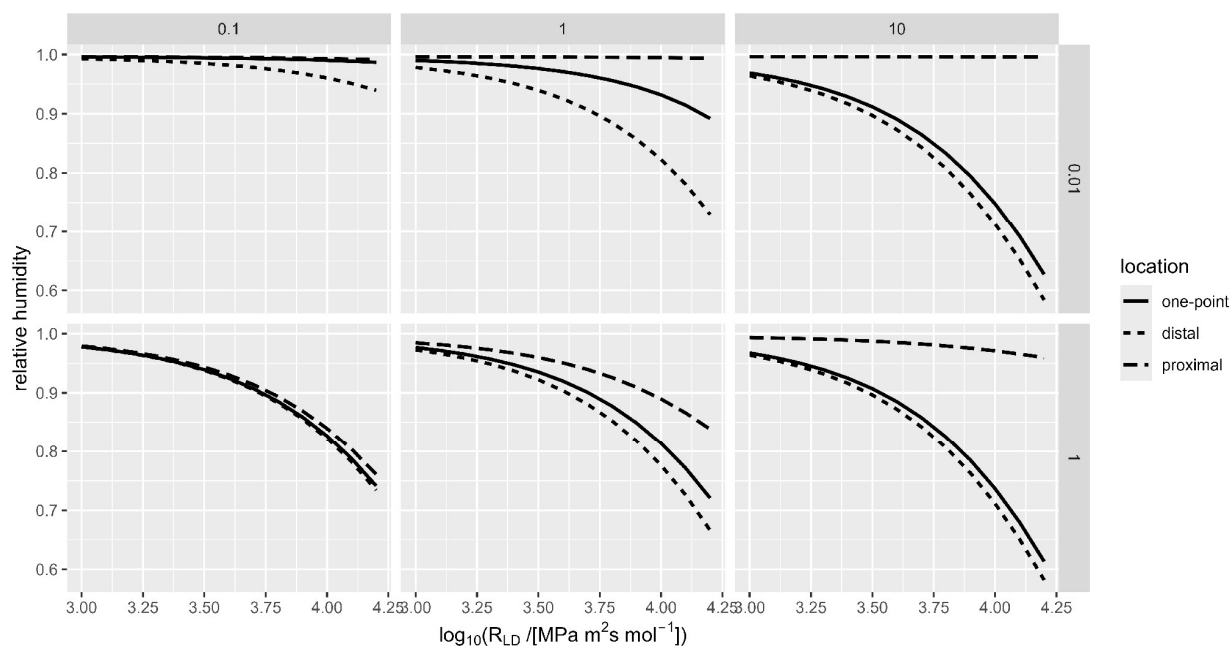


Figure S13. Simulated effects of helox on intercellular humidities. Long-dashed and short-dashed lines: simulations of relative humidity at the proximal (h_p) and distal (h_d) evaporating sites, respectively, from a two-point model of the leaf interior. Black: in nitox; red: in helox. ρ_v is shown in the grey bars at top; ρ_L is shown in the grey bars at right.

