Design and performance of unique field chamber and CO2 control system for the investigation of biological impacts on wheats grown under fluctuating CO2

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Abstract
The CO_2 concentration [CO_2] in the atmosphere is increasing, affecting plant growth and development. Free Air CO_2 Enrichment (FACE) technology is used for the study of plant development under elevated CO_2. However, criticism of the FACE technology is that it underestimates plant and crop responses to elevated [CO_2] because rapidly fluctuating [CO_2] within the FACE rings may force stomata to close more often and for longer periods than would occur under non-FACE conditions. Therefore, data from FACE rings cannot be used as validation for plant and crop responses in crop modelling. This report focuses on the engineering of chamber design to test constant vs. variable changes in [CO_2] levels on crops. Three chambers were built, and a non-replicated study was conducted during the 2015 and 2016 seasons to assess the methodology and chamber design. The chambers were set up to test the impacts of constant ambient [CO_2], elevated [CO_2] and variable [CO_2] on wheat biomass, yield and water use efficiency. The chamber design and control system performed as expected keeping 1 min average CO_2 concentration within 1.8 µmol/mol (insignificant variation) to the set point of 610 µmol/mol for elevated CO_2 treatments (fluctuating and constant).

Key Words
FACE System, fluctuating CO_2, field chamber, biological impact, wheat, Australia

Introduction
Naturally occurring CO_2 concentration [CO_2] in the atmosphere is increasing and currently is around 410 µmol/mol (Mauna Loa Observatory 2019). Elevated CO_2 (e[CO_2]) affects plant growth and development in various ways, most notably by increasing biomass, yields and water use efficiency (Leakey *et al* 2009, Taub 2010). Rapidly fluctuating [CO_2] within the FACE rings may force stomata to close more often and for longer periods than would occur under non-FACE conditions (Cardon *et al* 1994, Holtum and Winter 2003, Bunce 2012) leading to underestimates of plant and crop responses to elevated [CO_2] (Tubiello and Ewert 2002, Tubiello *et al* 2007, Ainsworth *et al* 2008). There have been a few studies (Hendry *et al* 1997, Holtum and Winter 2003, Bunce 2012) investigating whether high frequency fluctuations of [CO_2] affect plant response. Results varied between research teams.

To understand better the biological impact of high frequency fluctuations of [CO_2] on wheat, custom chambers were designed and built to mimic (as closely as possible) the fluctuations found inside the rings of the Australian Grains Free Air CO_2 Enrichment (AGFACE) facility. Baker et al (2009, 2014a, 2014b) field tested open system chambers and reported that the chambers were able to accurately estimate canopy transpiration for many field applications such as comparison of canopy gas exchanges and water use efficiencies amongst irrigation treatments. Consequently, in 2015, three chambers (Figure 1) were constructed based on the design theme of Baker et al (2014a & 2014b) and an experiment conducted for two seasons. Three treatments were included: 1) ambient [CO_2], 2) elevated constant [CO_2], and 3) elevated fluctuating [CO_2]. The details of chamber design and performance are presented in this article.

Methods
Chamber design
The chamber frames were made of 20 mm x 20 mm x 1.6 mm RHS welded in shape. The volume of the main chamber was 1 cubic metre with two inlet and outlet funnels connected (Figure 1). The whole chamber was clad with 2 mm thick solid clear UV resistant polycarbonate sheets (Laserlite Australia Pty Ltd Victoria Australia). A portable 300 mm diameter ventilator (MPV 300, Fanmaster, Sydney, Australia) with airflow capacity of 1300 L/s was used to suck the air through the chamber at a speed of about 1.3 m/s. A purpose-built cover protected the ventilator's electric wiring from harsh outdoor weather, especially the rain. This high volume, low pressure fan was fitted to each chamber to maintain the average air temperature inside the chambers within ±1˚C to ambient. Two clear 2 mm thick polycarbonate barriers were used at both inlet and outlet sides of the chamber to control the air flow and air distribution through the chamber. There was a 90

mm gap at the bottom of the barrier at the inlet side and a 90 mm gap at the top of the barrier at the outlet side. This arrangement was found to be the best for the desired airflow and air distribution after trying several ideas, including perforated clear plastic sheets with different sizes and orientations of holes. Spouts were made by a local engineering workshop to attach 300 mm diameter flexible ducting at the inlet side and the ventilator fan at the outlet side. The inlet duct was raised 2 m above the ground to minimise contamination from expelled CO_2 gas at the outlet side (Figure 1).

CO_2 Sensors

Chambers 1 & 2: The [CO_2] in chambers 1 & 2 were monitored by factory-calibrated infrared gas analysers (SBA-4model, PP Systems, USA) with the sampling head placed inside the chambers (Mollah *et al.* 2009).
Chamber 3: Open Path LI - 7500A CO_2/H_2O Gas analyser (LI-COR®, Inc, Lincoln, Nebraska 68504 USA) was used to monitor the fluctuating [CO_2] in chamber 3 (Figure 1). The analyser was set to sample 5 times per second. The high speed was needed so that the fluctuating CO_2 could be controlled quickly.

