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Exploring optimal stomatal control under alternative hypotheses for the regulation of plant sources and sinks

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Summary

- Experimental evidence that nonstomatal limitations to photosynthesis (NSLs) correlate with leaf sugar and/or leaf water status suggests the possibility that stomata adjust to maximise photosynthesis through a trade-off between leaf CO₂ supply and NSLs, potentially involving source-sink interactions. However, the mechanisms regulating NSLs and sink strength, as well as their implications for stomatal control, remain uncertain.
- We used an analytically solvable model to explore optimal stomatal control under alternative hypotheses for source and sink regulation. We assumed that either leaf sugar concentration or leaf water potential regulates NSLs, and that either phloem turgor pressure or phloem sugar concentration regulates sink phloem unloading.
- All hypotheses lead to realistic stomatal responses to light, CO₂ and air humidity, including conservative behaviour for the intercellular-to-atmospheric CO₂ concentration ratio. Sugar- and water-regulated NSLs are distinguished by the presence/absence of a stomatal closure response to changing sink strength. Turgor- and sugar-regulated phloem unloading are distinguished by the presence/absence of stomatal closure under drought and avoidance/occurrence of negative phloem turgor. Results from girdling and drought experiments on *Pinus sylvestris*, *Betula pendula*, *Populus tremula* and *Picea abies* saplings are consistent with optimal stomatal control under sugar-regulated NSLs and turgor-regulated unloading.
- Our analytical results provide a simple representation of stomatal responses to above- and below-ground environmental factors and sink activity.

Key words: non-stomatal limitation, optimisation model, phloem transport, photosynthesis, sink activity, stomatal conductance, xylem transport.

Introduction

The max-A hypothesis of optimal stomatal control: key uncertainties

Leaf photosynthesis is constrained by both stomatal and nonstomatal limitations (NSLs) (Buckley & Diaz-Espejo, 2015). Stomata limit leaf photosynthesis by restricting the diffusion of CO₂ from the atmosphere to the intercellular air spaces inside leaves. Additional limitations occur through restrictions to CO₂ diffusion from the intercellular air spaces to the sites of carboxylation in the mesophyll (*i.e.* mesophyll resistance), as well as through biochemical constraints on the light and dark reactions of photosynthesis; these additional limitations are referred to collectively as NSLs.

NSLs associated with decreased leaf water potential and/or increased leaf sugar concentration have been observed in several species (*e.g.* Jeannette *et al.*, 2000; Turnbull *et al.*, 2002; Franck *et al.*, 2006; Hüve *et al.*, 2006; Quentin *et al.*, 2013; Cano *et al.*, 2014; Kelly *et al.*, 2014; Ramalho *et al.*, 2014; Kitao *et al.*, 2015; Hölttä *et al.*, 2017; Salmon *et al.*, 2020). Because stomatal conductance indirectly affects leaf water and sugar status through changes in leaf water and carbon balance, these observations suggest that as stomata open (under given environmental conditions) there is a trade-off between increased CO₂ supply and increased NSLs, leading to the conjecture that stomata optimise this trade-off to maximize the rate of leaf photosynthesis (*A*) (Hölttä *et al.*, 2017; Dewar *et al.*, 2018; Gimeno *et al.*, 2019). We will refer to this conjecture as the maximum-*A* hypothesis (or max-*A* for short).

Currently the physiological mechanisms underlying the regulation of NSLs are not sufficiently well understood to conclude whether, under given environmental and physiological conditions, leaf sugar status or leaf water status is the main driver of NSLs. Moreover, leaf sugar and water status are tightly correlated, especially under drought stress – because lowered water content increases sugar concentration in the liquid phase – making it difficult to differentiate experimentally between these two variables as regulatory signals for NSLs. Previous modelling studies have shown that max-*A* leads to realistic predictions for stomatal behaviour – such as conservative behaviour for the ratio of intercellular-to-atmospheric CO₂ concentrations – whether it is assumed that NSLs are controlled through leaf sugar status (Hölttä *et al.*, 2017) or leaf water status (Dewar *et al.*, 2018). A formal comparison between the theoretical predictions of max-*A* based on these two possibilities may suggest ways in which they could be distinguished experimentally. However, such a comparison has yet to be performed and one key aim of this study is to address this gap.

Identifying the key drivers of NSLs is important for how max- A is implemented in plant models (Dewar *et al.*, 2018; Gimeno *et al.*, 2019). If leaf sugar status is the main transducer of NSLs, then implementing max- A requires phloem loading, transport and unloading at sink regions to be taken into account, because leaf sugar status is the outcome of all of these processes (Hölttä *et al.*, 2017). However, knowledge of the regulation of these additional processes is to varying degrees incomplete.

Phloem loading at the sources (leaves) can occur passively by diffusion through plasmodesmata in the symplast, actively across plasma membranes in the apoplast, or via a mechanism called polymer trapping involving symplastic one-way diffusion between the mesophyll and phloem cells (Lalonde *et al.*, 2003). In models, the simplest approach is to assume that phloem loading is not rate-limiting and so, within a steady-state approximation, can be set equal to the rate of photosynthesis (DeSchepper & Steppe 2010; Hölttä *et al.*, 2017).

The consensus view on phloem transport is encapsulated in the Münch flow hypothesis (Münch, 1930), according to which long-distance transport of carbon substrates (mainly, but not exclusively, sugars) occurs by mass flow down an osmotically-generated turgor pressure gradient between the sources, where sugars are loaded into the phloem, and the sinks where sugars are unloaded. Various mathematical representations of the Münch hypothesis have been developed (e.g. Goeschl *et al.*, 1976; Hölttä *et al.*, 2006; Payvendi *et al.*, 2014; Goeschl & Han, 2020), and anatomical studies lend empirical support to the underlying hypothesis, even for tall trees, although some questions remain unanswered (Savage *et al.*, 2017; Liesche & Schulz, 2018).

While active apoplastic phloem unloading can occur in tissues that accumulate high concentrations of non-structural carbohydrates, such as ripening fruits (Patrick, 1997; Lalonde *et al.*, 2003), phloem unloading is predominantly passive via the symplast (Liesche & Schulz, 2013). However, knowledge of the regulation of phloem unloading remains incomplete. Growth (a major sink for unloaded sugars) is known to be very sensitive to tree water status, and a growth response during drought is typically seen before reductions in stomatal conductance and photosynthesis rates (McDowell, 2011). The Lockhart equation (Lockhart, 1965), where cell enlargement during growth is controlled by turgor pressure, has been used to successfully model the cambial growth rate of trees (De Schepper & Steppe, 2010; Cabon *et al.*, 2020). Moreover, the growth-related synthesis of cell walls and proteins is known to depend on turgor pressure (Fatichi, 2014), and experimental evidence for symplastic phloem unloading by mass flow through plasmodesmata indicates turgor pressure-dependent phloem unloading (Ross-Elliott *et al.*, 2017; Milne *et al.*,

2018).

However, other experimental evidence indicates that growth rates also depend on tissue sugar concentration (Cooper & Thornley, 1976; Gifford & Evans, 1981). Sugar concentration-dependent growth rate forms the basis of several simplified plant growth models (*e.g.* Thornley & Johnson, 1990; Cannell & Dewar, 1994), while concentration-dependent unloading has also been assumed in more detailed models of phloem transport (*e.g.* Smith *et al.*, 1980; Thompson & Holbrook, 2003a; Mammeri & Sellier, 2017). The relative role of phloem turgor and phloem sugar concentration in controlling phloem unloading in sink regions remains unclear, mirroring the uncertainty in the drivers of NSLs in source regions. Therefore, a second aim of this study is to explore the implications of different controls on phloem unloading for stomatal behaviour within the *max-A* framework.

