INTRODUCTION

Cropland in Mendoza, Argentina, include arid and semiarid ecosystems with very high evapotranspiration during summer (in the range of 6 to 7 mm d⁻¹) (Catania et al., 2012; Uliarte et al., 2014). Water for irrigation is scarce and limits the expansion of agriculture in the region. Associated changes in climate during the coming decades, e.g. warming and reduced water availability, are expected to increase evaporative demand and actual transpiration (Hutchinson et al., 2007; IPCC, 2007; Labraga and Villalba, 2009; Lal, 2011). Therefore, the importance of developing techniques to accurately measure gas exchange of whole plants is imperative to anticipate irrigation requirements by herbaceous species in future climates.

Several portable chambers to measure plant gas exchange properties in the field have been developed in recent decades. Some chambers have been designed as closed or semiclosed systems (Pérez-Priego et al., 2010); plants are placed in a sealed enclosure and changes in CO₂ and water concentration are monitored with an infrared gas analyzer and temperature gradients are measured periodically with sensors. Air flow rates inside the chamber were 0.007, 0.012, 0.022, 0.047, and 0.072 m³ s⁻¹. Air flow rates did not modify substantially the natural environment within the chamber; measurements are close to real and exterior ones; temperature increased below 4 °C; photosynthetically active radiation (PAR) was reduced by 5%; and photosynthesis and evapotranspiration showed mean values with nonsignificant variations (22 ± 3.8 μmol CO₂ m⁻² s⁻¹, and 15 ± 4.0 mmol H₂O m⁻² s⁻¹, respectively). This chamber could be a useful tool to measure gas exchange of whole plants in herbaceous species under conditions of high evapotranspiration and for extended periods of time.

Key words: Carbon dioxide, evaporation, evapotranspiration, photosynthesis, temperature.

ABSTRACT

Much of our understanding about CO₂ and H₂O gas exchange in plants has been gained from studies at leaf level. Extrapolation of results to whole plant is difficult and not always accurate. In order to overcome this limitation, a chamber was designed to measure gas exchange at the whole plant level. The chamber developed in this work consisted on an acrylic cylinder 0.70 m high and 0.60 m wide. An incorporated blower was used to circulate air through the chamber and plant canopy from the bottom inlet upwards to the outlet tube providing a maximum flow of 0.072 m³ s⁻¹. Air CO₂ and water concentration were monitored with an infrared gas analyzer and temperature gradients were measured periodically with sensors. Air flow rates inside the chamber were 0.007, 0.012, 0.022, 0.047, and 0.072 m³ s⁻¹. A comparative study showed that 0.022 or 0.047 m³ s⁻¹ air flow rates did not modify substantially the natural environment within the chamber; measurements are close to real and exterior ones; temperature increased below 4 °C; photosynthetically active radiation (PAR) was reduced by 5%; and photosynthesis and evapotranspiration showed mean values with nonsignificant variations (22 ± 3.8 μmol CO₂ m⁻² s⁻¹, and 15 ± 4.0 mmol H₂O m⁻² s⁻¹, respectively). This chamber could be a useful tool to measure gas exchange of whole plants in herbaceous species under conditions of high evapotranspiration and for extended periods of time.

Key words: Carbon dioxide, evaporation, evapotranspiration, photosynthesis, temperature.
(Micro-Bowen ratio system) overcame this limitation but it did not estimate C gas exchange (Holland et al., 2013). Open chambers can be used to continuously measure whole plant gas exchange for experiments in small plots (Burkart et al., 2007). They are less expensive, easier to transport and assemble than micrometeorological systems (Burkart et al., 2007; Varela et al., 2010); however, there are secondary effects associated with the enclosure. Experimental artifacts in controlled environments often include (i) reduced total radiation (Müller et al., 2005; Burkart et al., 2007; Centinari et al., 2009), (ii) increased diffuse radiation (Müller et al., 2009), (iii) altered vapor pressure deficit (VPD) (Balogh et al., 2007) and wind speed (Bonada and Sadras, 2015). These changes in irradiance, in diffuse light and VPD because of the enclosure will affect gas exchange and C assimilation (Müller et al., 2005; Burkart et al., 2007; Centinari et al., 2009). There is possible, however, to minimize these undesirable effects with an appropriate air flow and chamber design (Balogh et al., 2007; Burkart et al., 2007; Bonada and Sadras, 2015).

