Analysing uncertainties in the calculation of fluxes using whole-plant chambers: random and systematic errors

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Abstract

Aims Gas exchange measurements on individual plants depend largely on chamber systems, and uncertainties and corrections in current flux calculation procedures require further assessment.

Methods We present a practical study with novel methods for analyses of flux uncertainties in an original chamber design excluding soil fluxes and allowing simultaneous measurements of whole-plant photosynthesis and transpiration.

Results Results indicate that random errors caused by IRGA noise and the lack of criteria to optimize the time window (TW) of chamber enclosure lead to significant flux uncertainties (12 %). Although enclosure should be rapid to minimize plant disturbances, longer TWs (3 min) increase confidence in flux estimates. Indeterminate stabilization periods in existing calculation protocols cause significant systematic errors. Stabilization times were identified via the change-point detection method, and flux uncertainties were reduced. Photosyn-

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thesis was overestimated by up to 28% when not correcting the evolving CO₂ molar fraction for water vapour dilution. Leakage can compromise flux estimates, but was negligible (ca. 2%) here due to the large chamber-headspace and relatively small values of both collar contact length and closure time.

Conclusions A bootstrapping, resampling-based flux calculation method is presented and recommended to better assess random errors and improve flux precision. We present practical recommendations for the use of whole-plant chambers.

Keywords Canopy chamber · Closed chamber · Flux calculation methods · Flux uncertainties · Shrublands

Introduction

Measurements of plant gas exchange by quantifying the change in molar fractions of the confined atmosphere within a chamber require adequate error treatment protocols and flux calculation methods. Here we will focus on inaccuracies and potential problems related to measurements. A variety of types of enclosures/configurations can be found such as steady-state (open dynamic) and non-steady-state (closed static or non-flow-through, and closed dynamic or flow-through systems; Livingston and Hutchinson 1995). Among chamber systems, accuracy and precision analysis of flux estimates are rare (Heinemeyer and McNamara 2011), and concurrent flux calculation methods need to determine random errors and improve flux precision (Levy et al. 2011; Pumpanen et al. 2004; Savage et al. 2008; Pihlatie et al. 2013). Pérez-Priego et al. (2010) adapted the typical closed canopy chamber design to enclose only aboveground vegetation by excluding soil fluxes, and enabling net photosynthesis ($F_{ph}$) and transpiration ($F_{tr}$) measurements of single canopy trees up to 20–30 m². Such whole-plant chambers are an appropriate means to investigate plant physiological processes on an intermediate scale between leaf-scale cuvette measurements and landscape-scale eddy covariance measurements.

In the assessment of flux uncertainties, some progress has been made particularly with respect to chamber disturbance effects and the correction of systematic errors. During measurement, both closed dynamic and static chambers alter state variables (temperature, humidity, CO₂), disturb plant physiology, and result in non-linear tendencies in progressions of chamber CO₂ and water vapour molar fractions (hereafter referred to as “progressions”). Nonetheless, representative fluxes can be inferred from these progressions by fitting non-linear regressions that describe fluxes at the initial time of chamber closure (Reicosky et al. 1990; Anthony et al. 1995; Wagner et al. 1997; Kutzbach et al. 2007; Langensiepen et al. 2012; Pihlatie et al. 2013). More relevant disturbances include alteration of radiation by the chamber walls, and ventilation affecting both air mixing and aerodynamic and leaf boundary-layer resistances (Denmead 1984; Leuning and Foster 1990; Denmead et al. 1993; Le Dantec et al. 1999; Hooper et al. 2002; Steduto et al. 2002; McLeod et al. 2004; Christiansen et al. 2011). Further major sources of systematic errors include:

i) chamber leakage, potentially causing flux underestimation (Held et al. 1990; Grau 1995; Steduto et al. 2002; Rodeghiero et al. 2007; Denmead 2008);
ii) inaccurate determination of effective chamber volume (Jassal et al. 2012);
iii) condensation of water vapour on chamber walls (Pérez-Priego et al. 2010);
iv) water dilution, particularly when the gas analyser does not compute the dry CO₂ molar fraction, as is the case for some Licor instruments (e.g., Li840/Li840A, Li820). In such cases, water vapour dilution must be corrected for since the addition of water molecules through transpiration lowers the CO₂ mole fraction (Webb et al. 1980) and can bias flux estimates (Hooper et al. 2002); and
v) determination of the starting time ($\delta$)—needed both to stabilise the chamber atmosphere following deployment and for transport of sample air from chamber to gas analyser—, which defines the initial slope of the non-linear fit.