Alternative source-sink hypotheses

Our overall objective was to explore optimal stomatal behaviour under alternative hypotheses for the regulation of NSLs and phloem unloading, in order to generate predictions that could be used to distinguish experimentally between them. Specifically, we compared the optimal stomatal conductance predicted by *max-A* when NSLs are mediated through either leaf sugar concentration or leaf water potential, in combination with phloem unloading mediated through either sink phloem turgor pressure or sink phloem sugar concentration. The resulting four sink-source hypotheses are summarised in Table 1. In view of the knowledge gaps surrounding the regulation of NSLs and phloem unloading, these hypotheses were explored in the spirit of ‘what-if’ thought experiments. We used data from new and previously reported girdling and drought experiments to evaluate the effectiveness of each source-sink hypothesis in explaining observed patterns in stomatal behaviour.

In reality, control of NSLs may involve some combination of leaf sugar and leaf water status, and similarly phloem unloading may depend on both turgor pressure and phloem sugar concentration (*e.g.* Goeschl & Han, 2020), perhaps with one type of control being more prevalent depending on environmental or physiological conditions. Nevertheless, many of the insights gained from studying the simple hypotheses in Table 1 can be extended to control by multiple signals (see Results).

Our approach was to derive analytical expressions for the optimal stomatal conductance predicted by *max-A* under these alternative hypotheses. To achieve this, we analysed a simple

model consisting of a single source (leaves) and a single sink (roots). Our motivation was that analytical solutions can provide greater insights into model behaviour than numerical simulations, by making mathematically transparent the key environmental and physiological determinants of stomatal conductance. Analytical solutions are also computationally more efficient than numerical solutions.

Hölttä *et al.* (2017) considered a model of optimal stomatal control based on sugar-dependent NSLs and turgor-dependent phloem unloading, whose solution could only be obtained numerically. Dewar *et al.* (2018) explored an analytically solvable model in which NSLs were mediated by leaf water status. Goeschl & Han (2020) explored alternative hypotheses for phloem unloading within a numerical model of Münch flow, but did not explore their consequences for stomatal control. The present study complements and extends these studies, by analytically solving optimal stomatal conductance across a wider range of hypotheses for the regulation of plant sources and sinks, thus allowing for the first time a formal comparison of their predictions within the max- A framework.

Materials and methods

Figure 1 depicts the modelled soil-plant-atmosphere system, showing the governing equations for water and carbon fluxes. All fluxes and conductances are expressed on a per unit leaf area basis. A list of symbol definitions, units and default parameter values is given in Table 2.

Photosynthesis, phloem loading and transpiration

We used the bi-substrate model of Thornley & Johnson (1990, their Eqn 9.7i) to describe the rate of leaf photosynthesis in the absence of NSLs (A_0) as a function of the intercellular CO_2 concentration (c_i) and leaf photosynthetic photon flux density (Q):

$$A_0 = \frac{\alpha Q g_c c_i}{\alpha Q + g_c c_i} \quad (1)$$

where α is the photosynthetic quantum yield (the initial slope of the A_0 - Q curve) and g_c is the carboxylation conductance (the initial slope of the A_0 - c_i curve) in the absence of NSLs. We assumed that NSLs reduce A_0 by a fraction φ , so the actual rate of leaf photosynthesis is

$$A = (1 - \varphi)A_0. \quad (2)$$

Following Table 1, the NSL cost fraction (φ) was assumed to be regulated by either leaf sugar

concentration (C_{leaf} ; Hölttä *et al.*, 2017) or leaf water potential (ψ_{leaf} ; Dewar *et al.*, 2018):

$$\varphi = \frac{C_{leaf}}{C_0} \quad \text{osmotic-driven NSLs (O)} \quad (3a)$$

or

$$\varphi = \frac{\psi_{leaf}}{\psi_0}, \quad \text{water potential-driven NSLs (W)} \quad (3b)$$

where C_0 and ψ_0 are, respectively, the critical values of C_{leaf} and ψ_{leaf} at which NSLs reduce A to zero. Adopting an overall NSL factor $1 - \varphi$ in Eqn 2 is equivalent to the assumption that NSLs reduce the photosynthetic quantum yield (α) and the carboxylation conductance (g_c) by the same fraction φ . Because A_0 is a function of intercellular rather than chloroplast CO_2 concentration, α and g_c are ‘apparent’ biochemical parameters, so that φ may reflect either actual (chloroplast) biochemical constraints, or reductions in mesophyll conductance, or both. Many studies have shown the occurrence of NSLs under even mild stress (*e.g.* Grassi & Magnani, 2005; Chaves *et al.*, 2009; Flexas *et al.*, 2012; Hölttä *et al.*, 2017; Salmon *et al.*, 2020).

Empirically, NSLs are correlated with both leaf osmotic concentration and leaf water potential (Salmon *et al.*, 2020). Although C_{leaf} and ψ_{leaf} are tightly correlated, especially under drought stress, Eqns 3a and 3b should be viewed not as equivalent empirical expressions but as theoretically distinct hypotheses for the regulation of NSLs, in which either C_{leaf} or ψ_{leaf} is assumed to be the key transducer, and from which different theoretical predictions follow (see Results).

For simplicity we ignored leaf mitochondrial respiration and boundary layer resistance. Then, in the steady state, photosynthesis is balanced by the diffusion of CO_2 between the atmosphere and leaf intercellular air spaces:

$$A = g_s(c_a - c_i) \quad (4)$$

where c_a is the atmospheric CO_2 concentration and g_s is the stomatal conductance for CO_2 diffusion. Photoassimilates were assumed to be loaded passively into the phloem, as is common in woody species (Rennie & Turgeon, 2009). Following Hölttä *et al.* (2017), we assumed phloem loading is not rate-limiting (*i.e.* infinite diffusion conductance) and set the steady-state phloem loading rate equal to A . Therefore, leaf and leaf phloem sugar concentrations are treated as equivalent from now on.

The rate of leaf transpiration (E) is given by

$$E = 1.6g_sD \quad (5)$$

where D is the water vapour pressure deficit (VPD) and the factor 1.6 is the ratio of the molecular diffusion coefficients for CO_2 and H_2O .

Xylem and phloem transport

We assumed steady-state plant water balance so that E is balanced by the flux of water from the bulk soil to the leaf (J_W):

$$E = J_W = K_{tot}(\psi_{soil} - \psi_{leaf}) \quad (6a)$$

where ψ_{soil} is the bulk soil water potential and K_{tot} is the total hydraulic conductance between the bulk soil and 1 m^2 of leaf. For conductances in series, K_{tot} satisfies

$$\frac{1}{K_{tot}} = \frac{1}{K_{soil}} + \frac{1}{K_x} \quad (6b)$$

where K_{soil} and K_x are, respectively, the bulk soil-to-root xylem and root xylem-to-leaf xylem hydraulic conductances. Then E can also be written as

$$E = J_x = K_x(\psi_{root} - \psi_{leaf}), \quad (6c)$$

where J_x is the xylem water flux and ψ_{root} is the root xylem water potential, or

$$E = J_{soil} = K_{soil}(\psi_{soil} - \psi_{root}), \quad (6d)$$

where J_{soil} is the rate of soil water uptake. K_{soil} is sensitive to soil water potential. Following Campbell & Norman (2000) and Duursma *et al.* (2008), we assumed

$$K_{soil} = K_{soil,sat} \left(\frac{\psi_{soil,sat}}{\psi_{soil}} \right)^a \quad (6e)$$

where $K_{soil,sat}$ and $\psi_{soil,sat}$ are the values of K_{soil} and ψ_{soil} for saturated soil, and the exponent a depends on soil type. The value of $K_{soil,sat}$ implicitly includes the hydraulic conductance of the soil-root interface (Duursma *et al.*, 2008).

