DOI: 10.1007/s11099-015-0086-5 PHOTOSYNTHETICA 53 (1): 72-84, 2015

Easy-to-make portable chamber for *in situ* CO_2 exchange measurements on biological soil crusts

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Abstract

Commercial chambers for *in vivo* gas exchange are usually designed to measure on vascular plants, but not on cryptogams and other organisms forming biological soil crusts (BSCs). We have therefore designed two versions of a chamber with different volumes for determining CO_2 exchange with a portable photosynthesis system, for three main purposes: (*1*) to measure *in situ* CO_2 exchange on soils covered by BSCs with minimal physical and microenvironmental disturbance; (*2*) to acquire CO_2-exchange measurements comparable with the most widely employed systems and methodologies; and (*3*) to monitor CO_2 exchange over time. Different configurations were tested in the two versions of the chamber and fluxes were compared to those measured by four reference commercial chambers: three attached to two respirometers, and a conifer chamber attached to a portable photosynthesis system. Most comparisons were done on biologically crusted soil samples. When using devices in a closed system, fluxes were higher and the relationships to the reference chambers were weaker. Nevertheless, high correlations between our chamber operating in open system and measurements of commercial respiration and photosynthetic chambers were found in all cases ($R^2 > 0.9$), indicating the suitability of the chamber designed for *in situ* measurements of CO_2 gas exchange on BSCs.

Additional key words: chamber; cyanobacteria; infrared gas analyzer; lichen; moss; net photosynthesis; soil respiration.

Introduction

In the context of increasing interest in more accurate local and global carbon balance measurements, many studies have analyzed the conditions under which different plant functional types, such as broadleaf and needle trees, shrubs, and herbaceous C_3 and C_4 plants, are carbon sources or sinks (*e.g.*, Cox *et al*. 2000, Sitch *et al*. 2003, Friedlingstein *et al*. 2006, Magnani *et al*. 2007). However, the relationship with carbon acquisition or loss by gas exchange of biological soil crusts (BSCs), defined as communities of cyanobacteria, algae, mosses, lichens, and fungi in differing proportions, living on top of or within the first few millimeters of the soil surface, and closely bound to soil particles (Belnap *et al*. 2003), is still little known (Bowling *et al*. 2011). BSCs are especially important in the global CO_2 gas exchange in dryland ecosystems where they predominate and vascular plant productivity is limited (Lange *et al*. 1992, Lange 2003). Indeed, there is evidence of their major contribution to soil respiration in semiarid ecosystems (Castillo-Monroy *et al*. 2011), as well as their photosynthetic capacity under

Received 6 December 2013, *accepted* 18 August 2014.
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Abbreviations: BSC(s) – biological soil crust(s); [CO_2] – CO_2 concentration; IRGA – infrared gas analyzer; RH – relative humidity.
Acknowledgements: The authors are grateful for the assistance provided by Victoria Ochoa, Beatriz Gozalo, Isabel Miralles and Emilio Rodríguez. We also thank AEMET for providing meteorological data. This research was carried out under the PREVEA (CGL2007-63258/BOS), CARBORAD (CGL2011-27493) and Bacarcos (CGL2011-29429) projects, funded by the Spanish National Plan for RD&I and by the European ERDF (European Regional Development Fund), BIOCOM (ERC Grant agreement 242658) funded by the European Research Council under the European Community's Seventh Framework Programme (FP7/2007-2013), as well as the COSTRAS (RNM-3614) and GEOCARBO (P08-RNM-3721) projects, both funded by the Junta de Andalucía (Autonomous Regional Government of Andalusia, Spain) and the European Union ERDF. M. Ladrón de Guevara and S. Chamizo received postgraduate fellowships from the Spanish National Research Council (CSIC, JAE-Pre Grants 2007-029) and the Spanish Government (BES-2007-15218), respectively.

appropriate moisture conditions (*e.g.*, Brostoff *et al*. 2005, Li *et al*. 2012). Global net carbon uptake by cryptogamic ground cover has been estimated at about 2.5 Pg yr^{-1} (Elbert *et al*. 2012), which corresponds to around 4.5% of net primary production by terrestrial vegetation.

Infrared gas analyzers (IRGAs) have been used for decades to study CO_2 exchange in several types of organisms, such as vascular plants, cyanobacteria, mosses, and algae (*e.g.,* Bloom *et al*. 1980, Dring and Brown 1982, Schipperges and Rydin 1998, Brostoff *et al*. 2002, Millan-Almaraz *et al*. 2009). There are two main IRGA operating systems: open (*i.e.*, where chamber air is renewed with ambient air from outside and CO_2 flux is calculated as the difference in CO_2 concentration ($[CO_2]$) between air in the chamber and incoming ambient air), and closed (*i.e.*, where air is not renewed from outside the system and CO_2 flux is calculated as the change in chamber $[CO_2]$ during the sample enclosure time). Both systems have advantages and disadvantages, which can be maximized and minimized, respectively, depending on a chamber design and configuration. The closed system was initially considered more accurate. It requires short measurement time, because, contrary to the open system, it does not need concentration gradient stabilization (Hutchinson and Mosier 1981, Sebacher and Harriss 1982, Mosier 1989, Vourlitis *et al*. 1993). This becomes more advantageous as the volume of the chamber increases (Hutchinson and Mosier 1981, Livingston and Hutchinson 1995). However, recently improved open system technology provides increased accuracy (*LI-COR* 2003, 2012), whereas the main disadvantage of the closed system, the gradual alteration of the initial concentration of gases inside the chamber over time persists (Nakayama 1990, Hutchinson *et al*. 2000, Davidson *et al*. 2002).

Although some studies have used chambers in closed systems (*e.g.*, Kershaw 1977, Snelgar *et al*. 1980), CO_2 fluxes in epiphytic, saxicolous, and terricolous lichens have been usually measured with chambers coupled to open system IRGAs, in *ex situ* controlled environments (*e.g.*, Carstairs and Oechel 1978, Lange *et al*. 1986, 1992; Friedmann *et al*. 1993, Zaady *et al*. 2000). These *ex situ* studies have provided valuable information for interpreting the responses of lichens and BSCs in their natural habitats (Lange *et al*. 2001), but they have not shown how far the laboratory response measurements differ from *in situ* responses. For instance, they require samples to be removed from their underlying soil, and there is little knowledge of how BSC removal (and often thallus clipping) affects the functioning of the component organisms. Even in field work, when the BSC removed from the soil is measured immediately in a cuvette, problems may linger due to the disruption of substrate water supply (Schroeter *et al*. 1999). In addition, these studies usually have a physiological approach, focusing only on BSC gas-exchange performance. However, in an ecological approach considering ecosystem implications, it is also necessary to take into account the contribution of the underlying soil, and therefore measurement of the whole soil profile becomes an advantage in quantifying the net ecosystem CO_2 balance. Despite these questions, there are few studies examining *in situ* net CO_2 exchange on biologically crusted soils, probably because the need for a more holistic approach to the subject has not come up until recent years.