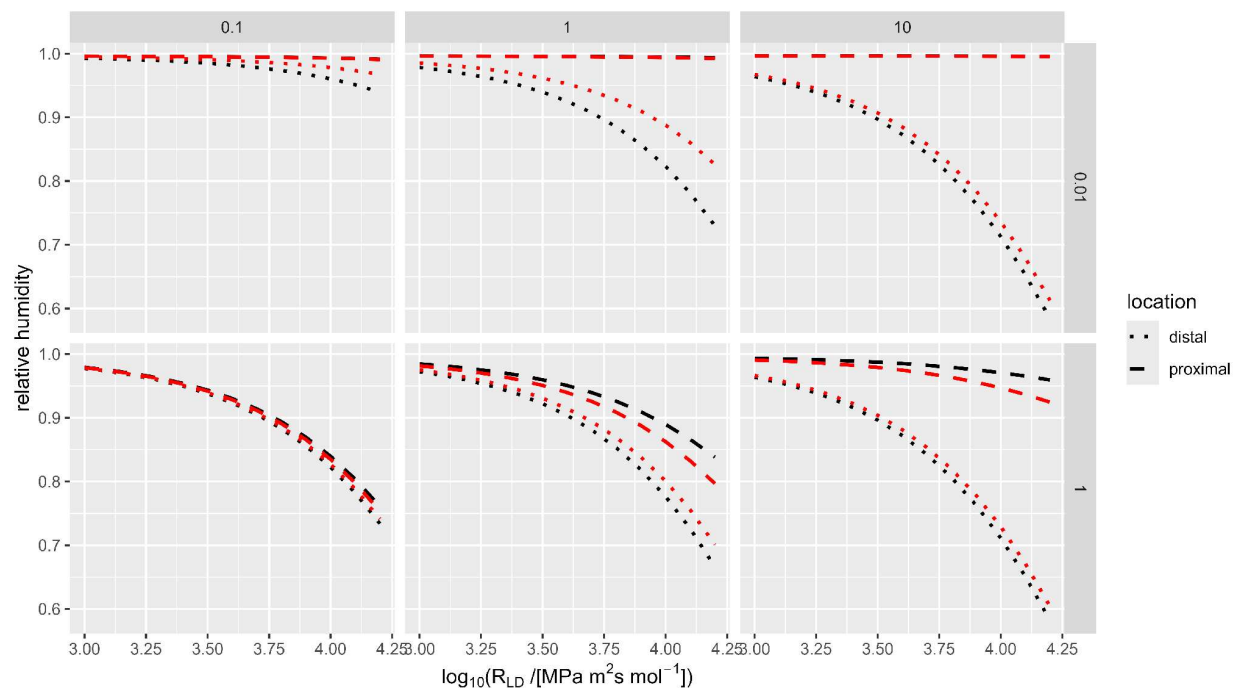


Figure S14. Simulated effects of within-leaf temperature gradient of 0.3 K. Long-dashed and short-dashed lines: simulations of relative humidity at the proximal (h_p) and distal (h_d) evaporating sites, respectively, from a two-point model of the leaf interior; solid lines: value of intercellular humidity (h) that would be inferred from experimental data using Eqn 5 in the main text, which treats the leaf interior as a single point. Red: proximal site is 0.3 K warmer than the distal site; black: proximal and distal sites are at equal temperatures. ρ_v is in the grey bars at top; ρ_L is shown in the grey bars at right.

