iForest
Biogeosciences and Forestry

Technical Report
doi: 10.3832/ifor2209-010
vol. 10, pp. 597-604

Canopy Chamber: a useful tool to monitor the CO_2 exchange dynamics of shrubland

Gabriele Guidolotti [1-2], Giovanbattista De Dato [1-3], Dario Liberati [1], Paolo De Angelis [1-4]

A transient state canopy-chamber was developed to monitor CO_2 exchange of shrubland ecosystems. The chamber covered 0.64 m^2 and it was modular with a variable height. Several tests were carried out to check the potential errors in the flux estimates due to leakages and the environment modifications during the measurements inside the chamber. The laboratory leakages test showed an error below 1% of the flux; the temperature increases inside the chamber were below 1.3 °C at different light intensity and small pressure changes. The radial blowers inside the chamber created different wind speed at different chamber height, with faster speed at the top of the chamber and the minimum wind speed that was recorded at soil level, preventing detectable effects on soil CO_2 emission rates. Moreover, the chamber was tested for two years in a semi-arid Mediterranean garrigue, identifying a strong seasonality of CO_2 fluxes with the highest rates during spring and lowest rates recorded during the hot dry non-vegetative summer.

Keywords: Canopy Chamber, CO_2 fluxes, *Cistus monspeliensis*, Shrubland, Semiarid Ecosystems, Mediterranean Garrigue

Introduction

In recent years, the quantification of ecosystem CO_2 fluxes has received increased attention by the scientific community, both at broader scale as well as at the plant-environment scale. In large areas with homogeneous topography and soil cover, micro-meteorological techniques, such as eddy covariance (Baldocchi et al. 1988), can measure ecosystem CO_2 fluxes without altering canopy environment (Foken et al. 2012). On the other hand, for heterogeneous multi-specific community like ecotonal and transitional areas, plot size experiments, or forest understory vegetation the canopy chamber technique can be a suitable alternative that provides reliable data (Steduto et al. 2002, Schaaf et al. 2005, Burkart et al. 2007).

Canopy chamber systems can be differentiated into "steady state" (open systems) and "non-steady state" (closed systems), according to the recirculation of the air inside the chamber (Livingston & Hutchinson 1995). In the "steady state" type, an external airflow passes through the chamber and the fluxes are calculated on the base of the differential CO_2 concentration of the incoming and outgoing air. In the "non-steady state" type, the fluxes are calculated on the basis of the CO_2 concentration changes in the air passing inside the chamber in a closed loop. In both systems, the measurement of CO_2 is repeated during short time intervals (seconds).

Enclosing a portion of an ecosystem inside a chamber produces several artifacts, caused by the chamber characteristics and deployment (chamber effect). The chamber walls affect light environment, air temperature and humidity, as well the CO_2 concentration inside the chamber. Although transparent materials are used, chamber walls absorb, reflect and therefore reduce the photosynthetically active radiation (PAR, 400-700 nm) reaching the ecosystem (Steduto et al. 2002), decreasing the photosynthetic rates of the plant community. The greenhouse effect produced by the walls and the consequent increase in air temperature and relative humidity (Leadley & Drake 1993) can change both photosynthetic and respiratory processes (Berry & Bjoerkman 1980, Atkin & Tjoelker 2003). The modified aerodynamic conditions due to fan functioning (Baldocchi et al. 1988) and the pressure variation occurring inside the chamber (Fang & Moncrieff 1998) can alter the soil CO_2 efflux, producing an error in the estimation of the different components of the ecosystem CO_2 fluxes (respiration and photosynthesis). Fans generally increase the CO_2 fluxes from the soil by disturbing the boundary layer above soil surface (Hanson et al. 1993, Pumpanen et al. 2004), while over-pressurization generate a mass transfer into the soil inhibiting the CO_2 efflux (Steduto et al. 2002, Takle et al. 2004). Additionally, the changes in CO_2 and H_2O concentration inside the chamber affect the natural gradient in the soil-atmosphere interface, thus the rates of processes such as photosynthesis, respiration or transpiration are biased from undisturbed rates (Lund et al. 1999, Pérez-Priego et al. 2010).

The choice of adequate wall material (Muller et al. 2009) and the use of non-steady state operating for short periods (Steduto et al. 2002), can limit the chamber

(1) University of Tuscia, Department for Innovation in Biological, Agro-Food and Forest Systems (DIBAF), v. S. Camillo de Lellis snc, I-01100 Viterbo (Italy); (2) National Research Council of Italy (CNR), Istitute for Agro-Environment and Forest Biology (IBAF), v. Salaria km 29.300, I-00015 Monterotondo Scalo, RM (Italy); (3) Council for Agricultural Research and Economics (CREA), Forestry Research Centre (CREA-SEL), v.le Santa Margherita 80, I-52100 Arezzo (Italy); (4) Global Change Research Centre, Division of Impact Studies and Physiological Analyses, Belidla 4a, CZ-60300, Brno (Czech Republic)

@ Gabriele Guidolotti (gabriele.guidolotti@ibaf.cnr.it)

Received: Aug 25, 2016 - Accepted: Mar 26, 2017

Citation: Guidolotti G, De Dato G, Liberati D, De Angelis P (2017). Canopy Chamber: a useful tool to monitor the CO_2 exchange dynamics of shrubland. iForest 10: 597-604. – doi: 10.3832/ifor2209-010 [online 2017-05-15]

Communicated by: Tamir Klein

effects due to walls, although longer periods of measurements seems to increase the confidence of the measurements (Pérez-Priego et al. 2015). Furthermore, the application of a venting tube on the chamber wall can help to balance the pressure between the inside and outside of the chamber (Savage & Davidson 2003). On the other hand, other systems based on air and microclimate conditioning have been tested to reduce the chamber effects, but these systems lack portability (Medhurst et al. 2006, Muller et al. 2009).

In the literature, a variety of chambers can be found with different configurations designed to respond to specific requirements such as: different ecosystem types and environments (*e.g.*, croplands, grasslands, orchards, forest understories – Steduto et al. 2002, Heijmans et al. 2004, Pérez-Priego et al. 2010), needs to modify the microclimatic conditions inside the chamber (Medhurst et al. 2006), and the accessibility of measurement areas (Muller et al. 2009).

In the context of a long-term climatic manipulation experiment on European shrubland (Beier et al. 2009), the objectives of this work were: (i) to develop a portable canopy chamber system designed to measure the CO_2 fluxes in shrubland ecosystems in remote areas; (ii) to assess the microclimatic modifications produced by the chamber system; and (iii) to test the suitability of the system to quantify the seasonal variation of photosynthetic and respiratory CO_2 fluxes in a Mediterranean, transitional ecosystem (garrigue).

