Title:

DYNAMISM: a low-cost automatic system for measurements of gas exchange at canopy scale in dynamic conditions

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Abstract

Background
Obtaining instantaneous gas exchanges data is fundamental to gain information on photosynthesis. Leaf level data are reliable, but their scaling up to canopy scale is difficult as they are acquired in standard and/or controlled conditions, while natural environments are extremely dynamic. Responses to dynamic environmental conditions need to be considered, as measurements at steady state and their related models may overestimate total carbon (C) plant uptake.

Results

In this paper, we describe an automatic, low-cost measuring system composed of 12 open chambers (60 x 60 x 150 cm; around 400 euros per chamber) able to measure instantaneous CO$_2$ and H$_2$O gas exchanges, as well as environmental parameters, at canopy level. We tested the system’s performance by simulating different CO$_2$ uptake and respiration levels using a tube filled with soda lime or pure CO$_2$, respectively, and quantified its response time and measurement accuracy. We have been also able to evaluate the delayed response due to the dimension of the chambers, proposing a method to correct the data by taking into account the response time ($t_0$) and the residence time ($\tau$). Finally, we tested the system by growing a commercial soybean variety in fluctuating and non-fluctuating light, showing the system to be fast enough to capture fast dynamic conditions. At the end of the experiment, we compared cumulative fluxes with total plant dry biomass.

Conclusions

The system slightly over-estimated (+7.6%) the total C uptake, even though not significantly, confirming its ability in measuring the overall CO$_2$ fluxes at canopy scale. Furthermore, the system resulted to be accurate and stable, allowing to estimate the response time and to determine steady state fluxes from unsteady state measured values. Thanks to the flexibility in the software and to the dimensions of the chambers, the system can be used for several applications and with different plant canopies by mimicking different (i.e. dynamic and static) environmental conditions.

Keywords

Growth chamber, canopy, low-cost, fluctuating light, dynamic photosynthesis
Background

Despite being the most important biological process on Earth, photosynthesis still presents mechanisms that are not deeply understood and it is considered a matter of priority interest for new pioneering research fields (Bahadur et al., 2015; Tanaka and Makino, 2009). By converting solar energy into chemical energy, plants accumulate biomass by which several human activities depend on (Hall, 2013; Vitousek et al., 1986). Due to the rise in food demands (Alexandratos and Bruinsma, 2012; South et al., 2019) and, more in general, in plant-derived products, the newest research is aiming to target those processes in photosynthesis that would improve the overall crop yield (Kromdijk et al., 2016; Long et al., 2006; Ort et al., 2015). This can be achieved in laboratories and tested in green houses, but translating these results into the field is highly demanding (Kaiser et al., 2018b; Matsubara, 2018). In fact, in natural environments, plants are affected simultaneously by several abiotic conditions and biological interactions, which could translate into high uncertainties (Annunziata et al., 2017; Köhl and Laitinen, 2015).

To facilitate the translation of information from the laboratory to the field, it is also necessary to mimic natural environmental conditions within growth chambers (Kaiser et al., 2018b). For example, simulating dynamic light conditions is necessary to retrieve canopy scale data that would reflect environmental variability. In fact, whereas most of the past experiments and models considered photosynthesis at the steady state (Farquhar and von Caemmerer, 1980; Farquhar et al., 2001; Kannan et al., 2019; Song and Zhu, 2012; Van Der Tol et al., 2009; Ye et al., 2013), the importance of considering some photosynthetic processes in their transient states has been recognized (Chazdon and Pearcy, 1986; Kaiser et al., 2018a; Morales et al., 2018; Taylor and Long, 2017; Tomimatsu and Tang, 2016). Plants are usually exposed to fluctuating irradiance due to the movements of clouds, the effect of wind and the gaps within the canopy (Pearcy, 1990; Retkute et al., 2015). How plants respond to these dynamic conditions affects carbon dioxide (CO₂) uptake and final biomass yield. Plants can adjust to the dynamic environmental conditions by regulating the stomata (Buckley, 2017; Matthews et al., 2018; Raines et al., 2017), by moving their chloroplasts within the leaves or by moving their leaves within the canopy (Kaiser et al., 2014), by regulating the photochemistry (Kaiser et al., 2018a), by activating the Calvin Cycle enzymes and by controlling photo-protective processes (Slattery et al., 2017).
Clearly, there is the need for adopting measuring systems able to take into account light dynamics and to obtain reliable and repeatable measurements at canopy scale. Therefore, an accurate determination of canopy photosynthesis is necessary to evaluate the observations and to test the results obtained in the laboratories.

Nowadays, plant net primary production (NPP) can be measured destructively by harvesting the whole plant and weighting its components (i.e. leaves, stem, roots) or can be assessed by measuring instantaneous CO$_2$ exchanges (Hall, 2013). Following this last approach, measurements are generally rapid and can be taken from leaf to canopy scale either in the laboratories or in the field.

