Thermodynamic balance of photosynthesis and transpiration at increasing CO₂ concentrations and rapid light fluctuations

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A B S T R A C T
Experimental and theoretical flux models have been developed to reveal the influence of sun flecks and increasing CO₂ concentrations on the energy and entropy balances of the leaf. The rapid and wide range of fluctuations in light intensity under field conditions were simulated in a climatic gas exchange chamber and we determined the energy and entropy balance of the leaf based on radiation and gas exchange measurements. It was estimated that the energy of photosynthetic active radiation (PAR) accounts for half of transpiration, which is the main factor responsible for the exportation of the entropy generated in photosynthesis (Sₑ) out of the leaf in order to maintain a functional photosynthetic machinery. Although the response of net photosynthetic production to increasing concentrations of CO₂ under fluctuating light is similar to that under continuous light, rates of transpiration respond slowly to changes of light intensity and are barely affected by the concentration of CO₂ in the range of 260–495 ppm, in which net photosynthesis increases by more than 100%. The analysis of the results confirms that future increases of CO₂ will improve the efficiency of the conversion of radiant energy into biomass, but will not reduce the contribution of plant transpiration to the leaf thermal balance.
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1. Introduction

Radiant energy is mainly consumed in the leaf to fuel photosynthesis and transpiration the estimations of which are essential for climate modelling and the understanding of vegetation (Berner, 1997; Chen et al., 2011) and water dynamics (Jasechko et al., 2013). The thermodynamics of the two processes have been frequently investigated both theoretically and experimentally (Ksenzhek and Volkov, 1998). From the agronomic perspective, attention has mainly focussed on the yield of chemical energy, from radiant energy, as an indicator of photosynthetic efficiency and on the concomitant water consumed and, mainly, lost in transpiration. In fact, the entropy production per unit of biomass synthesized is higher the lower the efficiency of energy use and the production of entropy in photosynthetic systems, as in microbial growth (von Stockart and Liu, 1999), reflects the Gibbs energy dissipation accompanying the production of biomass. However, formal estimations of the production of entropy are interesting in other aspects as well. As a measure of organization (Ksenzhek and Volkov, 1998; Marín et al., 2009; Davies et al., 2013), low entropy content is associated with the maintenance of leaf structure and function. Therefore, at least for a comparison with the magnitude of the low entropy associated with life, the production of entropy in the leaf is relevant. Photosynthesis and transpiration produce entropy that must be exported to maintain leaf structure and function, therefore increasing the global entropy as required for all irreversible processes (Schrödinger, 1944). However, due to the scant knowledge of entropy balances in photosynthesis and transpiration to date, a comparison with the estimations of negative entropy associated to cell organization is not possible.

On the other hand, most investigations so far have focussed on steady-state systems in which the leaf receives a constant light intensity at actual and free-air enriched CO₂ concentrations (Leakey et al., 2009). However, in the field, the leaf is very frequently exposed to rapid changes in light intensity (typically from 50 to about 1500 μmol photon m⁻² s⁻¹ PAR) due to transitory shadow produced by clouds, by other leaves fluttering in the wind and by animals (Küllheim et al., 2002; Smith and Berry, 2013) that could differently affect photosynthesis and stomata aperture and transpiration. In addition, the responses to fluctuating light should be affected by the concentration of CO₂, which is the main substrate of photosynthesis and a negative modulator of stomata aperture. Taking also into account the concern regarding increasing
concentrations of atmospheric CO₂, we have investigated how this affects the efficiency of energy use and the entropy production of the leaf under fluctuating light.

To investigate the thermodynamic parameters associated to photosynthesis and transpiration of leaves under rapid light intensity changes, we implemented a reference light fluctuation treatment previously assayed (Martín et al., 2009) consisting of 15 min of leaf acclimation at 130 µmol photon m⁻² s⁻¹ PAR, followed by four 6-min light phases of 870, 61, 870 and 130 µmol photon m⁻² s⁻¹ PAR at leaf surface. Tobacco leaves were used as the experimental model. The laboratory results were analysed with a thermodynamics model of fluxes that can be applied for field-based measurements.