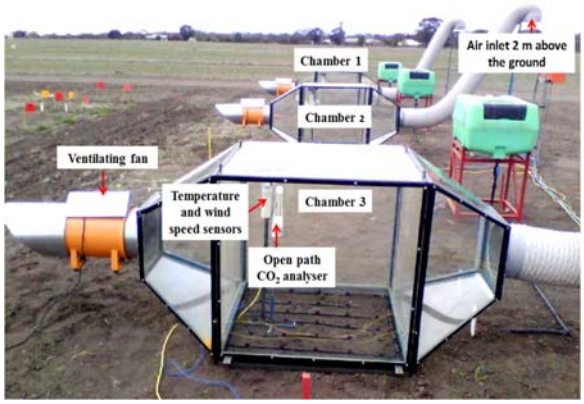

Figure 1. Chambers to study biological impacts of wheat grown under fluctuating CO_2.

CO_2 control system

To compare the biological impact of high frequency fluctuations of [CO_2] on plants, the criteria set out for the chambers in the field were: 1) No CO_2 injection and its control needed for Chamber 1 (ambient, (~400 µmol/ mol)), b) CO_2 injection and the control of CO_2 levels needed to maintain a set constant [CO_2] at 610 µmol/mol in Chamber 2, c) CO_2 injection and the control of CO_2 levels needed to maintain fluctuating [CO_2] between 400 – 1200 µmol/mol with a median value of 610 µmol/mol and 30 s periods. The architecture of the CO_2 control system consisted of the assembly of sensors, modems, computers etc. (Figures 2 & 3). The [CO_2], wind speed, %RH and temperature from each chamber and ambient temperature were received by the data hub every second (Figure 2) and then sent to a field office computer for logging (Figure 3). An alarm system was set up to send an email to a nominated account (configurable) if power failed and or wind-speed in any chamber dropped below a set limit.

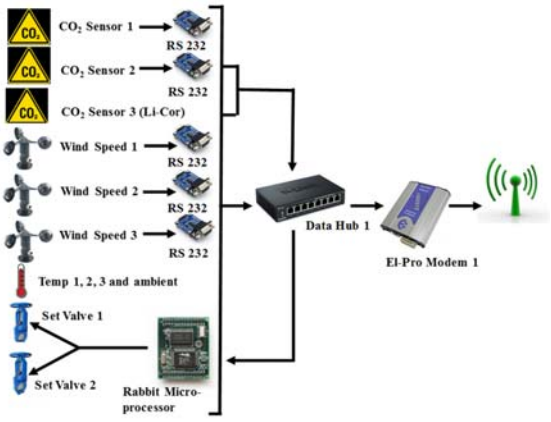

Figure 2. Schematic diagram of the components of the control system in the field.

Figure 3. Schematic diagram of the equipment architecture in the field office.

Rabbit Firmware and control valves

Rabbit firmware was used in Chambers 1 & 2 and consisted of: CPU SR9150, Backplane chassis SR9010, A/D converter. 0 - 10 V SR9300, Relay - 6 SR9500, LCD Keyboard/Display SR9010 +SR9050, FWT 18W SR9300 and Timer/Counter Card SSCTC-LS. The Rabbit Microprocessor (written in dynamic C) received [CO_2] from chambers 2 & 3 to operate the valves which were controlling the injection of CO_2 to chamber 2 & 3 as per the program. Two SMC ITV1030-011S5 valves with input 4~20mA DC, output 5 - 500 kPa were

used for the control of the [CO_2] in Chamber 2 & 3. One SMC VT307 - 5D -02, 0 - 0.9 MPa valve was used to shut down the CO_2 supply at night and turn on in the morning.

Understanding the CO_2 fluctuation in existing FACE Rings

To determine the target [CO_2] for the chambers, an open-path CO_2 analyser (Li-COR used in chamber 3) was placed half way between the edge and the ring-centre in an existing 12 m FACE ring to record CO_2 concentration @ 20 readings per second over a few days. A representative 5s recoding provided the basic understanding of wide CO_2 fluctuation inside an AGFACE ring. About 98% fluctuation happened between 400 and 1200 µmol/mol with a median CO_2 concentration value of 610 µmol/mol. This value was used as the set value for chamber 2 (constant CO_2) and the median value for the fluctuation profile (between 400 and 1200 µmol/mol) of chamber 3.

Data Logging and downloading

The data logging strategies were: a) Raw data (as observed) - one file per day, b) Raw data (as observed) – one file per hour, c) 1-second average data files - one file per day, d) 1-minute average data files - one file per day, e) 1-minute average cumulative data file - one file for whole season (append data to a file at midnight). The PC at the site office was accessed remotely via LogMeIn program for data downloading.