Gas exchange methods at leaf level are usually based on a leaf cuvette connected to an Infrared Gas Analyser (IRGA) measuring the difference among external and internal CO$_2$ concentration (closed systems) or between the inlet and the outlet air (open systems). These methods allow the estimation of several physiological parameters such as, for example, net photosynthesis and stomatal conductance (Kölling et al., 2015; Millan-Almaraz et al., 2013). When gas exchange measurements are combined with chlorophyll fluorescence, several other parameters related to photochemistry and the primary reactions of photosynthesis (i.e. light-harvesting and energy dissipation) can be retrieved (Baker, 2008; Maxwell and Johnson, 2000). Leaf level data are reliable and repeatable, but these data can be hardly scaled up at whole plant or whole canopy scale.

Canopy gas exchange measurements can be based on the use of micro-meteorological techniques or of growth chambers (Baldocchi, 2003; Matese et al., 2008; Wang et al., 2018). Open-field micro-meteorological techniques, such as eddy covariance, are appealing, but can suffer of three main weaknesses: i) difficulties in separating the target vegetation/canopy from the neighbor vegetation and different microclimates; ii) these methods do not provide a direct measure of the canopy CO$_2$ exchange per se (gross or net primary production), but rather of the whole community (i.e. net ecosystem production, NEP) (Flexas et al., 2011; Hall, 2013; Juszczak et al., 2012); iii) these methods usually need for particular environmental conditions in order to obtain reliable measurements (i.e. flat terrain, large footprint areas, atmospheric stability) (Acosta et al., 2017).

Growth chamber systems allow direct CO$_2$ gas exchange measurements at plant or small canopy scales. In open chambers, net carbon (C) exchange is estimated by measuring the inlet flux and the difference between inlet and outlet CO$_2$ concentrations; in the closed chambers, the change with time in CO$_2$ concentration within the chamber headspace is measured and the assimilation rate is then calculated (Hall, 2013; Wheeler, 1992). While open chambers can measure gas exchange for long time periods, closed chambers can be used only for short
time periods in order to avoid increase in air temperature or water condensation (Luo et al., 2018). Several growth chamber systems have been described in the literature (Andriolo et al., 1996; Hall, 2013; Mitchell, 1992), but some of them showed low ability to control environmental conditions (Miller et al., 1996; Smith et al., 2019), are not adapted to long-term continuous measurements (Andriolo et al., 1996) or are rather expensive (see Zabel et al., 2016 for a comprehensive review of space growth chambers).

Besides of the growth chamber systems, other systems have been developed in the last decades such as phytotrones (Kingsland, 2009) and the ‘exotic’ Biosphere 2 Laboratory (Rascher et al., 2004), with the idea of allowing a complete control of the environmental variables (Kingsland, 2009) and the scaling up of the measured values from the laboratory to model ecosystems (Nichol et al., 2012). Nevertheless, even if relevant tests have been performed, the conditions found within these systems are so dissimilar to natural conditions that it is, again, difficult to relate these results to field data (Köhl and Laitinen, 2015).

More recently, the remote sensing of solar-induced chlorophyll fluorescence (SIF), i.e. the passive retrieval of fluorescence deriving from the natural absorption of light by the vegetation, has been proposed as a method able to capture fundamental photosynthetic information (as photosynthetic light responses and net C exchange) at different spatial and temporal scales, and at high frequencies (Mohammed et al., 2019). Nevertheless, even if this method has given interesting results (Gu et al., 2019; Porcar-Castell et al., 2014), the physiological interpretation of SIF implies significant challenges; in particular, an accurate estimate of surface irradiance would be required (i.e. a 3D parameterization of the canopy structure) since the heterogeneous illumination within the canopy complicate the estimate of the actual surface irradiance, potentially affecting SIF accuracy (Pinto et al., 2016). Furthermore, nowadays it is still necessary to calculate C uptake at canopy level to validate this method (Mohammed et al., 2019).

Aware of the lack in systems able to retrieve relevant and repeatable data on canopy photosynthesis in conditions similar to the natural’s, in this study we describe a novel automatic, low-cost system based on 12 open chambers able to measure instantaneous CO$_2$ and H$_2$O gas exchange and environmental conditions (i.e. light and temperature) at canopy level. The system is flexible and allows to mimic different light conditions, either static or dynamic, as well as different CO$_2$ concentration levels if connected to a proper CO$_2$ source allowing a good characterization of canopy photosynthesis comparable to field data. To our knowledge, few other growth chamber systems have this ability to mimic natural environmental conditions and have been described
systematically including prices of the components, allowing a user-friendly reproduction of the system (Padmanabha and Streif, 2019).