2. Materials and methods

2.1. Plants culture

Assays were performed with tobacco (Nicotiana tabacum, cv. Petit Havana) plants cultivated as previously described (Martín et al., 2009). Seeds were sown in pots with compost soil substrate, germinated and grown in a glasshouse and regularly irrigated with Murashige/Skoog nutrient solution.

2.2. Measurement of net photosynthetic and transpiration rates and stomatal conductance

Assays were carried out in the glass house with intact attached fully-expanded healthy leaves (containing around 25 µg chlorophyll cm⁻²) of the mid-stem of tobacco plants at the beginning of flowering. Photosynthetic activity, transpiration and stomatal conductance were measured during a light sequence treatment (in µmol photon m⁻² s⁻¹ PAR, photosynthetic active radiation, at leaf surface) with abrupt changes in intensity according to the sequence: 15 min acclimation at 130, 6 min at 870, 6 min at 61, 6 min at 870 and 6 min at 130 µmol photon m⁻² s⁻¹ PAR. Data collected each min and at light intensity transitions were directly represented using the Origin software (Princeton, USA). Experiments were repeated two to ten times. Net photosynthetic rates (in µmol consumed of CO₂ m⁻² s⁻¹), transpiration (in mmol of H₂O m⁻² s⁻¹) and stomatal conductance (in mmol CO₂ m⁻² s⁻¹) were determined in the glasshouse at 25 °C in 6.25 cm² leaf sections fitted on the chamber of the LCpro+ portable photosynthesis system (ADC BioScientific Ltd., Hertfordshire, UK) as previously described (Martín et al., 2009), except for the CO₂ concentration which, having been programmed as fixed during the light sequence treatment, varied as indicated. Registered data indicated that the sub-stomatal CO₂ concentration was stabilized (<5% variation) from the end of 15 min acclimation through the following 24 min incubation. PAR light was provided by mixed red (peak at 660 nm) and blue (peak at 470 nm) LED array unit monitored with a silicon-based sensor, which adjusts the power for constant output at the value set. Changes in light intensity was completed in less than 15 s, which is the shortest possible time between two successive graphic lectures in the LPCpro+ photosynthesis system.

3. Results

3.1. Photosynthetic and transpiration rates under fluctuating light intensities. Effect of the concentration of CO₂

Net photosynthesis and transpiration rates varied for the same plant from one day and leaf to another. However, relative rates for different CO₂ concentrations were highly reproducible with differences that never exceeded 5%. Therefore, we determined rate responses for different CO₂ concentration assays carried out successively with the same leaf section by changing the CO₂ concentration. The 15 min acclimation was repeated for each CO₂ concentration and the order of the assays with different CO₂ concentrations (increasing or decreasing) did not affect the responses of photosynthesis and transpiration rates. Over 100 data of photosynthesis and transpiration rate data were collected in a single experimental session. Experiments were repeated without significant differences two to ten times and all curves in Fig. 1 correspond to one representative complete experiment.

Fig. 1A shows the evolution of photosynthetic rates during the four light phases after the 15 min acclimation of tobacco leaf at CO₂ concentrations ranging from 260 to 495 ppm. As expected, photosynthetic rates were greater at high than at low

![Figure 1](image.png)
light intensities and increased with the concentration of CO₂, especially in the range of 315–470 ppm and at the phases of high light intensity (870 μmol photon m⁻² s⁻¹), indicating that at the high light intensity the limiting factor of the rate of photosynthesis was the concentration of CO₂. The effect of the CO₂ concentration was less pronounced when the low light intensity (61 μmol photon m⁻² s⁻¹) became the limiting factor of photosynthesis. In general, the rates of photosynthesis change rapidly when light intensity changes, reaching a constant value between a few second and 2 min depending on the CO₂ concentration and the light value change transition. Within the range of CO₂ concentrations and light intensities assayed, photosynthetic rates varied strongly, between the minimum 2.4 and the 6.5-fold higher maximum 15.8 μmol CO₂ m⁻² s⁻¹.

