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Understand distribution of carbon dioxide to interpret crop growth data: Australian grains free-air carbon dioxide enrichment experiment

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Abstract. Carbon dioxide (CO_2) is the most important greenhouse gas, predicted to increase globally from currently 386 to 550 μmol mol^{-1} by 2050 and cause significant stimulation to plant growth. Consequently, in 2007 and 2008, Australian grains free-air carbon dioxide enrichment (AGFACE) facilities were established at Horsham (36°45′07″S lat., 142°06′52″E long., 127 m elevation) and Walpeup (35°07′20″S lat., 142°00′18″E long., 103 m elevation) in Victoria, Australia to investigate the effects of elevated CO_2, water supply and nitrogen fertiliser on crop growth. Understanding the distribution patterns of CO_2 inside AGFACE rings is crucial for the interpretation of the crop growth data. In the AGFACE system, the engineering performance goal was set as having at least 80% of the ring area with a CO_2 concentration [CO_2] at or above 90% of the target concentration at the ring-centre for 80% of the time. The [CO_2] was highly variable near the ring-edge where CO_2 is emitted and declined non-linearly with the distance downwind and wind speeds. Larger rings maintained the target [CO_2] of 550 μmol mol^{-1} at the ring-centres better than the smaller rings. The spatial variation of [CO_2] depended on ring size and the gap between fumigation and canopy heights but not on wind speeds. The variations in the inner 80% of the rings were found to be higher in smaller rings, implying that the larger rings had more areas of relatively uniform [CO_2] to conduct experiments.

Additional keywords: AGFACE, FACE, spatial variation, Australia.

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Introduction

Carbon dioxide (CO_2) is a colourless, odourless non-flammable gas and is the most prominent greenhouse gas in the earth's atmosphere, responsible for global warming. CO_2 is emitted naturally through the carbon cycle and through human activities. A large portion of CO_2 in the atmosphere is absorbed by the oceans, and other water bodies and some is used by forests and other vegetation for growth. In the past, this process kept a balance of CO_2 concentration ([CO_2]) in the atmosphere. However, the global atmospheric concentrations of CO_2 (a[CO_2]) began to increase at the beginning of the industrial revolution in the 18th century due to the burning of oil, coal and gas, and from deforestation. In recent decades, a[CO_2] has risen rapidly. The IPCC 2007 emissions scenario A1B indicates that globally a[CO_2] will increase to 550 μmol mol^{-1} from 386 μmol mol^{-1} by 2050 (Carter *et al.* 2007).

Australia and the globe are experiencing rapid climate change. Since the middle of the 20th century Australian temperatures have, on average, risen by ~1°C with an increase in the frequency of heatwaves and a decrease in the numbers of frosts and cold days. Rainfall patterns have also changed – the north-west of the continent has seen an increase in rainfall over the last 50 years while much of grain-growing regions of eastern Australia and the far south-west have experienced a decline (Australian Bureau of Meteorology 2010). These changes in climate will significantly challenge grain (including wheat) production in Australia. Wheat is a significant contributor to the Australian economy; it amounts to ~90% of the total value of grain production. It accounts for 3% of the total value of Australia's exports and 16% of Australia's total farm export, generating more than AUD$4 billion in export revenue (AWB 2010).

Consequently, in 2007 and 2008, Australian grains free-air carbon dioxide enrichment (AGFACE) facilities were established at Horsham (36°45′07″S latitude, 142°06′52″E longitude, 127 m elevation) and Walpeup (35°07′20″S latitude, 142°00′18″E longitude, 103 m elevation) in Victoria, Australia with an aim to investigate the effects of elevated atmospheric carbon dioxide concentration (e[CO_2]), water supply, temperature and nitrogen fertiliser on growth and development of wheat and other grain crops under future climate scenarios and Australian field conditions. Wheats were sown at different dates and locations to obtain various temperature effects during anthesis.