As BSCs are very sensitive to humidity and temperature pulses (Grote *et al*. 2010, Bowling *et al*. 2011), reducing the enclosure time is critical to alter these two environmental factors as little as possible. Commercial canopy chambers which can be coupled to the soil surface, such as *LICOR* and *PP-Systems*, cannot deal with this problem properly, as their volumes are not adapted to short measurement times. To overcome this drawback, we have designed a robust, easy-to-make chamber for short *in situ* measurements of net CO_2 gas exchange on undisturbed BSCs, and tested two versions with different diameters.

This matter is currently arousing considerable interest, as shown by the growing number of studies based on customized chambers to be coupled to the soil surface (Bremer and Ham 2005, Botting and Fredeen 2006, Langensiepen *et al*. 2012). However, their conclusions are unclear because their different methodologies are not fully comparable. Therefore, the aim of this study was to standardize the *in situ* gas-exchange measurements on biologically crusted soils by (*a*) describing and discussing chamber design, and (*b*) determining its specific measurement configurations by comparison with widely used commercial chambers. Specific steps for this purpose were: (*1*) to find the best combination of input airflow rate and fan speeds (internal system fan and additional fan inside the chamber if needed) as well as the best operation measurement system (closed or open); (*2*) then with the best chamber configuration, to verify the validity of the chamber design for measuring CO_2 fluxes by increasing the number of regression points in system comparisons and extending their ranges; and finally, (*3*) to examine any possible microenvironmental disturbances that could be caused by our chamber, especially keeping in mind that the BSC gas exchange is particularly sensitive to changes in temperature and humidity.

Materials and methods

Description of the chamber: An 8.5-cm-high transparent chamber was constructed from methacrylate tubes (supplied by *Rotufer S.L.*, Almería, Spain; and *Decorplax Metacrilatos S.L.*, Madrid, Spain). Most chambers used for this purpose are made of methacrylate because of its uniform 92% transmissivity at 400–800 nm wavelength light, and because it is lightweight, low-cost, shockproof, and easy to handle and to bond with solvent glue (Bloom

et al. 1980). However, its water adsorption is high, which is a problem if in addition to photosynthesis there is interest in evapotranspiration. Therefore, to avoid adsorption/desorption of water vapor, the inner chamber walls were covered with a *Teflon*® film, which is also highly transparent in the 300–900 nm range (Kesselmeier *et al*. 1996, Cocker *et al*. 2001). The top methacrylate surface was designed flat instead of the spherical shape used in other commercial chambers for easier manufacturing and handling in the field, and to reduce the measurement time due to the lower chamber volume. The top can also be made with propafilm (provided by *LI-COR Biosciences Inc.*, NE, USA), but we preferred a hard cover to make it more robust.

As an interface between the chamber and the IRGA (portable photosynthesis system *LI-6400*; *LI-COR Biosciences Inc.*, NE, USA), we used a *9864-157* mounting plate (*LI-COR Biosciences Inc.*, NE, USA) from a *Conifer Chamber 6400-05*. A flat frontpiece of the same size as the metal plate was placed on the outside of the acrylic cylinder, and a methacrylate reinforcement was added inside the cylinder. A 0.5-cm-thick rubber gasket was added to the plate to seal the connection. We included an E-type thermocouple (not encapsulated) to record the surface temperature required for calculation of certain variables (such as vapor pressure deficit, total conductance to CO_2 and H_2O, or intercellular CO_2), as this temperature cannot be well estimated from air temperature with energy balance parameters in BSCs. An exhaust tube was inserted in a female hose fitting to route the air from the chamber to the match valve. The exhaust tube was placed at a 90° angle from the metal plate, far away enough to avoid shading. The chamber was designed to work on top of PVC collars previously inserted in the soil (*see* Castillo-Monroy *et al*. 2011 and Escolar *et al*. 2012 for this procedure). To ensure that the chamber was sealed on the soil, a toroidal disk covered with an air-tight rubberized band was placed between the chambers and the PVC collars inserted in the soil.

The use of different chamber diameters provides different possibilities, mainly related to the plot size required for each study target, whether almost isolated BSC species (small chamber) or BSC communities (large diameter chamber) are to be sampled. We therefore made two versions of the chamber with different diameters (Fig. 1), 10 and 20 cm [668 and 2,385 cm^3, respectively]. The large chamber had an additional 5 × 5 cm^2 fan inside, also at a 90° angle from the metal plate (opposite the match exhaust tube), to minimize shading and optimize air circulation, and to ensure that air entering the chamber was well mixed. It was powered by a 12 V-7A battery connected to a voltage regulator so that the optimal fan speed could be selected. The holes for inserting the thermocouple and the fan cable in the chambers were sealed with *Terostat-81*®, which is neither gas source nor sink.

Comparison with commercial chambers using BSCs under laboratory conditions: As further precautions must be taken to operate with larger volumes (for example, measurement times and proper air mixing are more critical), we started the two comparative tests of this section on the larger chamber. The tests were also conducted in the small chamber to ensure that the selected configuration was appropriate for its volume, but with some abbreviations based on the previous results. As the core of these tests was the selection of the best chamber configuration for measuring on BSCs, they were performed under controlled laboratory conditions, using unaltered soil samples with three types of BSC: with a cover close to 100% of (*1*) *Diploschistes diacapsis* (Ach.) Lumbsch lichen, (*2*) *Cladonia convoluta* (Lam.) Anders lichen, and (*3*) mosses (*Tortula* sp., *Crossidium* sp., and *Didymodon* sp.). PVC cylinder diameters of 10 cm (four samples for each crust type) and 20 cm (three replicates) were used to extract 6-cm-thick samples previously moistened to minimize damage to the crust. A brief description of the sampling sites can be found in the supplementary material (Figs. 1S, 2S - *available online*).

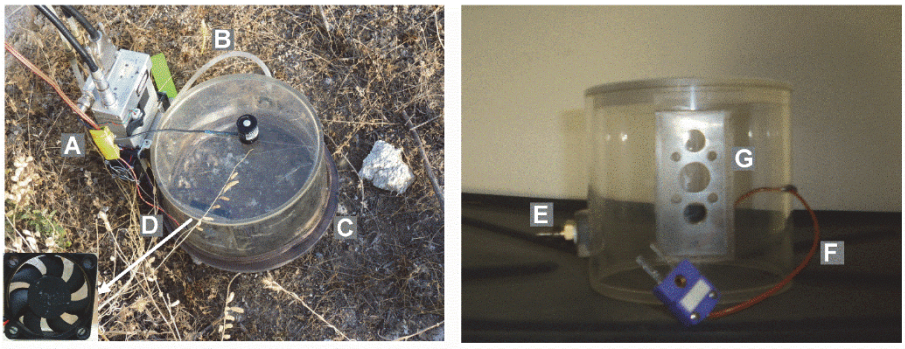

Fig. 1. *Left*: Large chamber with details of the fan voltage regulator (A), exhaust tube (B), toroidal disk (C), additional fan (D), with a detailed in the bottom left-hand corner insert. *Right*: Small chamber with details of the female hose fitting (E), thermocouple (F), and the flat frontpiece with three large holes matching the mounting plate, and another four for the screws (G).