An open chamber system for whole plants is essentially similar to a leaf chamber (Alterio et al., 2006). Leaf chambers do not take into account possible compensation between different leaf stages, types, and light exposure. Single leaves have different contribution on whole plant C and H2O balances; therefore using leaf chambers is impossible to calculate the transpiration of whole plants during long periods (Barton et al., 2010).

In the open chamber systems air is continuously blown into the enclosure and C exchange and water loss from plants can be estimated by monitoring the changes in humidity and CO2 concentration with an infrared gas analyzer (IRGA) (Burkart et al., 2007; Centinari et al., 2009). The difference between H2O and CO2 concentration of incoming and outgoing air allows for the estimation of E and CO2 exchange or net photosynthesis (Pn) (Alterio et al., 2006; Varela et al., 2010). The positive pressure generated within the chamber can minimize or even eliminate any CO2 efflux from soil due to respiration from roots or microorganisms (Burkart et al., 2007). Transpiration from herbaceous crops and water evaporation from soil occur simultaneously and the cumulative effect of both processes are quantified together with whole canopy chambers (Centinari et al., 2009).

This paper describes the construction and performance of a portable open chamber to measure gas exchange on cover crops or herbaceous plants in semiarid regions with high evaporative conditions.

**MATERIALS AND METHODS**

**Construction and operation of the chamber**

The cylindrical chamber of 0.28 m2 circular section and 0.70 m tall (0.196 m3) was made of 0.005 m thick transparent acrylic (polymethylmethacrylate) (Figure 1). Dimensions were chosen in order to achieve the lowest possible variability in gas exchange measurements (Czóbel et al., 2005). This acrylic is UV-resistant and impermeable, it has less than 10% of thermal transmission and only absorbs approximately 0.014% of water vapor per hour.

Ambient air was continuously blown into the chamber by an alternating current fan (Ital Air, Buenos Aires, Argentina). The air entered into the chamber through a plastic pipe (0.16 m diameter) located 0.10 m above the ground and exited through another pipe located on the top of the chamber. A baffle was placed on the outlet pipe to exclude direct incursion of wind. The air flow rate (F) was varied by replacing the inlet air pipe with others of different diameter (0.120, 0.050, 0.045, and 0.040 m) of at least 1.00 m long. All plastic pipes were white to reduce radiation absorption and temperature fluctuations of the incoming air (Burkart et al., 2007).

Gas samples from inlet and outlet tubes were taken by the infrared gas analyzer (IRGA, Portable Photosynthesis System CIRAS-2, PP Systems, Amesbury, Massachusetts, USA) to measure the corresponding CO2 and H2O concentrations. A small fan (0.075 m diameter; MJ 802512, Brushless, Hsin-Tien City, Taipei Hsien, Taiwan, China) was placed inside the chamber to obtain mixed and representative samples of air, and to prevent convection currents. Air flow through the chamber was determined using a hot-wire anemometer (series 471, Dwyer, Michigan City, Indiana, USA) in the cross section of the inlet tube (Balogh et al., 2007).

Two data loggers fitted with thermometers (TC1047A, Cavadevices, Buenos Aires, Argentina) and a quantum photosynthetically active radiation (PAR) sensors (cell: GaAsP with aluminum encapsulation) recorded information every minute and averaged it every 15 min. The inside PAR sensor was placed in the middle of the chamber at 0.60 m

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**Figure 1. Open chamber system prototype scheme.**

1: Acrylic cylindrical body, 2: alternating current fan, 3: plastic pipe for inner air, 4: plastic pipe for outlet air, 5: plastic baffle, 6: plastic reduction, 7: reference air sample, 8: analysis air sample, 9: infrared gas analyzer (IRGA).
Assessment of evaporation

A gravimetric method with Petri dishes filled with water was used to calibrate the evaporation from the chamber. The chamber was located on a white-waterproof surface. Six Petri dishes of 0.09 m diameter were filled with 25 g water and half of them placed either inside or outside the chamber. The inside evaporation was calculated by using a gravimetric method (IG) and chamber method (IC) (with the IRGA); and the outside evaporation was calculated with the gravimetric method (OG).

