Maximizing leaf carbon gain in varying saline conditions: An optimization model with dynamic mesophyll conductance

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SUMMARY

While the adverse effects of elevated salinity levels on leaf gas exchange in many crops are not in dispute, representing such effects on leaf photosynthetic rates (A) continues to draw research attention. Here, an optimization model for stomatal conductance (gc) that maximizes A while accounting for mesophyll conductance (gm) was used to interpret new leaf gas exchange measurements collected for five irrigation water salinity levels. A function between chloroplastic CO₂ concentration (cc) and intercellular CO₂ concentration (ci) modified by salinity stress to estimate gm was proposed. Results showed that with increased salinity, the estimated gm and maximum photosynthetic capacity were both reduced, whereas the marginal water use efficiency k increased linearly. Adjustments of gm, k and photosynthetic capacity were shown to be consistent with a large corpus of drought-stress experiments. The inferred model parameters were then used to evaluate the combined effects of elevated salinity and atmospheric CO₂ concentration (ca) on leaf gas exchange. For a given salinity level, increasing ca increased A linearly, but these increases were accompanied by mild reductions in gc and transpiration. The ca level needed to ameliorate A reductions due to increased salinity is also discussed using the aforementioned model calculations.

Keywords: Capsicum annum L., mesophyll conductance, osmotic pressure, photosynthetic impairment, salt stress, stomatal optimization.

INTRODUCTION

Irrigation is routinely used for maintaining and increasing food production worldwide. Irrigated agricultural lands produce 40–45% of the world’s food, and almost 90% of the global water use is for irrigation (Döll and Siebert, 2002). However, water scarcity remains the major limiting factor on expansion of irrigated agriculture in many parts of the world, especially in arid and semi-arid regions. In these regions, reliance on crops that require recycled irrigation water (e.g. saline water) is becoming necessary, which has an appreciable feedback on productivity and increased salinity (Runyan and D’Odorico, 2010). In some areas, up to 1200–1400 mm of saline water with salinity levels ranging from 2.2 to 3.7 dS m⁻¹ have been used to meet crop water requirements (Ben-Gal et al., 2008). Unsurprisingly, irrigation with high saline water does have adverse impacts on crop productivity, and its representation in mathematical models continues to draw research attention (Lycoskoufis et al., 2005; Qiu et al., 2017b).

High salinity induces both osmotic (short-term) and ionic (medium-to-long term) stresses that inhibit leaf photosynthetic (A) and transpiration (E) rates and stomatal conductance (gc) at differing time scales as discussed elsewhere (Hsiao et al., 1976; Morgan, 1984; Munns and Tester, 2008; Hossain and Dietz, 2016; Perri et al., 2018). Under changing environmental conditions, stomata adjust their opening dynamically to govern CO₂ and H₂O diffusion into and out of leaves (Manzoni et al., 2011). Open stomata result in liquid water molecules to experience a phase transition and escape into the atmosphere, while allowing for CO₂ molecules from the atmosphere to diffuse into the sub-stomatal (or internal) cavity. Under high salinity conditions, the short-term osmotic stress is assumed to act in a manner analogous to soil water stress and reduces gc (Munns and Tester, 2008), which then leads to a decreased E.
Simultaneously, the diffusion of CO\textsubscript{2} from the atmosphere into the leaves is also reduced – though \( A \) may be less affected than \( E \) because plants have the ability to reduce their internal CO\textsubscript{2} partial pressure. Another limitation of osmotic stress is restricting CO\textsubscript{2} diffusion towards the chloroplast, where this pathway is commonly represented by the leaf mesophyll conductance \((g_{\text{m}})\). A large body of evidence indicates that \( g_{\text{m}} \) is not only finite and of similar magnitude to \( g_{c} \) (Flexas et al., 2008), but is also reduced under soil water stress (Flexas et al., 2002, 2004; Delfine et al., 2005; Galmés et al., 2007; Nadal and Flexas, 2018) and salinity stress (Delfine et al., 1998, 1999; Centritto et al., 2003; Loreto et al., 2003). Hence, variation in \( g_{\text{m}} \) and \( g_{c} \) result in changes to \( A \), and the sum of both stomatal and mesophyll resistances set a limit for the overall conductance experienced by CO\textsubscript{2} uptake under saline conditions (Flexas et al., 2004; Volpe et al., 2011; Perri et al., 2019).

Experiments already report adverse effects of elevated salinity on gas exchanges (Chartzoulakis and Klapaki, 2000; Lycoskoufis et al., 2005; Azuma et al., 2010). However, what has resisted complete mathematical treatment is a phenomenonological link between elevated salinity and reductions in \( A \), \( E \), \( g_{\text{m}} \) and \( g_{c} \) (Flowers et al., 1977; Brugrenoli and Lauteri, 1991; Steduto et al., 2000), and this link frames the scope here. A stomatal optimization model for \( g_{c} \) is combined with a Farquhar photosynthesis model for \( C_{3} \) plants and diffusional mass transport to interpret the effects of salt stress on leaf gas exchange experiments. Within the context of coupled hydrological-biogeochemical models, this approach assumes that stomatal aperture is adjusted so as to maximize \( A \) for a certain amount of saline water uptake (Volpe et al., 2011). This hypothesis is compatible with the fact that uptaking saline water can cause measurable loss in carbon accumulation (Hsiao et al., 1976; Morgan, 1984; Munns and Tester, 2008). The model proposed here revises earlier studies (Volpe et al., 2011) by directly incorporating \( g_{\text{m}} \) and chloroplastic CO\textsubscript{2} concentration \((c_{\text{p}})\) using a proposed stress function discussed elsewhere (Dewar et al., 2018; Perri et al., 2019). The model provides a diagnostic of the relative impairment of the photosynthetic and hydraulic machinery using conventional gas exchange measurements. A literature review across a wide range of species is also conducted to assess similarities in impairment of the photosynthetic and hydraulic machinery of leaves due to salt- and drought stresses. Based on inferred parameters from gas exchange measurements, we also inquire as to whether and by how much elevated \( c_{a} \) might mitigate salinity stress in crops. The assessment of the magnitude of such compensation effect continues to be of interest in combined ecohydrological-climate change studies under saline conditions as well as phytoremediation efforts to reduce soil salinity in a fluctuating climate (Singh et al., 2006; Bonan et al., 2014; Jesus et al., 2015).