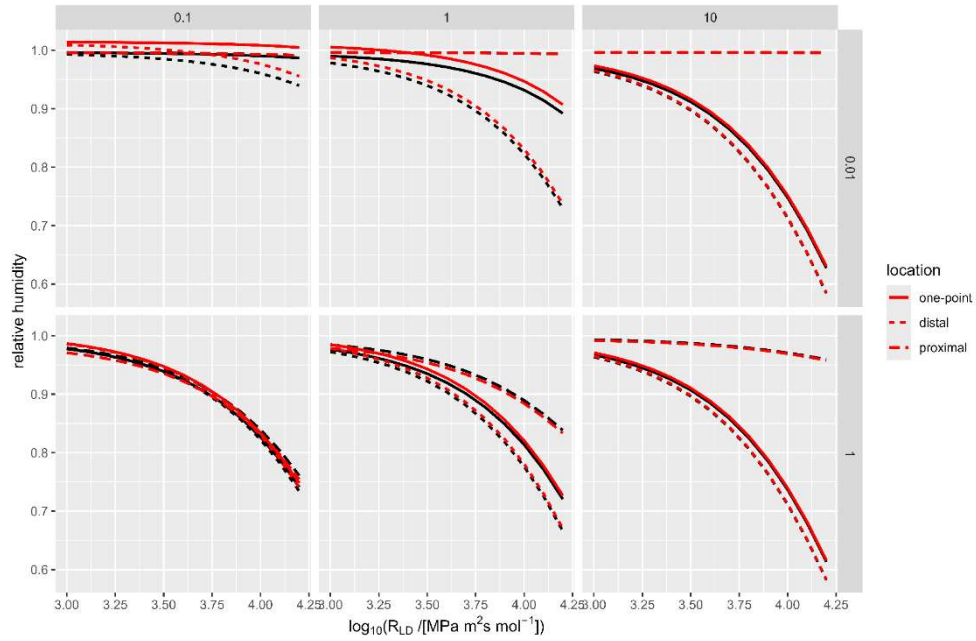
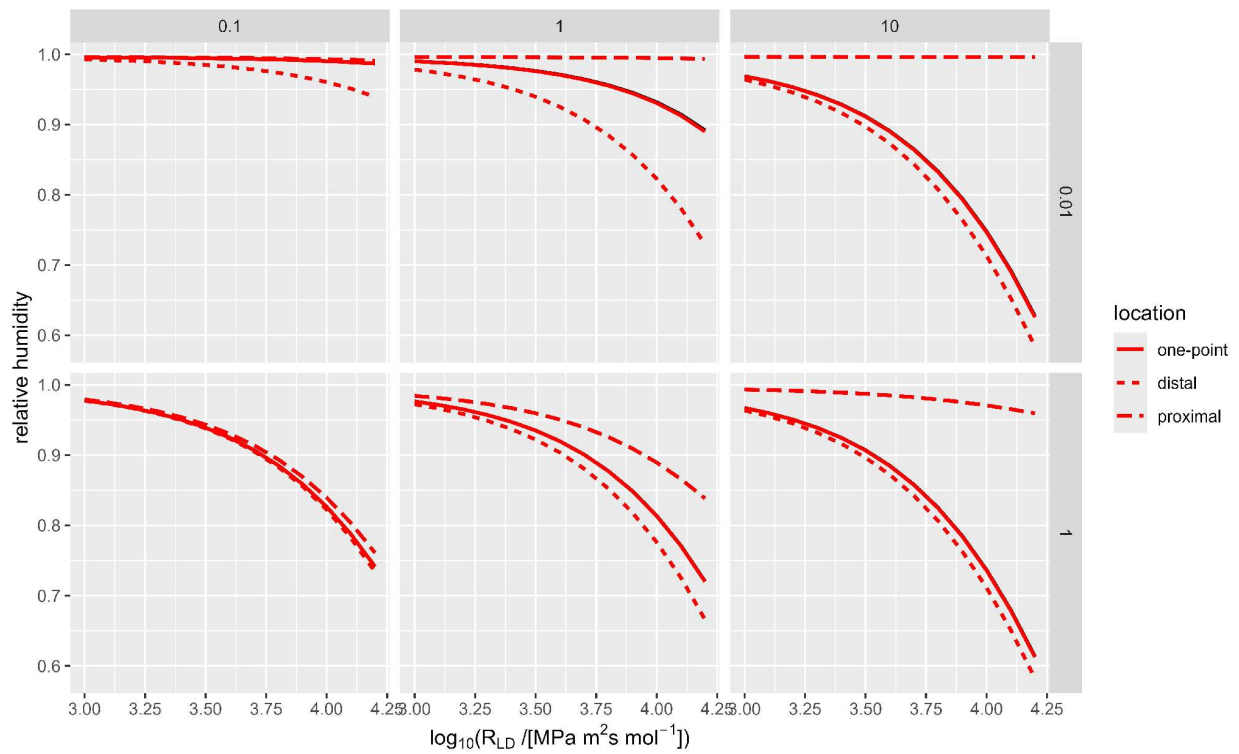


Figure S15. Simulated effects of temperature shift of -0.5 K upon shifting to helox. Long-dashed and short-dashed lines: simulations of relative humidity at the proximal (h_p) and distal (h_d) evaporating sites, respectively, from a two-point model of the leaf interior; solid lines: value of intercellular humidity (h) that would be inferred from experimental data using Eqn 5 in the main text, which is implicitly based on a model that treats the leaf interior as a single point. Red: leaf temperature is 0.5 K lower in helox than in nitox; Black (mostly obscured by the red lines): leaf temperature is identical in helox and in nitox. ρ_v is shown in the grey bars at top; ρ_a is shown in the grey bars at right.



Methods S1. Evaluation of potential bias in estimated intercellular humidity caused by a bias towards a small increase in transpiration rate in helox

Although we were able to keep transpiration rate (E ; symbols used in the SI are defined in Table S1) nearly constant when switching from nitox to helox, there was a very slight bias towards E being greater in helox than in nitox. This could have biased our estimates of intercellular humidity. Here we evaluate that potential bias.