Using a simple representation of the Münch flow hypothesis, the rate of phloem transport is

$$J_C = K_p C_{leaf} (P_{leaf} - P_{root}) \quad (7a)$$

where K_p is the phloem hydraulic conductance, and P_{leaf} and P_{root} are the leaf and root phloem turgor pressures. We assumed that the xylem and phloem are in hydraulic equilibrium (*e.g.* Thompson & Holbrook, 2003b) so that

$$\psi_{leaf} = P_{leaf} - RTC_{leaf} \quad (7b)$$

$$\psi_{root} = P_{root} - RTC_{root} \quad (7c)$$

where C_{root} is the phloem sugar concentration in the sink (root), R is the molar gas constant and T is temperature. For this study, we ignore the gravitational contributions to Eqns 7b,c.

Sink phloem unloading

Phloem unloading was considered to follow the symplastic pathway and be passive (Ayre *et al.*, 2003). Following Table 1, the rate of phloem unloading in the sink (U) was assumed to be proportional to either the root phloem turgor pressure (P_{root}) or the root phloem sugar concentration (C_{root}):

$$U = k_{sink}P_{root} \quad \text{turgor-driven unloading (T)} \quad (8a)$$

or

$$U = k_{sink}RTC_{root} \quad \text{osmotic-driven unloading (O)} \quad (8b)$$

where in each case the constant of proportionality k_{sink} is a sink strength rate constant that may depend on root temperature (Day *et al.*, 1991). The factor RT in Eqn 8b was introduced for convenience so that k_{sink} has the same dimensions as in Eqn 8a.

A simple proportionality between U and either P_{root} or C_{root} was assumed in order to make the model analytically tractable. In reality, phloem unloading may be a non-linear function of P_{root} (e.g. Hölttä *et al.*, 2017) and/or C_{root} (e.g. Goeschl & Han, 2020), so that Eqn 8a,b represent two extreme cases. However, it turns out that many of the insights gained here from assuming Eqn 8a or 8b can be extended to these more general cases (see Results). Finally, in the steady state we have the balance equation

$$A = J_C = U. \quad (9)$$

Optimal stomatal conductance

Under given environmental conditions, if stomatal conductance (g_s) is increased then greater CO_2 diffusion into the leaf leads to an increase in A_0 (Eqn1) due to an increase in c_i , while the simultaneous increase in leaf water loss leads to an increase in the NSL cost fraction φ (through an increase in leaf phloem sugar concentration and/or a decrease in leaf water potential, Eqn 3a or 3b). As a result, there is a maximum in A (Eqn 2) at an optimal value of g_s , reflecting a trade-off between the cost (increased NSLs) and benefit (increased CO_2 supply) of stomatal opening. According to the max- A hypothesis, stomatal conductance adopts this optimum value.

Simplifying assumptions for xylem and phloem hydraulic conductances

To obtain analytical solutions, xylem conductance (K_x) was assumed to be fixed (no xylem embolism). However, for comparison, we also solved the model numerically for variable K_x ,

assuming a simple ramp function of leaf water potential (see Discussion and (later) Methods S3).

We also assumed that the phloem hydraulic conductance (K_p) is very large, corresponding to a very small leaf-to-root phloem turgor pressure difference ($P_{leaf} - P_{root}$), such that the rate of phloem transport under the Münch flow hypothesis (Eqn 7a) remains finite (Methods S2, see later). Theoretical calculations taking into account changes in sieve element structure along the transport pathway indicate that, even in tall trees, relatively small pressure gradients of order 0.2–0.4 MPa are sufficient to maintain efficient carbohydrate transport (Savage *et al.*, 2017). This assumption is also supported by measurements of water potential and osmotic potential and estimates of the turgor pressure gradient along the phloem transport pathway in mature Scots pine trees (Paljakka *et al.*, 2017). As shown below (see Results), the key conclusions of our study do not depend critically on this approximation. Numerically, Hölttä *et al.* (2017) examined the case of finite K_p and found phloem transport limitations to be relevant only at very low values of K_p .

Girdling and drought experiments

We used data from new and previously reported girdling and drought experiments on saplings of *Pinus sylvestris* (Scots pine), *Betula pendula* (Birch), *Populus tremula* (Aspen), and *Picea abies* (Spruce) (Hölttä *et al.*, 2017; Salmon *et al.*, 2020), as well as literature data, to evaluate key predictions of the different hypotheses. For details see Methods S1.

Results

Analytical solutions reveal key similarities and differences between the four hypotheses

Table 3 summarises the analytical solutions for each hypothesis; for derivations see Methods S2. All four hypotheses predict the same generic relationships for the optimal stomatal conductance and optimal c_i/c_a ratio as functions of three dimensionless parameter combinations: β (a function of VPD and hydraulic conductance), θ (a function of soil water potential) and χ (a function of atmospheric CO₂ concentration, leaf irradiance and sink strength); the hypotheses differ only in how these dimensionless quantities depend on the model parameters.

Table 3 reveals that hypotheses W-T and W-O predict identical solutions for c_i/c_a and g_s ; thus for water-potential dependent NSLs, the solution is independent of the driver for phloem unloading. The analytical solution for W-T/W-O is consistent with the analytical results of Dewar *et al.* (2018; CAP optimization hypothesis; bi-substrate photosynthesis model, ignoring

photorespiration, *i.e.* $\Gamma^* = 0$).

Table 3 also reveals a close correspondence between the solutions for hypotheses O-T and W-T/W-O that was not apparent from previous studies (Hölttä *et al.*, 2017; Dewar *et al.*, 2018). Specifically, the solution for W-T/W-O can be obtained from that for O-T through the parameter replacements $RTC_0 \rightarrow |\psi_0|$, $k_{sink} \rightarrow \infty$. Moreover, the solution for O-O can be obtained from that for O-T through the replacements $K_{tot} \rightarrow K_x$, $\psi_{soil} \rightarrow 0$. These relationships reveal the key similarities and differences between the four hypotheses.

All four hypotheses predict similar stomatal responses to above-ground environment

Figure 2 illustrates the responses of optimal stomatal conductance to changes in light (Q), atmospheric CO₂ concentration (c_a), and VPD (D) predicted under hypothesis O-T; with the default parameters in Table 2, numerically identical responses are predicted under hypothesis O-O, while similar (but not identical) responses are predicted under hypotheses W-T/W-O (Methods S2, Fig. S2.2 therein). These responses reflect shifts in the optimal trade-off between the costs and benefits of stomatal opening. When photosynthesis is maximised at the optimal stomatal conductance, the gain in CO₂ supply from an infinitesimal increase in g_s is exactly offset by the increase in NSLs; increasing Q shifts this balance in favour of further stomatal opening, while increased c_a and D have the opposite effect.