Excluding $\delta$ determination, such systematic errors have been explored previously, but little progress has been made in characterizing random errors in chamber-based flux estimates. Furthermore, although micrometeorologists routinely apply “density corrections” to correct for water vapour dilution (Webb et al. 1980), some chamber studies still employ IRGAs that do not provide dry mole fractions (Dariah et al. 2014; Leiber-Sauheitl et al. 2014; Juszczak et al. 2013), and thus neglect this effect, highlighting a continuing need to revisit this issue and help to bridge knowledge across disciplines.
Statistical analysis is required to assess random errors and to better define standardised flux calculation protocols. Fluxes are inferred from the best parameter fit of the progressions by linear or non-linear regressions. Based on statistical tests of residuals, most authors agree that progressions are fit better by non-linear (e.g., exponential, asymptotic, or quadratic) than linear regression (Wagner et al. 1997; Levy et al. 2011; Kutzbach et al. 2007; Venterea et al. 2009; Pihlatie et al. 2013; but see Pedersen et al. 2010; Jassal et al. 2012). Although procedures that automatically detect the best fit among alternative regressions are valuable to reduce flux uncertainties (Pedersen et al. 2010), we draw the attention to how parameters from a particular non-linear fit are sensitive and may fluctuate largely depending on i) the starting time $\delta$ ii) time window (TW) used for flux calculation or number of gas samples (in the case of static chambers), and iii) “noise” inherent to the gas analyser. Regarding $\delta$, whereas the gas transport time can be assessed experimentally, the mixing time varies among measurements depending on random variations within the chamber. We anticipate that errors in fitted parameters decrease with the duration of sampling the progressions. The optimal TW used for flux calculation must be short to minimize disturbances, but long enough to estimate regression parameters with sufficient confidence, depending on natural within-chamber variability and gas analyser noise, chamber volume and the flux magnitude during enclosure (Pihlatie et al. 2013). Generally, there is a need for enhanced understanding of the causes of the uncertainty underlying flux calculation methods (Levy et al. 2011).

Here, we present a chamber and analysis of flux uncertainties based on $\delta$ definition, TW, and noise that goes beyond frequent discussions about goodness of fit and the choice of linear or non-linear flux calculation methods. In particular, we test a change-point detection algorithm earlier described by Hinkley (1970) to determine $\delta$, and a bootstrapping resampling-based method (BRM) for flux calculation which consists of making repeated flux calculations within measurement intervals. This method was also used to assess the effect of noise on parameter uncertainty. Additionally, we quantify the main sources of systematic error: $\delta$, water dilution, radiation attenuation by chamber walls and leakage. Finally, diurnal time courses of net whole-plant photosynthesis and transpiration rates are presented for the dominant species of a semi-arid shrubland, as well as practical recommendations for the use of whole-plant chambers of the closed dynamic type.

### Materials and methods

#### Chamber and site description

The chamber consists of a translucent sheet (“NRS90 clear”, Llumar®, Solutia Inc., Düsseldorf, Germany), stretched and fixed to a cubic aluminium frame structure (1×1×1 m) using adhesive tape. This film was chosen for its excellent broadband transmittance and absorptivity properties (see Appendix for more details about spectral transmittance). Photosynthetically active radiation ($F_{\text{PAR}}$) is measured outside with a quantum sensor (Li-190, Li-Cor, Lincoln, NE, USA). Air and canopy temperatures are measured with a thermocouple ($T_a$, Type T, 0.5 mm diameter) and an infrared thermometer ($T_c$, IRTS-P, Apogee, UT, USA), respectively, both installed near the chamber ceiling (Fig. 1).

The chamber operates as a closed dynamic system. A small pump circulates an air flow of 1 L min$^{-1}$ through the sampling circuit: air is drawn from inside the chamber—through many intake points included in three hanging tubes that are spatially distributed through the chamber headspace—to an infrared gas analyser (IRGA LI-840, Lincoln, NE, USA), which measures CO$_2$ and water vapour mole fractions at 1 Hz; sampled air is then returned to the chamber. Although the hanging tubes reduce the need to homogenise air during chamber deployment, two small fans (12 V, 0.14A) are fixed at 0.5 m on opposite sides of the chamber and angled 45° downward (Fig. 1).

On the soil, a 1-m$^2$ metal collar is installed around each plant, providing a flat surface on which the bottom of the chamber is set. A thick transparent plastic sheet sealed to the collar is tightly wrapped around the stem of the shrub or small tree, and thus excludes soil gas exchanges. The chamber is open and ventilated prior to measurement, so that initial air composition and temperature represent natural atmospheric conditions. For the flux measurement, the chamber is manually placed on the collar (closed position), and CO$_2$ and water vapour molar fraction progressions commence (Fig. 2). The rate of change of CO$_2$ and water vapour mole fractions (corrected for $\delta$, leakage and water dilution effects, see below) with respect to time is proportional to the apparent gas flux caused by canopy net photosynthesis ($F_{\text{Ph}}$) and transpiration ($F_{\text{tr}}$), respectively.

The chamber was tested at “El Llano de los Juanes” (Sierra de Gádor, Almería; 36.9283, −2.7505), a
shrubland in the semi-arid climate zone of Southeast Spain. Measurements of $F_{Ph}$ and $F_{Tr}$ were conducted on 24 July 2012 between 6:00 and 17:00 solar time on *Hormathophilla* sp., *Genista* sp., the two predominant shrub species whose average canopy height is 0.5 m. Chamber deployments lasted 180 s as a general rule. For more site details, see Serrano-Ortiz et al. (2007).