**RESULTS**

**Mathematical and modeling results**

For steady-state conditions, the mass transfer of CO\textsubscript{2} and water vapor between leaves and the atmosphere are described by

\[
A = g_{c}(c_{a} - c_{i}) = g_{m}(c_{i} - c_{c}),
\]

(1)

and

\[
E = 1.6g_{c}VPD_{L} = 1.6g_{c}(e_{1} - e_{a}).
\]

(2)

where \( A \) is the leaf photosynthetic rate, \( E \) is leaf transpiration rate, \( g_{c} \) and \( g_{m} \) are the stomatal and mesophyll conductances to CO\textsubscript{2}, respectively, \( c_{a} \), \( c_{i} \) and \( c_{c} \) are the ambient, intercellular and chloroplastic CO\textsubscript{2} concentrations, respectively, and \( VPD_{L} \) is the vapor pressure deficit with respect to leaf temperature \((T_{L})\) representing the driving force for transpiration \((e_{1} - e_{a})\), where \( e_{a} \) and \( e_{1} \) are the intercellular and ambient water vapor concentrations, respectively. During photosynthesis, CO\textsubscript{2} is first transferred from the atmosphere into the sub-stomatal internal cavity through stomata, and then from there to the chloroplast through the leaf mesophyll (Flexas et al., 2008). The effective conductance \((g_{\text{eff}})\) from the atmosphere to the chloroplast for photosynthesis can be determined from \( g_{m} \) and \( g_{c} \) using (Volpe et al., 2011)

\[
g_{\text{eff}} = \frac{g_{c}g_{m}}{g_{c} + g_{m}}.
\]

(3)

so that the overall mass transfer to CO\textsubscript{2} can be expressed as

\[
A = g_{\text{eff}}(c_{a} - c_{i}).
\]

(4)

Here, the aerodynamic conductance \( g_{a} \) is assumed to be much larger than \( g_{c} \) or \( g_{m} \), as common when interpreting leaf-gas exchange measurements (described later). In typical gas exchange experiments, the measured \( g_{c} \) is higher than 2 mol m\textsuperscript{-2} sec\textsuperscript{-1}, which is at least one order of magnitude larger than \( g_{m} \) reported in the literature. When mitochondrial respiration \((R_{d})\) is small compared with \( A \) (neglecting \( R_{d} \) to derive the analytical solution), the Farquhar photosynthesis model for \( C_{3} \) plants can be mathematically expressed as (Katul et al., 2010; Launiainen et al., 2011; Volpe et al., 2011)

\[
A = \frac{a_{1}(c_{p} - c_{2})}{a_{2} + c_{2}},
\]

(5)

where \( c_{p} \) is the CO\textsubscript{2} compensation point, \( a_{1} \) and \( a_{2} \) are parameters that depend on whether \( A \) is ribulose-1,5-bisphosphate carboxylase/oxygenase (hereafter Rubisco) or ribulose-1,5-bisphosphate (hereafter RuBP) limited. For Rubisco limited \( A, a_{1} \) is set to the maximum carboxylation.
capacity \( V_{\text{cmax}} \) and \( a_2 = K_c (1 + C_{\text{ca}}/K_c) \), where \( K_c \) and \( K_o \) are the Michaelis constants for \( \text{CO}_2 \) fixation and oxygen inhibition, respectively, and \( C_{\text{ca}} \) is the oxygen concentration in air (= 210 \text{ mmol}^{-1}). The \( c_p \), \( K_c \) and \( K_o \) have been shown to be \( T \) dependent (Ethier and Livingston, 2004; Sharkey et al., 2007). For RuBP limited conditions, \( a_1 \) is set to the electron transport rate \( (J_{\text{max}}) \) and \( a_2 = 2c_p \). When assuming steady-state conditions (i.e. every \( \text{CO}_2 \) molecule that enters through the leaf stomata from the atmosphere is assimilated), the Farquhar biochemical demand and atmospheric supply of \( \text{CO}_2 \) can be equated to yield

\[
\frac{c_a}{c_o} = \frac{1}{2} \frac{-a_1 - a_2 g_{\text{eff}} + \sqrt{a}}{2c_0 g_{\text{eff}}} \quad (6)
\]

and

\[
A = \frac{1}{2} \left[ a_1 + (a_2 + c_o) g_{\text{eff}} - \sqrt{a} \right] , \quad (7)
\]

where

\[
g_a = \left[ a_1 + (a_2 - c_o) g_{\text{eff}} \right]^2 + 4g_{\text{eff}} (a_1 c_o + a_2 c_o g_{\text{eff}}). \quad (8)
\]