Fig. 1B shows that transpiration rates were affected to a lesser degree than photosynthetic rates under the same fluctuating light conditions and CO₂ concentrations assayed: the minimum 1.47 mmol H₂O m⁻² s⁻¹ barely doubled to a maximum 3.17 mmol H₂O m⁻² s⁻¹. Responses of the transpiration rates to changes in light intensity were also slower than those of photosynthetic rates. In many cases, constant transpiration rates did not seem to be reached after the 6 min span of each light phase. As expected, transpiration rates were higher at high than at low light intensities. However, changes in the transpiration rates with the CO₂ concentrations were complex and varied from one light phase to another, probably because they are affected by the slow response to light intensity changes. In tobacco, as in most plants, the aperture of stomata and transpiration decreases with the concentration of CO₂ and increases with light intensity. However, frequently, the dependence of stomatal aperture on the concentration of CO₂ varies with the range of CO₂ concentrations and light intensity (Wheeler et al., 1999). The opening effect of light seems double: one direct (blue light), favouring the polarization of the guard cell membrane (Ueno et al., 2005) and the subsequent entry of K⁺ through open channels and another indirect, favouring photosynthesis and consequently decreasing the CO₂ concentration within the internal atmosphere of the leaf (Wong et al., 1979). The slower responses of transpiration rates to changes in light intensity with respect to photosynthetic rates suggest at least one of two possibilities: (1) the light signal transduction, between membrane polarization and K⁺ accumulation, is slower than the response of the photosynthetic machinery and (2) the CO₂-mediated response to light is more significant than the direct effect of light on guard cells.

Whatever the case, under conditions that simulate the rapid changes of light intensity in the field such as those in Fig. 1, there is not a continuous decrease of transpiration with increasing CO₂ concentrations. In fact, under the assay conditions, minimum transpiration was reached at an intermediate CO₂ concentration, around 315 ppm. Parallel measurements of stomatal conductance (Fig. 1C) also indicated a minimum value at around 315 ppm CO₂ and a slow response to the sudden changes of light intensity. The slow response of transpiration to light intensity changes could be in the origin of the complex effect of CO₂ and suggests that increasing concentrations of atmospheric CO₂ would have a minimal effect on total transpiration under field conditions subjected to light changes much more frequent than those of our assay model.

Photosynthesis and transpiration rates integrated over the 24 min assay period at different concentrations of CO₂ (Table 1) again show that, under rapidly fluctuating light, the concentration of CO₂ strongly affected net photosynthesis which more than doubled when the CO₂ concentration rose from 260 to 495 ppm. In comparison with the actual 375 ppm CO₂, net photosynthesis increased some 30% at 495 ppm CO₂ (Table 1), a result in the same range as the increase in biomass yield observed in cultivated rice when the concentration of CO₂ rose from 365 to 570 ppm (Costa et al., 2006). In contrast, transpiration was barely affected; the maximum (determined at 470 ppm CO₂) total transpiration rate calculated over the 24 min period exceeded the minimum determined at 315 ppm CO₂ by only 22%. Water use efficiency, as measured by the low mass ratio: transpired water/biomass (CH₂O) photosynthesized (last column of Table 1) still improved consider-ably with increasing CO₂ concentrations.

The different effect of light fluctuations on photosynthesis and transpiration and the present rise of atmospheric CO₂ concentrations, which also affects net photosynthesis and transpiration in different ways, prompted us to calculate the thermodynamic balance of the results described in Fig. 1 as a model to approach the effects of the frequent and wide ranges of light intensity variations in the field.