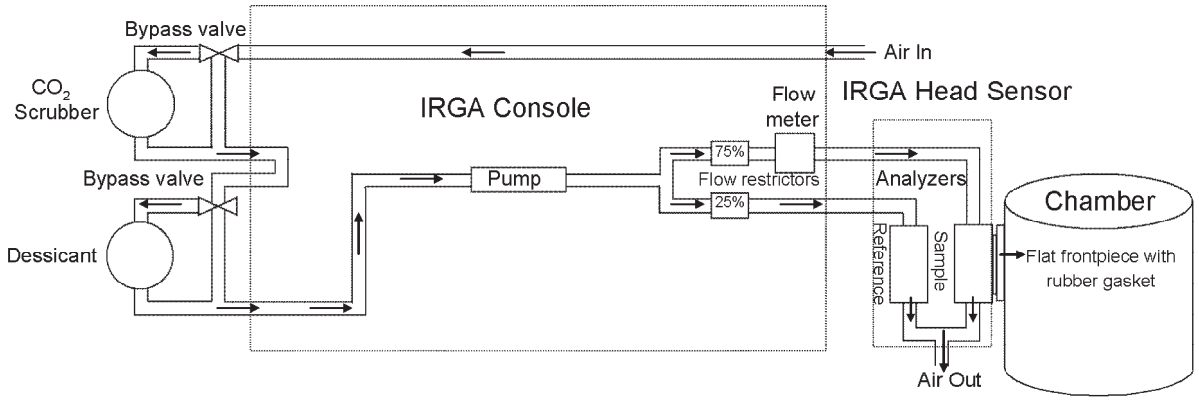

Fig. 2. Schematic flows in an IRGA connected to our chamber, based on *Li-6400/6400XT*. The IRGA system can modify CO_2 and H_2O concentrations in the incoming air by means of chemical tubes (circles). The pump speed controls the flow.

Prior to measurements, both small and large samples were moistened with 60 and 200 ml of distilled water, respectively, in order to enhance the biological activity of the soil, and thereby to gain a stronger respiratory signal. Measurements started at least 30 min after moistening to ensure steady CO_2 flux. *LI-6400* system flow and IRGA CO_2 zeros were calibrated before each test, and when this device was used in the open system (Fig. 2), sample and reference IRGAs were matched before each measurement. During these measurements, we covered the transparent chambers with an opaque cloth to prevent photosynthetic activity, and CO_2 flux recorded in each sample was related to the measurement immediately following it by an opaque commercial chamber attached to the soil respirometer *LI-8100* (*LI-COR Biosciences Inc.*, NE, USA) or *EGM-4* (*PP-Systems,* Hitchin, UK). The *LI-8100* was used for comparison to the two prototype chambers because it has two chambers with the same diameters as ours (10 and 20 cm). Only the small chamber was compared to the *EGM-4* respirometer, as this model only comes with a 10-cm-diameter chamber. Measurement time over the same sample was configured as 90 s, and data were stored every 5 s. The CO_2 flux measurements were adjusted to exponential and quadratic functions in the *LI-8100* and *EGM-4,* respectively, to acquire the final sample flux. The use of a linear function is not recommended, as gas diffusion theory predicts that the rate of gas exchange across the soil-atmosphere interface is not constant over the measurement period (Livingston *et al*. 2005). Absolute CO_2 flux was used for comparison between our chambers and commercial chambers, as the respiration and net photosynthesis systems have different signs.

Test 1. Determination of the best airflow and additional ventilation speed: With the larger chamber, always operating as an open system and with the internal fan at its highest speed, two airflow rates (300 and 700 μmol s^{-1}) were combined with three air speeds provided by the additional fan (0.7, 1.1, and 1.4 m s^{-1}). When the best combination of fan speed and airflow rate was found, based on the best fit of these measurements to the reference system (*LI-8100*), a last measurement was taken to check the effect of changing the speed of the sample IRGA's internal fan to low. Measurements were also performed without additional ventilation and with the best airflow rate previously found. In the small chamber, the airflow rate, additional and internal fan speed parameters were set according to the results of the large chamber comparisons, and the effect of additional ventilation was tested by comparing this chamber measurements, with and without fan, with those acquired by the *EGM-4* reference chamber.

Test 2. Open *vs.* closed system configuration: The respirometers used in this study operated in a closed system. In the IRGA attached to the prototype chambers (*LI-6400*), either open or closed system configurations may be selected. We tried both configurations with the samples, comparing their respiration responses measured by our custom chambers with those recorded by the *LI-8100* respirometer. The open system was configured with the best input airflow rate and fan speed combination found in the first test. All measurements in this system were taken after the gas exchange inside the chamber had stabilized (*i.e.*, the decimals in the photosynthetic rate were stable for 1 min). However, when measuring in the closed system, we used a procedure similar to that for the respirometers: the gas-exchange rate was recorded every 5 s for 90 s, and the final rate was found by regressing the individual rates over time. The fan speeds (additional and internal) were the same as for the open system, but in the closed system air was not recirculated by the pump.

Outdoor comparison tests: We carried out another two tests with the best chamber configurations found in the section above. The aim of these tests was to extend the data range of the regressions by measuring net photosynthesis under natural light conditions using the commercial *6400-05 Conifer Chamber* (*LI-COR Biosciences Inc.*, NE, USA) as the reference system, and increasing the number of points in the regressions using both respiration devices

for respiration measurements. The *LI-6400* calibration protocol and configuration of the respirometers were the same as in the section above.

Test 3. Net photosynthesis with our large chamber using vascular plants: As the *6400-05 Conifer Chamber* is unsuitable for soil measurements, we compared the CO_2 fluxes measured by our large chamber (without the opaque cloth) with this commercial chamber, operated by another *LI-6400* IRGA, using two vascular plants (*Ceratonia siliqua* L. and *Aspidistra* sp.). To close the custom chamber, a metal plate was placed on the bottom, and sealed with a gasket along the lower edge of the chamber wall. To eliminate the effect of any differences between the two *LI-6400s* in the comparison, both devices were previously compared using two of our large chamber prototypes with the same samples as in the preliminary comparison as well as with a vascular plant (*Aspidistra* sp.) to extend the measurement range. The small chamber was not used in this test.