Fig. 1 Schematic with the main elements of the canopy chamber. Pictures below show the chamber measuring in *Hormathophylla* sp. in “Llano de los Juanes”, Sierra de Gádor, Almería (Spain)

Fig. 2 Changes recorded in CO$_2$ (a) and water vapour (b) molar fractions during a measurement on 24 July 2012 (10:30) in *Hormathophylla* sp. and used for presenting flux calculation results inferred from windows using five different starting points (16–20 s) and durations (80, 100, 120, 140, and 160 s)
Systematic errors

Correction for water dilution effect

The increase of water vapour concentration in the headspace leads to one of the most important systematic errors affecting CO2 flux estimations when using closed chambers with infrared gas analysers that do not provide dry CO2 molar fractions. Processes causing this error are i) spectral cross-sensitivity due to absorption band broadening and inherent instrument cross-sensitivity, and ii) the water dilution effect. While the Li-840 corrects the cross-sensitivity error due to absorption band broadening (<0.1 μmol CO2 mol−1/mmol H2O mol−1), the CO2 molar fraction must be corrected for the water dilution effect as follows:

\[ C(t) = \frac{C'(t)}{1 - W(t)} \]

where \( C(t) \) is the water dilution-corrected or dry air CO2 mole fraction, and \( C'(t) \) and \( W(t) \) are molar fractions of CO2 and water vapour, respectively, simultaneously measured by the Li-840 gas analyser (Hupp 2011; Welles et al. 2001). We evaluated and quantified the magnitude of the water dilution error in the presented chamber type by estimating fluxes with and without correcting for the water dilution effect.

Attenuation of light by the chamber

The effect of incidence angle on the attenuation of radiation by the chamber was assessed using measurements of global photosynthetically active radiation (PAR, Li190SB, Li-Cor, Lincoln, NE, USA). Under clear-sky conditions, two inter-calibrated \( F_{\text{PAR}} \) sensors were installed, one covered by the chamber and the other uncovered. Photon counts at different solar zenith angles (14°, 30°, and 45°) were conducted during a sunny day (13 June 2013). Data collected at 1 Hz were averaged to 1 min.

During field measurements, light response curves were fit following the Michaelis-Menten model:

\[ F_{\text{ph}} = \frac{F_{\text{ph maxi}} \times F_{\text{PAR}}}{F_{\text{PAR}} + K_m} \]

where \( F_{\text{ph maxi}} \) is the light-saturated rate of canopy net photosynthesis, and \( K_m \) is the half-saturation constant. This is relevant to scale fluxes to undisturbed light conditions.

Effect of condensation of water vapour on chamber walls affecting the natural attenuation by the chamber was not observed.

Leakage test

Following the diffusion-based formulation proposed by Pérez-Priego et al. (2010), errors due to leakage were estimated by injecting a known amount of CO2 into the empty chamber. This test was carried out in the laboratory, where chamber enclosures were reproduced using a collar wrapped around a plastic trunk with the transparent plastic sheet used for soil exclusion. The decline in the CO2 molar fraction due to leakage was monitored over 15 min. As established by Pérez-Priego et al. (2010) but considering the collar contact length (\( l_c \), m) as a proxy for the leakage diffusion coefficient (\( \theta \), m² s⁻¹), this is deduced as:

\[ \theta = \frac{-V}{\Delta \ln(C_{t2}/C_{t1})} \]

where \( C_{t1} \) and \( C_{t2} \) (μmol CO2 mol⁻¹) are chamber CO2 molar fractions at times \( t_1 \) and \( t_2 \), respectively, \( \Delta t \) is the period considered (s), \( V \) is the chamber volume (m³), and \( C_a \) is the ambient CO2 molar fraction. Accordingly, the leakage error (\( \varepsilon_l \)) is time-dependent and calculated as:

\[ \varepsilon_l = \frac{\theta \times l_c \times \Delta t}{2V} \]

Determination of \( \delta \)

Although on-going processes (e.g., photosynthesis) drive changes in CO2 within the chamber just after chamber closure, there is an initial “stabilization time” before the CO2 progression begins (Fig. 2). Therefore, two subsets of data with different statistical distributions must be considered: 1) data before \( t = \delta \), which are excluded from flux calculations, and 2) the remaining data, defining the flux calculation time window (TW). Among measurements, \( \delta \) varies because the mixing time depends on random variation within the chamber. We used a change-point detection algorithm (Pruned Exact Linear Time (PELT) method; Killick et al. 2012), which uses a linear computational cost function under a
statistical criteria (Schwarz Information Criterion (SIC) Schwarz 1978) to seek an optimal $\delta$ as the change in the mean within normally distributed observations.

**Flux calculation approach and random uncertainties**

**Flux calculation**

Following Wagner et al. (1997), we use a quadratic least squares fit of the form:

$$C(t) = at^2 + bt + c,$$

where $a$, $b$, and $c$ are the optimised parameters. The canopy net photosynthesis rate ($F_{Ph}$) is calculated from the time rate of change of $C(t)$, given by the time derivative of Eq. (5):

$$\frac{\partial C}{\partial t} = 2at + b.$$  

(6)

The undisturbed (or minimum-disturbance) $F_{Ph}$ is estimated as $\frac{\partial C}{\partial t}$ at $t=0$ for the calculation window, which corresponds to the optimised fit parameter $b$ ($\mu$mol CO$_2$ mol$^{-1}$ s$^{-1}$). For disturbed conditions at time $t$, gas exchange must be estimated using the full equation. Scaling via the ideal gas equation, $F_{Ph}$ is finally calculated as follows;

$$F_{Ph}(\mu$mol CO$_2$s$^{-1}$ plant$^{-1}) = \frac{\partial C}{\partial t} \times \frac{(P-e_a)V}{RT_a},$$