Material and methods

Chamber design

The originally developed chamber was vertically modular and made of a minimum of 3 parts: a soil collar, a base and a lid (see Fig. S1 in Supplementary material). The chamber covers an area of 0.64 m^2 (0.8 × 0.8 m), and by piling up two or more bases, the height can vary from 0.6 to 1.3 m, depending on the plant height. To avoid leakages, all the junction parts are sealed by foam gaskets (RS 567-963, RS components).

The soil collar guarantees the insertion of the chamber to the ground in order to avoid leakeages of gases from the bottom. It is made of a stainless steel frame that can be fixed to the base, and it is equipped of a blade that enters 5 cm into the ground. Along the blade, every 5 cm, there are 5 mm-diameter holes that allow the roots colonization, and partially compensate for the disturbance occurring during soil collar installation. The base consists of an open top and open bottom parallelepiped with 4 transparent 2 mm-thick Lexan® polycarbonate (GE plastic, Pittsfield, MS, USA) walls, held together by an aluminium frame. H_2O and CO_2 showed a low permeation through Lexan®, which has often been used in recently developed gas exchange chambers for its transparency and low permeability (Bachman et al. 2010). In the lower part of the base two battery-operated radial fan blowers were fixed; the rotational speed of the blowers can be regulated using an external level potentiometer.

The lid is a bottom open parallelepiped made of five transparent walls made of the same materials as the base. Sensors of air temperature, air relative humidity (HydroClip S3 probe; Rotronic, Basserdorf, Switzerland) and photosynthetic photon flux density (PPFD; Apogee quantum sensor – Apogee Instruments Inc., Logan, UT, USA), together with a high-resolution differential pressure transducer sensor (TEPR 070, Tecno El, Rome, Italy) were mounted on the interior of the lid to monitor the chamber environment during measurements. Additionally, a curled venting tube (6 mm diameter, 70 cm length), passing through one of the wall of the lid, was inserted to equilibrate the pressure between the inside and the outside of the chamber.

The measurements of CO_2 and H_2O concentrations were made by an infrared gas analyzer LI-8100 (LI-COR, Lincoln, NE, USA), connected to the chamber by two polytetrafluoroethylene (PTFE) tubes (2 m in length), while the data originated from the lid sensors were recorded by the Auxiliary Sensor Interface (LI-COR, Lincoln, NE, USA). According to the purpose of this study, only the CO_2 fluxes were calculated as the temporal changes of the concentration in the air passing in a closed loop in the canopy chamber and in the analyzer. The flux computation can change depending on the regression type used to fit the data (typically linear or exponential fitting are used). An underestimation of the fluxes is often reported if the linear regression is used (Kutzbach et al. 2007), although this error is dependent on chamber volume to area ratio (chamber height), and experimental tests reported that it becomes negligible for a chamber height higher than 80 cm (Livingston et al. 2005).

In the present study, we used the LI-COR 8100 File Viewer 3.1.0 flux computation. The software performs at the same time both linear and exponential regression, providing the normalized sum of square residuals of fits. Because the linear fit performed better with our measurements, the fluxes presented here were calculated using the slope of the linear regression that fit the variation of CO_2 concentrations inside the chamber during measurement interval after the closure of the chamber.

The time duration of the measurement was chosen after preliminary tests, accounting for a duration that would simultaneously allow an adequate CO_2 concentration change as well as the linearity in gas concentration change during the time. We established that 90 seconds was the optimal duration time for the chamber presented here. This duration was similar to the 100 seconds used in another large chamber closed system (Pérez-Priego et al. 2010).

Chamber tests

Several tests were conducted in laboratory condition and outdoor, to evaluate the errors of our chamber system on flux measurements.

Chamber leakages test. This test was carried out in the laboratory to estimate the error produced on flux calculation caused by the leakages. The empty chamber was installed over an inert surface and pure CO_2 was injected inside the chamber to reach the concentration of 1000 ppm, maximizing the gradient between CO_2 concentration outside and inside the chamber, thus the leakages. The CO_2 concentration inside the chamber was monitored over time by the LI-8100 analyzer.

The flux error associated to the leaks was calculated by the following equations reported by Pérez-Priego et al. (2010) for a large canopy chamber (eqn. 1):

$$\frac{\partial Ci}{\partial t}=\frac{-\alpha(Ci-Ca)+FC}{V}$$

where (eqn. 2):

$$\alpha=-\frac{V}{\partial t}\ln\left(\frac{Ci_2-Ca}{Ci_1-Ca}\right)$$

In those equations α represents the leak coefficient of the chamber (m^3 s^{-1}), Ci_1 and Ci_2 represent the CO_2 concentration (ppm) inside the chamber at time 1 and 2, *Ca* is the CO_2 concentration outside the chamber measured discontinuously with the LI-8100 analyzer (ppm), *V* is the volume of the chamber (m^3), ∂*t* is the time (s) and *Fc* is the net exchange of the ecosystem (μmol CO_2 m^{-2} s^{-1}). When in the chamber there is nothing generating a CO_2 flux, *Fc* is zero, and α depends only on time and on the concentration gradient between the outside and the inside of the chamber. The flux error ε (%) associated to the leaks was estimated according to the eqn. 3:

$$\varepsilon=\frac{-\alpha(Ci-Ca)}{-\alpha(Ci-Ca)+FC'}=\frac{\alpha\,\partial t}{2V}$$

Temperature and relative humidity (RH) tests. These tests were based on concurrent measurements of temperature outside and inside the chamber (temperature test) and the measurement of RH increase inside the chamber (RH test) for the 282 field measurements (90 seconds length) carried out at different meteorological conditions. The soil and air temperatures were ranging from about 11 to 30 °C and about 20 and 34 °C, respectively; PPFD from about 600 to 1600 μmol photons m^{-2} s^{-1} and relative soil water content from about 6 to 55%.

Pressure test. The high-resolution pressure transducer installed on the chamber lid was used to monitor the difference of pressure (ΔP) between outside and inside the chamber (*Pi-Pa* where *Pi* is the pressure inside the chamber and *Pa* is the ambient pressure; the positive sign represents an overpressure in the chamber). The check of ΔP and its analysis was a standard procedure for all the 282 field chamber measurements.

Radiation test. To test the attenuation of solar radiation due to the chamber wall absorbance and reflectance, we monitored the solar radiation inside and outside the chamber in 6 moments along a sunny day in clear sky conditions (2011-06-28), integrating different sun angle. The irradiation measurements were done by a portable spectroradiometer (Field Spec Pro FR, Analytical Spectral Devices, Boulder, CO, USA). The instrument measured the irradiance in the wavelength range 350-2500 nm with a resolution of 1 nm.

Blowers test. To test the effect of rotational speeds on air circulation inside the chamber, we positioned the chamber on a bare sandy soil, where both wind speed inside the chamber (thermo-anemometer 2103-1 Delta-OHM, Italy) and soil CO_2 efflux rate were measured. The wind speed measurements were carried out at 6 different heights above the ground from 2 to 52 cm. In addition, wind speed measurements at 22 cm height were also performed at five different zenith angles (0°, 45°, 90°, 135°, 180°). All measurement were carried out at each level of blower speed potentiometer (6, 8, 10 and 12 Volts). Simultaneously, we tested how the blowers speed affected the rate of CO_2 efflux from bare soil. The results obtained for each level of blower speed (soil CO_2 efflux and wind speed) were tested by ANOVA and Fisher's *post-hoc* tests.