Hypotheses O-T and W-T/W-O predict a stomatal response to drought while hypothesis O-O does not

Figure 2 shows that, under hypothesis O-T, optimal stomatal conductance increases with increasing soil-to-leaf hydraulic conductance (K_{tot}). This response reflects the fact that the NSL cost fraction (ϕ) is an increasing function of E/K_{tot} (Methods S2, Eqn S2.5a therein). Specifically (Fig. S2.1), g_s increases approximately linearly with $\sqrt{K_{tot}}$ (Eqn S2.19a) except when K_{tot} is very small, when g_s is proportional to K_{tot} (Eqn S2.20a). Because the soil-to-root hydraulic conductance (K_{soil} , a component of K_{tot}) depends sensitively on soil water potential, these results imply a strong non-linear stomatal closure response to soil drying. In addition to this dependence of g_s on soil water potential mediated through K_{soil} , there is a direct linear dependence of g_s on soil water potential through the dimensionless quantity $\theta = 1 + \psi_{soil}/RTC_0$ (Table 3), due to the assumed dependence of unloading rate on phloem turgor.

Figure 3a shows the overall response of g_s to ψ_{soil} under hypothesis O-T, as well as the response assuming K_{soil} is fixed. A similar stomatal response to drought is predicted under hypotheses W-T/W-O (Fig. 3a), reflecting a similar dependence of the analytical solution on K_{tot} and ψ_{soil} (Table 3, Fig. S2.2). In contrast, the solution for O-O depends on K_x rather than K_{tot} and, moreover, there is no explicit dependence on ψ_{soil} ($\theta = 1$, Table 3). Consequently, since K_x is assumed fixed (but see Discussion), hypothesis O-O predicts no stomatal response to soil drying (Fig. 3a).

Under osmotic-dependent unloading, phloem turgor can become negative under drought

Figures 3b-e show the responses of leaf water potential (ψ_{leaf}), leaf phloem sugar concentration (C_{leaf}), leaf phloem turgor pressure (P_{leaf}) and the intercellular-to-atmospheric CO₂ concentration ratio (c_i/c_a) to soil drying under the four hypotheses.

Overall, hypotheses O-T and W-T predict similar responses. In particular, ψ_{leaf} (Fig. 3b) is maintained above the critical threshold value ($\psi_0 = -3$ MPa) at which NSLs reduce photosynthesis to zero, approaching it only in the limit $\psi_{soil} \rightarrow \psi_0$ when stomata close completely (Fig. 3a). At the same time C_{leaf} increases (because turgor-driven unloading in the sink declines as P_{root} declines) towards its critical value C_0 (Fig. 3c); the value of C_0 has been chosen so that $RTC_0 = -\psi_0$ (Table 2), making the predictions of O-T and W-T agree numerically in this limit. As a result, phloem turgor pressure remains above zero (Fig. 3d), approaching zero only when stomata close ($P_{leaf} \approx P_{root} \propto U = A \rightarrow 0$ as $g_s \rightarrow 0$).

In contrast, under hypotheses O-O and W-O phloem turgor can become negative under soil drying (Fig. 3d). Because C_{leaf} is independent of soil water potential under hypothesis O-O (Fig. 3c), leaf phloem turgor ($P_{leaf} = \psi_{leaf} + RTC_{leaf}$) decreases at the same rate as leaf water potential (Fig. 3b) and eventually drops below zero. Under hypothesis W-O, ψ_{leaf} tends to ψ_0 (identical to W-T); however C_{leaf} tends to zero (because $C_{root} \propto U = A \rightarrow 0$ as $g_s \rightarrow 0$) so that, like O-O, P_{leaf} can become negative.

Under all hypotheses except O-O, c_i/c_a declines under drought (Fig. 3e) through its dependence on K_{tot} (Table 3).

O-T and O-O predict that stomatal conductance depends on sink strength

Figure 2 shows that, under hypothesis O-T, optimal stomatal conductance increases with

increasing sink strength (k_{sink}), but saturates for sufficiently large values of k_{sink} . This response occurs because the NSL cost fraction (ϕ) increases linearly with A/k_{sink} (Eqn S2.5a), reflecting the dependence of the steady-state leaf phloem sugar concentration (C_{leaf}) on the balance between source and sink activity.

As Table 3 shows, the analytical solutions under hypotheses O-T and O-O share the same expression for the sink-related quantity χ , so that an identical response to sink strength is predicted under both hypotheses (Fig. 2). In contrast, hypotheses W-T/W-O predict that optimal stomatal conductance is independent of sink strength (Fig. S2.1), reflecting the fact that W-T/W-O is equivalent to O-T in the limit $k_{sink} \rightarrow \infty$, *i.e.* the plateau value of g_s as a function of k_{sink} shown in Fig. 2.

Figure 4a shows that, under the O-T and O-O hypotheses, the stomatal closure response to increasing atmospheric CO₂ concentration is faster at low sink strength (due to enhanced sugar accumulation) than at high sink strength, with hypotheses W-T/W-O (corresponding to $k_{sink} = \infty$) predicting the weakest stomatal response to CO₂. As a result, leaf photosynthesis increases less rapidly with CO₂ at low sink strength than at high sink strength (Fig. 4b).

Covariation of g_s with ψ_{leaf} and C_{leaf} under soil drying reflects a non-linear relationship between g_s and the NSL cost fraction

Figure 5 shows the covariation of stomatal conductance, leaf water potential and leaf osmotic potential under soil drying, predicted under hypothesis O-T. Recall that the NSL cost fraction ϕ is proportional to C_{leaf} under this hypothesis (Eqn 3a). Moreover, because C_{leaf} is tightly correlated with leaf water potential (ψ_{leaf}) (Fig. 5, inset), ψ_{leaf} is also closely correlated with ϕ .

Therefore, the covariation of g_s with ψ_{leaf} or RTC_{leaf} under soil drying effectively reflects a non-linear relationship between g_s and ϕ . This relationship is moderately affected by a doubling of VPD (Fig. 5). Methods S2 (Eqn S2.24) shows that the g_s vs. ϕ relationship is explicitly independent of K_{tot} , VPD, ψ_{leaf} and C_{leaf} , whose influence on g_s is therefore mainly captured through ϕ . However, the g_s vs. ϕ relationship does depend explicitly on ψ_{soil} and A/k_{sink} (Eqn S2.24a), which results in a shift in the curves under a doubling of VPD (Fig. 5). Thus, no unique relationship is predicted between stomatal conductance and leaf water potential under both soil drying and changes in VPD, as has been found experimentally in the sclerophyllous woody species *Nerium oleander* (Gollan *et al.*, 1985), although the predicted relationship is only

moderately sensitive to VPD.

Observational tests

Underlying the stomatal responses to light, CO₂ and VPD shown in Fig. 2 is the key prediction, common to all four hypotheses, that $c_i/c_a = 1/(1 + \beta)$. The parameter β is proportional to the square root of VPD ($\beta = \sqrt{D}/\xi$) (see Table 3), while the proportionality constant (written here as $1/\xi$) differs between hypotheses. This general relationship implies that photosynthesis and stomatal conductance are related by

$$g = \left(1 + \frac{\xi}{\sqrt{D}}\right) \frac{A}{c_a}, \quad (10)$$

which has been confirmed experimentally (Medlyn *et al.*, 2011; Dewar *et al.*, 2018; Gimeno *et al.*, 2019).