Test 4. Respiration with our chambers using ruderal and biologically crusted soils*:* Our large chamber was compared to the *LI-8100* chamber under natural conditions. PVC collars (16) with a diameter of 20 cm were installed in the soil in an area with ruderal vegetation (weeds growing in human-disturbed fields). This soil was selected to widen the respiration range, as the previous crusted soils collected from semiarid ecosystems are characterized by low CO_2 efflux (Raich and Schlesinger 1992, Rey *et al*. 2011). Herbaceous plants within the collars were removed, and soil respiration disturbance caused by collar installation was allowed to stabilize for 24 h. Half of the collar enclosures were left with their natural moisture, and the other half was moistened with increasing amounts of water from 100 to 1,000 ml to acquire a wide range of respiration. Measurement started after 12 h, alternating plots with and without moistening, first with the prototype chamber connected to the *LI-6400*, which was covered to ensure darkness like the opaque respirometer chamber, and then with the *LI-8100* chamber. The measurements were carried out from 09:00 to 19:00 h (local time) in a total of six measurement cycles.

We compared our small chamber to an *EGM-4* chamber under seminatural conditions, *i.e.*, unaltered samples were *ex situ*, but measured outdoors. Samples of soil (12) with four kinds of BSC were collected, with three replicates for each type characterized by having a cover close to 100% of: (*1*) *D. Diacapsis* lichen, (*2*) *Squamarina lentigera* (Weber) Poelt lichen, (*3*) well-developed cyanobacterial BSC, including pioneer lichens, and (*4*) incipient-cyanobacterial BSC. An analysis of the cyanobacterial community composition was not performed in this study, but based on a recent publication (Büdel *et al*. 2014), it is likely that *Microcoleus* sp. and *Nostoc* sp. were present in these samples. The measurements were taken for two days, the first with the samples watered to saturation, and the second with the remaining moisture after 24 h of outdoor evaporation. Respiration of each sample (one cycle per day) was recorded with the prototype chamber connected to the *LI-6400* (also covered with an opaque cloth), and immediately afterwards with the *EGM-4* chamber.

Testing the influence of the chamber on micrometeorological variables: As a change in natural air pressure can directly affect gas diffusion in the soil-atmosphere interface (Kanemasu *et al*. 1974, Schlesinger 1977), the relationship between airflow rate and/or additional ventilation and the pressure inside the large chamber must be carefully supervised. And, as mentioned above, BSCs are very sensitive to humidity and temperature micrometeorological variables, thus any effect of the sample enclosure on these variables should also be checked.

Test 5. Check for changes in air pressure inside the chamber: The pressure inside the large chamber was monitored under laboratory conditions by inserting an *MSR 145* pressure sensor (*MSR Electronics GmbH*, Henggart, Switzerland) in the chamber. The threshold, where pressure changes affect BSC CO_2 measurements, was also tested by generating an artificial overpressure inside the chamber. To cause this overpressure, an additional airflow introduced through the thermocouple orifice was provided by a bottle with 509.5 ppm [CO_2]. The [CO_2] of the flow generated by the *LI-6400* was regulated by the *6400-01* CO_2 injector, and was the same as the extra airflow to avoid changes in the reference [CO_2] due to the use of two different flow sources. The enclosure effect in the small chamber was tested by selecting the best configuration found in the previous tests and without artificial overpressure.

Test 6. Check for changes in air temperature and relative humidity (RH) inside the chambers: This test was performed in the large chamber under field conditions from dawn to noon on a summer day to cover wide natural temperature and solar radiation ranges. A *DS1923 iButton® Temperature/Humidity Logger* (*Dallas Semiconductor*, Texas, USA) was placed next to the large chamber and another one inside it, both directly over the BSC surface. Since soil water content on that test day was low (on average, 4% at a depth of 5 cm), and therefore, evaporation was also low, we used a series of measurements taken with the prototype chamber on several sunny days with the high soil water content (on average, 21% at a depth of 5 cm) as recorded on data loggers (model *U23-001, HOBO Pro V2 Temp/RH Data Logger*, *Onset Computer Corporation*, Bourne, MA, USA) placed 20 cm above the soil surface. In this case, the chamber air RH measured by the *LI-6400* was compared with data logger records. The temperature test was not done in the small chamber, since the most influential factors in its change

(material spectral properties and air renewal) were the same as in the larger one. RH changes in the small chamber were checked using the *LI-6400* RH sample sensor records from Test 4 and environmental RH data from a nearby weather station provided by the Spanish Meteorological State Agency *(*AEMET*)*.

Results

Test 1. Determination of the best airflow and additional ventilation speed: The use of a low airflow caused that the flux measured in our large chamber was lower than the *LI-8100* respirometer data, which was three-fold higher (Fig. 3). In all cases, the data acquired in our large chamber were lower than those with the *LI-8100* respirometer, but this difference could be reduced by raising the airflow to a ratio of 1.5:1. Increasing the speed of the additional fan worsened the correlation between the two CO_2 flux measurement systems. The configuration with a slow fan speed had the best R^2 using both high and low airflow rates. We selected a high airflow rate with slow fan speed as the optimum combination because the measurements were nearest the reference system, the R^2 was high, and the chamber stabilization time was half that required at the low airflow rate. Therefore, the effect of the sample IRGA mixing fan speed was tested only with these optimal parameters. Best results were found with the IRGA fan at high speed (Table 1S – *supplementary material available online*; Fig. 4*A*), since although its regression was below the 1:1 line, their slopes were closer. We also found that, when the internal fan speed was lowered, R^2 fell from 0.83 to 0.59. The regression models fitted well with either low additional fan speed or without a fan, but with the first, *LI-6400* data were slightly higher and closer to those of *LI-8100* (Fig. 4*A*). An additional fan placed inside the small chamber and set at slow speed generally did not raise the measurements compared to the mode without ventilation (Fig. 4*B*), indicating that there were no problems of homogenization of the sample air in the absence of an additional fan.