Chamber comparison. Our Canopy Chamber was compared with the commercial Soil Survey Chamber 8100-103 (LI-COR, Lincon, Nebraska, NE, USA). The comparison was made in the field on a bare sandy soil. Within the area defined for the canopy chamber measurement (0.64 m²), five PVC collars (ø 0.2 m, covering about 25% of the area) were inserted at 0.05 m depth for the measurements with the Soil Survey Chamber 8100-103. The comparison was performed by measuring in a short time lag the soil CO_2 efflux on the PVC collars by the Survey Chamber 8100-103 followed by measurement by the Canopy Chamber. In order to avoid any photosynthetic activity, an aluminum cloth darkened the canopy chamber during the measurements. The 5 measurements obtained by the Soil Survey Chamber 8100-103 were averaged for the comparison with the value measured using the chamber developed in this study. The comparison was carried out measuring the fluxes in 8 campaigns carried out in different seasons and environmental condition: soil and air temperature were ranging from about 11 to 30 °C and 20 and 34 °C, respectively; while relative soil water content was ranging from about 6 to 55%.

NEE, TER measurements and GP estimation in a Mediterranean garrigue

The net ecosystem exchange (NEE) and total ecosystem respiration (TER) fluxes were calculated according to the following equation (LI-COR 2010 – eqn. 4):

$$Fc=\frac{10\,V\,P_0\left(1-W_0/1000\right)}{RS\left(T_0+273.15\right)}\cdot\frac{\partial C'}{\partial t}$$

where P_0 is the initial pressure (kPa), W_0 is the initial concentration of water vapor (mmol mol^{-1}), S is the surface covered by the chamber (cm²), R is the universal gas constant (8.314 Pa m³ K^{-1} mol^{-1}), T_0 is the initial temperature (°C) and $\partial C'/\partial t$ is the change of CO_2 concentration during the measuring interval (µmol mol^{-1} s^{-1}).

The net ecosystem exchange was measured by positioning the base over the metallic soil collar, then the radial blowers were switched on, and finally the lid was positioned over the base. After the NEE measurement, the lid was pulled up to vent the chamber; then, after 2 minutes it was repositioned over the base and the entire chamber was darkened by an aluminum cloth to measure the TER. The gross photosynthesis of the ecosystem (GP) was calculated by subtracting TER from NEE.

In order to test the canopy chamber under variable field conditions, we measured the NEE, TER, GP and the environmental parameters sixteen times throughout the years 2010 and 2011 on a Mediterranean shrubland, located in the northeast of Sardinia (Italy – 40° 37′ N, 8° 10′ E). The soil is rocky and shallow and is covered mostly by a malacophyllous drought semi-deciduous species: *Cistus monspeliensis L.* The height of the plants is lower than 1 m. The climate is Mediterranean with a mean annual temperature of 16.8 °C and a mean annual rainfall of 640 mm. Further information on the site characteristics are reported in De Dato et al. (2008, 2010). Six chamber soil collars, delimiting six areas including at least one *Cistus* plant, were installed about 2 months prior to the start the measurements, in order to allow root re-colonization after the disturbance. To monitor soil water content and soil temperature two sensors (5TM – Decagon Devices, Pullman, WA, USA) were inserted inside each collar at 5 cm depth. The sensors measurements were recorded by an external data-logger (Em50 – Decagon Devices, Pullman, WA, USA).

Result and discussion

Chamber system tests

Chamber leakages

The CO_2 concentration of the empty chamber decreased regularly during the time of the test (in Fig. 1a is showed a part of the test). On average, the leak coefficient α (Fig. 1b) obtained solving the eqn. 2 was about $8\cdot10^{-6}$ m³ s^{-1}, while the average flux error ε estimated over the standard time of the measurements (90 s) was less than 1% per minute. The ε value estimated in this study was constantly in the range of 0.8-1% per minute, similar to the values reported for chamber of comparable volumes by Steduto et al. (2002) and Pérez-Priego et al. (2010), while larger errors are reported for the chamber developed by Held et al. (1990) and Grau (1995).

Temperature and relative humidity tests

The chamber walls altered the energy balance between the chamber and the outside environment, producing a slight increase of temperature inside the chamber (Tab. 1). The temperature increases (ΔT) were a function of PAR level (Fig. 2) and in the measurement interval (90 s), they were always below 1.3 °C (Tab. 1). The recorded ΔT was similar to the one reported from a larger canopy chamber (Bachman et al. 2010, Pérez-Priego et al. 2010) and considerably lower than the 2-4 °C found in many other closed chambers (Grau 1995, Reicosky 1990, Steduto et al. 2002). The lower

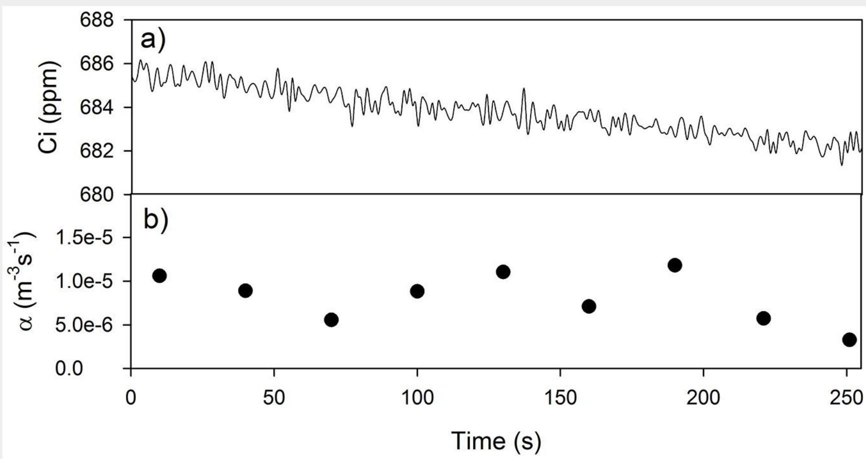
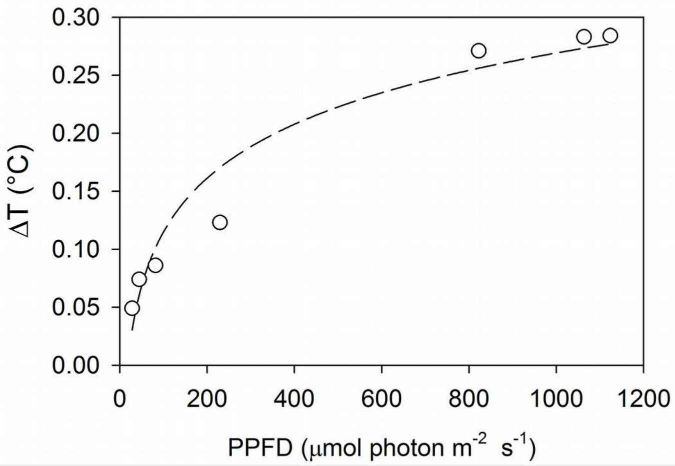


Fig. 1 - (a) Time evolution of CO_2 concentration inside the chamber; (b) leak coefficients α (black circles) estimated on a 30 seconds base. The measurement was carried out in laboratory condition.