DYNAMISM (DYNAMIC photoSynthesis Measurements) – a new low-cost and scalable whole plant gas exchange system

Description of the system

DYNAMISM is a low-cost open system composed of twelve 0.54 m$^3$ commercial growth chambers (60 x 60 x 150 cm; Secret Jardin, model Dark Dryer). The inlet ambient air is sucked into each chamber by a Blauberg inline mixed flow fan (diameter: 10 cm; flowrate: 102 m$^3$ h$^{-1}$) from a 4.5 m$^3$ buffer chamber (150 x 150 x 200 cm; Secret Jardin, model Dark Street DS150). The buffer is needed to keep inlet CO$_2$ and H$_2$O concentrations as stable as possible during measurements and to control air temperature and humidity inside the growth chambers using an air conditioner. The inlet flow rate is measured at each chamber using a miniature air flow transmitter (E+E Elektronik, model EE671) placed before the inline fan and can be easily regulated by opening/closing the holes at the top and at the side of the chamber. The overpressure created inside each chamber by the flow fan avoids possible CO$_2$ leakage or contamination during the measurements. Each air flow transmitter was calibrated against a reference mass flow meter before setting up the system (E+E Elektronik, model EE776; Additional file 1: Figure S1).

Air temperature inside each chamber is measured using a thermistor (Measurement Specialties, Inc., model 10K3A1 Series 1) placed above the LEDs, while inlet air pressure is measured at the inlet of the main pipeline using an integrated pressure sensor (Freescale Semiconductor, Inc., model MPX4115A). A schematic representation of the system is reported in Figure 1: the main pipeline starting from the buffer is made up of pipes with a diameter of 20 cm; chamber connecting pipes are 10 cm in diameter; the pipes connecting the buffer to outside the lab (outdoor) are 30 cm in diameter.

Instantaneous net canopy CO$_2$ flux (A; µmol CO$_2$ m$^{-2}$ s$^{-1}$) and instantaneous evapotranspiration (E; mol H$_2$O m$^{-2}$ s$^{-1}$) are measured as differences in CO$_2$ (µmol CO$_2$ mol$^{-1}$) and H$_2$O (mmol H$_2$O mol$^{-1}$), respectively, in the air stream flowing through each chamber using a LI-7000 gas analyzer (Licor, USA) in differential mode. Inlet CO$_2$ and H$_2$O concentrations (i.e. concentration inside the buffer) are measured by pumping the air through a
LI-840 gas analyzer and then to LI-7000 Cell A (reference). The outlet CO$_2$ and H$_2$O concentrations (i.e. concentration at the top of each chamber) are measured by pumping the air to the LI-7000 Cell B (sample) using an aquarium pump placed inside each chamber (Hailea, model ACO9602; flow rate: 7.2 l min$^{-1}$). Reference and sample CO$_2$ and H$_2$O concentrations, air temperature and air pressure are recorded by a datalogger (CR1000X, Campbell Scientific, USA) by parsing the digital output of the LI-7000.

The sequential sampling of air inside the chambers is electronically controlled by the CR1000X through a 16 channel AC/DC controller (SDM CD16-AC, Campbell Scientific, USA), which stimulates 24V solenoid valves connected to the aquarium pumps placed inside each chamber. Sampling frequency among the chambers, as well as sampling duration for each chamber, can be set by the user. A thirteen valve was connected to the main inlet within the buffer chamber allowing a periodic matching between Cell A and Cell B of the LI-7000. Such a matching is recommended in order to compensate for any differences in the two optical paths besides concentration differences. Outlet CO$_2$ and H$_2$O concentrations are thus corrected in post-processing and fluxes recomputed.

Each chamber is equipped with a 60 x 60 cm light system made up of 17 separate LED strips (Samsung SMD5630 “H-POWER”, 185W, 140 LED m$^{-1}$, CRI90, Natural White, 4000K). The light spectrum of the LEDs was measured using a fluorescence box (FloX, JB Hyperspectral Devices, Germany), and it well simulates the solar spectrum between 400 and 700 nm (Figure 2). LEDs can be moved up and down inside the chambers depending on canopy height, and light intensity within each chamber is independently controlled by the CR1000X through a Modbus to voltage output converter (4E+ Embedded Solutions, model DAT3028). The dimmer regulates the voltage signal (0-10 V) which determines the photosynthetic photon flux density (maximum PPFD = 1876 μmol m$^{-2}$ s$^{-1}$ at 10 cm distance when the number of LED strips per chamber is maximized).

The CR1000X can simulate daily solar radiation profile after the user sets the latitude and the longitude by computing solar elevation angle and knowing maximum PPFD or can simulate a fixed daily profile after the user chooses a fixed day of the year. Moreover, the user can simulate periodic light fluctuations around the hourly value by deciding the fluctuating range and the fluctuation period.
Transmitted radiation is measured using solar bars placed horizontally at the bottom of the canopy. Each bar is made of eight photodiodes in parallel (model S1087-01, Hamamatsu Photonics, Japan) with a 100 Ω resistance and was calibrated against a reference quantum sensor (Li-190R, Licor, USA) before setting up the system.