Test 2. Open *vs*. closed system chamber configuration: In the large chamber, with the airflow and fan speed optimized, the coefficient of determination between the *LI-6400* and the *LI-8100* (respirometer) was substantially higher in the open system than in the closed system. In the open system, the regression slope was similar to the

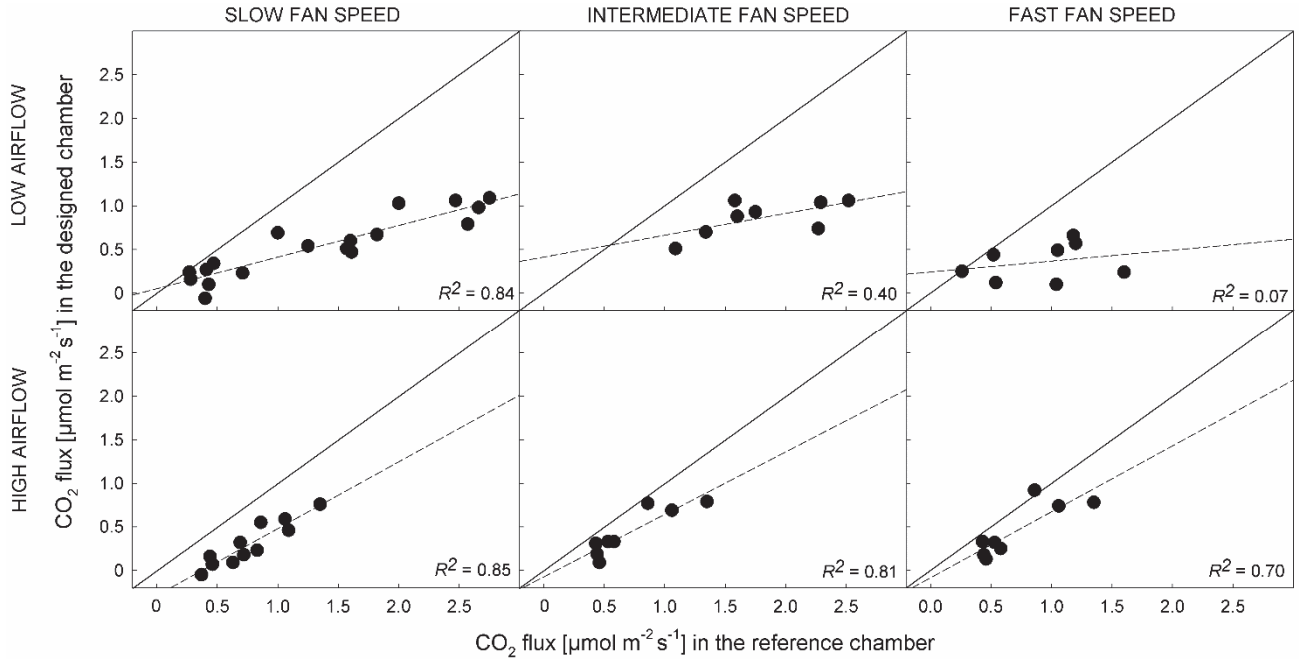

Fig. 3. Relationship between the respiration measured by our large chamber, attached to an *LI-6400*, and the reference system (respirometer *LI-8100*), at different airflow rates and additional fan speeds. *Dashed lines* – linear regressions, *solid lines* – ideal 1:1 relationships. Airflow rates were: low = 300 µmol s^{-1} and high = 700 µmol s^{-1}. The fan speeds were: slow = 0.7 m s^{-1}, intermediate = 1.1 m s^{-1}, and fast = 1.4 m s^{-1}. The measurements were performed under laboratory conditions (≈ 20ºC, moistened BSC samples), and with the sample IRGA internal fan set at high speed.

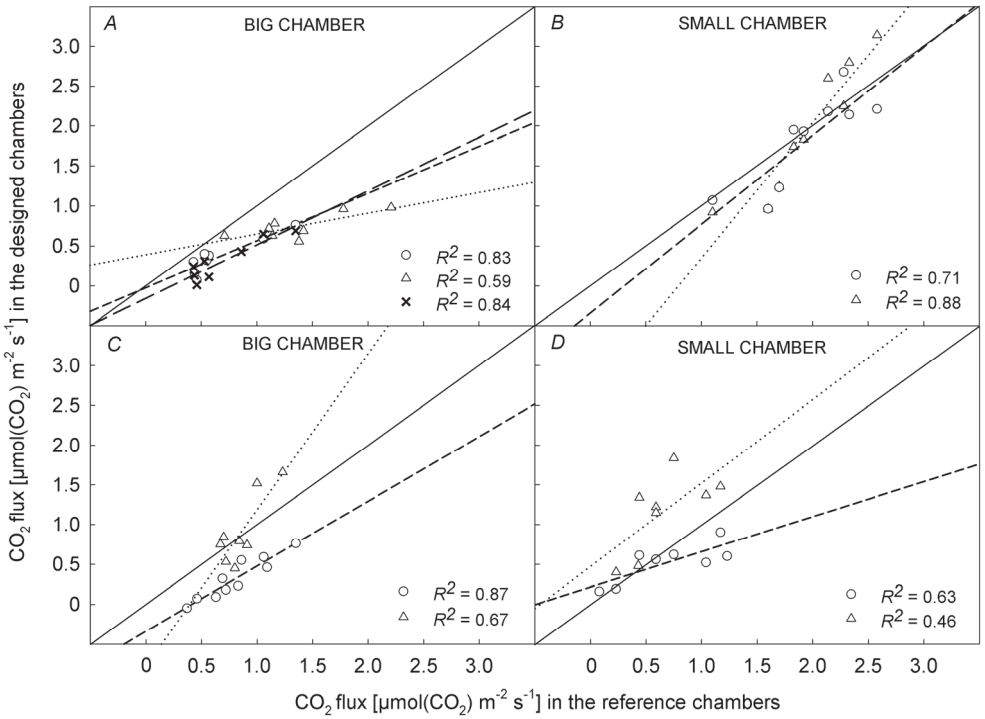

Fig. 4. Comparison of our in-house designed chambers attached to an IRGA *LI-6400*, and commercial chambers (reference chambers), under laboratory conditions (≈ 20ºC, moistened BSC samples). Solid lines – ideal 1:1 relationships. *A*: our chamber operating in the open system with the IRGA sample cell internal fan on high (*circles* and *short dashed line*) and low (*triangles* and *dotted line*) speed and the additional chamber fan on low (0.7 m s^{-1}). Measurements without the additional fan (*crosses* and *long dashed line*) and with the internal fan on high speed. *B*: the *LI-6400* in the open system with fan at low speed (*circles* and *dashed line*), without additional fan (*triangles* and *dotted line*), and internal fan on high speed. *C*: The *LI-6400* in open (*circles* and *dashed line*) and closed (*triangles* and *dotted line*) systems, both with fan on slow speed. *D*: the *LI-6400* in open (*circles* and *dashed line*) and closed systems (*triangles* and *dotted line*), both without fan. All open-mode measurements were done at an airflow rate of 700 µmol s^{-1}. The *LI-8100* respirometer was the reference system in *A*, *C*, and *D*, and the *EGM-4* device in *B*.

1:1 line, although the CO_2 flow was underestimated (Fig. 4*C*). In the small chamber, the R^2 coefficient was also higher in the open than that in the closed system (Fig. 4*D*). Nevertheless, CO_2 records in both systems were lower and higher, respectively, than respirometer data.