The evaporation measurements were performed during 30 min for each F (0.007, 0.012, 0.022, 0.047, and 0.072 m³ s⁻¹) and four replicates were performed for each one. The ratio between evaporation measured by chamber method and gravimetric method inside the chamber was calculated (IC/IG); and the ratio between the inner measurement determined by chamber method and the outer evaporation determined by gravimetric method was also estimated (IC/OG). Measurements were carried out under clear sky conditions between 10:00 and 16:00 h for 2 d in December 2012, and no water vapor condensation was observed on the walls of the chamber. Temperature and light inside and outside the chamber were recorded every 15 min. Data log of CO₂ and H₂O amounts were done every 15 s by the IRGA.

Effect of F on PN and E from a cover crop

Gas exchange experiment was conducted to select an appropriate F rate for a more accurate chamber operation. The study was performed on an irrigated cover crop that was 0.05 m tall and the coverage was composed of 79% *Cynodon dactylon* (L.) Pers. var. *dactylon* and 21% *Taraxacum officinale* F.H. Wigg agg., both species were at vegetative stage; groundcover was measured with the beaded-string method used by Uliarte et al. (2013). The experimental site was located at the agricultural research station of INTA in Mendoza, Argentina (33°00′21″ S, 68°51′53″ W).

Measurements of PN and E were done using each F (0.007, 0.012, 0.022, 0.047, and 0.072 m³ s⁻¹). The evaluations were repeated 4 times, and the order of use of F in each repetition was randomized. Determinations were carried out in December 2012, during 2 d with clear sky between 10:00 and 16:00 h. PN and E were calculated using averages of measured CO₂ and H₂O amounts over 30 min (Equations [1] and [2]). Data log of CO₂ and H₂O concentrations were done every 15 s by the IRGA. For each F, it was calculated the temperature difference (ΔT) between chamber interior and exterior, and the difference between VPD outside and inside the chamber (ΔVPD).

Gas exchange from a cover crop with a selected F

Measurements of gas exchange were carried out on the same cover crop as during comparison of air flow rates with F 0.022 m³ s⁻¹ and all parameters measured as before. There were calculated E and PN (Equations [1] and [2]) from the cover crop.

Determinations began at 08:30 h, and finished at 17:30 h in February 2013. There was an episode of clouds between 08:30 and 09:30 h. Then, the sky was completely clear until the end of the measurements. The maximum recorded temperature was 35.8 °C, and daily average relative humidity was 54%.

Statistical analysis

All data obtained during calibration, comparison of F and cover crop measurements were analyzed by ANOVA using statistical software InfoStat (2011). Normal distribution of data and variance homogeneity of data were tested. Linear regression analyses were performed between gas exchange fluxes, ΔT, and ΔVPD according to F. Pearson coefficient was calculated to assess the correlation between the gas exchange fluxes (E, PN) and F, T, and PAR light.

RESULTS AND DISCUSSION

Assessment of evaporation

During evaporation measurements with Petri dishes there were no appreciable fluctuations of PAR incidence, neither air vapor pressure; similar results were obtained by Müller et al. (2009) during their chamber assessment. Therefore, PAR
and air vapor pressure did not influence evaporation, \(E\), and \(P_N\) within the chamber. The inside evaporation was measured by using the gravimetric method (IG) and the chamber method with the IRGA (IC). As expected, an increase in the air flow rate inside the chamber increased evaporation, and no significant changes were registered in evaporation outside the chamber determined by gravimetric method (OG) with all \(F\) (Figure 2a).

Gravimetric method measurements were higher than IC ones until 0.05 m\(^3\) s\(^{-1}\), but OG remains almost constant. Evaporation inside the chamber followed a linear function reaching a maximum value of 5.6 g h\(^{-1}\) when it was determined by IG, and 6.8 g h\(^{-1}\) when measured by IC with \(F = 0.072\) m\(^3\) s\(^{-1}\). The maximum evaporation measured by IC (with 0.072 m\(^3\) s\(^{-1}\)) was 1.2 times higher than IG; but the minimum evaporation measured by IC (with 0.012 m\(^3\) s\(^{-1}\)) was 2.4 times lower than IG. IC and IG increased when \(F\) enhanced, it could be due to a decrease in resistance of the boundary layer on evaporation surface because of air velocity increasing and the turbulence within the chamber (Centinari et al., 2009). These increases in air velocity caused a rise in the water potential gradient between atmosphere and this surface, and evaporation rose too. The increased wind speed within the chamber may increase evaporation from soil surface. Stannard and Weltz (2006) registered large evaporation values from chamber method compared with eddy-covariance method.