Suppose E increases by a proportion ϵ in helox as compared to nitox:

$$E' = \epsilon E \quad (S1)$$

where the prime denotes a value in helox. Using $E \approx g_{tw}(hw_s - w_a)$, this gives

$$\eta g_{tw}(h'w'_s - a') = \epsilon g_{tw}(hw_s - a) \quad (S2)$$

If $E' > E$, then the apoplastic water potential in helox should be more negative than that in nitox, and therefore h' should also be lower than h . That is, $h' = h + \delta h$, where δh is the change in h caused by a change in water potential; $E' > E$ would imply $\delta h < 0$. Thus

$$\eta g_{tw}((h + \delta h)w'_s - a') = \epsilon g_{tw}(hw_s - a) \quad (S3)$$

This leads to the following solution for h :

$$h = \frac{\eta(-w'_s\delta h + \eta a' - a)}{\eta w'_s - \epsilon w_s} = \frac{\eta(\eta a' - a)}{\eta w'_s - \epsilon w_s} - \frac{\eta w'_s\delta h}{\eta w'_s - \epsilon w_s} = \hat{h} - \frac{\delta h}{1 - \frac{\epsilon w_s}{\eta w'_s}} \quad (S4)$$

where $\hat{h} = \eta(\eta a' - a)/(\eta w'_s - \epsilon w_s)$ is the 'naive' estimate of h that arises from assuming, as we did for the calculations presented in the main text, that $\delta h = 0$. Then

$$\hat{h} = h + \frac{\delta h}{1 - \frac{\epsilon w_s}{\eta w'_s}} \quad (S5)$$

Thus, a bias such that $E' > E$ on average ($\delta h < 0$) should lead $\hat{h}$ to underestimate the true value of h , or equivalently, to overstate the degree of unsaturation. To estimate the value of δh , we can write

$$\delta h \approx \frac{\partial h}{\partial \psi_{apo}} \cdot \frac{\partial \psi_{apo}}{\partial E} \cdot \delta E = \frac{\partial h}{\partial \psi_{apo}} \cdot \frac{\partial \psi_{apo}}{\partial E} \cdot E(\epsilon - 1) \quad (S6)$$

Since $h = \exp(\psi_{apo}/y)$, it follows that $\partial h / \partial \psi_{apo} = h/y$; and since $\psi_{apo} = \psi_s - R_p E$, where ψ_s is source water potential and R_p is the resistance for liquid phase water transport from the source to the evaporating site, it follows that $\partial \psi_{apo} / \partial E = -R_p$. Thus

$$\delta h \approx -R_p \frac{h}{y} \cdot E(\epsilon - 1) \quad (S7)$$

Applying S7 to S5 gives

$$h = \hat{h} + h \cdot \frac{RE}{y} \cdot \frac{\epsilon - 1}{1 - \frac{\epsilon w_s}{\eta w'_s}} \quad (S8)$$

which can be solved for the 'true' value of h (i.e., corrected for the small change in h caused by a small change in E in helox) as

$$h_{corrected} = \frac{\hat{h}}{1 + \frac{\psi_s - \psi_{apo}}{y} \cdot \frac{\epsilon - 1}{1 - \frac{\epsilon w_s}{\eta w'_s}}} \quad (S9)$$

We computed $h_{corrected}$ as given by Eqn S9 for every nitox-helox switch, assuming $\psi_s \ll \psi_{apo}$. The results showed that in all but two cases, $h_{corrected}$ was within 1% of $\hat{h}$, and moreover, that $\hat{h}$ was actually greater than $h_{corrected}$ in most cases (Fig S9). We omitted the two cases in which $h_{corrected}$ and $\hat{h}$ differed by more than 1%.

Methods S2. Two-point model of the leaf interior, and implications for estimating intercellular humidity

The Egorov and Karpushkin (EK) method assumes there are no significant vapor gradients within the leaf. That assumption is convenient because it implies that the pathway for water transport consists of liquid- and vapor-phase paths that are strictly in series, not in parallel, which in turn implies that helox does not affect water transport proximal to the evaporating site. If correct, this in turn would imply that h should be unaffected by helox, provided E is constant. However, if instead the sites of evaporation are distributed throughout the leaf interior, then some portion of total water transport must occur in the vapor phase – from more-proximal evaporating sites to more-distal evaporating sites. Helox would decrease the resistance of those vapor-phase pathways, therefore altering the distribution of flows among the liquid phase pathways proximal to the sites of evaporation. That would, in turn, alter the water potentials at the evaporating sites, and hence the relative humidity of air immediately adjacent to them. This would contradict the assumption, tacit in Equation 5 in the main text, that h is unaffected by helox.