Finally, in Table 1 we report the list of all the major parts of the system, their technical specification and prices. The overall system cost is 5.000 euro (only 417 euro per chamber), without considering the reference sensors for calibrations, and the analyzers (LI-840 and LI-7000). One of the strengths of DYNAMISM is that any number of chambers is possible in the multiplexer mode, thus allowing to have a high number of replicates with a limited cost; nevertheless, if only a multiplexer is used, it will go in a repeated cycle.

| Sensor                  | Model and Manufacturer          | Technical Specification                      | Prices    | Sources                                                                 |
|-------------------------|---------------------------------|-----------------------------------------------|-----------|-------------------------------------------------------------------------|
| Growth chamber          | Secret jardin – Dark street     | 60x60x150 cm (12 small chambers) 150x150x200 (buffer chamber) | 84.4 €*12 | https://www.idroponica.it/growbox-c-22/secret-jardin-s-311/dark-street-ds-secret-jardin-36855.html |
| LED                     | Samsung LED strip 5630 “H-POWER”| 185 W; 140 LED/m SMD5630; Natural white: 4000K 5-meter length | 106.4 € *12 | https://store.ledpro.it/prodotti-led/strisce-led/striscia-led-5630-h-power-5-metri-185w-140-led-m-smd5630-samsung-bianco-naturale-4000k.html |
| Flowmeter for air flux  | EE671 - Miniature Air Flow transmitter – E+E electronica | Measuring range: 0-5 m/s; 0-10 m/s; 0-20 m/s; Response time: 4s | 177.1 € *12 | https://eu-shop.epluse.com/collections/air-velocity/products/355065 |
| Flowmeter for scrubbing | SFM4100 - Sensirion             | Digital gas flow meter for gases              | 187 €     | https://www.sensirion.com/en/flow-sensors/mass-flow-meters-for-high-precise-measurement-of-gases/mass-flow-meter-for-medical-gas-measurements/ |
| Ventilator              | Tube In-line fans – Blauberg ventilatoren | Diameter: 10cm Energy Supply: 220V AC. Maximum air flow:102 m³/h. | 17.1 € *12 | https://www.idroponica.it/cavo-alimentazione-200cm-con-spina-schuko~1146.html |
| Air pump                | Hailea ACO9602                  | Pump speed: 7.2L/min                         | 14.7 € *12 | https://www.amazon.it/Pompa-dAria-Regolabile-Hailea-ACO9602/dp/B01GO80XE4 (not available in idroponica at the moment) |
| Pressure sensor         | MPX4115 - freescale semiconductor | Integrated Silicon Pressure Sensor           | 20 €      | https://www.nxp.com/docs/en/datasheet/MPX4115.pdf                      |

Table 1. Description and technical specifications of all the system’s components. Prices and companies (webpages) are also listed. All prices are indicated excluding VAT.
Gas exchange calculations

E and A are computed according to the following equations:

\[
E = \text{air}_{\text{flow}} \times \frac{H_2O_{\text{chamber}} - H_2O_{\text{in}}}{S \times (1000 - H_2O_{\text{out}})} \quad \text{Equation 1}
\]

\[
A = \text{air}_{\text{flow}} \times \frac{CO_2_{\text{chamber}} - CO_2_{\text{in}}}{S} - CO_2_{\text{out}} \times E \quad \text{Equation 2}
\]

where \(H_2O_{\text{in}}\) and \(CO_2_{\text{in}}\) are the \(H_2O\) and \(CO_2\) concentrations within the buffer chamber (inlet) and \(H_2O_{\text{chamber}}\) and \(CO_2_{\text{chamber}}\) are the concentrations in each chamber; \(\text{air}_{\text{flow}}\) is the air flux entering the chamber (mol s\(^{-1}\)) and \(S\) is the chamber area (0.36 m\(^2\)). We adopted the micro-meteorological convention to indicate \(CO_2\) uptake (net photosynthesis, negative value) and release (respiration, positive value) Air flow from the miniature air flow transmitter is converted from m s\(^{-1}\) (flow) to mol s\(^{-1}\) (air flow) according to the equation:

\[
\text{air}_{\text{flow}} = \frac{\text{flow} \times S_{\text{tube}} \times P}{R \times (T_{\text{chamber}} + 273.15)} \quad \text{Equation 3}
\]

where \(S_{\text{tube}}\) is the tube area (0.10 x 0.10 m\(^2\)), \(P\) is the inlet air pressure (Pa) and \(T_{\text{chamber}}\) is the air temperature inside the chamber (°C) and \(R\) is the universal constant of gases (8.3144998 m\(^3\) Pa K\(^{-1}\) mol\(^{-1}\)).