Tab. 1 - Average initial measurement temperature (Initial T Air) and relative humidity (Initial RH) values and temperature increase (ΔT Air) and relative humidity increase (ΔRH) values for each field measurement campaign (totally 282 measurements).

Date	Initial T Air (°C)	ΔT Air (°C)	Initial RH (%)	ΔRH (%)
2010-Feb-26	22.38	0.34	62.31	15.42
2010-Mar-16	22.19	0.67	54.16	19.70
2010-Apr-28	29.46	0.60	60.26	18.74
2010-Jun-07	32.45	0.77	47.33	17.53
2010-Jul-02	33.55	1.27	65.95	15.38
2010-Jul-28	34.56	0.62	47.63	6.39
2010-Sept-10	32.02	0.46	46.02	4.93
2010-Oct-10	34.07	0.31	35.26	9.10
2010-Nov-06	26.85	0.43	57.15	15.09
2011-Feb-12	20.50	0.32	58.50	15.20
2011-Mar-24	27.70	0.72	62.10	12.19
2011-May-05	30.68	0.38	44.09	17.56
2011-Jun-15	29.61	0.82	63.73	15.42
2011-Jul-15	34.78	0.48	39.34	6.41
2011-Oct-19	28.81	0.68	41.53	4.36
2011-Nov-13	22.79	0.90	53.11	13.97

Fig. 2 - Relationship between the increase in air temperature inside the chamber (ΔT) and incident light (PPFD) measured at the end of 90 seconds period. The line is the logarithmic equation fitting the data.



values measured in our chamber may be explained by both the short duration of measurements and the lower infrared reflectance properties of the Lexan material constituting the chamber walls. Moreover, we noticed that the aluminum cloth (used for the respiration measurement) slightly reduced the temperature increase (0.2 °C, overall average). Relative humidity (RH) increase during the measurements ranged between 4 and 20 % with an average value of 13% (Tab. 2). No condensation inside the chamber was observed, in any of the sampling campaigns. Although limited, the observed increase may affects the evaporation processes (out of the scope of our measurements), but considering the short time of the measurements and a certain delay in the stomata responses (Vico et al. 2011), no major effect on the CO_2 exchanges could be assumed.

Tab. 2 - Imposed blowers voltage (V), soil CO_2 efflux (µmol CO_2 m^{-2} s^{-1}) and coefficient of variation (CV %) of the flux computation. Values are means (± standard error) of 5 different measurements carried out on a bare sandy soil. For each column different letters indicate significant differences (p<0.05) after one-way ANOVA and Fisher's *post-hoc* test.

Blowers voltage (V)	Soil CO_2 efflux (µmol CO_2 m^{-2} s^{-1})	Coefficient of variation (%)
0	4.07 ± 0.57 ^{a}	3.17 ± 0.52 ^{b}
6	4.49 ± 0.77 ^{a}	1.63 ± 0.33 ^{a}
8	4.06 ± 0.83 ^{a}	1.39 ± 0.11 ^{a}
12	4.10 ± 0.79 ^{a}	1.38 ± 0.08 ^{a}

Pressure test

Considering all the 282 measurements, the maximum ΔP was on average +4 ± 0.4 Pa (positive sign means overpressure in the chamber). Generally, spikes of 4-5 Pa occur only occasionally during a measurement and were not influenced by temperature increase and H_2O concentration inside the chamber (an example of ΔP variation during a typical measurement is reported in Fig. S2 – Supplementary material). Steduto et al. (2002) explained these short time variations by the functioning of the air pump located inside the analyser, while Takle et al. (2004) showed that also wind could induce pressure fluctuation. In our case, due to the coastal location of the experimental site, wind could play a major role on chamber pressure fluctuations. Positive or negative pressure perturbations could be dependent on wind intensity and direction as previously demonstrated (Kutsch et al. 2001).

Considering all the 90 seconds of the measurement, ΔP showed a net variation of +0.2 Pa. Although the effect of pressure is connected to the degree of soil permeability (Takle et al. 2003), these levels of over pressurization should not inhibit the natural soil CO_2 efflux (Lund et al. 1999) and thus we can exclude sensible errors on the ecosystem respiration measurements.

Radiation test

The reduction of the radiation available for the ecosystem is unavoidable, and the degree of this reduction depends on chamber wall material properties and on the shade of the holding frame as well as on the solar angle. As reported in Fig. 3a, the Lexan transmittance of the radiation was close to 0 in the UV region (< 400 nm), while reached about the 90% in the photosynthetic active region (PAR, 400-700 nm). Moreover, the transmittance in this region was affected neither by the different levels of radiation nor by the different radiation angle (changing with the hours of the measurement – Fig. 3b). Out of the PAR, the transmittance became lower, reaching values of about 20% in the SWIR region (short wave infrared, about 1600 nm) that did not influence the photosynthetic activity in the short term (Fig. 3a). These values of transmittance are similar (Pickering et al. 1993) or higher than the transmittance reported for others chambers (Held et al. 1990, Steduto et al. 2002, Bachman et al. 2010).

Blowers test

Inside the chamber, wind speed increased with chamber height, with the minimum wind speed recorded at 2 cm height (soil level) and the maximum between 42 and 52 cm height (Fig. 4b). Moreover, the wind speed was maximum at the zenith angle of 90° (perpendicular to the soil layer), while the minimum was found at 0° and 180° (parallel to the soil layer) (Fig. 4a). Several studies reported a change of the boundary layer at the soil level as a

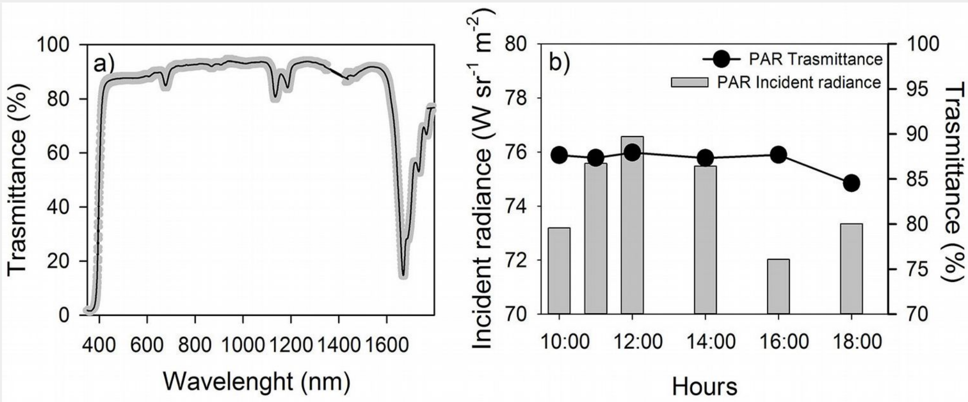


Fig. 3 - (a) Solid black line and gray band represent the averaged solar radiation transmitted by chamber walls and standard deviation (n=6), respectively. The wavelength transmittance was averaged based on six measurements carried out at different times in 2011-06-28. (b) Incident PAR radiance (integrated value between 400-700 nm) outside the chamber (gray bars) and percent of radiance transmitted inside the chamber (black circles) during different times during 2011-06-28.