Understanding the distribution pattern (spatial and temporal variations) of [CO_2] within an AGFACE ring is crucial for treatment allocation inside the ring and interpretation of crop responses to e[CO_2]. Over the past 3 years several 4-m (Walpeup 2008 and 2009), 12-m (Horsham 2007 and 2008) and 16-m (Horsham 2009) AGFACE rings were used in separate experiments with the target [CO_2] of 550 µmol mol^{-1} set for ring-centres. Monitoring of these rings showed that while engineering performance criteria were met in all of these rings, there were variations in [CO_2] within the rings (Mollah *et al*. 2009). These variations were similar to other free-air carbon dioxide enrichment (FACE) rings used elsewhere (Miglietta *et al*. 1997, 2001; Okada *et al*. 2001; Hendrey and Miglietta 2006).

In this paper we present [CO_2] and the extent of its variation at different locations within horizontal and vertical planes within AGFACE rings and discuss its possible consequences.

Materials and methods

AGFACE system

The AGFACE system is based on the principle of direct injection of pure CO_2 in the atmosphere. In this system CO_2 is injected into the atmosphere on the upwind side, through 0.30-mm laser-drilled holes (on stainless steel tubes) at supply line pressures up to 500 kPa. This CO_2 is then quickly mixed with air and transported across the ring by the prevailing wind. CO_2 was turned off at night when plants were not photosynthesising and if the wind speed exceeded 8 m/s in daytime. Each AGFACE ring consists of eight unconnected stainless steel fumigation tubes forming an octagon. CO_2 concentration is measured at the centre of each ring using an infrared gas analyser (IRGA) to facilitate the ring control system to maintain the target [CO_2] at the ring-centre. For detailed design and performance of the AGFACE system consult the previously published article (Mollah *et al*. 2009).

Multi-port IRGA

A multi-port IRGA was used to measure the spatial distribution of CO_2 within the rings. The sensor is the same model (SBA-4, PP Systems International, Inc., Amesbury, MA, USA) as used at each ring-centre (Mollah *et al*. 2009). The multi-port IRGA uses 16 diaphragm air pumps (Schego, Germany, Aquatic Life Aquariums, Berkley Vale, NSW, Australia) powered by 12-V direct current to draw air samples. Two-way valves split the inlets of 16 pumps into 32 inlets (ports). Air is drawn continuously through 1 of the 2 ports of a particular pump at a time. Any number of ports between 1 and 16 (inclusive) can be selected for use at any particular time. Also, 32 ports can be selected for use but not any number of ports between 16 and 32. For example, if 9 ports are selected, then the algorithm allows using a single port from each of the 9 pumps (pumps 1–9) and the loop continues until stopped. For 5 ports, 5 pumps, for 12 ports 12 pumps and so on for up to 16 ports. When 32 ports are selected, the sampling starts at port 1 on pump 1 and continues up to port 16 on pump 16. When sampling at port 16 is finished, the two-way valves inside each pump automatically change their direction closing the used ports and opening the unused ports. Again sampling starts at pump 1 through its second inlet (port 17) and continues until it reaches port 32 on pump 16. After sampling through port 32, two-way valves change direction again and the loop continues until stopped. The sampling, analysis and line purging takes ~10 s for each port, therefore it takes ~320 s (5.33 min) to complete a set of data collection from 32 different locations.

Spatial variation in horizontal plane – 2008

In 2008, to assess the horizontal distribution of [CO_2] within the AGFACE rings, two rings from an early sowing and two rings from late sowing treatments in wheat were selected as study rings at Walpeup (4-m rings) and Horsham sites (12-m rings). Locations for data collection were pre-determined and marked inside each ring. Data were collected manually using a portable IRGA analyser (EGM-4 Environmental Gas Monitoring for CO_2, PP Systems International, Inc., Amesbury, MA, USA). The sampling head of EGM-4 was mounted on a purpose-built frame so that the sampling head was at least 1.5 m in front of and upwind of the operator to avoid contamination from the operator's breath. A single reading (1-s instantaneous value) was taken at each of the sampling locations (22 for 4-m rings and 32 for 12-m rings) at the same height as the central IRGA's sampling head after a time lapse of at least 13 s to ensure an adequate purging of previous air-sample from the IRGA. The measurements were repeated 7 times from all the selected rings (8 rings in total) throughout the season between 25 July and 8 November 2008.