Performance and accuracy of DYNAMISM

Before testing the system with a real plant canopy, we simulated six different photosynthesis levels \((A_{\text{sim}}; \mu\text{mol CO}_2 \text{ m}^2 \text{ s}^{-1})\) at five different air flux velocities (from 0.73 to 2.73 m s\(^{-1}\)) in order to assess its performance and accuracy. We did this by using a tube filled with soda lime connected to a pump (Hailea ACO9602) and placed inside one of the chambers, according to the equation:

\[
A_{\text{sim}} = 1000 \times \frac{\text{scrub}_{\text{flux}} \times P}{R \times (T_{\text{chamber}} + 273.15)} \quad \text{Equation 4}
\]
where scrub\(_{\text{flux}}\) is the scrub’s pump speed (1 s\(^{-1}\)) measured using a flowmeter (Sensirion SFM4100), P is the air pressure (constant at 101300 Pa), T is air temperature (°C) and R is the universal constant of gases (8.3144598 m\(^3\) Pa K\(^{-1}\) mol\(^{-1}\)). The pump was turned on for 10 minutes at a first level of scrub’s pump speed (0.021 s\(^{-1}\)), then the scrub’s pump speed was increased at the second target velocity for another 10 minutes, and so on for all the six levels of simulated photosynthesis. When the maximum level of scrub flux (pump speed =0.21 s\(^{-1}\)) was reached, the same procedure was applied from the highest value to the lowest. Final measured net CO\(_2\) fluxes were calculated from the system’s acquired data for the last 60 seconds of each step according to equation 2.

When comparing all the five air flux velocities, we expect that the steady state is reached faster at higher fluxes without affecting the steady state itself. In order to compare measured values at different speed of the scrub pump, we normalized the data by multiplying the \(\Delta CO_2\) values for \(S_x/(S_0 - S_x)\) where \(S_x\) is the CO\(_2\) scrubbed flux and \(S_0\) is the CO\(_2\) flux at time 0; then we further rescaled the data through a min-max normalization. As expected, the results of these tests clearly show that higher the air flux, faster the steady state is reached (Additional file 1: Figure S2).

We also simulated five respiration levels (\(R_{\text{sim}}\)) by injecting pure CO\(_2\) inside the chambers at two different air flux velocities (0.71 and 1.71 m s\(^{-1}\)), following the same procedure (steps) described above for photosynthesis, using a gas mass flow controller for low flow rates (Bronkhorst, model F-201CV-100_RAD-00-Z). \(R_{\text{sim}}\) was computed according to the following equation:

\[
R_{\text{sim}} = \frac{CO_2^{\text{injected}} \times P}{S \times (T_{\text{chamber}} + 273.15) \times R}
\]

\text{Equation 5}

where \(CO_2^{\text{injected}}\) is the CO\(_2\) injected flux (ml CO\(_2\) s\(^{-1}\)), P is air pressure (101300 Pa), S is chamber area (0.36 m\(^2\)), \(T_{\text{chamber}}\) is chamber temperature (°C) and R is the universal constant of gases (8.3144598 m\(^3\) Pa K\(^{-1}\) mol\(^{-1}\)).

The accuracy of DYNAMISM was finally assessed by comparing measured values with simulated ones (Equation 4 and 5) using a simple linear regression. The system slightly overestimated CO\(_2\) fluxes (+7%) over the range from -10 to 10 μmol CO\(_2\) m\(^2\) s\(^{-1}\) (slope: 1.06; intercept: -0.85; \(R^2=0.98\); p<0.001; Figure 3).
Performance evaluation through a mass balance model

To further assess DYNAMISM accuracy, we performed a comparison among the measured $\Delta CO_2$ values ($CO_{2\text{in}} - CO_{2\text{chamber}}$), obtained after scrubbing the air or injecting pure CO$_2$, with a physical model based on a mass balance approach: the change in CO$_2$ concentration with time inside the chamber depends on the CO$_2$ entering the chamber from the buffer ($CO_{2\text{in}}$ in ppm) at a certain flux (F in mol s$^{-1}$) minus the CO$_2$ consumed (or released) by the photosynthesis (or respiration) ($Sx$ in $\mu$mol CO$_2$ s$^{-1}$) and the internal concentration within the chamber ($CO_{2\text{chamber}}$ in ppm). Therefore, the physical model can be described by the following equation:

$$\frac{dCO_{2\text{chamber}}}{dt} = \frac{FCO_{2\text{in}} - Sx}{V} - \frac{FCO_{2\text{chamber}}}{V}$$

Equation 6

By integrating this differential equation and by assuming perfect mixing within the chamber, the following equation is obtained:

$$\Delta CO_2 = \frac{1}{F} \cdot \left( Sx + (S_0 - Sx) \cdot e^{-\frac{F}{V}(t-t_0)} \right)$$

Equation 7

where $S_0$ is the CO$_2$ flux at time 0, V is the chamber volume (19.3 mol) and $t_0$ is the delay due to chamber dimension (in seconds).