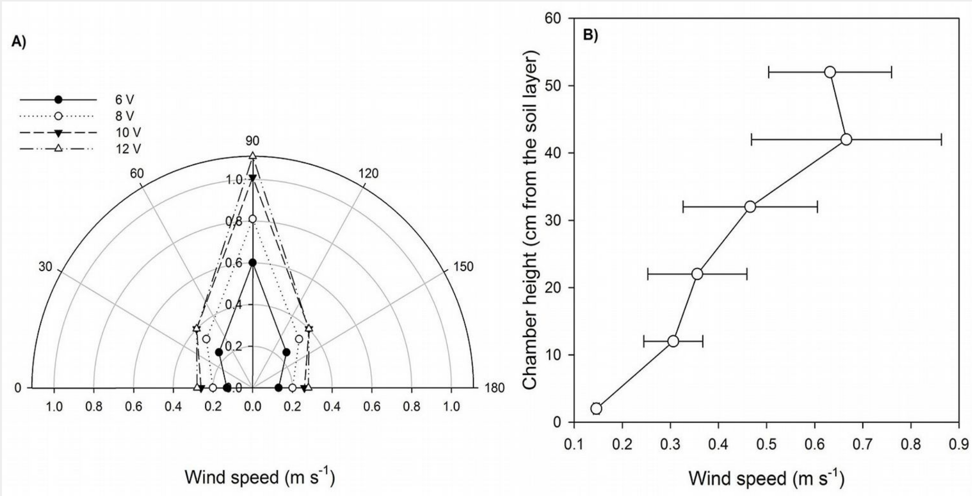


Fig. 4 - (a) Wind speed measured at different zenith angles for different blowers power. Black circles, white circles, black triangles and white triangles represent the wind speed (m s^{-1}) measured at 6, 8, 10 and 12 volts blowers voltage respectively. The rotation speed of the blowers was function of power supply. The measurements were carried out inside the chamber at 22 cm height. (b) Wind speed profile. White circles represent the wind speed measured across the chamber height profile with the blowers at 10 volts. Bars represent the standard error of the wind speed mean over 5 different zenith angles.

consequence of the fans functioning, causing an overestimation of soil CO_2 effluxes (Baldocchi et al. 1988, Hanson et al. 1993, Pumpanen et al. 2004). In our chamber, the lowest wind speed was recorded in the vicinity of the soil and the blowers speed did not sensibly affect the soil CO_2 emission rates. On the contrary, it improved significantly the precision of the measurement expressed as the coefficient of variation of the flux (Tab. 2).

Chamber comparison

The result of the comparison showed a non-significant deviation between the fluxes measured by the Canopy Chamber and the Soil Survey Chamber 8100-103, as demonstrated by the linear regression with a not significant intercept (p = 0.82) and not different from the 1:1 line (Fig. 5). Although the comparison was conducted on bare soil, the small deviation resulting between the averaged points and the Canopy Chamber is probably due to the high spatial variability generally present in soil CO_2 efflux (Stoyan et al. 2000).

CO_2 flux measurements in a Mediterranean garrigue

Over the two years of field measurements, the environmental factors (photosynthetically active radiation, air temperature, soil water content and soil temperature) showed high temporal variability, with the typical trends of the Mediterranean climate: the highest values of air, soil temperature and radiation were measured together with the lowest value of soil water content and *vice versa* (Fig. 6A and Fig. 6B). During the same period, the CO_2 fluxes measured by the Canopy Chamber showed a significant seasonal variability (Repeated measures ANOVA, p<0.0001 – Fig. 6C), similar between the two years: the gross photosynthesis (GP) increased almost continuously from the winter period (11.98 ± 0.84 µmol CO_2 m^{-2} s^{-1}) until the early

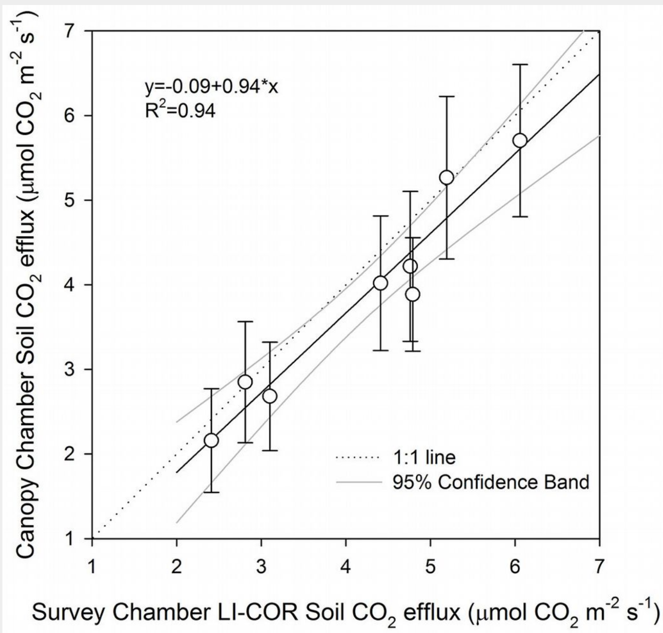


Fig. 5 - Relationship between soil CO_2 efflux measured by Canopy Chamber and Soil Survey LI-COR 8100-103 chamber. The dotted line represent the 1:1 line, the black line represent the linear regression and grey lines the 95% confidence band. The vertical bars represent the standard deviation of the mean (n=5).