(7)

where the term $(P - e_a)$ refers to partial pressure of dry air, $P$ is the atmospheric pressure (Pa), $e_a$ is the partial pressure of water vapour (Pa), $R$ is the ideal gas constant (8.314 m$^3$ J mol$^{-1}$ K$^{-1}$), and $V$ is the effective chamber volume (m$^3$). All variables are taken at time 0. The transpiration rate ($F_{tr}$) is estimated following the same procedure but using water vapour progressions. In this study, plant volume represents <0.2 % of the headspace volume of the chamber (1 m$^3$) and errors due to air volume displacement by plants were neglected.

**Random uncertainties; the bootstrapping resampling-based method (BRM)**

An initial analysis was performed to assess the sensitivity of the optimised $b$ parameter with regard to both the uncertainty in $\delta$ and the time window (TW) used for flux calculation. Different starting points were used within a small range of 5 s following the $\delta$ defined by the change-point detection algorithm, with subsequent duration 80, 100, 120, 140 and 160 s. Thus, fluxes were inferred from the regressed data points taken within measurement intervals determined by five points after $\delta$ and five TWs, for 25 permutations. To demonstrate using a single measurement, the CO$_2$ and water vapour progressions during a measurement on Hormathophilla sp. at 10:30 (solar time) were used to elucidate flux uncertainties using different starting points and TW.

Based on the initial analysis, an automated Bootstrapping Resampling Method (BRM, Hall and Martin 1988) was used as an automatized flux calculation procedure. This consists of generating a variety of 80 combinations by varying randomly both starting point (within 0–10 s) after $\delta$ and TW (from 81 to 160 s) for fitting the progression. From these cases, the mean is taken as the best estimate of the flux, and the standard deviation ($\sigma$) to represent uncertainty associated with the selection of the TW for regression.

Additionally and also based on the results of the initial analysis, we maximize the TW to reduce errors in fit parameters and assess flux uncertainties through a Bootstrapping Resampling Method with Noise (BRMN) resampling, with random noise added reiteratively to the data. This approach consists of:

1. Selecting the longest available TW (180 s - $\delta$);
2. Adding artificial, random noise to the data, with 0 mean and standard deviation corresponding to both the IRGA specifications and measurements in an empty chamber;
3. Calculating $\frac{\partial C}{\partial t}$ at the window start as regression parameter $b$ of Eq. (6); and
4. Repeating steps 2–3 500 times with resampling.

The best estimate of the flux and its uncertainty are inferred from the mean and standard deviation of the mean ($\sigma$) of the bootstrap dataset ($N$=500).

**Chamber disturbance of plant physiology**

When a plant is enclosed in a chamber, the humidity is expected to increase initially at a rate that is determined by stomatal conductance ($g_s$), which can be defined as follows (Jones 1998);

$$g_s = P / (D \times \rho_a) \times F_{tr}$$

(8)

where $\rho_a$ is the air density, $P$ the air pressure, $D$ is the
Plant-to-air vapour pressure deficit calculated as the difference between saturated (stomatal; \( e_s = 0.61078 \times \exp[(17.269 \times T_p)/(237.3+ T_p)] \)) and ambient (\( e_a = W \times P \)) partial pressures of water vapour, and \( T_p \) is the plant temperature. Combining Eqs. (6) and (8), and using \( \rho_a \) and \( D \) at any time (t) of measurement, the stomatal response to environmental modifications (temperature, humidity) was analysed over chamber deployments as:

\[
g_s = P \left/ \left( D \times \rho_a \right) \times \left( 2\alpha + b \right) \right. \tag{9}
\]

**Software**

The change-point detection method was reproduced in the change-point R package (Killick and Eckley 2010), and implemented in the BRM algorithm, which was developed using R’ software, and calculations and statistical analyses were computed using the Statistics Toolbox of RStudio Version 0.97.551-© 2009–2012 RStudio, Inc.

**Results**

Flux calculation and uncertainties analysis

Chamber flux estimates using common calculation protocols, evaluating goodness of fit, are subject to significant uncertainties. To understand the main source of uncertainties, we performed a test for one chamber campaign on 24 July 2012 (10:30) in *Hormathophylla* sp., where net photosynthesis (\( F_{ph} \)) and transpiration (\( F_{tr} \)) rates were repeatedly computed from CO2 and water vapour progressions, varying both the starting point and time window (TW; Table 1; progressions correspond to data in Fig. 2). The value of \( \delta \) determined by the change-point method resulted in 16 s, after which the subsequent 5 s and selected TW were considered for analysis. Results indicate that fluxes calculated using different starting points fluctuate markedly as the TW is reduced, although all quadratic regressions fit the data well. Note that all of the data in Table 1 were well represented by the quadratic fit \( R^2 > 0.92 \). For durations of 140 s or more, fluxes converged to relatively consistent values and uncertainty was dramatically reduced; the value of \( \sigma \) for shorter TW was several times larger, for both \( F_{ph} \) and \( F_{tr} \). These results show that both systematic errors, particularly those associated with the determination of \( \delta \), and random errors such as IRGA noise contribute to flux uncertainty, particularly for shorter TW. Thus, determining the most appropriate \( \delta \) and TW is essential for reducing random uncertainties when using quadratic methods.