### 3.2. Thermodynamic background and balance model

The scheme in Fig. 2 shows the photosynthesis-related energy flows for the determination of thermodynamic parameters in the leaf. To determine the incident PAR energy (Eᵢ), the PAR reading at leaf surface was multiplied by 0.93 to subtract the 7% which is transmitted, as determined in complementary assays with the LI-COR quantum detector on the opposite side of the leaf and Eᵢ accounts for the sum of absorbed and reflected energy. In the steady state, the energy inflow, Eᵢ, equals Eᵢ (the energy outflow) which is the sum of Eᵢ, the chemical energy of the net photosynthesis products stored as biomass, and Eother which includes energies of reflected light, fluorescence emission and the heat produced by non-photochemical quenching (Ptushenko et al., 2013) and energy dissipated in the physical and chemical processes from net photons used to net biomass produced.

\[ Eᵢ = Eᵢ = Eᵢ + Eᵢ \]

(1)

Photosynthetic energy efficiency (η, the fraction of absorbed radiant energy converted to biomass chemical energy) is:

\[ \eta = 100Eᵢ/Eᵢ \]

In contrast to energy, the entropy is not conserved in the steady-state; according to the second principle of thermodynamics, more entropy outflows (Sᵢ) than enters (Sᵢ), and the difference (Sᵢ – Sᵢ = Sᵢ) is the entropy generated by the photosynthetic leaf in the use of PAR. Therefore, the balance of entropy is:

\[ Sᵢ + Sᵢ = Sᵢ = Sᵢ + Sᵢ \]

(2)

where Sᵢ and Sᵢ are, respectively, the entropy production associated to biomass production and to the flows included under “other” (reflected, fluorescence and heat) in Fig. 2. The incident radiation different from PAR and heat transmission and convec tion balances are part of the whole heat flow, which may, at least
Table 1
Net photosynthesis and transpiration under fluctuating light. Indicated values were calculated by integration over the 24 min period shown in Fig. 1 of net photosynthesis and transpiration at the different concentrations of CO₂ (left column). The efficiency of the use of water was indicated in the far-right column as the ratio of g of transpired water to g of biomass (CH₂O) synthesized.

<table>
<thead>
<tr>
<th>[CO₂] ppm</th>
<th>Net photosynthesis, mmol CO₂ fixed m⁻²</th>
<th>Transpiration, mol H₂O m⁻²</th>
<th>Transpiration/photosynthesis ratio, g H₂O/g CH₂O</th>
</tr>
</thead>
<tbody>
<tr>
<td>260</td>
<td>7.36</td>
<td>3.36</td>
<td>274</td>
</tr>
<tr>
<td>315</td>
<td>9.36</td>
<td>3.27</td>
<td>210</td>
</tr>
<tr>
<td>375</td>
<td>12.15</td>
<td>3.68</td>
<td>182</td>
</tr>
<tr>
<td>470</td>
<td>15.43</td>
<td>3.99</td>
<td>155</td>
</tr>
<tr>
<td>495</td>
<td>15.78</td>
<td>3.72</td>
<td>141</td>
</tr>
</tbody>
</table>

partially, also be dissipated as the latent heat of water evaporation in transpiration. All uncertainties related to the relative flows are not a problem when we consider only energy and entropy flows derived from the PAR influx according to Eq. (1). Hence:

\[
S_{\text{other}} = \frac{E_{\text{other}}}{T} = \frac{E_{\text{in}} - E_{\text{BI}}}{T}
\]

(3)

where \( T \) is the absolute temperature.

By considering a \( \lambda \), PAR mean of 550 nm and applying the equivalence radiant energy \( J = 119.3 \times 10^6 / \lambda \) (nm), the \( E_{\text{PAR}} \) was estimated to be 140.2 kJ m⁻² for the four light phases totaling 24 min (0.695 mol photon m⁻²). Their associated entropies were determined as that of non-direct sunlight (Ksenzhek and Volkov, 1998) by \( S_{\text{in}} = E_{\text{in}}/5000 = 28.0 \) K·m⁻².