Test 3. Net photosynthesis measured with the large chamber *vs.* a conifer photosynthesis chamber: The large custom chamber measuring photosynthesis in the open system fitted well (R^2 = 0.959) to the commercial conifer chamber, with very similar measurements on the same samples, albeit slightly higher (Fig. 5*A*).

Test 4. Respiration under natural and seminatural conditions: The larger number of replicates and wider data range improved the fit between the two systems, resulting in a better correlation. The CO_2 flux in the *LI-6400* continued to be lower than in the *LI-8100*, although less than in the preliminary tests (Fig. 5*B*).

As in the test with the large chamber, the increase in replicates and respiration range by using samples with different moisture levels over time improved the fit between our small custom chamber measurements and the *EGM-4* respirometer (Fig. 5*C*). Measurements in both chambers were very similar even though the devices were from different manufacturers and operated in different configurations (open and closed systems).

Test 5. Changes in air pressure inside the chambers: The pressure variation in the chambers did not exceed 150 Pa when the IRGA system operated with its own airflow system, and with the fan at low speed (large chamber) or without the additional fan (small chamber). Pressures ranged (including the initial pressure without closing the chambers) from 101.47×10^3 to 101.58×10^3 Pa in the larger chamber, and from 100.82×10^3 to 100.85×10^3 Pa in the small chamber. Thus a high airflow in this system did not cause any pressure deficit or overpressure. When air from outside the system forced a pressure increase, the CO_2 flow remained unaltered as long as it did not exceed 200 Pa, but when it reached 300 Pa, the net CO_2 flow rate increased (Fig. 6*A*). The variation in pressure at different additional fan speeds were far from achieving the threshold needed to alter natural CO_2 flow rates (Fig. 6*B*).

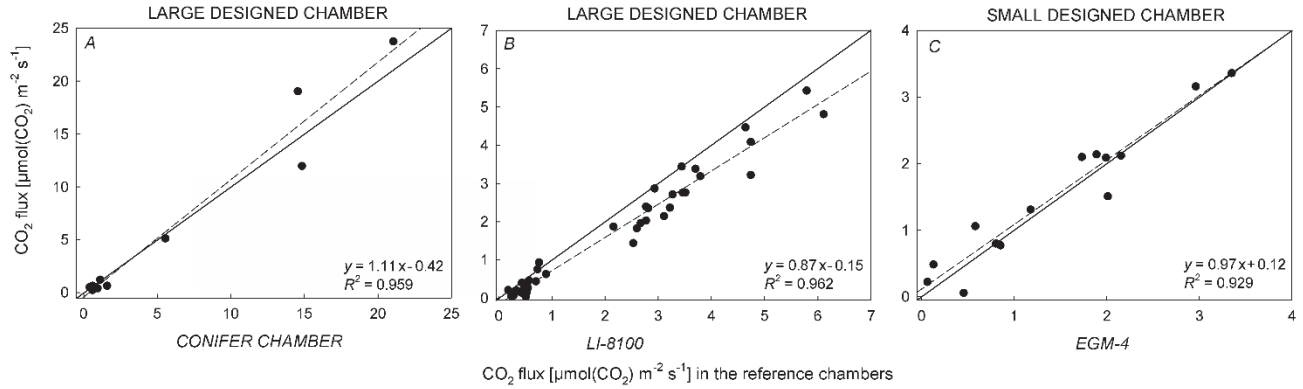

Fig. 5. Comparison of our in-house designed chambers attached to an IRGA *LI-6400*, and commercial chambers (reference systems) at outdoor conditions. *Dotted lines* – linear regressions; *solid lines* – ideal 1:1 relationships. *A*: comparison in the net photosynthesis range with vascular plants. *B*: comparison of respiration in the range extended by using ruderal soil samples. *C*: comparison of respiration using BSC samples. Selected *LI-6400* configurations: in *A* and *B*, IRGA sample cell internal fan at the highest speed, additional fan at low speed (0.7 m s^{-1}), and airflow rate at 800 µmol s^{-1}; in *C*, IRGA sample cell internal fan at the highest speed, without additional chamber fan, and airflow rate at 700 µmol s^{-1}.

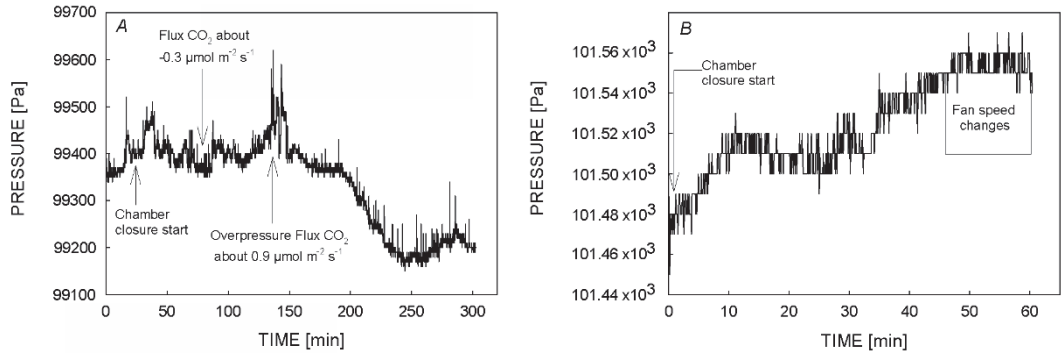

Fig. 6. Air pressure test. *A*: Effect of increased pressure on CO_2 flux. Airflow rate in the *LI-6400* was 800 µmol s^{-1}. *B*: Pressure in the large chamber during enclosure with changes in additional fan speed and 800 µmol s^{-1} airflow rate. Note that gas exchange is net assimilation: positive values imply a reduction in CO_2 concentration inside the chamber headspace, and negative values an increase.

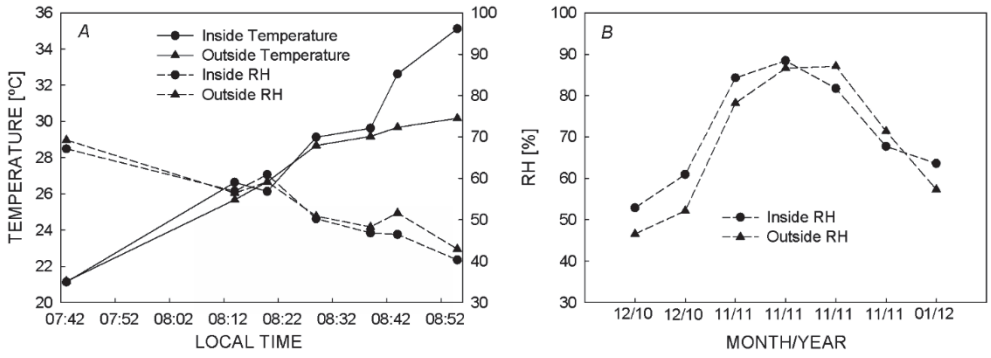

Fig. 7. Temperature and RH test. *A*: Temperature and RH inside and outside the large chamber with stabilized CO_2 concentrations (on average in 4 min) measured on a sunny summer day. *B*: RH inside and outside of the large chamber on sunny days and with high soil water content.