The Bootstrapping Resampling Method (BRM) was used in an algorithm to automatically establish flux estimates and characterize their uncertainties due to noise over the accepted window. No significant differences were found for the means obtained with both approaches BRM and BRMN (with noise added). However as was expected, fluxes inferred from BRM showed lower spread than those with BRMN in almost all cases (Fig. 3). Overall, while BRM gave a less precise estimation of the flux, the means did not differ and thus the same results for BRM and BRMN can be obtained for a variety of environmental conditions throughout the day. The value of \( \delta \) determined by the change-point method ranged from 16 to 42 s for measurements of whole-plant net photosynthesis taken in *Genista* sp. and *Hormathophylla* sp. in Sierra de Gador (Almeria) throughout 24 July 2012. No relations were found between times required for mixing and environmental conditions within the chamber (e.g., temperature).

Precise measurements of net photosynthesis (\( F_{ph} \)) and transpiration (\( F_{tr} \)) using this whole-plant chamber and flux calculation procedure allow detailed evaluation of plant responses between species. Characteristic diurnal trends of \( F_{ph} \) (Fig. 4a) and \( F_{tr} \) (Fig. 4b) by the two dominant species of the Mediterranean shrubland (*Hormathophylla* sp., and *Genista* sp.) during a typical summer day (24 July 2012) exhibit different physiological strategies. Overall, \( F_{ph} \) showed an asymmetric pattern with a rapid increase after sunrise, a peak between 8:00 and 11:00 h, afternoon declines during high vapour pressure deficits (\( D > 4 \) kPa), and finally lower values around sunset with limited light. On the other hand, \( F_{tr} \) followed the diurnal trend in \( D \) (data not shown), with low values in the morning and high in the afternoon. The maximum \( F_{tr} \) was near 1.6 mmol H2O plant\(^{-1}\) s\(^{-1}\) in *Hormathophylla* sp. and lower (1 mmol H2O plant\(^{-1}\) s\(^{-1}\) ) in *Genista* sp. While the same environmental conditions were shared for all plants, stomatal constraint of \( F_{ph} \) and \( F_{tr} \) was stronger in *Genista* sp. than *Hormathophylla* sp. as is evident in the daily time courses presented in the Fig. 4.
The impact of chamber disturbances on plant gas exchange during the measurement depends largely on initial environmental conditions and plant physiological status, independent of the period of chamber closure. We examined changes in stomatal conductance ($g_s$, Eq. 9) during chamber deployments to observe if reductions in transpiration rates are physiologically controlled. State variables showed evidence of disturbance after chamber closure. In particular, canopy temperature ($T_c$) rose in the afternoon by up to 2.4 °C per minute, but less in the morning (~0.5 °C, data not shown). Overall, the rise in $T_c$ consistently increased the vapour pressure deficit ($D$), whereas chamber disturbances consistently reduced $F_{tr}$. This behaviour is explained by stomatal control of transpiration. However, this effect varied widely depending on physiological and environmental conditions. This is clear if we focus on the measurements taken at 8:00 (morning) versus those taken at 14:00 (afternoon) in the case of the *Hormathophylla* sp. (Fig. 5). For both measurements, both environmental ($\Delta T$ and $\Delta D$, Fig. 5a–f) and physiological ($\Delta g_s$ and $\Delta F_{tr}$, Fig. 5c–h) changes showed that the chamber impact was small early in the morning and stronger in the afternoon. At 8:00, $D$ appeared to be steady around 2.6 kPa with a slight $D$ increase of up to 0.4 kPa (Fig. 5b) and small changes were observed in both progressions of stomatal conductance ($g_s$ decreased 2 mm s$^{-1}$, Fig. 5c) and transpiration ($F_{tr}$ stayed stable around 0.8 mmol H$_2$O plant$^{-1}$ s$^{-1}$) over the measurement time (Fig. 5d). By contrast, at 14:00 with high $D$ increase by up to 1.6 kPa (Fig. 5f), strong decreases of $g_s$ were observed over the closure (from 8.5 to 3.8 mm s$^{-1}$) and consequently $F_{tr}$ decreased from 1.55 to 0.85 mmol H$_2$O plant$^{-1}$ s$^{-1}$ (Fig. 5h). These afternoon results indicate that plant physiology rapidly responds to chamber closure, and disturbances in $F_{tr}$ or $F_{ph}$ must be proportional to closure times as explained by Eq. (6). Thus, the impacts of disturbances that result in non-linear water vapour molar fraction progressions depended on initial environmental and physiological conditions.