According to (2) and (3)

\[
S_{\text{S}} = S_{\text{out}} - S_{\text{in}} = S_{\text{BI}} + S_{\text{other}} - S_{\text{in}} = S_{\text{BI}} + \frac{E_{\text{other}}}{T} - \frac{E_{\text{in}}}{5000}
\]

(4)

In addition to the energetic efficiency defined above (\( \eta = 100E_{\text{BI}}/E_{\text{in}} \)) as a positive indicator of the photosynthetic performance, \( S_{\text{other}}/E_{\text{BI}} \) and, especially, \( S_{\text{in}}/E_{\text{BI}} \) ratios provide negative indicators of the photosynthetic performance that are related to \( \eta \) by:

\[
\frac{S_{\text{other}}}{E_{\text{BI}}} = \frac{E_{\text{in}} - E_{\text{BI}}}{E_{\text{BI}}T} = \frac{E_{\text{in}}/E_{\text{BI}} - 1}{T} = \frac{100}{\eta - 1} \text{ K}^{-1}
\]

(5)

\[
\frac{S_{\text{S}}}{E_{\text{BI}}} = \frac{S_{\text{in}}}{E_{\text{BI}}} = \frac{E_{\text{in}}/E_{\text{BI}} - 1}{T} = \frac{E_{\text{in}}}{5000E_{\text{BI}}}
\]

\[
= \frac{S_{\text{in}}}{E_{\text{BI}}} - \frac{1}{T} \frac{100}{\eta(1/T - 1/5000)} \text{ K}^{-1}
\]

(6)

\( S_{\text{in}}/E_{\text{BI}} \) (K⁻¹ unit) is the value of the entropy generated per J of generated chemical energy.

\( E_{\text{BI}} \) and \( S_{\text{BI}} \) were calculated from net CO₂ fixation data, dimensions of the experimental design, Gibbs free energy and entropy values in data banks and conventional thermodynamics.

Hence, \( CO₂ \) consumed is converted to C-equivalent biomass (CH₂O) according to the reaction:

\[
CO₂(gas) + H₂O(liquid) \rightarrow 1/6 C₆H₁₂O₆(solid) + O₂(gas)
\]

(7)

and

\[
E_{\text{BI}} = \Delta G = \Delta G^0 + RT \ln \left( \frac{P_{O₂}}{P_{CO₂}} \right)
\]

\[
= 475.900 - RT \ln(10^6 |CO₂|) \text{ mol}^{-1}
\]

(8)

Calculated with \( \Delta G^0 = 479.8 \text{ kJ mol}^{-1} \), \( R = 8.314 \text{ J mol}^{-1} \cdot \text{K}^{-1} \), \( T = 298 \text{ K} \), \( P_{O₂} = 0.21 \text{ bar} \) and where (in the last equation) \( |CO₂| \) is in ppm. Eq. (8) allows calculation of the energy (\( E_{\text{BI}} \)) stored as biomass per mol of CO₂ fixed.

For each photosynthesis assay, the integrated net CO₂ consumed over the last 24 min of light phases were referred to leaf square metre and multiplied by \( E_{\text{BI}} \) determined by (8) to obtain the \( E_{\text{BI}} \) stored as biomass per square metre.

Similarly, the associated production of entropy (\( S_{\text{BI}} \)) for one CO₂ mol in reaction (7) obeys:

\[
S_{\text{BI}} = \Delta S = \Delta S^0 - R \ln \left( \frac{P_{O₂}}{P_{CO₂}} \right)
\]

\[
= -30.2 + R \ln(10^{-6} |CO₂|) \text{ K}^{-1} \text{ mol}^{-1}
\]

(9)

\( S_{\text{BI}} \) is negative as it corresponds to the decrease of entropy in the chemical reaction converting \( CO₂ \) and \( H₂O \) to biomass \( CH₂O \) plus \( O₂ \).