Test 6. Changes in temperature and RH inside the chambers: The paired measurements (outside-inside the chamber) showed that the air temperature inside the large chamber did not change with exposure to direct sunlight at temperatures below 30ºC, even though chamber closure time was over 4 min (Fig. 7*A*). However, when outside temperatures reached 30ºC, with the same closure time, the air temperature inside the chamber rose by as much as 5ºC. No relevant changes in air RH were observed inside the large chamber, where the average increase was only 3% in

summer, late autumn, and winter (Fig. 7*A*,*B*). However, in the small chamber, a considerable increase in RH was observed at high evaporation rates and when the soil moisture content was high, close to saturation; RH inside the chamber ranged from 37 to 94%, while the environmental RH only varied from 33 to 68%. See the supplementary material for a table summarizing all test results (Table 2S – *available online*).

Discussion

We have designed a versatile, robust, portable, and transparent custom-built chamber for *in situ* net CO_2 exchange on soils covered by BSCs in two different sizes, and compared it with other systems. Internal air movement and system mode (open or closed) were configured to standardize their measurements with other commercial chambers. The configurations with the highest determination coefficients were considered the best, their gas exchange rates could be equated with those of commercial chambers by a correlation equation, and with minimal instrumental error.

The CO_2 exchange measured in our chambers for soil respiration or net photosynthesis in vascular plants is quite similar to the measurements with various widely used commercial chambers. The laboratory and seminatural conditions to which the biologically crusted soil samples were exposed can be compared in terms of temperature and moisture (the most important environmental variables for the soil respiratory activity) with the growing season in their areas of origin. The ranges of the reference systems in our tests carried out on BSCs [0.1–3.3 μmol(CO_2) m^{-2} s^{-1}] were similar to those reported for *in situ* measurements during the growing season of one of these areas. For example, Rey *et al*. (2011) measured soil respiration on the same type of soil as our samples, in a range of 0.5 to 2.4 μmol(CO_2) m^{-2} s^{-1}. On a soil moisture gradient from near 0 to 25%, and including different types of soil in this area, the respiration flux varied from 0.6 to 3.8 μmol(CO_2) m^{-2} s^{-1} (Oyonarte *et al*. 2012). These ranges are lower than, for example, those found in subhumid forest ecosystems (*e.g.*, Rey *et al*. 2002, Tang *et al*. 2009, Arevalo *et al*. 2010), but similar to those observed in semiarid regions (Chen *et al*. 2008, Almagro *et al*. 2009, Castillo-Monroy *et al*. 2011). Therefore, according to the reference systems, our chambers were configured within a range of naturally-occurring dryland fluxes.

We discuss the results of our comparative tests further below, in the context of the conclusions of several previous studies. Although the literature describing the design of chambers for measuring soil respiration is quite abundant, there is not as much on design for net photosynthesis. We also address some special considerations which must be taken into account for these measurements.

Additional ventilation and airflow configuration: The optimal CO_2 flux measurement was found with high air intake (700–800 μmol s^{-1}) and the additional fan at low speed (0.7 m s^{-1}), but with IRGA sample cell ventilation at high speed (Figs. 3, 5). Our additional fan at high speed caused the worsened correlation between the two systems, possibly due to the generation of turbulence causing increased measurement randomness. Other authors have also recommended the use of a low fan speed (*e.g.,* Norman *et al*. 1997), as the turbulent airflows generated by high speeds decrease the soil boundary layer (Le Dantec *et al*. 1999, Janssens *et al*. 2000, Pumpanen *et al*. 2004). However, it is important to ensure adequate air mixing in the chamber. In the large chamber, the data recorded without ventilation were slightly lower than when moderate ventilation was used, showing that the additional fan in the chamber improved air mixing. In the small chamber, additional ventilation for proper air mixing was unnecessary, since data were no lower without fan action (Fig. 4*B*). In fact, the measurements with added ventilation had a lower R^2 and a stronger bias with respect to the *EGM-4*, possibly because the fan may have altered the aerodynamic conditions inside the chamber. This agrees with the *LI-COR Biosciences Inc.* recommendation that additional ventilation should be used when the volume of the chamber is over 2 L (*LI-COR* 2003).

In the open system, the steady-state [CO_2] gradient inside the chamber always differ from the outside natural conditions, but this disturbance can be minimized by optimizing the airflow generated by the system (Livingston and Hutchinson 1995). Although high airflow rates may lower these gradient differences (Gao and Yates 1998), they may also reduce measurement accuracy (*LI-COR* 2003). However, according to our results, the highest *LI-6400* airflow rate did not cause this problem with the chamber volumes employed, as demonstrated by their high correlation with the respirometers (Fig. 5). This was probably because the volumes were large enough in both cases to prevent the excessive air renewal that could cause CO_2 sample dilution to exceed sensor sensitivity levels (Ryden *et al*. 1978). Lower CO_2 fluxes, when low airflow was used in our large chamber, also reflected an air mixing problem. In addition, the high airflow rate shortened stabilization times (on average 2 and 4 min for the small and large chambers, respectively). This is especially important in poikilohydric organisms, for which enclosure times must be limited to a minimum due to the sensitivity of their metabolism to microenvironmental changes (Lange *et al*. 1997).

Comparison of systems under indoor conditions: We found a better correlation between the closed system *LI-8100* and our chamber in the open than in the closed system (Fig. 4*C*,*D*). CO_2 flux found with the *LI-6400* in

the closed system was higher than that in the open system. This was not consistent with the results found by Pumpanen *et al*. (2004) or Norman *et al*. (1997), who reported lower values in closed systems than in open systems. We do not think the lower CO_2 flux found in the open system was a result of poor air mixing, as we also found slightly lower values than with the *LI-8100* at a high additional fan speed. In addition, we consider the basis for closed systems inadequate for measuring the response of photosynthesis in a wide range of environmental conditions, precisely because of impossibility to maintain those environmental conditions constant as a consequence of the absence of air renewal (Field *et al*. 1989, Gao and Yates 1998). For example, in soil respiration, if the sample residence time is too long for a particular C efflux, the natural gas exchange in the soil-atmosphere interface decreases because of accumulation of the target gas in the chamber space (Rolston *et al*. 1976). This is one of the main problems of closed systems, and has been discussed in several articles (Ryden *et al*. 1978, Hutchinson and Mosier 1981, Mosier 1989, Rolston *et al*. 1976, Nakayama 1990, Freije and Bout 1991, Hutchinson *et al*. 2000, Davidson *et al*. 2002).