2009 experiments

In 2009, eight, 16-m-diameter AGFACE rings were installed on 7.5 ha of land at Horsham (36°45′07″S latitude, 142°06′52″E longitude, 127 m elevation). Of these eight similarly performing rings, one ring was selected for continuous logging of [CO_2], wind speeds, and wind directions from multiple locations within the ring using a multi-port IRGA.

There were 24 treatment plots of wheat inside the AGFACE ring. The centres of these plots and 250 mm inward from the middle of each of the eight sectors of the octagon were pre-marked as the sampling points (Fig. 1). The *x–y* coordinates of each location from the centre of the 16.25-m (actual dimension)-diameter octagonal ring were estimated to the nearest 0.1 m using trigonometry. The sampling height at each location for multi-port IRGA was set at the same height as the central IRGA sampling head, which was 50 mm below the CO_2 fumigation height (550 mm) to ensure that [CO_2] was measured inside the blanket of CO_2 above the canopy. This experiment was set to assess the spatial variation in a horizontal plane within an AGFACE ring. The multi-port IRGA logged [CO_2], wind speeds, and wind directions every 5.33 min from each of the 32 locations between 5 and 10 November 2009.

On 11 November 2009 inside the same ring, six sampling points were selected along the ring diameter (x – x line) and at each location five sampling heads of multi-port analyser were placed at five different heights (Fig. 1). The extra two sampling heads were placed at ring-centre at 750 mm (fumigation height) and at 1.0 m above the ground. The crop height was 550 mm. The multi-port IRGA logged [CO_2], wind speeds, and wind directions every 5.33 min from each of the 32 locations between 11 and 20 November 2009.

Statistical methods and contour mapping

In 2008 there were 616 (for 4-m ring) and 703 (for 12-m ring) data points (collected manually) available for analysis. However, in 2009 we collected a large amount of data using the multi-port analyser. There were 28 213 data points for Expt 1 and 13 565 data points for Expt 2 available for analysis.