Calculated with \( \Delta S^0 = -43.2 \text{ kJ mol}^{-1} \), \( R = 8.314 \text{ J mol}^{-1} \cdot \text{K}^{-1} \), \( T = 298 \text{ K} \), \( P_{O₂} = 0.21 \text{ bar} \) and where (in the last equation) \( |CO₂| \) is in ppm. Multiplication of \( S_{\text{BI}} \) by the integrated moles of fixed \( CO₂ \) per square metre equals the entropy (\( S_{\text{BI}} \)) of the biomass generated per leaf square metre.

By substituting in Eq. (4) and with the \( E_{\text{in}} \) (140.2 kJ m⁻²) and \( S_{\text{in}} \) (28.0 K·m⁻²) values estimated above for the 24 min incubation, the entropy generated in one square metre of leaf associated to the photosynthetic process, at 25 °C (approximately 298 K) is:

\[
S_{\text{S}} = S_{\text{BI}} + \frac{E_{\text{in}} - E_{\text{BI}}}{298} - 28.0 = 442 + S_{\text{BI}} - \left( \frac{E_{\text{BI}}}{298} \right) \text{ K} \text{ m}^{-1}
\]

(10)

The photosynthetic energy efficiency is:

\( \eta = 100 E_{\text{BI}}/E_{\text{in}} = 0.71 E_{\text{BI}} \), with \( E_{\text{BI}} \) in kJ m⁻².

According to Eqs. (8) and (9), Table 2 shows that \( E_{\text{BI}} \) slightly decreased and \( S_{\text{BI}} \) moderately increased (less negative entropy) when the concentration of \( CO₂ \) increased. As the moles of biomass photosynthesized per square metre strongly increased with the concentration of \( CO₂ \) (Table 1), \( E_{\text{BI}} \) (positive) and \( S_{\text{BI}} \) (negative) more than doubled their absolute values when the concentration of \( CO₂ \) increased from 260 to 495 ppm (Table 2). The negative \( S_{\text{BI}} \) is a minor contribution to \( S_{\text{S}} \), see Eq. (10), whose value only slightly decreased when the concentration of \( CO₂ \) increased (last column of Table 2).

In transpiration, standard references show the corresponding molar values for evaporation at 25 °C: \( \Delta H_{\text{evap}} = 44.0 \text{ kJ mol}^{-1} \) and \( \Delta S_{\text{evap}} = 147.6 \text{ J K}^{-1} \text{ mol}^{-1} \) (obviously independent of the concentrations of \( CO₂ \)) that must be multiplied by the moles of \( H₂O \) transpired to obtain energy and entropy flows associated to transpiration.

3.3. Energy and entropy flows

As expected, the photosynthetic efficiency \( \eta \), or percentage of the energy of incident PAR (\( E_{\text{in}} \)) that is converted into chemical energy of biomass, increased with the concentration of \( CO₂ \) (Fig. 3) whereas the entropy generated per unit (J) of chemical energy stored (\( S_{\text{BI}}/E_{\text{BI}} \)) decreased. The increase of photosynthesis at high \( CO₂ \) concentrations drives more \( E_{\text{in}} \) to biomass and hence \( E_{\text{BI}}, \) decreasing derivations to \( E_{\text{other}} \), which, in different ways, is the main source of generated entropy \( S_{\text{S}} \) (Eq. (4)). However, \( E_{\text{BI}} \) is a minor fraction of \( E_{\text{in}} \) and the relative decrease of \( E_{\text{other}} \) is low when \( CO₂ \) increases. Therefore, the relative decrease of \( S_{\text{BI}}/E_{\text{BI}} \) is only slightly more pronounced than expected for the sole increase
Table 2