In fact, the open system has been used mainly for measuring photosynthesis in plants, whereas the closed system, cheaper and easier to handle, is used more frequently for soil gas fluxes (Healy *et al*. 1996, Conen and Smith 2000). This is probably because stronger changes in CO_2 and H_2O concentrations inside the chamber in the closed system affect the physiology of plants (*e.g*., stomatal conductance and photosynthetic rates) more than soil respiration measurements. Thus, we conclude that the open system is the best operating mode for measuring net photosynthesis with our chambers.

Comparison of systems under outdoor conditions: The similarity of the *LI-6400* (operating in the closed system) and the *LI-8100* soil respiration measurements has already been confirmed (Madsen *et al*. 2008). However, this study showed that this similarity continues to be valid when the *LI-6400* is operated with our custom chambers in the open system, considered a more appropriate method for measuring photosynthesis. Our two chambers were found to be consistent with the commercial chambers for both respiration and positive net photosynthesis over a wide range. The small chamber data were neither under nor over *EGM-4* respirometer measurements. However, the large chamber data were slightly lower than the *LI-8100* respirometer. Nevertheless, a high correlation between our chambers and the commercial respirometers and photosynthetic chamber measurements was found in all cases (Fig. 5, $R^2 > 0.9$), thus indicating the suitability of the custom chambers designed for measurement of CO_2 gas exchange on biologically crusted soils. Examples of its usefulness for measuring *in situ* net and gross photosynthesis can be found in Maestre *et al*. (2013) and Ladrón de Guevara *et al*. (2014).

Changes in micrometeorological conditions inside the chamber: One of the main open-system biases in the measurement of CO_2 flux could be caused by the generation of pressure differences between the chamber space and the ambient due to air circulation inside the system (Rayment and Jarvis 1997, Gao and Yates 1998, Welles *et al*. 2001). Soil porosity enables CO_2 to escape from the soil, where it is much more concentrated than in the air (from 2,000 to 10,000 ppm in soil, compared to 350–380 in the air, according to Welles *et al*. 2001). But, although CO_2 flux inside the chamber is less sensitive to overpressure than to pressure deficit, overpressure could lead to substantial underestimation of natural CO_2 flux (Fang and Moncrieff 1998). This can be seen in Fig. 6*A*, where overpressure caused a positive net assimilation flux, which indicates a reduction of $[CO_2]$ inside the chamber headspace. It should also be considered that both overestimation and underestimation of CO_2 flux are higher in dry soils (Hutchinson and Mosier 1981, Lund *et al*. 1999), because water in soil prevents gas transport through the profile by advection, and greatly reduces molecular diffusion (Livingston and Hutchinson 1995). It is therefore particularly important to consider whether the chamber operating configuration increases or decreases air pressure when used on dry soils.

No relevant pressure change was observed when measuring in the open system (Fig. 6*B*), which shows that pressure deficit problems can be avoided by placing the pump before the air inlet into the chamber (such as in the *LI-6400*, *see* Fig. 2). But the most important factor that prevents important pressure changes is that the *LI-6400* pump is underpowered for this. Although the optimal airflow found in this comparison was at the pump upper limit (1.34 L min^{-1}), according to Fang and Moncrieff (1996), this airflow hardly alters the atmospheric pressure inside the chamber, regardless of whether by suction or pumping, or the length and diameter of air inlet and outlet tubes. Moreover, we found that the use of additional small fans with a slow speed in the large chamber did not cause any change in pressure.

Temperature rises inside the chambers because of the greenhouse effect of the material used, which can potentially directly and indirectly affect photosynthetic activity. Such increases change the affinity for O_2 of the primary carboxylating enzyme, Rubisco, and increases evaporation from the soil surface, decreasing the magnitude and duration of BSC hydration (Grote *et al*. 2010). However, in our 4-min-long field measurements, on sunny days, the temperature inside our custom chamber only increased when the outside temperature was over 25ºC. When the maximum outside temperature reached 30ºC, the temperature in the chamber was about 5ºC higher (Fig. 7*A*). The smaller temperature increase in this study may be due to ventilation and air renewal at a high airflow (800 μmol s^{-1}), as the use of a static-closed chamber could cause an increase in air temperature of about 15ºC in only three minutes (Matthias *et al*. 1980).

Lichens with green algae as the photobiont have been found to be able to recover metabolic activity at high relative air humidity without any other source of water (Lange *et al*. 1986, 1992). Thus, when soil evaporation is greater than the air renewal within the chamber, an increase in RH takes place in it, potentially causing artificial metabolic activation of some species in the BSC. Chamber measurements may thus overestimate the photosynthesis of poikilohydric organisms, as found by Wilske *et al*. (2008) in field measurements on BSC. It is therefore important to control RH when small chamber volumes are used. In our large chamber, no relevant changes in RH were observed due to the stronger air recirculation, and to the chamber volume, which acted as a buffer (Fig. 7*A*,*B*). However, under high evapotranspiration conditions, the small chamber was close to air water vapor saturation. The humidity input in the small chamber therefore needs to be regulated under extremely high moisture content and solar radiation conditions, which can be achieved by turning the *LI-6400* desiccant adjustment screw to scrub position, keeping H_2O vapor from entering the circuit (Fig. 2).

Conclusions and usefulness of the chamber: Our chamber, measuring in the open system mode, at high airflows and with the sample IRGA mixing fan at the highest speed, was demonstrated to be very suitable for measuring CO_2 exchange on soils with BSCs in a wide range of both positive and negative values. Custom manufactured chambers, such as those proposed here, have five main advantages: (*1*) they are cheaper than commercial chambers; (*2*) chamber diameters can be adapted to the best plot size for the study target (isolated species or communities); (*3*) the chamber volume can be minimized for faster measurement and fewer changes in the environment near the sample surface; (*4*) due to the shortened measurement time, more complete experiments with larger sample sizes can be designed; (*5*) and furthermore, our chamber can also be used as a respirometer simply by covering it with an opaque cloth. On the other hand, different chamber sizes also require different precautions for their proper application. For example, our larger chamber has to have additional low-speed ventilation for adequate air sample homogenization; and as our smaller chamber is more sensitive to changes in humidity, it requires manual adjustment of the humidity input for measuring under a high soil water content and evapotranspiration demand. Our results also demonstrated that this methodology is consistent and appropriate for use under field conditions, and the good correlations found with commercial respirometers mean that, for example, these two systems may be used simultaneously to estimate gross photosynthesis.

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