Solving equation 7 for $Sx$ results in:

$$Sx = \frac{\Delta CO_2 F}{1 - e^{-\frac{F}{V}(t-t_0)}} - \frac{S_0 e^{-\frac{F}{V}(t-t_0)}}{1 - e^{-\frac{F}{V}(t-t_0)}}$$

Equation 8

In order to understand the error associated to $Sx$ measurements due to errors in the measured variables ($F$, $S_0$, $t_0$ and $\Delta CO_2$), we made a sensitivity analysis. The total error was then computed according to Jordan and Sewell (1975) by considering the partial derivatives of $Sx$ per each measured variable:
\[
T = \sqrt{\left(\frac{\partial S_x}{\partial F}\overline{F}\right)^2 + \left(\frac{\partial S_x}{\partial S_0}\overline{S_0}\right)^2 + \left(\frac{\partial S_x}{\partial \Delta CO_2}\Delta CO_2\right)^2 + \left(\frac{\partial S_x}{\partial t_0}\overline{t_0}\right)^2}
\]

Equation 9

where \(\overline{F}, \overline{S_0}, \Delta CO_2\), and \(\overline{t_0}\) indicate the range in parameters for which the partial derivative is computed being

\(\overline{F} = [0.2 - 0.6] \text{ mol s}^{-1}, \overline{S_0} = [0 - 5] \text{ \mu mol CO}_2 \text{ s}^{-1}, \overline{t_0} = [0 - 100] \text{ s}, \Delta CO_2 = [0 - 10] \text{ ppm.}\)

In Figure 4, we reported the total error (T) and the errors related to F and \(\Delta CO_2\) only, as those due to \(S_0\) and \(t_0\) were smaller than 1% and thus negligible. According to our sensitivity analysis, the major source of error in the measurements of CO2 fluxes with DYNAMISM is related to F, especially at the highest air flux velocity (0.6 mol s\(^{-1}\)), underlying the need to use an accurate flowmeter to assess it.

**Correct for delays**

The model described in equations 7 and 8 allows to mathematically compute the delay of the measuring system \((t_0)\) due to the lengths of the tubes and the volume of the chamber, and the residence time \(\tau\), which applies in case of no perfect mixing. In fact, in such last case, equations 7 and 8 need to be changed by adding \(\tau\) to the exponent value, which reads as \(\tau(t - t_0)\). By first fitting the \(\Delta CO_2\) calculated according to equation 7 with the \(\tau\) correction to the measured data and then using the fitted parameters values to compute \(Sx\) based on equation 8 (i.e. perfect mixing, no \(\tau\) correction), it is possible to estimate \(\tau\) and \(t_0\). If this procedure is repeated at least once per day in a chamber by scrubbing/injecting CO\(_2\), it is possible to have an estimate of both \(\tau\) and \(t_0\) and correct the measured data for the delays (Figure 5). In fact, as the structure of the canopy itself changes over time affecting the mixing within the chamber, this procedure allows having a daily correction of the data taking into account the delays.

**Estimating steady state \(\Delta CO_2\)**

The described modelling framework also allows to determine steady state fluxes from unsteady state data by fitting equation 7 with the \(\tau\) correction to \(\Delta CO_2\) measured values. To test this, we grew a soybean variety inside the chambers with fluctuating light conditions and measured the changing canopy photosynthetic rates. As the light was fluctuating (with a period of 1 minute) it determined a continuous change in the \(\Delta CO_2\) due to canopy
carbon assimilation (i.e. photosynthesis). Since fluctuations were very frequent, the measured values never reached steady state (Figure 6A). The fitting procedure though allowed to have an estimate of the steady state values by fitting unsteady state $\Delta CO_2$ values (Figure 6B), showing that steady state is reached only after about 300 seconds (as also evident from Figure 5).

Quantifying whole plant gas-exchange under fluctuating conditions

To test the accuracy of DYNAMISM in real conditions, we used a commercial soybean variety (Eiko, Asgrow, USA). Plants were sown in 96 pots (13 x 13 x 18 cm) with siliceous sand in order to have an inert substrate and to zeroing heterotrophic respiration (Rh). We used six chambers for the experiment, and we placed 16 pots chamber$^{-1}$. In three chambers, the LED system was set to simulate a fixed daily profile (June 21$^{st}$) in Udine, Italy (latitude: 46.07 N; longitude: 13.23 E) with a maximum PPFD of 1000 $\mu$mol m$^{-2}$ s$^{-1}$ at noon (non-fluctuating light treatment, NF). In the other three chambers (fluctuating light treatment, F), light was fluctuated $\pm$50% with a period of 60 seconds around the hourly value measured in NF. By doing this, plants grown either in fluctuating or non-fluctuating light received the same cumulative light intensity throughout the day. According to the light curve reported by Sakowska et al. (2018), these fluctuations at midday (500-1500 $\mu$mol m$^{-2}$ s$^{-1}$) fall within the logistic range of the curve, therefore the highest values of light are saturating. It is than predictable that the cumulative average value (1000 $\mu$mol m$^{-2}$ s$^{-1}$) would entail a higher C assimilation than the cumulative fluctuating values. This though is not the case when the oscillations of light fall into the linear range of the light curve, as in the first (and last) hours of the day. In this case, we expect the average value of light to be translated into a similar accumulation of CO$_2$.