To proceed further, a model for \( g_{\text{eff}} \) (or its two constituents \( g_a \) and \( g_{\text{m}} \)) is needed. Two approaches are now used and the mathematical results that emerge from implementing them are highlighted. One approach is sufficiently general and accommodates both RuBP and Rubisco limitations to \( A \), whereas the second approach is only applicable to Rubisco limitations. Inherent to both approaches is the hypothesis that leaves autonomously maximize their carbon gain for a given amount of water by altering \( g_c \) (Givnish and Vermeij, 1976; Cowan and Farquhar, 1977; Cowan, 1978; Hari et al., 1986; Katul et al., 2009, 2010; Launiainen et al., 2011; Manzoni et al., 2011; Volpe et al., 2011; Vico et al., 2013). Mathematically, this hypothesis can be translated into a Hamiltonian to be maximized with respect to the independent variable \( g_c \) given as

\[
H(g_c) = A - \dot{E} = \frac{1}{2} \left[ a_1 + (a_2 + c_o) g_{\text{eff}} - \sqrt{a} \right] - 1.6 \dot{g_c} \text{VPD_L.} \quad (9)
\]

where \( \dot{E} \) is the cost of losing water in \( \text{CO}_2 \) units (i.e. linking the carbon and water economies of the plant) and is mathematically equivalent to the Lagrange multiplier (i.e. constant over time scales where \( g_c \) changes). Differentiating equation (9) with respect to \( g_c \), setting \( \partial H(g_c)/\partial g_c = 0 \), and assuming \( \partial g_{\text{m}}/\partial g_c = 0 \) yields

\[
\frac{\partial H(g_c)}{\partial g_c} = \frac{1}{2} \left[ (a_2 + c_o) g_{\text{eff}}^2 + \frac{2g_{\text{eff}}}{a_c} a_1 (c_o - 2c_p - a_2) - \frac{2g_{\text{eff}}}{a_c} (a_2 + c_o)^2 \right] \frac{1}{\sqrt{a}} - 1.6 \dot{g_c} \text{VPD_L} = 0. \quad (10)
\]

This \( g_c \) or \( g_{\text{eff}} \) formulation is implicit and the analytical solution can be derived (not shown here). Combined with equations (3) and (10), the \( g_c \) can be solved and this solution is referred to as the non-linear optimization model.

To provide analytical results that can be discussed in general terms, a simpler solution is also presented that is restricted to Rubisco limitations. Expression \( a_2 + c_o \) is written as \( a_2 + (c_o/c_o)c_o = a_2 + sc_o \), where \( s = c_o/c_o \). Inserting this expression into equation (5) yields

\[
A = \frac{a_1 (c_o - c_p)}{a_2 + sc_o}. \quad (11)
\]

A linearization is now conducted that is only plausible when \( a_2 > sc_o \) (i.e. Rubisco limitations on photosynthesis). For the case where \( a_2 > sc_o \), small variations in \( s \) have a minor impact on \( a_2 + sc_o \) and can be ignored. Thus, \( s \) is treated only as a constant in the expression \( a_2 > sc_o \) resulting in a linear \( A-c_o \) biochemical demand function. For RuBP limitations on \( A, a_2 = 2c_o < 80 \text{ \mu mol}^{-1} \), but \( c_o > 300 \text{ \mu mol}^{-1} \) is usually high so that \( a_2 < c_o \) and the aforementioned approximation cannot hold. However, for Rubisco limitations on \( A, a_2 > 550 \text{ \mu mol}^{-1} \) and \( c_o < 300 \text{ \mu mol}^{-1} \) so that \( a_2 > sc_o \), and treating \( s \) as a constant in the expression \( a_2 + sc_o \) results in a linear biochemical demand function as expected in Rubisco limited \( A \) (Katul et al., 2010; Volpe et al., 2011). To be clear, \( s \) is treated as a constant only in the denominator of equation (11) but, everywhere else, it is allowed to vary. Upon combining equations (11) and (4),

\[
\frac{c_o}{c_o} = \frac{a_1 c_o + g_{\text{eff}} (a_2 + sc_o)}{a_1 + g_{\text{eff}} (a_2 + sc_o)}. \quad (12)
\]

and

\[
A = \frac{a_1 g_{\text{eff}} (c_o - c_p)}{a_1 + g_{\text{eff}} (a_2 + sc_o)}. \quad (13)
\]

Now equations (12) and (13) can be used in the Hamiltonian of equation (9) to yield:

\[
H(g_c) = A - \dot{E} = \frac{a_1 g_{\text{eff}} (c_o - c_p)}{a_1 + g_{\text{eff}} (a_2 + sc_o)} - \dot{E} (1.6 \text{ g_v \text{VPD_L}}). \quad (14)
\]

Upon differentiation with respect to \( g_c \), setting \( \partial H(g_c)/\partial g_c = 0 \) and solving for \( g_c \) results in:

\[
\frac{\partial H(g_c)}{\partial g_c} = \frac{1}{2} \left[ (a_2 + c_o) g_{\text{eff}}^2 + \frac{2g_{\text{eff}}}{a_c} a_1 (c_o - 2c_p - a_2) - \frac{2g_{\text{eff}}}{a_c} (a_2 + c_o)^2 \right] \frac{1}{\sqrt{a}} - 1.6 \dot{g_c} \text{VPD_L} = 0. \quad (10)
\]
Combining equations (3), (13) and (15), an expression for \( A \) as a function of \( g_c \) is given as:

\[
A = g_c \sqrt{1.6 \cdot \psi_{L,max} - 1}. \tag{16}
\]

Hereafter, the result in equation (16) is referred to as the linear optimization model. The \( g_m \) is one of the key factors in the non-linear and linear optimization models. This study also provides a mechanism to model \( g_m \), which constitutes one of the main results of the work here.