We addressed this concern through modeling and scenario analysis. First, we derived a formal mathematical description for liquid and vapor phase water movement in the leaf interior, for a model containing two evaporating sites: one located close to the xylem (the proximal site) and one located just inside the stomata (the distal site). This model leads to predictions for the relative humidities at each site in both nitox and helox, but requires iterative numerical solution. Then, we solved the model numerically to simulate the implications of various assumptions regarding the partitioning of liquid and vapor phase resistances within the leaf, and compared the resulting predictions with the value of intercellular relative humidity that we would estimate using the single-point model (Eqn 5 in the main text). Below, we derive the model and then describe the simulations.

Two-point model of the leaf interior

First consider a single-point model of the leaf interior, in which liquid water arrives at the site of evaporation via liquid-phase pathways from the xylem, then evaporates and diffuses out through the stomata. h is the relative humidity of air immediately adjacent to those evaporating sites. In the limit of vanishingly small distances from the liquid surface, h approaches equilibrium with the water potential of apoplastic liquid water at the evaporating site (ψ_{apo}). At steady state, transpiration rate is equal to the rate of water transport from the xylem to the evaporating site, through a liquid-phase resistance R_L :

$$E = \frac{\psi_x - \psi_{apo}}{R_L} \quad (S10)$$

which gives $\psi_{apo} = \psi_x - R_L E$. Since R_L is liquid-phase, it is unaffected by helox; and since E is also unaffected by helox by design in the EK method, it follows that helox should not change ψ_{apo} or h . This allows h to be estimated using Eqn 5 in the main text.

Now consider a two-point model of the leaf interior (Fig S10), with two evaporating sites (proximal and distal) fed by liquid-phase resistances from the leaf xylem (R_{LP} and R_{LD} , respectively) and a vapor-phase resistance r_v between the proximal and distal sites. All flow must occur through the two evaporating sites, and hence through the two liquid-phase pathways proximal to those sites, so that, at steady-state,

$$E = \frac{\psi_x - \psi_p}{R_{LP}} + \frac{\psi_x - \psi_d}{R_{LD}} \quad (S11)$$

where ψ_p and ψ_d are the apoplastic water potentials at the proximal and distal evaporating sites, respectively. Vapor-phase flow from the proximal site to the distal site occurs at a rate

$$F_V = \frac{h_p w_{sp} - h_d w_{sd}}{r_V} \quad (S12)$$

where h_p and h_d are the relative humidities of air immediately adjacent to the proximal and distal evaporating sites, respectively, and w_{sp} and w_{sd} are the corresponding the saturated water vapor mole fractions. h_p and h_d are related to ψ_p and ψ_d , respectively, by

$$h_p = \exp\left(\frac{\psi_p}{y}\right), \quad h_d = \exp\left(\frac{\psi_d}{y}\right) \quad (S13)$$

where $y \equiv RT/V_w$ (R being the gas constant, T the absolute temperature, and V_w the molar volume of liquid water). F_V must also equal the liquid-phase flow to the proximal evaporating site, so

$$\frac{w_{sp} \exp\left(\frac{\psi_p}{y}\right) - w_{sd} \exp\left(\frac{\psi_d}{y}\right)}{r_V} = \frac{\psi_x - \psi_p}{R_{LP}} \quad (S14)$$

Solving Eqn S14 for ψ_d gives

$$\psi_d = y \ln \left[\frac{w_{sp}}{w_{sd}} \exp\left(\frac{\psi_p}{y}\right) - \frac{r_V}{w_{sd}} \frac{\psi_x - \psi_p}{R_{LP}} \right] \quad (S15)$$

Solving Eqn S11 for ψ_d gives

$$\psi_d = \psi_x - R_{LD} \left(E - \frac{\psi_x - \psi_p}{R_{LP}} \right) \quad (S16)$$

Setting S15 and S16 equal gives an expression for ψ_p :

$$\psi_x - R_{LD} \left(E - \frac{\psi_x - \psi_p}{R_{LP}} \right) = y \ln \left[\frac{w_{sp}}{w_{sd}} \exp\left(\frac{\psi_p}{y}\right) - \frac{r_V}{w_{sd}} \frac{\psi_x - \psi_p}{R_{LP}} \right] \quad (S17)$$