LEDs were manually moved up inside the chambers as canopy grew thus to be at a constant distance of 13 cm above the plants throughout the experiment.

Each chamber was sampled for 290 seconds and A was calculated as average between 110 and 290 seconds thus to not consider the tube’s purging after chamber switch ($t_0 = 110$ s). The matching procedure with the thirteenth valve was done every hour in order to compute the difference in CO$_2$ and H$_2$O concentration among the cell A and B of the LI-7000, thus correcting the data based on this value. Measurements were run for four
weeks during which plants were regularly watered with the addition of a Hoagland solution twice per week (Table 2).

| Components          | Stock (g/L) | mL stock/30L |
|---------------------|-------------|--------------|
| **Macro-nutrients**  |             |              |
| 1M KNO$_3$          | 101         | 150          |
| 1M Ca(NO$_3$)$_2$ 4H$_2$O | 236         | 150          |
| Fe-EDTA             | 15          | 30           |
| 2M MgSO$_4$ 7H$_2$O | 123         | 120          |
| 1M KH$_2$PO$_4$     | 136         | 30           |
| **Micro-nutrients** |             |              |
| H$_3$BO$_3$         | 2.86        |              |
| MnCl$_2$ 4H$_2$O    | 1.81        |              |
| ZnSO$_4$ 7H$_2$O    | 0.22        |              |
| CuSO$_4$ 5H$_2$O    | 0.08        |              |
| H$_2$MoO$_4$ H$_2$O | 0.09        |              |

Table 2. Nutrients (mL) for a 100% Hoagland solution. For Soybean we used a half strength solution: nutrients for 30L diluted in 60L of distilled water per week. pH of 6.47.

At the end of the experiment, we harvested four plants per chamber. Leaf area was measured using a LI-3000 (Licor, USA), stem and leaves were separated from roots and these lasts were gently washed to remove sand. Leaves, stems and roots were then dried at 70°C for 48 hours and then weighted. Because of the inert substrate used in the pots (no heterotrophic respiration, Rh), the measured CO$_2$ flux corresponds to net primary production (NPP = A) instead of net ecosystem production (NEP = NPP – Rh), allowing a direct comparison between the cumulative A flux (gC m$^{-2}$) at the end of the experiment and the total produced biomass (i.e. total dry weight; g m$^{-2}$) by assuming a C content of 46.8% (Sakowska et al. 2018).

Considering the response time due to the dimension of the chambers ($t_0 = 110$ s) the system was clearly able to detect instantaneous changes in A related to light fluctuations, while it measured stable A in non-fluctuating light conditions (Figure 7).

On an hourly basis, the system responded as expected: from a positive CO$_2$ flux during night (respiration) to a maximum net uptake (negative flux) at midday with a small variability among chambers (Figure 8A). At the end of the experiment, cumulative fluxes slightly over-estimated (+7.6%) the total plant dry biomass measured...
at harvest (Figure 8B), even though the difference was not significant, confirming the applicability of DYNAMISM to measure canopy CO₂ fluxes.

**Discussion and conclusions**

Several approaches have been used in the literature to obtain reliable measurements of CO₂ assimilation. Leaf level data are mainly reliable but the scaling to plant or canopy scale is rather difficult. On the other hand, canopy scale methods exist and can capture CO₂ exchange dynamics at bigger scales but suffer from several weaknesses (Acosta et al., 2017; Pinto et al., 2016). Therefore, to overcome these issues, many growth chamber systems have been developed in the last decades, but most of them lack the ability to measure dynamic environmental conditions, such as those generally occurring in the field, and/or are extremely expensive. On the contrary, DYNAMISM can be used to simulate dynamic environmental conditions in terms of light, temperature, humidity and, eventually, higher atmospheric CO₂ concentrations at a limited cost, it is user friendly and can be built using commercial sensors and equipment (Table 1).

The main strength of DYNAMISM relies on its accuracy and stability (Figures 3, 4 and 5), on the possibility to accurately estimate the response time and to correct for the intrinsic delays of the system (Figure 6) and to determine steady state fluxes from unsteady state measured values (Figure 7). Thus, it is able to efficiently capture the effect of fast fluctuating light on instantaneous CO₂ gas exchanges (Figure 8).