When \( g_m \) is not directly measured, a stress function between \( c_i \) and \( c_0 \) is now proposed to estimate \( g_m \), and is given by

\[
\frac{c_i - c_{\text{min}}}{c_i} = r \approx r_{\text{max}} \left[ 1 - \left( \frac{EC_{\text{dw,max}}}{EC_{\text{dw}}} \right)^2 \right] = r_{\text{max}} \left[ 1 - \left( \frac{\psi_L}{\psi_{L,\text{max}}} \right)^2 \right], \tag{17}
\]

and

\[
g_m = \frac{A}{(1 - r)c_i - c_{\text{min}}} \tag{18}
\]

where \( \psi_{L,\text{max}} \) is the maximum leaf water potential, \( EC_{\text{dw,max}} \) is the maximum electrical conductivity of drainage water, which reflects the level of drainage water salinity that would lead to no appreciable yield (or \( A = 0 \)). Based on a threshold-slope linear salinity response model (Maas and Hoffman, 1977), the calculated \( EC_{\text{dw,max}} \) was ~43 dS m\(^{-1}\) determined from an expansive data set described elsewhere (Qiu et al., 2017b). However, a theoretical \( EC_{\text{dw,max}} \) may be deemed infinite if a sigmoidal-shape salinity response model is adopted (Van Genuchten and Hoffman, 1984). As a compromise between these two end-members, the \( EC_{\text{dw}} \) was set to 60 dS m\(^{-1}\) here, where the relative yield (or \( A \)) was reduced to 20% in a sigmoidal-shaped model.

The \( r_{\text{max}} \) and \( c_{\text{min}} \) are the maximum ratio and minimum \( c_0 \), respectively, estimated from the measured \( A - c_i \) curves under Rubisco limitations. Alternatively, this \( c_{\text{min}} \) can be estimated from the linearized Farquhar biochemical demand model as \( c_{\text{min}} = c_0 - c_p \), where \( c_p \) is a proxy for the \( c_i \) compensation point. From equation (1), below \( c_p \), \( A < 0 \), thus \( c_p > c_i \) and \( c_{p} > c_{\text{min}} \). In fact, these two parameters have the following relation (Flexas et al., 2007): \( c_p = c_0 - R_d/g_m \). Combining these two equations results in:

\[
c_{\text{min}} = (1 - r)c_p + \frac{R_d}{g_m} \tag{19}
\]

The evaluation of this result (i.e. equation 19) is featured in the Discussion section.

In equation (17), the use of leaf water potential instead of \( EC_{\text{dw}} \) is preferred because \( EC_{\text{dw}} \) is an indirect measure of leaf water status. Because the leaf water potential was not measured, an equilibrium approximation between salinity concentration in the leaf and the soil must be adopted to proceed further. It is assumed that when salinity in the drainage water is proportional to salinity concentration in the leaf (as common when using filtration theory with constant filtration efficiency), then \( EC_{\text{dw}} \) describes the expected osmotic potential in the leaf. Evidence and plausibility arguments for the link between \( EC_{\text{dw}} \) and leaf water status are described elsewhere (Perri et al., 2018). The mathematical form of expressions (17) and (18) and its justification when stomata are not the only limiting factor has been the subject of a recent investigation described elsewhere (Dewar et al., 2018).

**Salinity effect on \( g_m \), \( V_{\text{cmax}} \), \( J_{\text{max}} \) and \( \lambda \)**

From measured \( A - c_i \) curves on hot pepper seedlings, \( g_m \), \( V_{\text{cmax}} \), \( J_{\text{max}} \) and \( A \) were determined using a non-linear regression method with matlab software for different irrigation water salinity (\( EC_{\text{iw}} \)) levels. Generally, estimated \( g_m \), \( V_{\text{cmax}} \), \( J_{\text{max}} \) decreased as the electrical conductivity of drainage water (\( EC_{\text{dw}} \)) increased some 27 days after transplanting (DAT; Figure 1a–c). The \( g_m \), \( V_{\text{cmax}} \), \( J_{\text{max}} \) at \( EC_{\text{iw}} \) of 7.3 dS m\(^{-1}\) decreased by 41.2%, 38.7%, 31.2%, respectively, compared with \( EC_{\text{dw}} \) of 0.9 dS m\(^{-1}\). The salinity stress has little impact on the relation between \( c_i \) and \( c_0 \), respectively, from all data sets (Figure 1d), which were then used when analyzing the gas exchange data set in equations (17) and (18) at ambient (\( c_a \)).

For the gas exchange data set, the \( g_m \) and \( V_{\text{cmax}} \) estimated based on equation (18) and the non-linear optimization model, respectively, also exhibited a decreasing trend with increasing \( EC_{\text{dw}} \) from 2.4 dS m\(^{-1}\) sampled 23 DAT (Figure 2a,b). These findings are consistent with the result from the independently measured \( A - c_i \) curves earlier discussed. The different values of estimated \( g_m \) and \( V_{\text{cmax}} \) between \( A - c_i \) curve experiments (described later) and the gas exchange data set are due to leaf temperature (\( T_L \)) differences. The \( V_{\text{cmax}} \) exponentially increases with increasing \( T_L \) (the equation is shown in Table S1). The \( g_m \) showed a similar trend as \( V_{\text{cmax}} \) with a certain range of \( T_L \), but decreased at high \( T_L \).
Sharkey et al., 2007). The $r$ value calculated from equation (17) showed minor reduction (from 0.49 to 0.48) as $EC_{dw}$ increased from 1.0 to 6.4 dS m$^{-1}$ (Figure 2c), which is in line with the result from the independent $A$–$c_i$ curves measured 27 days after transplanting (DAT). For reference, the gas exchange data with no water, salinity and nitrogen stresses from Delfine et al. (2001) for bell pepper, from a greenhouse experiment for hot pepper in 2008–2009 ($A$–$c_i$ curve, not published) and from data for other crops are shown in (d). The regression function for peppers in (d) was with a coefficient of determination $R^2 = 0.97$. The data in the insets of (a), (b) and (c) are mainly derived from a large number of experiments on drought stress where leaf water pressure was reduced due to soil moisture reductions. Some salt stress experiments have been reported and included as well in the insets (Data S1). Note the collapse of the data sets despite the differing measuring methods, crop type and replication.