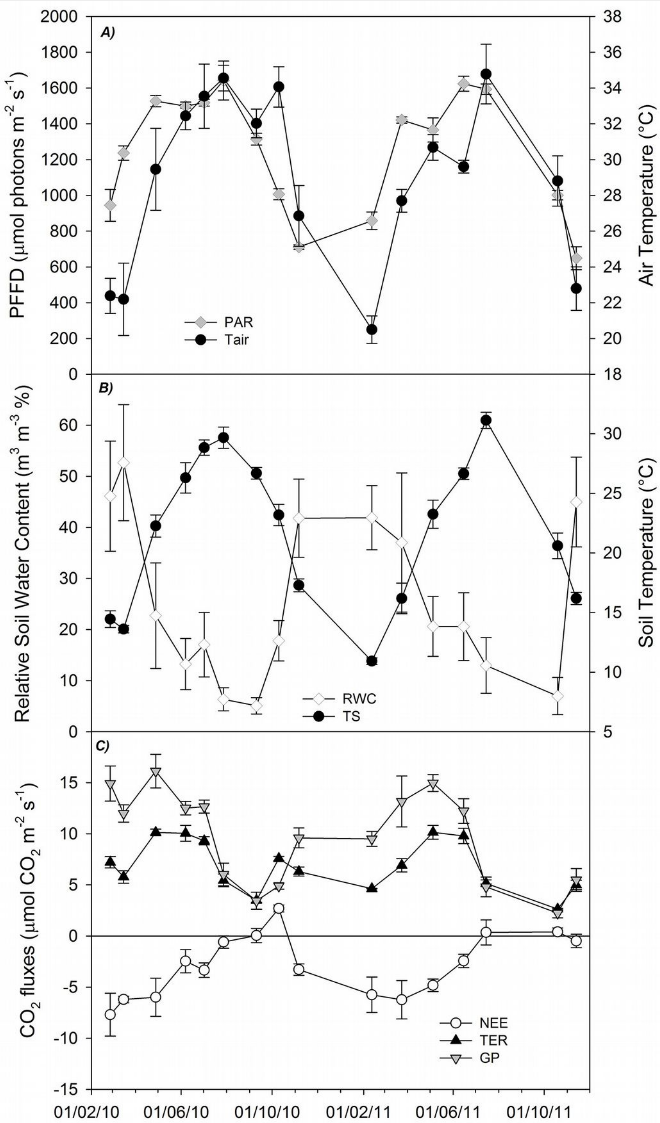


Fig. 6 - Measured values recorded during the field application of the chamber. (A) Air condition inside the chamber: the grey diamonds represent the incident radiation while the black circles represent the air temperature (°C). (B) Soil condition: white diamond and black circle represent soil water content and soil temperature at 5 cm depth, respectively. (C) Gross photosynthesis (GP; grey triangles), Ecosystem Respiration (TER; black triangles) and Net Ecosystem Exchange (NEE; white circle). The bars represent the standard error of the mean (n=6).

spring (16.12 ± 1.64 µmol CO_2 m^{-2} s^{-1}). Afterwards, following the decrease of soil water content, GP started to decline until it reached its minimum in summer (3.45 ± 0.83 µmol CO_2 m^{-2} s^{-1}). Total ecosystem respiration (TER) showed a similar temporal trend (Fig. 6C) increasing from the winter value of 7.22 ± 0.55 µmol CO_2 m^{-2} s^{-1} to the maximum of 10.13 ± 0.33 µmol CO_2 m^{-2} s^{-1} recorded in spring; then gradually decreasing until the minimum of 3.49 ± 0.20 µmol CO_2 m^{-2} s^{-1} recorded at the end of the summer.

Consequently, NEE showed a maximum uptake of CO_2 during late winter-spring (about -7.69 ± 1.48 µmol CO_2 m^{-2} s^{-1}); after this period it gradually reduced, reaching a balance between the GP and TER during the summer (Fig. 6C). This seasonal variation of GP, TER and NEE is similar to that observed in other Mediterranean ecosystems (Xu & Baldocchi 2004, Pereira et al. 2007, Allard et al. 2008) where productivity rates are higher in spring followed by a strong summer reduction connected both to the stomatal and non-stomatal limitation to the photosynthesis (Galmés et al. 2007, Grassi et al. 2009) and to the drought semi-deciduous habitus of *Cistus* species (Werner et al. 1999, De Dato et al. 2013).

The autumnal rains re-activated both photosynthetic and respiratory processes, but to different extent: TER peaked at 7.58 ± 0.21 µmol CO_2 m^{-2} s^{-1}, while GP reached only 4.63 ± 0.03 µmol CO_2 m^{-2} s^{-1}. As a result, the ecosystem became a net source of CO_2 (+2.69 ± 0.32 µmol CO_2 m^{-2} s^{-1}).

The delay of the autumnal recovery of the photosynthesis is due to the summer semi-deciduous habits of *Cistus* in semi-arid environment; meanwhile, the availability of respiratory substrates, derived from leaves fall and fine roots dead during the summer drought (Kuzyakov & Gavrichkova 2010), coupled with favourable soil water conditions (Fig. 6B), substantially increased the respiratory fluxes, as already observed in other arid and semi-arid ecosystems (Jarvis et al. 2007, Pereira et al. 2007, Unger et al. 2012). A comparison of the fluxes measured by our Canopy Chamber with those deriving from comparable or different measuring techniques, in similar environment is limited because of the lack of data. However, GP and TER reported by our investigation are similar to the fluxes measured with a chamber system in a cork oak understorey in Portugal, and mainly composed by *Cistus crispus* and *Cistus salvifolius* (Correia et al. 2014). Moreover the fluxes measured in this study were within the values measured by eddy covariance and reported by Unger et al. (2010) for a savannah-like woodlands during drought period and Reichstein et al. (2002) for a *Juniperus phoenicia L.* shrubland located near our experimental site.

Conclusion

The developed modular Canopy Chamber was a useful tool to measure the CO_2 fluxes at ecosystem level. The chamber tests showed microclimatic disturbances within the range (and in some case outperformed) of other chambers reported in the literature. The error due to the chamber leaks is estimated in 1% per minute; the temperature increment was always below 1.3 °C, the visible radiation reduction due to chamber walls was about 10% and the pressure variations between outside and inside the chamber were in such a range that the gas exchange measurements were not critically altered. In addition, we found that the use of radial ventilators, oriented to move the air in vertical direction, guaranteed an optimal air mixing with the minimum interference with the soil CO_2 effluxes.

Furthermore, the open field application showed the suitability of this instrument to monitor the CO_2 fluxes (NEE, TER and estimated GPP) in remote areas, with relative small and complex plant canopies as a semi-arid Mediterranean garrigue. There, the Canopy Chamber was able to detect CO_2 fluxes in a wide range of environmental conditions, evidencing the high seasonality of the CO_2 fluxes and giving the opportunity to obtain both NEE and TER during daytime. These positive tests and application indicated that this Canopy Chamber could be used in various experimental situations and could give the opportunity to know the dynamics of CO_2 fluxes of shrubland vegetation as well the contribution of understory shrubland vegetation to the whole forest ecosystem fluxes.

Acknowledgements

The authors thank the financial support by the European Union FP7 - Infrastructure-2008-1 project INCREASE, contract no. 227628.