Finally, DYNAMISM can be used for several applications: different plant canopies can be monitored thanks to the flexibility in the software and to the dimension of the chambers, allowing to answer relevant biological questions. Furthermore, even if DYNAMISM has been tested for measuring CO₂ exchange, it can also be used for measuring transpiration, stomatal conductance, temperature and humidity changes and respiration, not only in plants but for all the kind of organisms one might be interested in.

The system is not only able to simulate dynamic light conditions, but, with some simple upgrades, could be used to simulate dynamic environmental CO₂ concentrations and/or dynamic temperatures. Thus, it can be potentially used to induce abiotic stresses, by simulating, for example, drought conditions, high light conditions (inducing photo-inhibition) and high environmental CO₂ levels. Moreover, as a future development, we think to couple DYNAMISM with real-time fluorescence measurements to investigate photochemistry and the
primary reactions of photosynthesis in dynamic environments as well as to use it for photosynthesis phenotyping (Keller et al., 2019).

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**Declarations**

**Ethics approval and consent to participate**
Not applicable

**Consent for publication**
Not applicable

**Competing interest**
The authors declare that they have no competing interests

**Availability of data and materials**
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

**Authors' contributions**
NS and GA constructed the growth chamber system and performed the experiments; NS wrote the article; AP and GA planned the experiments; OM and UR provided the facilities and gave suggestions on the experiments. All the authors agreed on the final version of the manuscript.

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List of Figures

Figure 1. (A) Schematic representation of DYNAMISM. The 12 chambers (not all represented here) are connected to the bigger chamber that acts as a buffer. The buffer is itself connected to outdoor and has an air conditioning inside to keep the temperature and humidity more stable, and a pressure sensor. The air flows from the buffer to the chambers. Air is sampled within each chamber and analysed by the Licor-7000 (IRGA). Each chamber is equipped with a LED system, a mass flow meter to measure the inlet flow rate, a solar bar, a thermistor and an aquarium pump placed at the top chamber. Chamber sampling and data acquisition is made through a CR1000X datalogger which itself controls a multiplexer and a relay controller (SDM CD16-AC). (B) Example of the control of the CR1000X output variables through the RTMC software. In this case, in the main screen are shown the CO$_2$ and H$_2$O changes in real-time in the sampled chamber, as well as other environmental parameters. Then in each chamber the desired parameters can be monitored, here we have set an alarm for chamber temperatures higher than 40° and a slider input to change incident PPFD.

Figure 2. Light spectrum of the LED panels measured with FLoX at 10 cm distance (constant PPFD at 1876 ± 30 μmol m$^{-2}$ s$^{-1}$). The solid line is the mean, the grey shadow represents mean ± standard deviation (n=6).

Figure 3. Preliminary test results: measured CO$_2$ fluxes after scrubbing inlet CO$_2$ (negative values) or after injecting pure CO$_2$ (positive values) versus modeled fluxes calculated using Equation 4 and 5 for photosynthesis and respiration, respectively.

Figure 4. Total percentage error (T) and percentage errors due to changes in air flux velocity (F) and in ΔCO$_2$ (Δ) calculated from the partial derivation of equation 8. The parameter changes (x axis) are shown as normalized values (i.e. percentage change [0-100]) but the actual ranges of parameters are: F=[0.2 : 0.6] mol s$^{-1}$ and ΔCO$_2$=[0 : -10] ppm. The boxplots show the aggregated values for all 12 chambers.
**Figure 5.** Example of the scrubbing of CO$_2$ with an air flux velocity (F) of 0.34 l s$^{-1}$ (red line, measured data). The black line indicates the ΔCO$_2$ corrected for the delay and residence time ($\tau$ and $t_0$, respectively). The lines represent 5 seconds averaged values.

**Figure 6.** A: ΔCO$_2$ changes due to fluctuations in light intensity. The vertical red dashed lines indicate the data used for fitting the model. B: Fitting of the data highlighted in A through equation 7 and estimation of steady state ΔCO$_2$.

**Figure 7.** Soybean CO$_2$ fluxes in non-fluctuating (above) and fluctuating light conditions (below). Data are instantaneous measurements during one session (25th July at 10:00 am). Red lines represent photoflux density (PPFD), dots represent CO$_2$ fluxes. CO$_2$ fluxes data are corrected for the delayed response ($t_0=110$ s). The lines represent 4 seconds averaged values. More negative values of A at higher PPFD values corresponds to higher photosynthesis (micro-meteorological convention).

**Figure 8.** A: Daily course of net primary production (NPP) measured five weeks after sowing. Closed and open symbols are fluctuating (F) and non-fluctuating (NF) light conditions, respectively. In the inner panel, the daily course of PPFD is reported. Negative NPP values denote C uptake following the micro-meteorological convention. B: total final biomass derived from fluxes and from plant dry weights at harvest for the two considered treatments. Any significant difference was found at harvest. Vertical bars are standard error (n=3).