Effects of elevated CO2 and salinity on gas exchange

The results from the linearized optimization model are now used to assess how much elevated $c_a$ is required to buffer plants against salinity in the foreseeable future. For this purpose, all $EC_{nw}$ levels were considered along with the

(coefficient of determination $R^2 = 0.99$ based on the pooled data from all gas exchange data sets). The linear optimization model only underestimated $\lambda_{NL}$ by about 2.6%, indicating that $\lambda_{LI}$ can still provide acceptable estimates of $\lambda$ for operational purposes or parameter constraints.
values of kinetic constant for photosynthesis \( a_1 = V_{c_{\text{max}}} \) and \( g_m \) estimated from 23 DAT. The premise here is that \( a_1 \) and \( g_m \) values are not altered by elevated \( c_a \) (Katul et al., 2010; Volpe et al., 2011). This assumption is likely to be an overestimate for the effects of elevated \( c_a \) on \( A \) as downregulation can reduce \( a_1 \) with increasing \( c_a \). Long-term exposure to CO\(_2\) enrichment plants may also adjust their morphology, which is not considered here. The value of parameter \( s \) (long-term \( c_c/c_p \)) used in the linearized optimization model [equations (11)–(15)] was calculated from the long-term \( c_c/c_p \) and \((c_c -37)/c_a = 0.49\) derived from the \( A-c_c \) curve, and this ratio is assumed to be not sensitive to \( c_a \). The elevated \( c_a \) (from 360 to 500 \( \mu \text{mol mol}^{-1} \)) has a minor impact on \( s \) in the linear model. To simplify, the \( c_a \) was set to 430 \( \mu \text{mol mol}^{-1} \) when evaluating \( s \) values (i.e. 0.483, 0.483, 0.478, 0.474 and 0.459, respectively, for different \( EC_{\text{iw}} \) levels). The mean values of measured \( VPD_L \) were used in all calculations because salinity had little effect on \( VPD_L \) (10\% variation among treatments). The dependence of \( k_{LI} \) on \( c_a \) was assumed linear for Rubisco limited photosynthesis as discussed elsewhere (Katul et al., 2010; Manzoni et al., 2011; Volpe et al., 2011; Vico et al., 2013), and was given by: \( k_{LI} = k_{LI0} \frac{c_a}{c_a0} \), where \( c_{a0} = 360 \mu \text{mol mol}^{-1} \). The compensation point, \( c_{p} \), is defined as the concentration when \( A = 0 \) [equation (16)]. The values of \( \lambda_{LI} \) estimated from 23 DAT for different \( EC_{\text{iw}} \) levels were used to calculate the effects of elevated \( c_a \) on \( \lambda_{LI} \).

Figure 2. Effects of drainage water salinity \( (EC_{\text{dw}}) \) on estimated (a) mesophyll conductance \( (g_m) \), (b) maximum carboxylation capacity of Rubisco \( (V_{c_{\text{max}}}) \), (c) \( r \) and (d) marginal water use efficiency estimated from a linear \( (\lambda_{LI}) \) and a non-linear \( (\lambda_{NL}) \) optimization model for \( g_c \). The data were from gas exchange measurements 23 days after transplanting (DAT). The inset in (d) includes data from drought stress studies described in Data S1.

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The modeled \( A \) increased linearly with elevated \( c_a \) for all \( EC_{iw} \) levels (Figure 3a), partially offsetting the adverse impacts of salinity stress. The slope of the linear relation between \( A \) and \( c_a \) decreased as \( EC_{iw} \) levels increased. That is, the gains in \( A \) with increasing \( c_a \) are ameliorated by increased salinity. In fact, an increase of 1.4-fold \( c_a \) for \( EC_{iw} \) of 7.0 dS m\(^{-1}\) can only attain ~ 57% of the same value of \( A \) as under \( EC_{iw} \) of 0.9 dS m\(^{-1}\) in ambient CO\(_2\). However, at low salinity levels, future increases in \( c_a \) may offset the short-term osmotic effects of salinity on \( A \). Interestingly, the \( g_c \), \( c_c/c_a \) and \( E \) are all mildly reduced as \( c_a \) and \( EC_{iw} \) increased (Figure 3b–d). However, the reductions in \( g_c \), \( c_c/c_a \) and \( E \) with increasing \( c_a \) are minor and are only 3.4%, 2.1–4.5% and 3.4%, respectively, when \( c_a \) increased by as much as 40%.

**DISCUSSION**

New experiments on leaf-gas exchange from hot pepper seedlings were conducted and interpreted using a model for stomatal adjustment based on optimization principles described in Results. This combination of data and model results allows for the evaluation of the relative impairment of photosynthetic and hydraulic machinery of leaves with increased salinity. In this interpretation, photosynthetic properties were represented by \( g_m \) and \( a_1 \), while hydraulic properties were represented by the marginal water use efficiency \( \lambda \) (Volpe et al., 2011).