For the O-T and W-T/W-O hypotheses, ξ is proportional to $\sqrt{K_{tot}}$ (see Table 3, with $\beta = \sqrt{D}/\xi$), so that ξ is positively related to soil water status through the below-ground component of K_{tot} ; this prediction has been confirmed by Gimeno *et al.* (2019). Other experimental studies have also highlighted the key role of below-ground hydraulic conductance (K_{soil}) in mediating stomatal responses to drought and soil temperature for trees (Lintunen *et al.*, 2019) and to drought for tomato plants (Abdulla *et al.*, 2020). For large enough K_{tot} , the second term in parentheses in Eqn 10 dominates the first term, so that $g_s \propto \sqrt{K_{tot}}$; this prediction is supported quantitatively by drought and notching experiments on trees (Salmon *et al.*, 2020).

The drought responses of stomatal conductance, leaf water potential, leaf osmolality and leaf turgor (Fig. 3, O-T and W-T hypotheses) are broadly consistent with those observed in our drought experiment (Fig. 6). Moreover, Salmon *et al.* (2020) found that the drought response of stomatal conductance under W-T/W-O (equivalent to the model of Dewar *et al.*, 2018) was quantitatively close to observations (as noted above). The predicted covariation of stomatal conductance with leaf water potential and leaf osmotic potential under soil drying (Fig. 5, O-T hypothesis) is qualitatively consistent with observations (Fig. 7). A more quantitative comparison is not possible here because estimates of leaf turgor are subject to considerable uncertainty in the amount of dilution by apoplastic water during the osmolality measurements (Tyree, 1976; Gersony *et al.*, 2020; Methods S1), leading to some negative turgor estimates under severe drought stress. Nevertheless, the observed trends (Figs. 6 and 7) match those predicted under hypotheses O-T and W-T/W-O (Figs. 3 and 5).

A key prediction of hypotheses O-T and O-O is that stomatal conductance and photosynthesis decrease in parallel under a decline in sink strength (k_{sink}), such that the ratio c_i/c_a is independent of k_{sink} (Table 3). This prediction is supported by our girdling experiments: approximately 100 minutes after girdling, A and g_s begin to decline (Fig. 8a,b) while c_i/c_a remains relatively constant (Fig. 8c); as g_s decreases, leaf water potential and leaf osmolality generally increase, consistent with reduced transpiration and reduced sink strength (Fig. 8d,e). Some variation in c_i/c_a is observed on longer timescales, although there is no consistent trend across species (Fig. 8c). Theoretically (Table 3), c_i/c_a could vary through longer-term adjustments in hydraulic conductance (K_{tot}) and/or carboxylation conductance (g_c). It should also be emphasised that model predictions represent steady-state rather than dynamic responses.

Other sink manipulation experiments have also shown no significant response of c_i to girdling (Urban *et al.*, 2004; Cheng *et al.*, 2008), pruning (Fabre *et al.*, 2019) or soil temperature (Anbebe *et al.*, 2009). However, some studies have reported both decreases and increases after girdling (Batista *et al.*, 2012; Sellin *et al.*, 2013) or in response to increased soil temperature (Day *et al.*, 1991; Dang & Cheng, 2004; Zhang & Dang, 2005), although responses may depend on timescale (Zhang & Dang, 2005) and cultivar (Quentin *et al.*, 2013). According to our model, soil temperature could alter c_i through the predicted dependence of c_i/c_a on the below-ground component of K_{tot} (Table 3), as found experimentally (Lintunen *et al.*, 2019).

Numerous studies support the prediction of the O-T hypothesis (Fig. 4b) that sink capacity modulates the response of photosynthesis to elevated CO₂ (Ainsworth & Rogers, 2007; Davey *et al.*, 2006). We have found no studies reporting a corresponding modulation of stomatal responses to CO₂ (Fig. 4a). Intriguingly, however, stomatal conductance is significantly more sensitive to CO₂ in angiosperm than gymnosperm tree species (Klein & Ramon, 2019). The O-T hypothesis offers a possible explanation of this difference, in light of the generally higher concentrations of above-ground non-structural carbohydrates observed in angiosperms (Piper *et al.*, 2019), implying lower sink strength and hence higher sensitivity to CO₂ (Fig. 4a). Additionally, the greater hydraulic efficiency of vessels vs. tracheids (Lusk *et al.*, 2013), implying higher K_{tot} for angiosperms, would also confer greater stomatal sensitivity to CO₂ (through the interaction between parameters β and χ , see Table 3).

Discussion

Our results show that max- A predicts qualitatively different stomatal behaviours under the four source-sink hypotheses in Table 1. The key differences are the presence/absence of a stomatal response to changes in sink strength, and the presence/absence of a stomatal response to drought. In view of experimental evidence for stomatal sensitivity to both sink strength and soil water availability, we can eliminate three of these hypotheses (O-O, W-T, W-O). Theoretically, a combination of W-T/W-O and O-O could lead to responses to both drought and sink strength; however, O-T is the simplest hypothesis that does so.

For osmotic-driven NSLs, max- A predicts stomatal closure under soil drying only if phloem unloading has some dependence on phloem turgor pressure

Our results indicate that, for osmotic-driven NSLs, purely osmotic-driven phloem unloading (O-O hypothesis) is unrealistic, because it implies that stomatal conductance is insensitive to drought. As a result, phloem turgor can become negative. In contrast, with turgor-driven phloem loading (O-T hypothesis), stomata close under soil drying and turgor pressure remains positive.

These conclusions are based on the simplifying approximations that the phloem unloading rate U under hypotheses O-O and O-T depends linearly on C_{root} and P_{root} , respectively, and that the phloem hydraulic conductance (K_p) is infinite, allowing the model to be solved analytically. However, further analysis (Methods S2) shows that the same conclusions hold when U is any arbitrary function of C_{root} or P_{root} alone, and when K_p is finite, although the solution is no longer analytically solvable. More generally (Methods S2), with osmotic-driven NSLs, stomatal closure under drought requires U to have *some* dependence on P_{root} – including the case $U = f(P_{root}, C_{root})$ – but that P_{root} remains positive only if U vanishes as P_{root} tends to zero. This general insight is consistent with the numerical simulations of Goeschl & Han (2020), who modelled U as a product of Michaelis-Menten functions of sink phloem turgor pressure and sugar concentration; Eqns 8a and 8b correspond to the extreme cases in which one or other of these variables is the limiting factor. Our analytical results confirm the numerical simulations of Goeschl & Han (2020), and extend them to include their implications for optimal stomatal control within the max- A framework.

Osmotic- vs. water potential-driven NSLs imply the presence/absence of a stomatal response to changes in sink strength

Hypotheses O-T and W-T/W-O are distinguished by the presence/absence of a dependence of

stomatal conductance on sink strength (k_{sink}), but otherwise they predict similar stomatal behaviours. Many studies of stomatal conductance have been made where soil and air water availability are limiting. Under these conditions, osmotic- and water potential-driven NSLs are difficult to distinguish because osmotic potential (or sugar content) and water potential typically correlate strongly and negatively with each other (Salmon *et al.*, 2020). However, girdling experiments and manipulation of source-sink imbalance (*e.g.* Iglesias *et al.*, 2002) allow these two factors to be teased apart. Our girdling experiments and numerous other sink manipulation experiments (Results, *Observational tests*) support the presence of a sink feedback effect on photosynthesis and stomatal conductance consistent with the O-T hypothesis.