References

Allard V, Ourcival JM, Rambal S, Joffre R, Rocheteau A (2008). Seasonal and annual variation of carbon exchange in an evergreen Mediterranean forest in southern France. Global Change Biology 14: 714-725. - doi: 10.1111/j.1365-2486.2008.01539.x

Atkin O, Tjoelker MG (2003). Thermal acclimation and the dynamic response of plant respiration to temperature. Trends in Plant Science 8:

343-351. - doi: 10.1016/S1360-1385(03)00136-5

Bachman S, Heisler-White JL, Pendall E, Williams DG, Morgan JA, Newcomb J (2010). Elevated carbon dioxide alters impacts of precipitation pulses on ecosystem photosynthesis and respiration in a semi-arid grassland. Oecologia 162: 791-802. - doi: 10.1007/s00442-009-1511-x

Baldocchi DD, Hincks BB, Meyers TP (1988). Measuring biosphere-atmosphere exchanges of biologically related gases with micrometeorological methods. Ecology 69 (5): 1331-1340. - doi: 10.2307/1941631

Beier C, Emmett BA, Tietema A, Schmidt IK, Penuelas J, Láng EK, Duce P, De Angelis P, Gorissen A, Estiarte M, De Dato G, Sowerby A, Kröel-Dulay G, Lellei-Kovács E, Kull O, Mand P, Petersen H, Gjelstrup P, Spano D (2009). Carbon and nitrogen balances for six shrublands across europe. Global Biogeochemical Cycles 23: GB4008. - doi: 10.1029/2008GB003381

Berry J, Bjoerkman O (1980). Photosynthetic response and adaptation to temperature in higher plants. Annual Review of Plant Physiology 31: 491-543. - doi: 10.1146/annurev.pp.31.060180.002423

Burkart S, Manderscheid R, Weigel H (2007). Design and performance of a portable gas exchange chamber system for CO_2- and H_2O-flux measurements in crop canopies. Environmental and Experimental Botany 61: 25-34. - doi: 10.1016/j.envexpbot.2007.02.007

Correia A, Costa E, Silva F, Correia A, Hussain M, Rodrigues A, David J, Pererira J (2014). Carbon sink strength of a Mediterranean cork oak understorey: how do semi-deciduous and evergreen shrubs face summer drought? Journal of Vegetation Science 25: 411-426. - doi: 10.1111/jvs.12102

De Dato G, De Angelis P, Sirca C, Beier C (2010). Impact of drought and increasing temperatures on soil CO_2 emissions in a Mediterranean shrubland (gariga). Plant and Soil 327: 153-166. - doi: 10.1007/s11104-009-0041-y

De Dato G, Micali M, Abou Jaoudé R, Liberati D, De Angelis P (2013). Earlier summer drought affects leaf functioning of the Mediterranean species *Cistus monspeliensis* L. Environmental and Experimental Botany 93: 13-19. - doi: 10.1016/j.envexpbot.2013.03.007

De Dato G, Pellizzaro G, Cesaraccio C, Sirca C, De Angelis P, Duce P, Spano D, Scarascia Mugnozza G (2008). Effects of warmer and drier climate conditions on plant composition and biomass production in a Mediterranean shrubland community. iForest - Biogeosciences and Forestry 1 (1): 39-48. - doi: 10.3832/ifor0418-0010039

Fang C, Moncrieff JB (1998). An open-top chamber for measuring soil respiration and the influence of pressure difference on CO_2 efflux measurement. Functional Ecology 12: 319-325. - doi: 10.1046/j.1365-2435.1998.00189.x

Foken T, Aubinet M, Leuning R (2012). The Eddy covariance method. In: "Eddy Covariance: a Pratical Guide to Measurement and Data" (Aubinet M, Vesala T, Papale D eds). Springer, Dordrecht, Netherlands, pp. 438. - doi: 10.1007/978-94-007-2351-1_1

Galmés J, Medrano H, Flexas J (2007). Photosynthetic limitations in response to water stress and recovery in Mediterranean plants with different growth forms. New Phytologist 175: 81-93. - doi: 10.1111/j.1469-8137.2007.02087.x

Grassi G, Ripullone F, Borghetti M, Raddi S, Magnani F (2009). Contribution of diffusional and non-diffusional limitations to midday depression of photosynthesis in *Arbutus unedo* L. Trees 23: 1149-1161. - doi: 10.1007/s00468-009-0355-7

Grau A (1995). A closed chamber technique for field measurement of gas exchange of forage canopies. New Zealand Journal of Agricultural Research 38: 71-77. - doi: 10.1080/00288233.1995.9513105

Hanson PJ, Wullschleger SD, Bohlman S, Todd DE (1993). Seasonal and topographic patterns of forest floor CO_2 efflux from an upland oak forest. Tree Physiology 13: 1-15. - doi: 10.1093/treephys/13.1.1

Heijmans M, Arp W, Chapin III F (2004). Carbon dioxide and water vapour exchange from understory species in boreal forest. Agricultural and Forest Metereology 123: 135-147. - doi: 10.1016/j.agrformet.2003.12.006

Held AA, Steduto P, Orgaz F, Matista A, Hsiao TC (1990). Bowen ratio/energy balance technique for estimating crop net CO_2 assimilation, and comparison with a canopy chamber. Theoretical and Applied Climatology 42: 203-213. - doi: 10.1007/BF00865980

Jarvis P, Rey A, Petsikos C, Wingate L, Rayment M, Pereira J, Banza J, David J, Miglietta F, Borghetti M, Manca G, Valentini R (2007). Drying and wetting of Mediterranean soils stimulates decomposition and carbon dioxide emission: the "Birch effect". Tree Physiology 27: 929-940. - doi: 10.1093/treephys/27.7.929

Kutsch W, Staack A, Wotzel U, Middelhoff U, Kappen L (2001). Field measurement of root respiration and total soil respiration in an alder forest. New Phytologist 150: 157-168. - doi: 10.1046/j.1469-8137.2001.00071.x

Kutzbach L, Schenider J, Sachs T, Giebels M, Nvkanen H, Shurpali N, Martikainen P, Alm J, Wilmking M (2007). CO_2 flux determination by closed-chamber methods can be seriously biased by inappropriate application of linear regression. Biogeosciences 4: 1005-1025. - doi: 10.5194/bg-4-1005-2007

Kuzyakov Y, Gavrichkova O (2010). Time lag between photosynthesis and carbon dioxide efflux from soil: a review of mechanisms and controls. Global Change Biology 16: 3386-3406. - doi: 10.1111/j.1365-2486.2010.02179.x

Leadley PW, Drake BG (1993). Open top chambers for exposing plant canopies to elevated CO_2 concentration and for measuring net gas exchange. Vegetatio 104-105: 3-15. - doi: 10.1007/BF00048141

LI-COR (2010). Theory of operation (chapt. 4). In: "LI-8100A Automated Soil CO2 Flux System LI-8150 Multiplexer Instruction Manual". LI-COR, Lincoln, NE, USA, pp. 2-12.