Estimated \( g_m \) from \( A-c_i \) curves measurements decreased with increasing salinity after a moderate salinity level was crossed. Similar results were reported for *Spinacia*

![Figure 3](https://example.com/figure3.png)

**Figure 3.** Effects of elevated CO\(_2\) concentration \( (c_a) \) on modeled (a) photosynthetic rate \( (A) \), (b) stomatal conductance \( (g_c) \), (c) relation between \( c_c/c_a \) and \( c_a \) and (d) leaf transpiration rate \( (E) \) under different irrigation salinity levels \( (EC_{iw}) \). The ambient CO\(_2\) is set to 360 \( \mu \)mol mol\(^{-1}\).
oleracea (intermediate salt tolerance) and *Olea europaea* (mild salt tolerance; Deline *et al.*, 1998, 1999; Centritto *et al.*, 2003; Loreto *et al.*, 2003). The modeled \( g_m \) from gas exchange measurements using the proposed equation (18) here captured the trends in the aforementioned studies. Based on literature survey, there are three methods to estimate \( g_m \), i.e. gas exchange and chlorophyll fluorescence method, carbon isotope discrimination method and curve fitting method (Flexas *et al.*, 2007, 2008). Only the curve fitting method was used here with limited replications of the \( A-c_i \) curve for each \( EC_{iw} \) level. However, the limited replication for each \( EC_{iw} \) was necessary to ensure a wide range of \( EC_{iw} \) values. Nonetheless, the limited replications of the \( A-c_i \) cannot preclude some bias in the estimation of \( g_m \). Notwithstanding this replication issue, the overall results for pepper seedlings here are shown to be consistent with many other studies on other crops (see literature review) where \( g_m \) was determined by the other two methods, lending some confidence to the robustness of the results here.

A model-data finding is a connection between \( r \) and \( c_{min} \) derived from the relation between \( c_c \) and \( c_r \) (equation (17); Figure 1d), which is an expansion of a previous model presented in Volpe *et al.* (2011). A similar mathematical form of equation (17) was recently reported, where linear reduction of \((c_c - c_r) / (c_c - c_p)\) was defined as \( \psi_L \) increased (Dewar *et al.*, 2018). In equation (17), the \( r \) value was affected by two factors. The first is the value of \( EC_{cw} \) (or \( \psi_{Lmax} \)), which affects the gradient of \( g_m \) induced by salinity (or \( \psi_L \)). The second is the \( r_{max} \) which affect the initial value of \( g_m \). Higher \( r_{max} \) results in higher \( g_m \). From the linear correlation between \( c_c \) and \( c_r \) found here, the intercept can be obtained and appropriately labeled as \( c_{min} \) (obtained when \( c_r = 0 \)). Equation (1) suggests that there should be a concentration gradient between \( c_c \) and \( c_r \) whenever a steady-state \( CO_2 \) flux through the leaf is established (Ethier and Livingston, 2004). That is, any \( c_c \) should be higher than \( c_{min} \) because \( c_r > 0 \) and \( c_c > c_r > 0 \) from equation (1) when \( A < 0 \) and \( c_r > 0 \). This \( c_{min} \) could also be derived with aforementioned equation (19). Upon pooling data for all \( A-c_i \) curves, the average \( T_a \) was 32.3°C resulting in an average \( c_p \) of 47 \( \mu \)mol mol\(^{-1}\). The average values of \( R_d \) and \( g_m \) were 4 \( \mu \)mol m\(^{-2}\) sec\(^{-1}\) and 0.15 mol m\(^{-2}\) sec\(^{-1}\), respectively. Using these data together with \( r = r_{max} = 0.49 \), the calculated \( c_{min} \) from equation (19) was 37 \( \mu \)mol mol\(^{-1}\). This value is in agreement with the value obtained from the correlation between \( c_c \) and \( c_r \). For different crops, the values of \( c_{min} \) and \( r_{max} \) may be varied, but their relation is similar to the one shown here. For instance, assuming the same \( c_{min} \) of 37 \( \mu \)mol mol\(^{-1}\), the calculated \( r_{max} \) varied from 0.42 to 0.60 for no stress plants, where data for \( c_c \) and \( c_r \) are shown in Figure 1d). The minor change in the value of \( r \) in this study indicated that the reduction of \( c_c \) and \( c_r \) is nearly synchronous for young leaves of hot pepper, and the reduction of \( g_m \) is mainly due to the faster reduction in \( A \) than \( c_r \) (equation (18)). However, several studies show that lower \( \psi_L \) did induce a lower value of \( c_r \) than \( c_c \) under drought experiments, in turn leading to lower \( r \) (Warren *et al.*, 2004; Delfine *et al.*, 2005).

The reductions in \( g_m \) and \( g_c \) for higher salinity lead to a lower \( c_r \) (see Figure 3c at ambient \( c_r \)), which limited \( A \) (Deline *et al.*, 1998; Loreto *et al.*, 2003). Except for those inhibitions of \( CO_2 \) diffusion (linked to \( g_m \) and \( g_c \)), the photosynthetic capacity of leaves, \( a_n \), decreased after a moderate salinity level is crossed, which also caused a reduction in \( A \).

The hydraulic properties encoded by \( \lambda \), calculated from linear and non-linear optimization models both increased with increased salinity. Recalling that \( \lambda_{LI} \sim (WUE_{int})^2 \) [reversing equation (16) and assuming VPD\(_L\) is not significantly affected by salinity (supported by the data here)], the increase in \( \lambda \) as salinity increased is not surprising given the definition of intrinsic water use efficiency \( WUE_{int} = A/g_c \). A high \( WUE_{int} \) in high salinity is expected because \( g_c \) is reduced faster than \( A \) as salinity increased, a known result supported by many experiments (Chartzoulakis and Klapani, 2000; Azuma *et al.*, 2010; Fernández-Garcia *et al.*, 2014). This severe reduction in \( g_c \) and corresponding \( E \) reduces salt loading into leaves and avoids irreversible (or plastic) damage (Koyro, 2006; Volpe *et al.*, 2011) commonly associated with ionic stresses. Although \( \lambda \) increased monotonically with increasing salinity within the range of salinity levels considered here (corresponding to linear increased \( WUE_{int} \)), additional data on more severe salinity levels are necessary to evaluate the correlation between \( \lambda \) and salinity. For instance, when recalculating data from Chartzoulakis and Klapani (2000), there was only a small increase in \( WUE_{int} \) when \( EC_{iw} \) increased from 12.6 to 17.8 dS m\(^{-1}\), indicating \( \lambda \) did not appreciably increase for the aforementioned study. The correlation between linear and non-linear optimization models shows that \( \lambda_{LI} \) of hot pepper can be approximated by \( \lambda_{LI} \) which is independent of \( g_m \) and can be readily calculated from measured \( A \), \( g_c \) and VPD\(_L\) [reversing equation (16)]. This correlation is in line with other studies on trees (e.g. *Pinus taeda* and *Pinus sylvestris*) and spinach (*Spinacia oleracea*; Katul *et al.*, 2010; Launainen *et al.*, 2011; Volpe *et al.*, 2011), although overestimation of \( \lambda \) in the linear optimization model was observed in prior studies.