**Table 1** Net photosynthesis ($F_{ph}$, μmol CO$_2$ plant$^{-1}$ s$^{-1}$, grey columns), and transpiration ($F_{tr}$, mmol H$_2$O plant$^{-1}$ s$^{-1}$, white columns) inferred from the quadratic regression to the CO$_2$ and water vapour molar fraction progressions, presented in Fig. 2

<table>
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<th>Time (s)</th>
<th>Windows</th>
<th>80s</th>
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<td>$F_{tr}$</td>
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</table>

Flux calculations were performed using windows after a defined $\delta$ with five different starting points (16–20 s) and durations (80, 100, 120, 140, and 160 s). The $\delta$ time in this measurement resulted in 16 s using the change-point method. Mean and standard deviation ($\sigma$) of fluxes calculated varying starting points with same durations are also presented. Data were taken during a measurement on 24 July 2012 (10:30 am.) in *Hormathophylla* sp.

Fig. 3 Molar CO$_2$ fluxes inferred from progressions using bootstrapping resampling-based method (BRM) and BRMN (noise added, $y=1.01x - 0.0004$, $R^2=0.99$). Bars represent standard deviation of the optimised parameters obtained from repeated flux calculations. Data correspond to the CO$_2$ mole fraction progressions taken in *Genista* sp. and *Hormathophylla* sp. in Sierra de Gador (Almería) on 24 July 2012.
Fig. 4 Day-time courses of canopy net photosynthesis (a) and transpiration (b) measured in *Genista* sp. (black circles), and *Hormatophylla* sp. (white circles) in Sierra de Gador (Almería) on 24 July 2012. Fluxes were estimated using the BRM and bars represent standard deviation of the optimised parameters obtained from repeated flux calculations.

Fig. 5 Changes in environmental and physiological variables following chamber closure in two typical measurements, morning (a–d) and afternoon (e–h) taken in a representative plant of *Hormatophylla* sp. in Sierra de Gador (Almería) on 24 July 2012. Relative changes to initial conditions of the chamber deployment in a, e air temperature, b, f plant-to-air vapour pressure deficit $D$, c, g stomatal conductance $g_s$, and d, h transpiration $F_{tr}$ are presented.
Systematic errors

Independent of $\delta$ and random error, water dilution was identified as the most important source of systematic errors. Regressions were performed on both the CO$_2$ water dilution-corrected (C, referred to dry air) and the CO$_2$ non water dilution-corrected (C', referred to wet air) mole fractions taken in Genista sp. and Hormathophylla sp. over a typical day in July 2012 to assess the error owing to water dilution effects. Results showed that the water dilution effect always made the slope of the CO$_2$ progression more negative, such that net photosynthesis was overestimated when no correction was applied (Fig. 6, $y=1.15x$, $R^2=0.95$, $n=13$).

When not correcting for dilution, CO$_2$ fluxes were overestimated by 5 % early in the morning and 28 % late in the afternoon.

Light is attenuated slightly by the chamber walls throughout the day, but this effect is most important for net photosynthesis during light-limited conditions. The chamber wall transmitted between 90 and 91 % of $F_{\text{PAR}}$ throughout a sunny day (Table 2). The lowest transmittance values correlate with the largest solar zenith angles of which the maximum value was 45°, due to the cubic morphology of the canopy chamber. Photosynthetic light response models made for Hormathophylla sp. and Genista sp. on 24 July 2012 were used to quantify the effect of this light attenuation by the chamber wall on net photosynthesis (Table 3). The model predicts that when light is attenuated by 10 %, net photosynthesis by Hormathophylla sp. is greatly reduced under non-light-saturating conditions (Fig. 7). Net photosynthesis can be reduced by 20 % at $F_{\text{PAR}} \approx 250$ $\mu$mol m$^{-2}$ s$^{-1}$ but only about 3 % at $F_{\text{PAR}}$ close to 1500 $\mu$mol m$^{-2}$ s$^{-1}$ and even less in the afternoon hours under light-saturating conditions. This pattern was similar but less pronounced for Genista sp. (5 % underestimation in the morning, negligible during afternoon; Fig. 7).

For the chamber examined here, leakage errors proved to be relatively unimportant. The CO$_2$ molar fraction (C, referred to dry air) decreased steadily after CO$_2$ injection into the empty chamber (Fig. 8) from an initial C value near 1000 $\mu$mol CO$_2$ mol$^{-1}$ (versus the surrounding atmospheric C$\approx 550$ $\mu$mol CO$_2$ mol$^{-1}$). Since the initial environmental parameters (temperature, pressure, gas mixture) were in equilibrium with the environment outside the chamber, the difference of 450 $\mu$mol CO$_2$ mol$^{-1}$ caused diffusional leakage of CO$_2$, depleting C from 1000 to 900 $\mu$mol CO$_2$ mol$^{-1}$ during the almost 20 min of monitoring. The leakage coefficients ($\Phi$) calculated with Eq. (3) for 120 s intervals ranged between $6.5 \times 10^{-5}$ and $1 \times 10^{-4}$ m$^2$ s$^{-1}$. According to Eq. (4) and considering the 1 m$^3$ headspace and the relatively small collar contact length (4 m) and measurement time (3 min), the relative error resulted in just 2 % of the flux.