**Figure 2.** A. Evaporation measured by gravimetric method (IG: inside, OG: outside) and by chamber method (IC: inside). B. IC/IG and IC/OG ratios.

![Graph of Evaporation vs. Air Flow Rate](image)

The ratio between inner evaporation determined by chamber method and gravimetric method (IC/IG) was linearly correlated with the \(F\) \((p < 0.001)\) (Figure 2b). The IC/IG ratios were 0.29, 0.49, 0.74, 1.11, and 1.61 for 0.007, 0.012, 0.022, 0.047, and 0.072 m\(^3\) s\(^{-1}\), respectively (Figure 2b). IC/IG ratios close to 1 were obtained by using 0.022 and 0.047 m\(^3\) s\(^{-1}\). The mean IC/OG ratios were 0.31, 0.58, 1.13, 1.88, and 2.50, for 0.007, 0.012, 0.022, 0.047, and 0.072 m\(^3\) s\(^{-1}\), respectively. As above, the IC/OG ratios close to 1 were obtained by using 0.022 and 0.047 m\(^3\) s\(^{-1}\). IC/IG and IC/OG ratios close to 1 were obtained by using the chamber with 0.022 and 0.047 m\(^3\) s\(^{-1}\). Therefore with these two \(F\), the evaporation determined by the chamber (IC) would be close to the real (IG) and similar to the exterior (OG) without modifying conditions within the chamber. The IC/IG values were then used to correct \(E\) measurements of plants to each \(F\).

After calibration we can ensure that chamber method underestimates the actual gravimetric water loss (with \(F < 0.05\) m\(^3\) s\(^{-1}\)). Centinari et al. (2009) also found that measurements from their chamber method slightly underestimate the water loss calculated by gravimetric method. They stated that material of chamber construction does not absorb air moisture, so neither does it the chamber prototype developed during this work.

**Effect of \(F\) on \(P_N\) and \(E\) from a cover crop**

The \(\Delta T\) values obtained for 0.022, 0.047, and 0.072 m\(^3\) s\(^{-1}\) were lower than 5 °C (Figure 3a). Low \(\Delta T\) is desirable to make the environment within the chamber representative of the surrounding environment of plants evaluated. The largest \(\Delta T\) was 6.74 and 7.08 °C corresponding to \(F = 0.007\) and 0.012 m\(^3\) s\(^{-1}\), respectively. The \(\Delta T\) should be below 5 °C to avoid increasing evapotranspiration due to increasing sensible heat, and prevent substantial modification of natural surrounding environment of plants evaluated within the chamber (Burkart et al., 2007). The \(\Delta T\) could be kept below this limit at \(F = 0.022\) m\(^3\) s\(^{-1}\) or higher. Changes in \(F\) did not modify the irradiance reaching the plants inside the chamber. The mean decreasing irradiance within the chamber was about 5% for all \(F\) tested \((p = 0.650)\). Other authors reported percentages from 10.5% to 20% (Burkart et al., 2007; Baker et al., 2009; Centinari et al., 2009). Finally, \(\Delta VPD\) was negatively correlated with \(F\) \((p < 0.001)\) (Figure 3a). As expected and due to the increased of temperature, increasing \(F\) caused the decrease of the difference of VPD between chamber outlet and inlet; Balogh et al. (2007) found similar results with their chamber. According to these authors, the level of \(F\) should be high to increase reliability of chamber measurements to match those under natural conditions.

The values of \(P_N\) and \(E\) of the cover crop were obtained with low scatter, and both increased almost three-fold and five-fold (respectively) throughout the range of air flow tested in a linear trend with similar slopes (Figure 3b). The correlation between of \(P_N\) and \(E\) with \(F\) were 0.93 and 0.96, respectively (Pearson coefficient = r). However, Centinari et al. (2009) registered a exponential model of \(E\) when...
incrementing $F$ in grass cover crops ($R^2 = 0.99$). Poni et al. (2014) found a gravimetric vine water loss highly correlated with chamber-derived vine transpiration ($r = 0.95$).