In helox, r_V is reduced 2.33-fold, modifying Eqn S17 to

$$\psi_x - R_{LD} \left(E - \frac{\psi_x - \psi'_p}{R_{LP}} \right) = y \ln \left[\frac{w_{sp}}{w_{sd}} \exp\left(\frac{\psi'_p}{y}\right) - \frac{r_V}{2.33 w_{sd}} \frac{\psi_x - \psi'_p}{R_{LP}} \right] \quad (S18)$$

Equations S13 and S16-S18 comprise an implicit model for h_d , h_p , h'_d and h'_p , which we solved numerically in simulations described below. With h_d (or h'_d), and with an assumed value for ambient humidity in nitox (w_a), the equality between transpiration rate in nitox and helox (Eqn 3 in the main text) can be inverted to calculate the implied ambient humidity in helox (w'_a):

$$w'_a = \frac{\epsilon \eta h'_d w'_{sd} (2 - w_{sd} - w_a) - (h_d w_{sd} - w_a) (2 - h'_d w'_{sd})}{\epsilon \eta (2 - h_d w_{sd} - w_a) - (h_d w_{sd} - w_a)} \quad (S19)$$

This value can then be applied to the expression used to estimate h experimentally in this study (Eqn 5), along with values for η , w_s , w_s' , and w_a , for which we used 2.33, w_{sd} , w_{sd}' , and the input value of w_a , respectively. In this way, we can use the two-point model of the leaf interior to predict the value of h that would be inferred from experimental measurements using the one-point model of the leaf interior. The rationale for using w_{sd} and w_{sd}' , rather than w_{sp} and w_{sp}' , as the experimental estimates for w_s and w_s' , respectively, is that the latter are experimentally calculated from the temperature reported by an ultrafine thermocouple appressed to the lower leaf surface, which should be closer to the true temperature of the distal evaporating site than to that of the proximal evaporating site.

Vapor-phase resistance

Note that $e^x \approx 1 + x$ for x close to zero. Thus, for water potentials greater than -30 MPa, $\exp(\psi/y)$ can be approximated as $1 + \psi/y$ with less than 3% error (assuming $T = 298.15$ K). Using this approximation, the vapor flow from the proximal to the distal site can be written as

$$\frac{w_{sp} \exp\left(\frac{\psi_p}{y}\right) - w_{sd} \exp\left(\frac{\psi_d}{y}\right)}{r_V} \approx \frac{\psi_p - \psi_d}{yr_V/w_{sd}} \quad (S20)$$

assuming $w_{sp} \approx w_{sd}$. We did not apply this approximation in practice; we present it merely to suggest the following definition:

$$R_V \equiv \frac{yr_V}{w_{sd}} \quad (S21)$$

This transformation makes the resistance of the vapor pathway within the leaf, r_V , dimensionally commensurable with the two liquid-phase resistances, which in turn allows for convenient comparisons of the orders of magnitude of R_{LD} , R_{LP} and R_V . Specifically, we define the ratios $\rho_L \equiv R_{LP}/R_{LD}$ and $\rho_V \equiv R_V/R_{LD}$.

Simulations

For a range of values of R_{LD} , ρ_L and ρ_V , and other conditions as well (described in Table S3), we solved Eqns S17 and S18 numerically for ψ_p , and then applied the results to Eqn S15 or S16 to calculate ψ_d and ψ_d' . Finally, we applied those results to Eqn S13 to calculate h_d , h_p , h_d' and h_p' .

Simulation set I: Simulations to determine reasonable and relevant ranges of unknown parameters

Figure S11 shows results with R_{LD} varying by 5 orders of magnitude, and ρ_L and ρ_V both varying by 6 orders of magnitude. Rows are values of ρ_L (shown in grey bars on the right side) and columns are values of ρ_V (shown in grey bars on the top). Most change in the effect of ρ_L occurs between $\rho_L = 0.01$ and $\rho_L = 1$. Most change in the effect of ρ_V occurs between $\rho_V = 0.1$ and 10. For R_{LD} , intercellular humidity is very close to saturating for $R_{LD} \leq 10^3$ MPa m² s mol⁻¹, and is below 50% in most cases for $R_{LD} > 10^{4.25}$. Thus, for subsequent simulations, we choose these three ranges: $R_{LD} \in [10^3, 10^{4.25}]$ MPa m² s mol⁻¹, $\rho_L \in [0.01, 1]$, and $\rho_V \in [0.1, 10]$.