Livingston G, Hutchinson G (1995). Enclosure-based measurement of trace gas-exchange: applications and sources of error. In: "Biogenic Trace Gases: Measuring Emissions from Soil and Water" (Matson PA, Harris RC eds). Blackwell Scientific Publication, London, UK, pp. 14-51. [online] URL: http://books.google.com/books?id=WDjpgK7IQAgC

Livingston G, Hutchinson G, Spartalian K (2005). Diffusion theory improves chamber-based measures of trace gas emissions. Geophysical Research Letters 32: L24817. - doi: 10.1029/2005GL024744

Lund CP, Riley WJ, Pierce LL, Field CB (1999). The effects of chamber pressurization on soil-surface CO_2 flux and the implications for NEE measurements under elevated CO_2. Global Change Biology 5: 269-281. - doi: 10.1046/j.1365-2486.1999.00218.x

Medhurst J, Parsby J, Linder S, Wallin G, Ceschia E, Slaney M (2006). A whole-tree chamber system for examining tree-level physiological responses of field-grown trees to environmental variation and climate change. Plant Cell and Environment 29: 1853-69. - doi: 10.1111/j.1365-3040.2006.01553.x

Muller J, Eschenroder A, Diepenbrock W (2009). Through-flow chamber CO_2/H_2O canopy gas exchange system - construction, microclimate, errors, and measurements in a barley (*Hordeum vulgare* L.) field. Agricultural and Forest Meteorology 149: 214-229. - doi: 10.1016/j.agrformet.2008.08.007

Pereira JS, Mateus JA, Aires LM, Pita G, Pio C, David JS, Andrade V, Banza J, David TS (2007). Net ecosystem carbon exchange in three contrasting Mediterranean ecosystems - the effect of drought. Biogeosciences 4: 791-802. - doi: 10.5194/bg-4-791-2007

Pérez-Priego O, Testi L, Orgaz F, Villalobos FJ (2010). A large closed canopy chamber for measuring CO_2 and water vapour exchange of whole trees. Environmental and Experimental Botany 68: 131-138. - doi: 10.1016/j.envexpbot.2009.10.009

Pérez-Priego O, Lopez-Ballesteros A, Sanchez-Canete E, Serrano-Ortiz P, Kutzbach L, Doming F, Eugster W, Kowalski A (2015). Anaysing uncertainties in the calculation of fluxes using whole plant chambers: random and systemaic errors. Plant and Soil 393: 229-244. - doi: 10.1007/s11104-015-2481-x

Pickering N, Jones J, Boote K (1993). Evaluation of the portable chamber technique for measuring canopy gas exchange by crops. Agricultural and Forest Meteorology 63: 239-254. - doi: 10.1016/0168-1923(93)90062-M

Pumpanen J, Kolari P, Ilvesniemi H, Minkkinen K, Vesala T, Niinistö S, Lohila A, Larmola T, Morero M, Pihlatie M, Janssens I, Yuste JC, Grünzweig JM, Reth S, Subke J, Savage K, Kutsch W, Ostreng G, Ziegler W, Anthoni P, Lindroth A, Hari P (2004). Comparison of different chamber techniques for measuring soil CO_2 efflux. Agricultural and Forest Meteorology 123: 159-176. - doi: 10.1016/j.agrformet.2003.12.001

Reichstein M, Tenhunen J, Roupsard O, Ourcival JM, Rambal S, Dore S, Valentini R (2002). Ecosystem respiration in two Mediterranean evergreen Holm Oak forests: drought effects and decomposition dynamics. Functional Ecology 16: 27-39. - doi: 10.1046/j.0269-8463.2001.00597.x

Reicosky DC (1990). Canopy gas exchange in the field: closed chambers. Remote Sensing Reviews 5: 163-177. - doi: 10.1080/02757259009532127

Savage KE, Davidson EA (2003). A comparison of manual and automated systems for soil CO_2 flux measurements: trade-offs between spatial

and temporal resolution. Journal of Experimental Botany 54: 891-899. - doi: 10.1093/jxb/erg121
Schaaf S, Dämmgen U, Grünhage L, Burkart S (2005). The assessment of water vapour and carbon dioxide fluxes above arable crops - a comparison of methods. Meteorologische Zeitschrift 14 (2): 151-155. - doi: 10.1127/0941-2948/2005/0016
Steduto P, Cetinkökü Ö Albrizio R, Kanber R (2002). Automated closed-system canopy-chamber for continuous field-crop monitoring of CO_2 and H_2O fluxes. Agricultural and Forest Meteorology 111: 171-186. - doi: 10.1016/S0168-1923(02)00023-0
Stoyan H, De-Polli H, Böhm S, Robertson GP, Paul EA (2000). Spatial heterogeneity of soil respiration and related properties at the plant scale. Plant and Soil 222: 203-214. - doi: 10.1023/A:1004757405147
Takle E, Brandle JR, Schmidt RA, Garcia R, Litvina IV, Massman WJ, Zhou X, Doyle G, Rice C (2003). High-frequency pressure variations in the vicinity of a surface CO_2 flux chamber. Agricultural and Forest Meteorology 114: 245-250. - doi: 10.1016/S0168-1923(02)00174-0
Takle ES, Massman WJ, Brandle JR, Schmidt RA, Zhou X, Litvina IV, Garcia R, Doyle G, Rice C (2004). Influence of high-frequency ambient pressure pumping on carbon dioxide efflux from soil. Agricultural and Forest Meteorology 124: 193-206. - doi: 10.1016/j.agrformet.2004.01.014
Unger S, Maguas C, Pereira J, Aires L, David T, Werner C (2010). Disentangling drought-induced variation in ecosystem and soil respiration using stable carbon isotopes. Oecologia 163: 1043-1057. - doi: 10.1007/s00442-010-1576-6
Unger S, Máguas C, Pereira JS, David TS, Werner C (2012). Interpreting post-drought rewetting effects on soil and ecosystem carbon dynamics in a Mediterranean oak savannah. Agricultural and Forest Meteorology 154-155: 9-18. - doi: 10.1016/j.agrformet.2011.10.007
Vico G, Manzoni S, Palmroth S, Katul G (2011). Effects of stomatal delays on the economics of leaf gas exchange under intermittent light regimes. New Phytologist 192: 640-652. - doi: 10.1111/j.1469-8137.2011.03847.x
Werner C, Correia O, Beyschlag W (1999). Two different strategies of Mediterranean macchia plants to avoid photoinhibitory damage by excessive radiation levels during summer drought. Acta Oecologica 20: 15-23. - doi: 10.1016/S1146-609X(99)80011-3
Xu L, Baldocchi DD (2004). Seasonal variation in carbon dioxide exchange over a Mediterranean annual grassland in California. Agricultural and Forest Meteorology 123: 79-96. - doi: 10.1016/j.agrformet.2003.10.004

Supplementary Material

Fig. S1 - The canopy chamber system.

Fig. S2 - Time course of differences between inside-outside chamber pressure (ΔP), H_2O concentration and chamber temperature during the 90 seconds of a typical measurement.

Link: Guidolotti_2209@suppl001.pdf