Discussion

Our results highlight the uncertainties associated with current flux calculation procedures, as well as the need to determine random errors and improve flux precision. Two numerical approaches based on the change-point method, and a bootstrapping resampling-based method (BRM) provide the statistical means to examine the spread of flux estimates and characterize some of the uncertainties as a function of $\delta$, the data time window (TW) used for flux calculation, and inherent system noise. Regarding systematic errors, we identify two important sources of error: i) the water-dilution effect and ii) attenuation of the photosynthetically active radiation by the chamber wall. In addition to photosynthetic uptake of CO$_2$, the CO$_2$ mole fraction progression is forced by water dilution (28 %). The water dilution correction is particularly relevant when using IRGAs that do not provide mole dry fraction. When net photosynthesis is measured under non-light-saturating conditions, modest light attenuation by the chamber (10 %)
can lead to greater reduction of the net photosynthesis. The light attenuation effect by chamber walls on net photosynthesis is a general issue concerning any chamber set-up but solved when using net photosynthetic light response curves to extrapolate net photosynthesis to undisturbed light conditions. For the chamber examined here, leakage represents a minor error (2 %) due to the large chamber volume.

Once $\delta$ is properly determined using the change-point detection method, uncertainty in the flux estimates is reduced when using longer chamber closure times, and undisturbed fluxes can be inferred even though environmental and physiological variables are modified during measurements. We found that chamber flux measurements may be subject to important uncertainties since the data immediately following $\delta$ are high-leverage points, and thus the slope at time 0 inferred from quadratic regression is highly sensitive to noise, even with acceptable goodness of fit ($R^2>0.9$). Changes in physiology during chamber closure do not preclude the estimation of undisturbed fluxes when non-linear regressions are used. Disturbance effects on $F_{tr}$ and $F_{Ph}$ are assumed to be proportional to closure time, as explained by Eq. 6, depending on physiological and environmental conditions as shown in Fig. 5. The same assumption was made by Pérez-Priego et al. (2010), who found proportionality between chamber closure time and the reduction in sap velocity. Errors due to water vapour condensation on the chambers walls were either absent or too small to be detectable in such large chambers. In such dry environments, this is not surprising as condensation is expected to be a function mainly of the leaf area and head space volume and the chamber closure time, thus it must be low when plant volume represents less than 0.2 % of the head space volume of the chamber and relatively short closures are conducted (3 min). However, this artefact of error must be considered in humid environments. Chamber closure times of less than 90 s are common practice (Reicosky et al. 1990; Reicosky 1990; Pickering et al. 1993; Grau 1995; Wagner et al. 1997; McLeod et al. 2004), following earlier recommendations favouring short deployments (Wagner and Reicosky 1992; Wagner et al. 1997). By contrast, as long as no unwanted biological disturbances occur, we recommend prolonging measurements (to 3 min) to improve confidence in regression parameters and the use of BRM method to assess random errors. Prolonged periods are essential particularly when measuring low fluxes and signal-to-IRGA noise may bias flux estimation.

The magnitude of water dilution error for these measurements achieved values up to 28 % (Fig. 6). Adjustment for the displacement of CO₂ by water vapour, which generally reduces the CO₂ density, is a commonly corrected for in the eddy covariance community (Webb et al. 1980). We note that IRGAs that incorporate a water vapour channel, with measurements of cell temperature and pressure computed internally for dry CO₂ molar fraction (e.g., Licor 7000, 7200, 6400), require no additional density corrections. However, although Li-840/840A measures water vapour pressure and cell temperature and pressure, it does not provide dry mole fractions, and thus water dilution is relevant. By contrast, the change in density due to concurrent evaporative flux must be quantified with independent sensors, e.g., via measurements of relative humidity and temperature, when using an IRGA with no water vapour channel (e.g., Li-820). Finally, when scaling by air density, that of dry air must be specified; the term $P-e_{av}$ rather than $P$, must be included in Eq. (7).

### Table 2

<table>
<thead>
<tr>
<th>Solar time (hour:minute)</th>
<th>Zenith angle (°)</th>
<th>Photon flux density Plastic covered ($\mu$mol m⁻² s⁻¹)</th>
<th>Photon flux density Non-covered ($\mu$mol m⁻² s⁻¹)</th>
<th>Transmittance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12:15</td>
<td>13.8</td>
<td>1838±4</td>
<td>2013±5</td>
<td>91.321±0.004</td>
</tr>
<tr>
<td>14:18</td>
<td>30.3</td>
<td>1661±3</td>
<td>1820±3</td>
<td>91.265±0.004</td>
</tr>
<tr>
<td>15:38</td>
<td>45.5</td>
<td>1318±4</td>
<td>1459±5</td>
<td>90.315±0.006</td>
</tr>
</tbody>
</table>