Max-A and the avoidance of xylem embolism

The simultaneous decline in stomatal conductance and leaf water potential observed under drought or high VPD is commonly interpreted as a stomatal strategy to avoid catastrophic xylem embolism at low water potentials (Tuzet *et al.*, 2003; McDowell *et al.*, 2008; Brodribb & McAdam, 2017). Recent stomatal optimization models have also related the cost of stomatal opening to the risk of hydraulic failure due to excessive embolism (*e.g.* Wolf *et al.*, 2016; Sperry *et al.*, 2017; Andregg *et al.*, 2018; Lu *et al.*, 2020; Wang *et al.*, 2020). In contrast, Figure 5 shows that the decline of g_s with ψ_{leaf} under soil drying can also be interpreted as a stomatal strategy for maximising photosynthesis subject to the cost of NSLs. Regardless of whether NSLs are controlled by leaf sugar status or leaf water potential, ψ_{leaf} is closely correlated with the NSL cost fraction ϕ , so that the g_s vs. ψ_{leaf} effectively depicts a curve of g_s vs. ϕ .

Nevertheless, the max-A hypothesis is not inconsistent with avoidance of embolism. If the critical leaf water potential ($-RTC_0$ or ψ_0 , hereafter denoted $\psi_{c,NSL}$) at which NSLs reduce photosynthesis to zero is greater than or equal to the threshold potential for the onset of xylem cavitation, then max-A predicts avoidance of embolism because stomata shut at $\psi_{leaf} = \psi_{c,NSL}$, before embolism occurs. This case is consistent with our assumption that xylem conductance (K_x) is fixed. This assumption may be justified by the fact that during drought the soil is the main hydraulic bottleneck, its conductance (K_{soil}) changing by orders of magnitude while changes in xylem conductance (K_x) due to embolism are much smaller (Carminati & Jarvaux, 2020; Corso *et al.*, 2020).

In Methods S3 we examine the alternative possibility that xylem embolism can occur at leaf

water potentials greater than $\psi_{c,NSL}$; in this case, the optimal solution must be obtained numerically. For simplicity we assumed a ramp function for K_x as a function of ψ_{leaf} (with $K_x = 0$ for $\psi_{leaf} < \psi_{c,emb}$, Fig. S3.1). When complete loss of xylem conductance occurs before complete loss of photosynthesis due to NSLs ($\psi_{c,emb} > \psi_{c,NSL}$), the behaviour of the model changes significantly, with complete stomatal closure under soil drying occurring earlier, at $\psi_{soil} = \psi_{c,emb}$ rather than at $\psi_{soil} = \psi_{c,NSL}$ (Fig. S3.3a-c) under all four hypotheses. In this case, xylem hydraulic resistance ($R_x = 1/K_x$) contributes a significant fraction of the total soil-to-leaf resistance ($R_{tot} = 1/K_{tot}$) (Fig. S3.4a, Table S3.1). In particular, the O-O hypothesis now predicts stomatal closure under drought, in contrast to the lack of response in the absence of embolism (Fig. S3.3c).

On the other hand, when xylem conductance and photosynthesis decline to zero at the same leaf water potential ($\psi_{c,emb} = \psi_{c,NSL}$), the behaviour of the model under hypotheses O-T and W-T/W-O is almost indistinguishable from the case where K_x is fixed (Fig. S3.3d-e) because soil hydraulic resistance dominates R_{tot} (Fig. S3.4b, Table S3.1). In contrast, under hypothesis O-O, embolism introduces a stomatal closure response to drought (Fig. S3.3f). Unlike the other hypotheses, embolism is a necessary requirement for a stomatal drought response under the O-O hypothesis.

Theoretically, therefore, xylem embolism may alter the model behaviour if it is significantly more sensitive than NSLs to drought stress ($\psi_{c,emb} \gg \psi_{c,NSL}$). Empirically, however, comparisons across different species in different biomes reveal that the water potential levels at 50% loss of xylem conductivity due to cavitation ($\psi_{50,X}$) and 50% reduction of apparent photosynthetic capacity ($\psi_{50,M}$) are tightly correlated (Manzoni, 2014, their Fig. 1b). Thus we would expect that $\psi_{c,emb}$ and $\psi_{c,NSL}$ would be closely co-ordinated, with higher values of $\psi_{c,emb}$ (corresponding to higher $\psi_{50,X}$) being associated with higher values of $\psi_{c,NSL}$ (corresponding to higher $\psi_{50,M}$).

Recent studies using direct, non-invasive X-ray microtomography and optical visualisation techniques to monitor embolism within plants suggest that major embolism formation only occurs after stomatal closure (Martin St-Paul *et al.*, 2017; Lamarque *et al.*, 2018; Creek *et al.*, 2020), suggesting avoidance of embolism. Embolism avoidance may be strategically important as embolism may be less reversible than NSLs (Choat *et al.*, 2019; Rehschuh *et al.*, 2020). Following the removal of drought stress, any remaining embolism would limit stomatal conductance because the xylem would then be the main hydraulic bottleneck (Fig. S3.3). The relationship between the critical thresholds for NSLs and embolism ($\psi_{c,NSL}$ and $\psi_{c,emb}$) merits further study to examine

whether NSLs are more sensitive than xylem embolism to drought.

Model limitations

To obtain analytical solutions we made many simplifying assumptions. In adopting a steady-state approach, internal storage of water and carbon has been neglected. For water, this is not a critical concern because steady-state models have been quite accurate in characterising tree water relations, except perhaps under severe drought (Klein *et al.*, 2016) and for very large trees (Scholtz *et al.*, 2009).

For carbon, sugars may be temporarily converted to starch when sink strength is (momentarily) insufficiently strong to use the sugars produced in photosynthesis. However, starch content most likely does not directly affect either source or sink processes, as has been shown for photosynthesis in coffee shrubs (Avila *et al.*, 2020). However, under extreme conditions (*e.g.* removal of all sinks) starch accumulation has been suggested to negatively affect photosynthesis by damaging the thylakoids (Schaffer *et al.*, 1986), and we cannot rule this out as a contribution to the decline in photosynthesis observed in our girdling experiment (Fig. 8a). Our steady-state assumption also neglects the day/night regulation of phloem loading, which tends to minimise variability in the diurnal cycle of photosynthesis (Smith & Stitt, 2007; Scialdone *et al.*, 2013).

The model assumes that all soluble sugars and other dissolved compounds have an equal effect on stomatal and non-stomatal limitations to photosynthesis. Previous studies (Hölttä *et al.*, 2017; Salmon *et al.*, 2020) suggest that this assumption is likely true in many situations. However, it is possible that under transient conditions an imbalance between the types of sugar present might occur, in which case the gas-exchange response might depend on the concentration changes of individual sugars. For example, hexoses may be particularly important in such situations as they are sensed by hexokinase which in turn has been shown to regulate leaf-gas exchange (*e.g.* Kelly *et al.*, 2013) and mesophyll conductance (Kelly *et al.*, 2014). Effects of phloem sugar concentration on sap viscosity have also been ignored; these are included in the numerical model of Hölttä *et al.* (2017).

We assumed that NSLs are linearly related to leaf sugar or water status (Eqns 3a,b) whereas in the experiments of Salmon *et al.* (2020) in some cases the relationship was significantly non-linear (see also Drake *et al.*, 2017). However, Salmon *et al.* (2020) found that stomatal conductance is proportional to the square root of soil-to-leaf hydraulic conductance, as predicted by max-*A* under both the O-T and W-T/W-O hypotheses (Methods S2). Therefore our linear

approximation appears to be a reasonable one for practical purposes.