The \( g_m \), \( V_{cmax} \), \( J_{smax} \) and \( \lambda \) variations across different salinity levels for hot pepper were compared against values reported across many drought experiments for several crops for varying \( \psi_L \). The overall patterns in \( g_m \) reductions and \( \lambda \) increases with decreased \( \psi_L \) (whether drought or salinity induced) are broadly comparable (Figures 1 and 2). Likewise, the decrease in computed \( V_{cmax} \) and \( J_{smax} \) with decreased \( \psi_L \) (drought or osmotically induced) appear
comparable and are suggestive of photosynthetic impairment (Figure 1). Hence, osmotic (and not ionic) stress is the dominant factor responsible for the adjustments in $g_{m}$, $\lambda$ and photosynthetic capacity. This finding may not be a surprise as ionic stress requires longer salt accumulation duration and may be a factor in adult plants, not the seedlings analyzed here. Many studies also showed that early response to drought and salinity stress has been mostly identical (Munns, 2002; Chaves et al., 2009).

The $c_h$ has increased since the pre-industrial era, and is projected to increase further in the future (Pachauri et al., 2014). Hence, an extension of this study is to assess the combined effects of elevated salinity and $c_h$ on gas exchange of young hot pepper leaves. The linear optimization model is used for illustration, though the findings here also apply for the non-linear optimization model. An assumption that $\lambda$ linearly increased with increasing $c_h$ was adopted, which is confirmed by prior studies for the case of Rubisco limitation (Katul et al., 2010). Unsurprisingly, salinity stress inhibits $A$, $g_{c}$, $E$ and $c_{i}/c_{a}$ (Figure 3 under ambient $c_{i}$). The elevated $c_{i}$ ameliorated the $A$, but only under low salinity levels. There was a similar positive effect of elevated $c_{i}$ on $A$, while negative effects on $g_{c}$ or $E$ were reported in O. europaea (Volpe et al., 2011) and P. taeda (Katul et al., 2010) when using $\lambda \propto c_{i}$. Manzoni et al. (2013) also demonstrated an increased $A$ but no reductions in $g_{c}$ for elevated $c_{i}$ using a dynamic optimization scheme where transients in soil moisture were considered. The results here suggest that the inhibition of $A$ by salinity stress may be partly buffered by elevated $c_{i}$ but only for low salinity levels. The model results derived here are intended to serve as conjectures or hypotheses to be tested in future experiments with elevated CO₂ and salinity.

**EXPERIMENTAL PROCEDURES**

**Experiment**

The details of the experimental setup are presented elsewhere (Qiu et al., 2017a, 2018). Briefly, the experiment was conducted using pots positioned in a rain shelter at the Agro-Meteorology Research Station located in Nanjing city, China (32.2°N, 118.7°E, altitude 14.4 m). Five irrigation water salinity ($EC_{iw}$) characterized by electrical conductivity levels (i.e. 0.9, 1.6, 2.7, 4.7 and 7.0 dS m$^{-1}$) with four replications at a leaching fraction (the fraction of amount of drainage water relative to amount of irrigation water) of 0.29 were used. Irrigation water salinity was increased by adding 1:1 milli equivalent concentrations of NaCl and CaCl₂ to fertilizers (a half-strength Hoagland solution; Qiu et al., 2017a), which added an electrical conductivity of 0.9 dS m$^{-1}$ by adding 1:1 milli equivalent concentrations of NaCl and CaCl₂ to fertilizers (a half-strength Hoagland solution; Qiu et al., 2017a). When varying $c_{i}$ from 20 to 1500 µmol mol$^{-1}$, each measured A-ci curve spans both Rubisco and RuBP limitations (Figure 4). Hence, it is possible to estimate $g_{m}$, $V_{cmax}$, $J_{max}$ and $R_{d}$ from each A-ci curve using a non-linear regression method (see Appendix S1 and Table S2 for approach, comparison with other published methods). These inferred parameters can then be related to the salinity levels experienced by the seedling. The basic equations used in parameter inferences were (Sharkey et al., 2007):

$$A = \frac{A_{1}(c_{i} - c_{d})}{a_{2} + c_{a}} - R_{d}; c_{i} = c_{1} - \frac{A}{g_{m}}$$

where $A$ and $c_{i}$ are measured. When fitting the A-ci curve, a critical $c_{i}$ of 300 µmol mol$^{-1}$ was found to separate Rubisco and RuBP
limits on $A_i$ where Rubisco limits $A$ for $c_i < 300 \mu$mol mol$^{-1}$, whereas RuBP limits $A$ for $c_i > 300 \mu$mol mol$^{-1}$. However, when measured $A$ variations drop below 2% with increasing $c_i$, it was assumed that triose phosphate utilization (TPU) limits $A$ (i.e. $\partial A/\partial c_i \approx 0$). TPU limitations occurred at high $c_i > 1200 \mu$mol mol$^{-1}$. The parameters here obtained using matlab software (MathWorks, MA, USA) were then compared with another standard method (made public via a downloadable spreadsheet requiring $A$–$c_i$ curve) described elsewhere (Sharkey et al., 2007; Table S2), though similar methods are also available (Gu and Sun, 2014; Sun et al., 2014). Deviations among fitted parameters here and the standard approach (Sharkey et al., 2007) were below 10%. The calculations were repeated by commencing the search for optimal parameter combinations using several initial conditions so as to ensure that the search along the parameter manifold is not prematurely terminated at a local minimum for reasons discussed elsewhere (Gu and Sun, 2014). The usage of the proposed non-linear parameter inference method here permits routine analysis of numerous $A$–$c_i$ curves in a self-consistent manner. The Rubisco limited $A$ in the $A$–$c_i$ curves (Figure 1) were used to analyze relations between $c_i$ and $c_j$ for different salinity levels.