<table>
<thead>
<tr>
<th>[CO₂] ppm</th>
<th>E_m, kJ mol⁻¹</th>
<th>S_m, J K⁻¹ mol⁻¹</th>
<th>E_n, J m⁻²</th>
<th>S_n, J K⁻¹ m⁻²</th>
<th>S_g, J K⁻¹ m⁻²</th>
</tr>
</thead>
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<tr>
<td>260</td>
<td>496.4</td>
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<td>-0.73</td>
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</tr>
<tr>
<td>315</td>
<td>495.9</td>
<td>-97.24</td>
<td>4641</td>
<td>-0.91</td>
<td>426</td>
</tr>
<tr>
<td>375</td>
<td>495.4</td>
<td>-95.78</td>
<td>6020</td>
<td>-1.16</td>
<td>421</td>
</tr>
<tr>
<td>470</td>
<td>494.9</td>
<td>-93.91</td>
<td>7636</td>
<td>-1.45</td>
<td>415</td>
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<td>495</td>
<td>494.8</td>
<td>-93.47</td>
<td>7807</td>
<td>-1.48</td>
<td>415</td>
</tr>
</tbody>
</table>

Fig. 3. Effects of the concentration of CO₂ on η and S_m/E_m under fluctuating light. As detailed in the text, η is the photosynthetic efficiency: the percentage of incident PAR energy (E_m) stored as biomass chemical energy (E_m). S_m/E_m represents the entropy generated in the photosynthetic process (S_m) per unit of chemical energy stored as photosynthesized biomass.

of E_m when the concentration of CO₂ increases (Fig. 3 and Table 2). Over the 24 min of the four light phases, about 5.5% of the incident PAR energy (E_m, 140.2 kJ m⁻²) is converted to chemical energy of biomass when the concentration of CO₂ is near 500 ppm. The percentage is still far from the theoretical maximum, around 30%, when 8 photons are assumed sufficient to convert one molecule of CO₂ to one equivalent of biomass (CH₂O). Efficiencies of near 20% were determined (results not shown) when integration only spanned the low intensity phase (61 μmol photon m⁻² s⁻¹ PAR) at 495 ppm CO₂ (see Fig. 1A) at which the limiting factor of photosynthesis is light intensity.

As indicated, the entropy generated in association with the photosynthetic process (S_g) slightly but consistently decreased with increasing concentrations of CO₂. However, mirroring the changes in transpiration (Table 1), the trend of the entropy associated to transpiration (S_t) is to increase, although somewhat erratically, with the concentration of CO₂ (Fig. 4). S_t was always higher than S_g but, in contrast to the high mass ratio transpired water/net biomass synthesized (between 141 and 274, Table 1), the contribution of transpiration to entropy export never exceeded the entropy generated by the PAR-dependent photosynthesis by more than 50%. The ratio S_t/S_g varied between a 1.13 minimum at 315 ppm CO₂ to a maximum 1.4 at 479 ppm CO₂.

4. Discussion and conclusions

The response of net photosynthetic production to increasing concentrations of CO₂ under fluctuating light (Fig. 1 and Table 1) is similar to that expected under continuous light (at least qualitatively), mainly because there is a rapid adjustment of the rate of photosynthesis to light changes. However, rates of transpiration respond slowly to changes in the intensity of light. In most cases, a constant rate of transpiration was not reached within the 6 min of each experimental light phase (Fig. 1B). Similar differences between photosynthesis and stomatal conductance responses to light intensity changes were reported by Way and Pearcy (2012) in assays at a fixed CO₂ concentration with forest understory plants.

For this research, we selected (Martín et al., 2009) the frequency of light fluctuations (each 6 min) such that the rate of photosynthesis reached the constant final value within each light phase. We only tested four amplitude of light intensity changes (130–870, 870–61, 61–870 and 870–130 μmol photon m⁻² s⁻¹ PAR), which are within the range that leaves receive in field. The results of this first approach indicate the convenience of further thorough investigations with a wide range of frequencies and amplitude intensities at constant root mean square intensity. These should provide a more precise estimation of the thermodynamics parameters in field conditions. Preliminary extrapolation of the results described here to field conditions suggests that the magnitude of the transpiration rate continuously oscillates with low amplitude around a mean value under the frequent situation of short light phases of different intensity. Under these conditions, the magnitude of transpiration (integrated over a period of oscillating light) would be barely affected by the concentration of CO₂ in the range of 260–495 ppm, as in the experimental model described here. The efficiency in the use of water, as measured by the transpired water/biomass synthesized (last column of Table 1), will rise with future increases of atmospheric CO₂ as found in temperate and boreal forests (Keenan et al., 2013). However, this is mainly due to an increase of net photosynthesis and not to significant decreases of transpiration, which, in fact, slightly increases, at least under fluctuating light. Leakey et al. (2006) reported that the stomatal conductance is not affected by long-term acclimation at elevated CO₂. Therefore, at least where fluctuating light is common, future increases of CO₂ should not significantly reduce the contribution of plant transpiration to the thermal balance of the leaf and the environment.