Conclusion

Predictions of the max- A stomatal optimization hypothesis are sensitive to assumptions regarding the regulation of plant sources and sinks. Control of NSLs by leaf sugar and water status are distinguished by the presence/absence of stomatal responses to changes in sink strength. In the case of osmotic-driven NSLs, a stomatal response to drought requires some degree of turgor-driven sink phloem unloading. Our girdling and drought studies are consistent with optimal stomatal control under sugar-regulated NSLs and turgor-regulated unloading. Our analytical results provide a simple yet comprehensive representation of stomatal responses to above- and belowground environmental conditions, including the effects of source-sink interactions.

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Author Contributions

RD, TH and YS developed the model hypotheses. RD derived the analytical solutions and wrote the manuscript with the help of TH and YS. YS performed the analysis of experimental data.

Data availability

The data that supports the findings of this study are available in the supplementary material of this article.

Table 1. Summary of the four source-sink hypotheses explored in this study, together with the relevant model equations (see Materials and Methods section).

	Osmotic-driven NSLs (O), Eqn 3a	Water potential-driven NSLs (W), Eqn 3b
Turgor-driven unloading (T), Eqn 8a	O-T	W-T
Osmotic-driven unloading (O), Eqn 8b	O-O	W-O

Non-stomatal limitations to photosynthesis (NSLs) are mediated through either leaf sugar concentration (osmotic-driven, O) or leaf water potential (water potential-driven, W). Phloem unloading is mediated through either sink phloem turgor pressure (turgor-driven, T) or sink phloem sugar concentration (osmotic-driven, O).

Table 2. List of symbol definitions, units and default parameter values used in the main text.

Symbol	Definition	Units/default value
a	Soil parameter	2.75 ⁽¹⁾
A	Leaf area-specific rate of photosynthesis	$\text{mol m}^{-2} \text{s}^{-1}$
A_0	Value of A in the absence of NSL	$\text{mol m}^{-2} \text{s}^{-1}$
c_a	Atmospheric CO_2 concentration	$400 \times 10^{-6} \text{ mol mol}^{-1}$
c_i	Leaf intercellular CO_2 concentration	mol mol^{-1}
C_{leaf}	Leaf phloem sugar concentration	mol m^{-3}
C_{root}	Root phloem sugar concentration	mol m^{-3}
C_0	Critical value of C_{leaf} at which $A = 0$	1230 mol m^{-3} ⁽²⁾
D	Atmospheric water vapour pressure deficit	$0.01 \text{ mol mol}^{-1}$
E	Leaf area-specific rate of transpiration	$\text{mol m}^{-2} \text{s}^{-1}$
g_c	Carboxylation conductance in absence of NSLs	$0.5 \text{ mol m}^{-2} \text{s}^{-1}$ ⁽¹⁾
g_s	Stomatal conductance for CO_2 diffusion	$\text{mol m}^{-2} \text{s}^{-1}$
k_{sink}	Sink strength parameter	$4 \times 10^{-5} \text{ mol m}^{-2} \text{s}^{-1} \text{ MPa}^{-1}$ ⁽³⁾
K_p	Leaf area-specific phloem hydraulic conductance	$\text{m s}^{-1} \text{ MPa}^{-1}$
K_{soil}	Leaf area-specific soil hydraulic conductance	$\text{mol m}^{-2} \text{s}^{-1} \text{ MPa}^{-1}$
K_{tot}	Total leaf area-specific hydraulic conductance	$0.01 \text{ mol m}^{-2} \text{s}^{-1} \text{ MPa}^{-1}$ ⁽⁴⁾
K_x	Leaf area-specific xylem hydraulic conductance	$0.01 \text{ mol m}^{-2} \text{s}^{-1} \text{ MPa}^{-1}$ ⁽⁴⁾
$K_{soil,sat}$	Value of K_{soil} for saturated soil	$10^4 \text{ mol m}^{-2} \text{s}^{-1} \text{ MPa}^{-1}$ ⁽⁴⁾
J_C	Leaf area-specific rate of phloem sugar transport	$\text{mol m}^{-2} \text{s}^{-1}$
J_{soil}	Leaf-specific rate of soil water uptake	$\text{mol m}^{-2} \text{s}^{-1}$
J_W	Leaf-specific soil-to-leaf water flux	$\text{mol m}^{-2} \text{s}^{-1}$
J_x	Leaf-specific xylem water flux	$\text{mol m}^{-2} \text{s}^{-1}$
P_{leaf}	Phloem leaf turgor pressure	MPa
P_{root}	Phloem sink (root) turgor pressure	MPa
Q	Leaf photosynthetic photon flux density	$500 \times 10^{-6} \text{ mol m}^{-2} \text{s}^{-1}$
R	Molar gas constant	$8.314 \text{ J K}^{-1} \text{ mol}^{-1}$
T	Temperature	293 K
U	Leaf area-specific sink phloem sugar unloading rate	$\text{mol m}^{-2} \text{s}^{-1}$

α	Photosynthetic quantum yield in absence of NSLs	0.1 mol mol ⁻¹ ⁽¹⁾
β	Dimensionless quantity in generic solution	-
θ	Dimensionless quantity in generic solution	-
φ	NSL cost fraction	-
χ	Dimensionless quantity in generic solution	-
ψ_{leaf}	Leaf water potential	MPa
ψ_{root}	Root water potential	MPa
ψ_0	Critical value of ψ_{leaf} at which $A = 0$	-3 MPa ⁽⁵⁾
$ \psi_0 $	Absolute value of ψ_0	3 MPa
ψ_{soil}	Soil water potential	-2×10^{-3} MPa
$\psi_{soil,sat}$	Value of ψ_{soil} at saturation	-2×10^{-3} MPa

NSLs, non-stomatal limitations to photosynthesis. Parameter values (based on *Pinus sylvestris* in Boreal conditions) are illustrative only and do not affect the key conclusions of this study.

¹Illustrative example (Dewar *et al.*, 2018). ²Based on values of 1000–1500 mol m⁻³ measured on Scots pine (Hölttä *et al.*, 2017), adjusted to give $RTC_0 = -\psi_0$ so that hypotheses O-T and W-T/W-O predict the same limiting leaf water potential as $\psi_{soil} \rightarrow \psi_0$. ³Chosen to give realistic values for phloem sugar concentration, ca. 300 mol m⁻³ as measured on Scots pine (Hölttä *et al.*, 2017). ⁴Based on Duursma *et al.* (2008) for Scots pine. ⁵Hölttä *et al.* (2017).

Table 3. Analytical solutions for the optimal c_i/c_a ratio and optimal stomatal conductance (g_s), for the four source-sink hypotheses in Table 1, as functions of the three dimensionless parameter combinations β , θ and χ .

Generic solution for the optimal c_i / c_a ratio and optimal stomatal conductance (g_s):			
	$\frac{c_i}{c_a} = \frac{1}{1 + \beta}$ $g_s = g_c \frac{\theta}{\beta \left[1 + \beta + \frac{\chi}{1 + \beta} \right]}$		
Source-sink hypothesis	β	θ	χ
O-T	$\sqrt{\frac{1.6Dg_c}{K_{tot}RTC_0}}$	$1 + \frac{\psi_{soil}}{RTC_0}$	$g_c c_a \left(\frac{1}{\alpha Q} + \frac{1}{k_{sink} RTC_0} \right)$
O-O	$\sqrt{\frac{1.6Dg_c}{K_x RTC_0}}$	1	$g_c c_a \left(\frac{1}{\alpha Q} + \frac{1}{k_{sink} RTC_0} \right)$
W-T and W-O	$\sqrt{\frac{1.6Dg_c}{K_{tot} \psi_0 }}$	$1 - \frac{\psi_{soil}}{\psi_0}$	$\frac{g_c c_a}{\alpha Q}$

See Table 2 for symbol definitions.