Therefore, considering an increase of temperature within the chamber < 4 °C, low values of ΔVPD, and evaporation (IC) with low variability and near to actual (IG) and to the exterior (OG), the appropriated $F$ for accurate operation of the chamber were 0.022 and 0.047 m$^3$ s$^{-1}$. Chamber may have some limitations if it would be used with less than 0.022 m$^3$ s$^{-1}$; internal temperature could rise above 5 °C, internal VPD could increase, and gas exchange fluxes would be unreliable.

**Gas exchange from a cover crop with a selected $F$**

Measurements of cover crop gas exchange during a day were made using $F = 0.022$ m$^3$ s$^{-1}$. This assessment was carried out on February 2012 under high environmental evaporative demands (high temperature and low relative humidity). Temperature inside the chamber was higher than outside, especially between 13:00 and 15:00 h, where it reached a 3.4 °C averaged difference (Figure 4a); although the mean ΔT was 2.4 °C. The cloudiness episode caused a decrease of both temperature and radiation received, and consequently gas exchange of the cover crop was affected during 1 h.

The PAR light inside the chamber was generally lower than outside. The $E$ evolved very similar to the inner temperature (Figure 4b). Correlation between $E$ and temperature and between $E$ and PAR were both high: $r = 0.94$ and $r = 0.83$, respectively ($p < 0.001$). Maximum $E$ was achieved at 15:30 h; it was 4.3 mmol H$_2$O m$^{-2}$ s$^{-1}$.

Evapotranspiration was highly correlated with temperature, but maximum temperature was recorded at 16:30 h and maximum $E$ was achieved 1 h earlier. On the other hand, $P_N$ was highest at midday and it followed a very similar trend to that of PAR with a high and significant correlation ($r = 0.96$); the correlation between $P_N$ and $T$ was lower but significant ($r = 0.77$). The maximum $P_N$ was achieved a few minutes before 14:00 h; it was almost 3.5-fold higher to that of $P_N$ early in the morning (25.7 vs. ~ 7.3 µmol m$^{-2}$ s$^{-1}$, respectively) (Figure 4b).

During measurements taken under the cover crop for a day with $F = 0.022$ m$^3$ s$^{-1}$ the $E$ (edited by IC/IG value) was highly correlated with temperature and PAR; Burkart et al. (2007) also found a close relationship between temperature and $E$. The increase in temperature may have caused an increase in the deficit of water vapor in the atmosphere, and thereby increased the $E$ of plants. PAR recorded within the chamber was generally less than ambient except when sun was at its highest position. At that time, acrylic reflection within the chamber may have affected these PAR measurements. Lambers et al. (2008) claim that the daily
$P_N$ is proportional to received PAR; and in this test $P_N$ and PAR evolved similarly. Burkart et al. (2007) also found high correlation coefficients between these two variables on sugar beet and wheat ($r = 0.92$ and 0.98, respectively). They found the correlation coefficients slightly lower between $E$ and temperature inside the chamber for the same crops ($r = 0.75$ and 0.71, respectively). The maximum $P_N$ and the highest record of PAR light were registered at the same time.

The correlation between $E$ and PAR was elevated; around 16:00 h $E$ decreased while $T$ was still very high, therefore the reduction $E$ may have been related with PAR decreasing. Apparently when leaves began a stress episode (high temperature, high VPD, or water deficiency) they lessen the photosynthesis (Lammers et al., 2008). However, stomata closure occurs later; therefore $E$ did not decrease until a little later and temperature slightly decreases.

CONCLUSIONS

With these obtained results, we recommended using this prototype gas exchange chamber with air flow rate ($F$) 0.022 or 0.047 m$^3$ s$^{-1}$ even under high evaporative conditions, like on warmest months in Mendoza, Argentina. The evaluated microclimatic conditions inside the chamber do not change substantially (PAR decreased 5% and temperature increased < 4 °C), and the evaporation within the chamber was close to real and outer ones. The chamber could be useful to measure evapotranspiration flux and CO$_2$ exchange or net photosynthesis for long periods of time in whole herbaceous plants. We recommended corroborating these results in other environmental conditions before starting measurements.

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