With a total transpiration ranging, for the different concentrations of CO₂ assayed, between 3.27 and 3.99 H₂O moles per
square metre during the 24 min incubation (Table 1), multiplication by the latent water evaporation heat (\(\Delta H_{\text{evap}} = 44.0 \text{kJ mol}^{-1}\)) indicates an energy consumption for transpiration between 144 and 175 kJ m\(^{-2}\), approximately 14% higher than incident PAR (\(E_{\text{in}} = 140.2 \text{kJ m}^{-2}\)). Of course, electromagnetic waves other that PAR contribute to the energy balance of the leaf in a fraction that is affected by several factors (Wang et al., 2013). Taking also into account that the leaf exchanges heat with the LCpro+ chamber as adjusted for isotherm and that fluorescent and reflected light energies (Fig. 2) are not used for water evaporation, the contribution of PAR energy to transpiration is difficult to evaluate. The magnitude of energy used in transpiration is of the same order as that of incident PAR and only a low percentage (\(\eta\) between 2.5 and 5.5%) of incident PAR is stored as chemical energy \(E_{\text{Bi}}\) (Fig. 3). Thus, assuming a minor contribution of light reflected and fluorescence emission, it may be conjectured that the Heat box of Fig. 2 (\(E_{\text{H}}\)) makes up the main fraction of incident PAR energy and was finally used in evaporation of water for transpiration. Under this assumption, incident PAR could account for around half of the heat required for transpiration and most of \(S_{\text{g}}\) is yet included in \(S_{\text{g}}\). In the field, the suggested approximate and relative magnitude of energy flows may be similar, as heat conduction and convection with the environment and other parts of the plant could play a similar role to that of the isothermal experimental chamber.

At actual CO\(_2\) concentrations of between 350 and 400 ppm, under fluctuating light, \(S_{\text{g}}\) is around 420 J K\(^{-1}\) m\(^{-2}\) (Fig. 4) and \(S_{\text{g}}/E_{\text{Bi}}\) around 0.08 K\(^{-1}\) (Fig. 4). Assuming that one square m of leaf contains 2 \(\times 10^{-3}\) m\(^{2}\) of tissue volume, an entropy production of 10\(^3\) J K\(^{-1}\) associated to the photosynthetic process is estimated to be produced per min and m\(^3\) of leaf tissue. For a comparison, the entropy associated to metabolite compartmentalization within cell organelles (that is presumably the main negative entropy of eukaryotic cells) is estimated to be 10\(^5\) J K\(^{-1}\) m\(^{-3}\) (Marín et al., 2009). Although roughly estimated, these values indicate that the entropy produced during photosynthesis might be rapidly exported outside the leaf to maintain it functional. Although not only \(S_{\text{g}}\) accounts for \(S_{\text{g}}\) and other factors of the energy balance contribute to transpiration (and hence to \(S_{\text{g}}\)), transpiration is probably the main vehicle for the necessary entropy exportation. As Fig. 4 shows, the slight decrease of \(S_{\text{g}}\) and the slight increase of \(S_{\text{g}}\) raises the excess of \(S_{\text{g}}\) over \(S_{\text{g}}\) when the concentration of CO\(_2\) increases and suggests that the potential of transpiration to export the generated entropy could be compromised at low CO\(_2\) concentrations, thus becoming a factor by which leaf cells become disorganized and die at low CO\(_2\) concentrations.

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**References**