### Table 3

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Hormatophylla sp.</th>
<th>Genista sp.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Estimate</td>
<td>Std. error</td>
</tr>
<tr>
<td>$F_{Ph,max}$</td>
<td>4.4</td>
<td>1.0</td>
</tr>
<tr>
<td>$K_m$</td>
<td>433.4</td>
<td>370.8</td>
</tr>
<tr>
<td>$R_d$</td>
<td>−1.0</td>
<td>0.3</td>
</tr>
</tbody>
</table>
Another important matter to consider in the use of all existing chambers is the suitability of the translucent sheet. Rather than gas-permeability, one of the most important factors influencing the selection of the cover material is light transmittance. In this context, a wide variety of materials are used to build canopy chambers, such as acrylic glass (Langensiepen et al. 2012), transparent polycarbonate (Steduto et al. 2002), transparent low-density polyethylene (Corelli-Grappadelli and Magnanini 1993), fluorinated ethylene propylene (Breuninger et al. 2012) or biaxially oriented polypropylene (Pérez-Peña Tarara 2004). The transmittance of these materials may range between 80 and 97 %. The transparent sheet used to build our chamber, Llumar®, is impermeable to CO$_2$ and water vapour, has low water vapour adsorption (Pérez-Priego et al. 2010) and 90 % $F_{PR}$ transmittance (see Appendix), and is resistant and light enough for field use. Regarding geometrical characteristics, this cubic chamber has a height of 1 m, and enclosed plants are around 0.5–0.8 m tall. For solar zenith angles lower than 45°, non-diffuse radiation passes through the top of the chamber; otherwise it passes through a lateral wall. In the latter situation the relevant angle is the solar elevation angle, which is the complement of the solar zenith angle. Accordingly, spherical chambers appear to be the most correct in...
terms of transmittance; however, this geometry presents obvious practical disadvantages regarding chamber construction. Generally, light attenuation is an important concern at times when net photosynthesis is not light-saturated.

Rather than the airtight design of the chamber, the chamber headspace geometry determined the low magnitude of the error due to CO$_2$ leakage flux. Therefore, this value is negligible when relatively short time periods are required to obtain a measurement (e.g., 3 min). According to Eq. (4), this error is inversely proportional to the chamber headspace volume. Therefore, leakages should be accounted for in measurements taken with typical clamp-on leaf cuvettes (Flexas et al. 2007), whose volume can be up to four orders of magnitude smaller than our chamber. Otherwise, flux estimates and subsequently photosynthetic model parameters are susceptible to large errors (up to 40 % overestimation of daytime respiration; Flexas et al. 2007; Rodeghiero et al. 2007). By contrast, insignificant leakage errors have been found for large canopy chambers. Held et al. (1990) and Steduto et al. (2002) estimated a leak flow error of 4 % min$^{-1}$. Likewise, Grau (1995), with an open-bottom chamber inserted on a bed of sterilised sand, evaluated an under-estimation error of 5 %. However, although tricky to evaluate, under field conditions, pressure waves prompted by wind gusts around the chamber walls may intensify leaks by advection processes. This process induces abrupt changes in CO$_2$ and water vapour concentration inside the chamber and can be discernible with peaks and chaotic trends in the progressions.

Conclusions

An overview of the importance of each error type examined here as shaped by different environmental conditions and chambers configurations is presented (Fig. 9). As a major issue concerning all chamber configurations, random errors associated with the selection of the calculation time window (TW) together with (instrument or natural) noise lead to important flux uncertainties, and hence statistical analyses such as

![Fig. 9](https://example.com/fig9.png)

**Fig. 9** A summary of the magnitude of relative flux errors in the calculation of net photosynthesis for the set-up used here and estimated as a random errors (%), resulting of the error of the optimized parameter of the polynomial fit for 20 selected time windows between 80 and 160 s relative to the net photosynthesis estimate using BRM method ($F_{BRM}$) as follows Error (%) = $sd/F_{BRM}$, b dilution error as affected by the increase of water vapour within the chamber and shaped by transpiration rates, c leakage error, computed considering different volumes of the chamber with the leaks coefficient obtained here, and d the effect of attenuation on photosynthesis measured in the *Hormatophylla* sp. within the chamber if we extrapolate the results to outside conditions using light-response curve.
those presented here are required (Fig. 9a). Likewise, water dilution represents a relevant error when using IRGAs that do not provide dry molar fractions (Fig. 9b). In such cases, density corrections must be applied explicitly in the calculation of the whole-plant net photosynthesis. For the set-up used here, errors due to leakages were a minor error but can compromise fluxes when using chamber volumes lower than 0.2 m$^3$ (Fig. 9c). Net photosynthesis was inhibited by the effect of light attenuation by the chamber walls, particularly in light-limiting conditions (Fig. 9d). The photosynthetic light response curve is an adequate means to correct for light attenuation when comparing/extrapolating results to specific environmental conditions.

Prolonged closure times reduce uncertainties in fitting parameters. Nevertheless, flux values inferred from molar fraction progressions may fluctuate due to random errors even when regression fits have acceptable goodness of the fit. Chamber disturbances prompted by longer enclosures do not preclude undisturbed flux estimations. Correcting for systematic errors ($\delta$, water-dilution, light attenuation and leakage) and characterizing random errors, whole-plant gas exchanges can be properly determined using a portable closed dynamic chamber. This chamber design, which excludes soil fluxes, is suitable for characterizing water use and carbon uptake by plants and studying ecological functioning of species within ecosystems.

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Compliance with ethical standards

Conflict of interest All authors state that there are no conflicts of interest.

Human and animal rights and informed consent In this work no research involving human participants or animals were conducted.

Appendix

Fig. 10

Fig. 10 Spectral transmission of the translucent film “NRS90 clear” measured with a Li-Cor 1800-12 integrating sphere (Li-Cor, Lincoln, NE, USA) coupled to a fiber optic spectrometer (Ocean Optics model USB2000 spectrometer, Ocean Optics, Dunedin, FL, USA)

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