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Supporting Information

- Methods S1** Analytical solutions for the optimal c_i/c_a ratio and optimal stomatal conductance
- Methods S2** Girdling and drought experiments
- Methods S3** Numerical solutions including xylem embolism

Figure captions

Fig. 1 Schematic diagram of the soil-plant-atmosphere system, showing fluxes of water (blue) and carbon (red), and their governing equations. See Table 2 for symbol definitions and units.

Fig. 2 Responses of optimal stomatal conductance (g_s) to changes in leaf irradiance (Q , orange), atmospheric CO₂ concentration (c_a , red), vapour pressure deficit (D , blue), sink strength (k_{sink} , gray) and soil-to-leaf hydraulic conductance (K_{tot} , dashed black), predicted under hypothesis O-T (Table 3). Each variable was varied independently while all other parameters were fixed at their default values (Table 2; $Q = 500 \mu\text{mol m}^{-2} \text{s}^{-1}$, $D = 10^{-2} \text{mol mol}^{-1}$, $c_a = 400 \mu\text{mol mol}^{-1}$, $k_{sink} = 4 \times 10^{-5} \text{mol m}^{-2} \text{s}^{-1} \text{MPa}^{-1}$, $K_{tot} = 0.01 \text{mol m}^{-2} \text{s}^{-1} \text{MPa}^{-1}$). Numerically identical responses are predicted under hypothesis O-O, with K_{tot} replaced by the xylem hydraulic conductance, K_x (Table 3).

Fig. 3 Responses of stomatal conductance (g_s , a), leaf water potential (ψ_{leaf} , b), leaf phloem sugar concentration (C_{leaf} , c), phloem turgor pressure ($P_{leaf} \approx P_{root}$, d) and the intercellular-to-atmospheric CO₂ concentration ratio (c_i/c_a , e) to changes in soil water potential (ψ_{soil}), predicted under hypotheses O-T, O-O, W-T and W-O. Also shown is the linear response of g_s to ψ_{soil} under hypothesis O-T when the soil-to-root hydraulic conductance (K_{soil}) is fixed (a, dashed red line). All other parameters were fixed at their default values (Table 2). For O-T and W-T, ψ_{leaf} and C_{leaf} approach their critical values ($\psi_0 = -3 \text{MPa}$ and $C_0 = 1230 \text{mol m}^{-3}$) as $\psi_{soil} \rightarrow \psi_0$. The value of C_0 has been chosen so that $RTC_0 = -\psi_0$ (Table 2), making O-T and W-T agree numerically in this limit.

Fig. 4 Responses of stomatal conductance (a) and leaf photosynthesis rate (b) to atmospheric CO₂ concentration under hypothesis O-T at three values of sink strength: $k_{sink} = 4 \times 10^{-5} \text{mol m}^{-2} \text{s}^{-1} \text{MPa}^{-1}$ (default value, solid red), $k_{sink} = 4 \times 10^{-6} \text{mol m}^{-2} \text{s}^{-1} \text{MPa}^{-1}$ (dashed red), k_{sink} infinite (blue, corresponding to hypothesis W-T/W-O). All other parameters were fixed at their default values (Table 2). Numerically identical responses are predicted under hypothesis O-O.

Fig. 5 Covariation of stomatal conductance (g_s) with leaf water potential (ψ_{leaf} , blue) and leaf

phloem osmotic pressure (RTC_{leaf} , red) under soil drying (range of ψ_{soil} as in Fig. 3), predicted under hypothesis O-T for VPD = 1 kPa (solid lines) and 2 kPa (dashed lines). The horizontal distance between the g_s - ψ_{leaf} and g_s - RTC_{leaf} curves is equal to the leaf phloem turgor pressure (P_{leaf}). Inset: corresponding covariation of RTC_{leaf} and ψ_{leaf} showing tight correlation. All other parameters were fixed at their default values (Table 2).

Fig. 6 Stomatal conductance (a), leaf water potential (b), leaf osmolality (c) and leaf turgor pressure (d) at the time of leaf gas exchange measurement, as functions of predawn water potential for well-watered (circles) and drought-stressed (triangles) saplings of *Populus tremula* (Aspen, orange), *Betula pendula* (Birch, blue), *Pinus sylvestris* (Pine, green) and *Picea abies* (Spruce, yellow). The raw data were previously published in Salmon *et al.* (2020), except for the predawn water potentials. Loess regression curves have been added to help visualise the trends in the data (Methods S1). Compare the general trends with Fig. 3.

Fig. 7 Stomatal conductance as a function of the absolute value of leaf water potential (ψ_{leaf} , blue) and osmotic potential (RTC_{leaf} , orange) for well-watered (circles) and drought-stressed (triangles) saplings of *Populus tremula* (Aspen, a), *Betula pendula* (Birch, b), *Pinus sylvestris* (Pine, c) and *Picea abies* (Spruce, d). The raw data were previously published in Salmon *et al.* (2020). Power-law regression curves ($y \sim x^a$) have been added to help visualise the trends in the data (Methods S1). Compare the general trends with Fig. 5.

Fig. 8 The dynamics of net assimilation (a), stomatal conductance (b), the ratio of intercellular (c_i) to atmospheric (c_a) CO₂ concentration (c), leaf water potential (d), and leaf osmolality (e) after girdling the phloem stem on young saplings of *Populus tremula* (Aspen, orange), *Betula pendula* (Birch, blue) and *Pinus sylvestris* (Pine, green). Girdling was applied immediately after the first measurement point. For each species, three to four saplings were girdled (shown by different symbols). Time is shown on a log scale. Straight lines between consecutive points have been added to help visualise the trends in the data. Some of the data for pine were previously published in Hölttä *et al.* (2017).

Transpiration:
 $E = 1.6g_s D$

Stomatal CO₂ diffusion:
 $A = g_s(c_a - c_i)$

Photosynthesis (with NSL):

$$A = (1 - \phi) \frac{\alpha Q g_c c_i}{\alpha Q + g_c c_i}$$

$$\phi = \frac{c_{leaf}}{c_0} \quad \text{or} \quad \frac{\psi_{leaf}}{\psi_0}$$

Xylem water flux:
 $J_x = K_x(\psi_{root} - \psi_{leaf})$

Phloem sugar transport:
 $J_C = K_p C_{leaf} (P_{leaf} - P_{root})$

Soil water uptake:
 $J_{soil} = K_{soil}(\psi_{soil} - \psi_{root})$

$$K_{soil} = K_{soil,sat} \left(\frac{\psi_{soil,sat}}{\psi_{soil}} \right)^a$$

Sink phloem unloading:

$$U = k_{sink} P_{root}$$

or

$$U = k_{sink} R T C_{root}$$

Xylem-phloem hydraulic equilibrium:

$$\psi_{leaf} = P_{leaf} - R T C_{leaf}$$

$$\psi_{root} = P_{root} - R T C_{root}$$