For the gas exchange data set, $c_j$ did not vary from its ambient value. Optimal $g_w$ in equations (10) and (16) were employed separately. Both formulations require the parameters $g_m$, $a_2$, $C_p$, $a_1$ and $\lambda$. The parameter $g_m$ was estimated from the aforementioned equation (18) with measured $A$ and $c_i$ and estimated $r$ and $c_{\text{min}}$. The parameters $a_2$ and $c_2$ were determined, as before, from temperature adjustment equations (Sharkey et al., 2007) shown in Table S1. The parameter $a_1$ was estimated by inverting equation (5) with measured $A$, $T$, and estimated $c_j$ from equation (17). The cost parameter $\lambda$ of individual leaves was determined by inverting equations (10) and (16) for both the non-linear and linear optimization models, respectively.

**Figure 4.** An example of Rubisco and RuBP limited photosynthesis fitted to measured $A$–$c_i$ curves under different irrigation water salinity levels. The low and high irrigation water salinity levels were 0.9 and 7.0 dS m$^{-1}$, respectively, used in this study. The photosynthetic rate at any $c_i$ is the minimum of these potential limitations.

**Literature review**

Many studies show that plant response to salinity stress resembles drought stress at the early stages (Munns, 2002; Chaves et al., 2009). Specifically, the early and rapid decline in growth among plants is often linked to osmotic instead of ionic stresses (Perri et al., 2018). Hence, the effects of salt stress on reducing $g_m$, $V_{c_{\text{max}}}$, $J_{c_{\text{max}}}$ and increasing $\lambda$ here are compared with other salt stress experiments as well as drought experiments on several plant species. Data sets were compiled from the literature that feature reductions in $g_m$, $V_{c_{\text{max}}}$, $J_{c_{\text{max}}}$ and increases in $\lambda$ relative to a reference unstressed (or well-watered) condition. In drought experiments, changes in $g_m$, $V_{c_{\text{max}}}$, $J_{c_{\text{max}}}$ and $\lambda$ are presented as a function of reduced plant water potential $\psi_w$. In the case of salinity stress for well-irrigated crops (as is the case here), the plant water potential was not measured but was inferred as follows. The $EC_{\text{sat}}$ was converted to $\psi_w$ assuming: (i) osmotic pressure dominates the overall total water potential (no gravitational or pressure potentials) in the leaf (a reasonable assumption for well-watered short plants); and (ii) a 50% dilution ratio of salt concentration in leaves when compared with the drainage water salinity (a reasonable assumption because electrical conductivity of soil saturated paste extract ($EC_p$) is $\sim 0.5EC_{\text{sat}}$ measured at the end of the experiment). The van’t Hoff equation $n = M_i/R/T$ is used to relate osmotic pressure ($P_i$ in atm) to solute molar concentration ($M_i$ in mol L$^{-1}$) and temperature ($T_i$ in K), where $R$ is the gas constant ($\sim 0.08206$ L atm mol$^{-1}$ K$^{-1}$). Estimation of $M_i$ from $EC$ measurements of soil salinity is based on the approximate linear expression $M_i = bEC + c$, where $b$ and $c$ are based on instrument calibration. To relate soil salinity to salinity in the leaf needed for, it is assumed that salinity concentration in the leaf is in quasi-equilibrium with salinity concentration in the soil-root system (though the soil and leaf pressures differ). That is, the state of equilibrium is restricted to chemical potentials not mechanical pressures. The linear relation between soil and leaf salinity is
often used in models based on filtration arguments where the filtration efficiency varies linearly with external salinity concentration (for review, see Perri et al., 2018). Continuous monitoring of soil water salinity, which is far more desirable than EC\text{cist}, is made difficult because of the intrusive effects of such measurements on the rooting system.

**DATA STATEMENT**

The data set from the experiment and the literature review are available in Data S1. The matlab code for inferring parameters from A\text{-ci} curve was shared freely in the Github: https://github.com/shuilibite?tab=repositories.

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**AUTHORS’ CONTRIBUTIONS**

RQ designed and conducted most of the experiment, analyzed the data and wrote the draft manuscript. GK provided the new insights for the model and markedly improved the manuscript. All authors discussed the results and approved the final version of the manuscript.

**CONFLICT OF INTEREST**

The authors declare that there are no conflicts of interest.

**SUPPORTING INFORMATION**

Additional Supporting Information may be found in the online version of this article.

**Table S1.** The values of scaling constant and activation energy describing the temperature adjustment for Rubisco limited photosynthesis.

**Table S2.** Comparison of values of \( V_{\text{cmax}}, R_{\text{d}}, J_{\text{m}} \) and TPU under different irrigation water salinity based on the proposed matlab program here and Sharkey et al.'s model.

**Appendix S1.** Literature used and introduction for Data S1.

**Appendix S2.** Introduction of matlab program for solving A\text{-ci} curve when \( g_{m} \) was unknown.

**Data S1.** Data set used for Figures 1 and 2.

**REFERENCES**