Simulation set II: Simulations to examine relation of relative humidity (h) inferred using single-point model to values at proximal and distal evaporating sites in a two-point model

Fig S12 shows h simulated for the proximal and distal evaporating sites (h_p and h_d , respectively) for a range of within-leaf resistances, and the corresponding values of relative humidity that would be

inferred experimentally using our solution given in the main text (h). ρ_V is shown in the grey bars on top, and ρ_L is shown in the grey bars on the right. The value of h always lies between h_p and h_d . When the vapor-phase resistance between the proximal and distal sites is large relative to the xylem resistance to the distal site ($\rho_V = 10$, right column of panels), h is much closer to h_d . When vapor-phase resistance is equal to the liquid-phase resistance to the distal site ($\rho_V = 1$), but the proximal site is fed by liquid water at a low resistance ($\rho_L = 0.01$), h is roughly midway between h_p and h_d . When R_{LD} is very large but R_V is much smaller ($\rho_V = 0.1$) and R_{LP} is much smaller still ($\rho_L = 0.01$), moderate unsaturation could occur at the distal site ($h_d = 0.94$) but would not be detected by the experimental method ($h > 0.98$).

Simulation set III: Effect of helox on within-leaf humidity gradients

Figure S13 shows the same simulations as in Fig S12, but this time with h at the proximal and distal sites *in helox* overlaid in red (and the predicted experimental estimate not shown), to illustrate the depression of the within-leaf vapor gradient caused by helox despite constant transpiration rate. When ρ_L is low, the proximal humidity is negligibly altered by helox. Similarly, when ρ_V is large, the distal humidity is only slightly increased in helox. The largest effects of helox on h_d and h_p occur when $\rho_V = 1$ or $\rho_L = 1$. For example, when $\rho_V = 1$ and $\rho_L = 0.01$, helox causes h_d to recover by more than one-third; when $\rho_V = 10$ and $\rho_L = 1$, helox causes the depression of h_p below saturation to roughly double.

Simulation set IV: Effect of within-leaf temperature gradients on intercellular humidity

Figure S14 shows the simulated effects of a within-leaf temperature gradient of 0.3 K (the upper end of the range previously simulated by Buckley *et al.*, 2017). Compared to simulations with no gradient, the effects on h were mostly very small. For the scenario in which the proximal site is always essentially saturated ($\rho_L = 0.01$), the outcomes depended sensitively on the vapor-phase resistance. When R_V was large ($\rho_V = 10$), the temperature gradient had negligible effects. When R_V was equal to the liquid-phase resistance to the distal site ($\rho_V = 1$), the effect on h_d was also very small, but a positive artefact of 1-2% was introduced into the experimental estimate (h); however, h remained either between h_d and h_p or very close to h_p . When both ρ_V and ρ_L were low (i.e., the proximal site was nearly saturated and R_V was quite low), an interesting phenomenon was predicted under low R_{LD} : namely, super-saturating (condensing) conditions occurred at the distal site. This occurs because anisothermal vapor transport (vapor flow driven by temperature gradients, independent of water potential gradients) from the proximal site to the distal site exceeds the rate of transpiration, causing water to condense at the distal site and then flow in the opposite direction back towards the proximal site. In these conditions, the experimental method would infer a slightly super-saturated intercellular humidity ($h > 1$, but < 1.02).

Simulation set V: Effect of a systematic shift in leaf temperature upon switching to helox

In our experiments, leaf temperature often drops by about 0.3-0.6 K after switching to helox in high light, despite no change in transpiration rate. We believe this is due to the five-fold increase in thermal conductivity of helox as compared to nitox, which more effectively couples the leaf to the air in the chamber, and the chamber air to the temperature-controlled chamber core (controlled by water flow from a recirculating water bath through channels in the chamber body; see *Methods* in the main text). It is impossible to change the chamber-core temperature rapidly enough for this experiment. However, we can use simulations to assess the likely impact of these temperature shifts on our experimentally-estimated value of intercellular relative humidity, and its relation to the actual humidities within the leaf. Figure S15 shows simulations comparing a -0.5 K shift in helox with no such shift. These shifts had negligible impact on either the actual humidities in the leaf or our experimental estimation thereof.

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