Monitoring system performance

System performance was regularly monitored using a dashboard (written in LabView) on computer screen (Figure 4) and alarms received on mobile phone. There was a provision to access the dashboard remotely to facilitate monitoring from a PC away from the field site. The dashboard also had a specific button to monitor the performance of chamber 3, where CO_2 was set to fluctuate within a given range. This was very useful as the set up for CO_2 was affected by the growth of the canopy inside the chamber (Figure 5

Figure 4. Dashboard on computer screen to monitor system performance and trouble shooting.

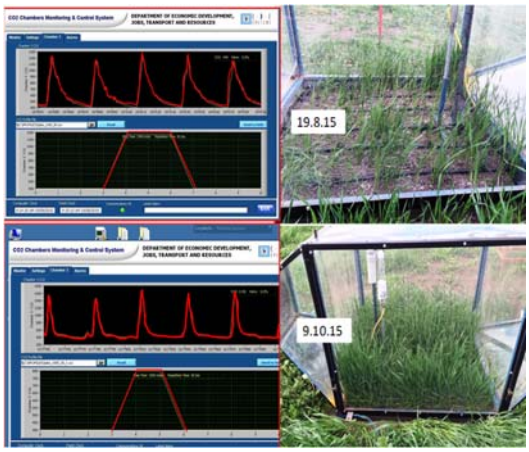

Figure 5. Chamber 3 performance at different growth stage

Results

The system was able to maintain 1-min average [CO_2] very close to the set point of 610 µmol/mol for chambers 2 (constant, 611.8 µmol/mol) & 3 (fluctuating, 610.6 µmol/mol) [Figure 6]. There were no significant differences between the chambers for temperature and relative humidity (RH) and as expected humidity reduced with increasing temperature (Figure 7). The temperatures and RH (Figure 7) in the chambers closely followed the ambient temperature and ambient RH (results not shown). These results suggest that the ventilator chosen to suck the air through the chamber at a speed of about 1.3 m/s was sufficient and kept the chamber temperature and RH close to the ambient. The control of CO_2 levels in the chambers (outlined in Methods) worked as expected. The CO_2 fluctuation profile in Chamber 3 was created based on the actual data collected from the field. The fluctuation profile varied with canopy growth, so occasional adjustment in CO_2 fluctuation valve was needed for Chamber 3. The air flow in the chambers caused the plants to bend in the early growth stages but became upright with time (Figure 5). For unknown reasons, the PP Systems IRGAs (close path CO_2 analyzers, see CO_2 sensors section) used in chamber 1 & 2 showed some undesirable fluctuations in the CO_2 readings especially at night time, but managed to keep the

median CO_2 value at 610 µmol/mol. Crops grew well inside the chambers (Figure 5) without any heat damage and matured normally.

Conclusion

Chambers had no harmful effects on crop growth and kept the temperature and RH close to the ambient, providing confidence in the chamber design and CO_2 control systems. These chambers with their CO_2 control systems are suitable for testing the biological impacts of fluctuating CO_2 on crops in the field.

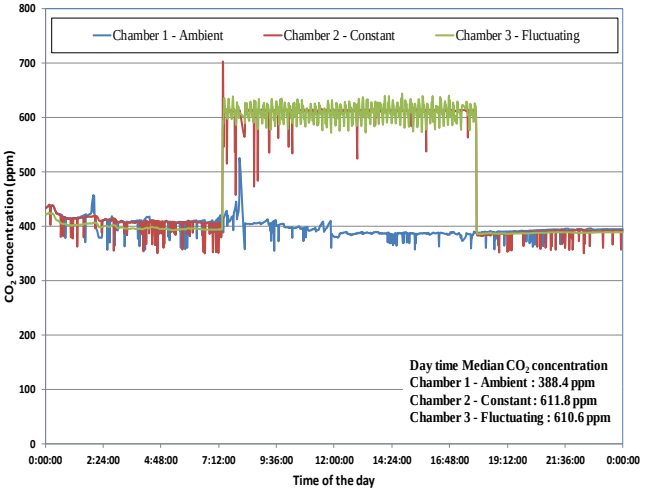

Figure 6. Typical performance of three chambers on 9 August 2015.

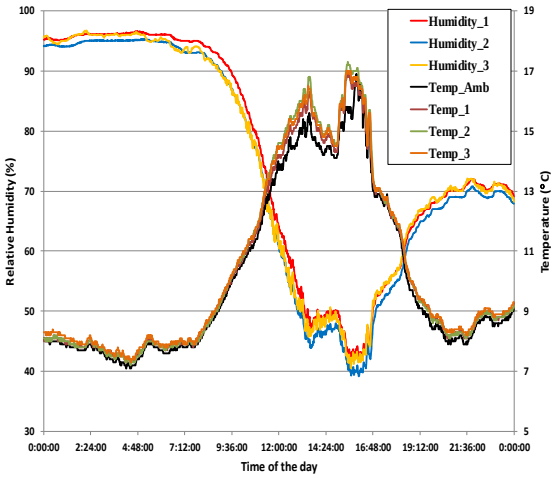

Figure 7. Relative humidity and temperature inside three chambers on 9 August 2015

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