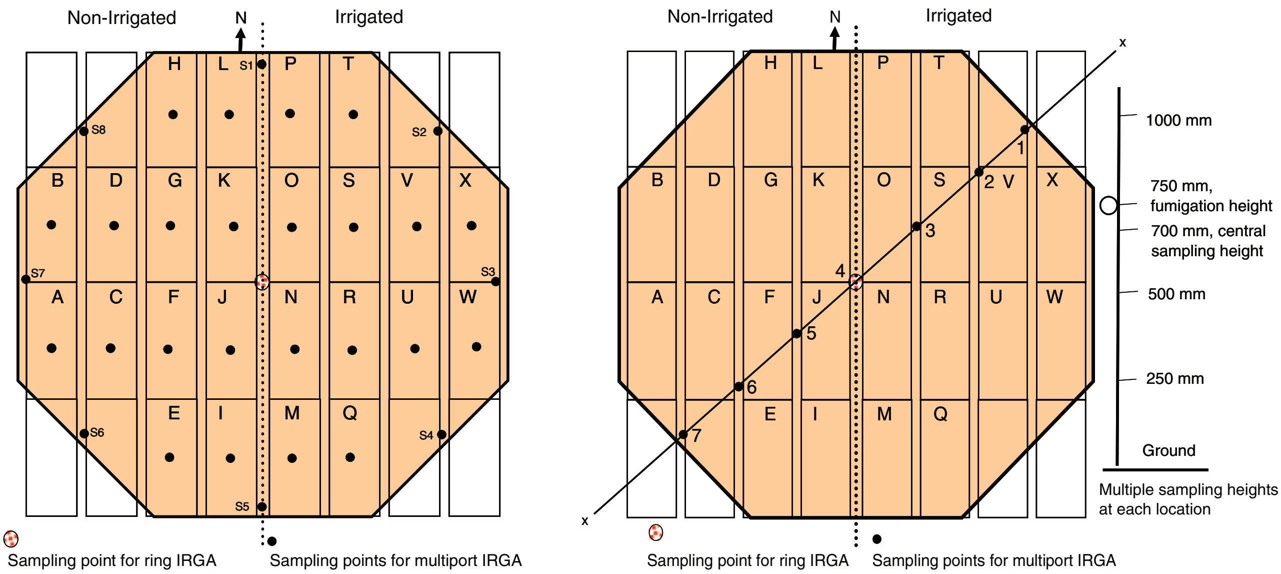

Fig. 1. Sampling locations for monitoring CO_2 concentrations with the multi-port.

The data collected by the multi-port analyser and IRGA at ring-centres were categorised according to whether the CO_2 flow was on or off and summarised using means, medians, standard deviations and mid-95% ranges. The 1-min average [CO_2] logged from the ring-centres were used to calculate the percentage of time when the ring-centres recorded [CO_2] within various ranges. To assess spatial variation of [CO_2] across the ring, the data collected from each experiment were used to develop a linear regression of the [CO_2] against distance downwind from the CO_2 source. The [CO_2] was logarithm-transformed and the terms tested in the model were the logarithm of the distance downwind from the CO_2 source to the sampling point, [CO_2] at the centre of the ring and wind speed. The *x*–*y* coordinates of each location within the rings from the ring-centres were estimated. The distance downwind from the CO_2 source for each sampling location was calculated (for details see Mollah *et al*. 2009). Regression analyses were carried out using GENSTAT statistical software, version 12 (Payne *et al*. 2009).

The spatial interpolation method of kriging (point type) employing a linear variogram model was used to draw contour maps of spatial variation of [CO_2] within each ring. The software Surfer version 7 (1999) (www.goldensoftware.com/) was used.

Results

[CO_2] declines significantly with distance downwind

Both manual and automatic (using multi-port IRGA) monitoring have shown that [CO_2] is much more variable at high concentrations near the ring-edge where the CO_2 is injected and declines non-linearly with the distance downwind of the ring-edge (see Mollah *et al*. 2009; for details) corroborating the findings of others (Miglietta *et al*. 2001; Hovenden *et al*. 2006).

The average median of [CO_2] at the 16-m ring-centre (location 4, Fig. 1) was significantly ($P = 0.5$) lower than most of the locations (Table 1). During 11–20 November 2009 (Expt 2), the wind mostly blew between 135 and 270° implying that the sector near location 7 (ref. Fig. 1) was the prominent fumigation tube. This was reflected in the results (Table 1) as the average median of [CO_2] gradually decreased from location 7 (near the fumigation tube) down to the centre of the ring (location 4). The [CO_2] varied between 350 and 375 μmol mol^{-1} in control (ambient CO_2) areas during day time depending on the photosynthetic conditions and minor contamination from elevated CO_2 rings. The figure often exceeded 550 μmol mol^{-1} during night times (no injection of CO_2) most probably due to respiration from canopy and soil.

[CO_2] is much more variable around ring-perimeter

The standard deviations of [CO_2] at each measurement locations were plotted in a contour map (Fig. 2), derived by kriging the point data. The figures around the ring-edges are relatively high, suggesting high variability close to ring-perimeter. These results corroborate other studies (Miglietta *et al*. 1997, 2001; Okada *et al*. 2001; Hendrey and Miglietta 2006; Mollah *et al*. 2009).

[CO_2] is high on upwind side of the ring

As expected, the average median of [CO_2] in 2009 (Fig. 3), were relatively higher inside the plots close to the fumigation tubes

Table 1. Average median of [CO_2] at different locations within a 16-m Australian grains free-air carbon dioxide enrichment ring
Medians followed by the same letter are not significantly different at $P = 0.05$

Location (refer to Fig. 1)	1	2	3	4	5	6	7
Average median [CO_2] (μmol mol^{-1})	593a	546b	541b	539b	584a	639c	822d

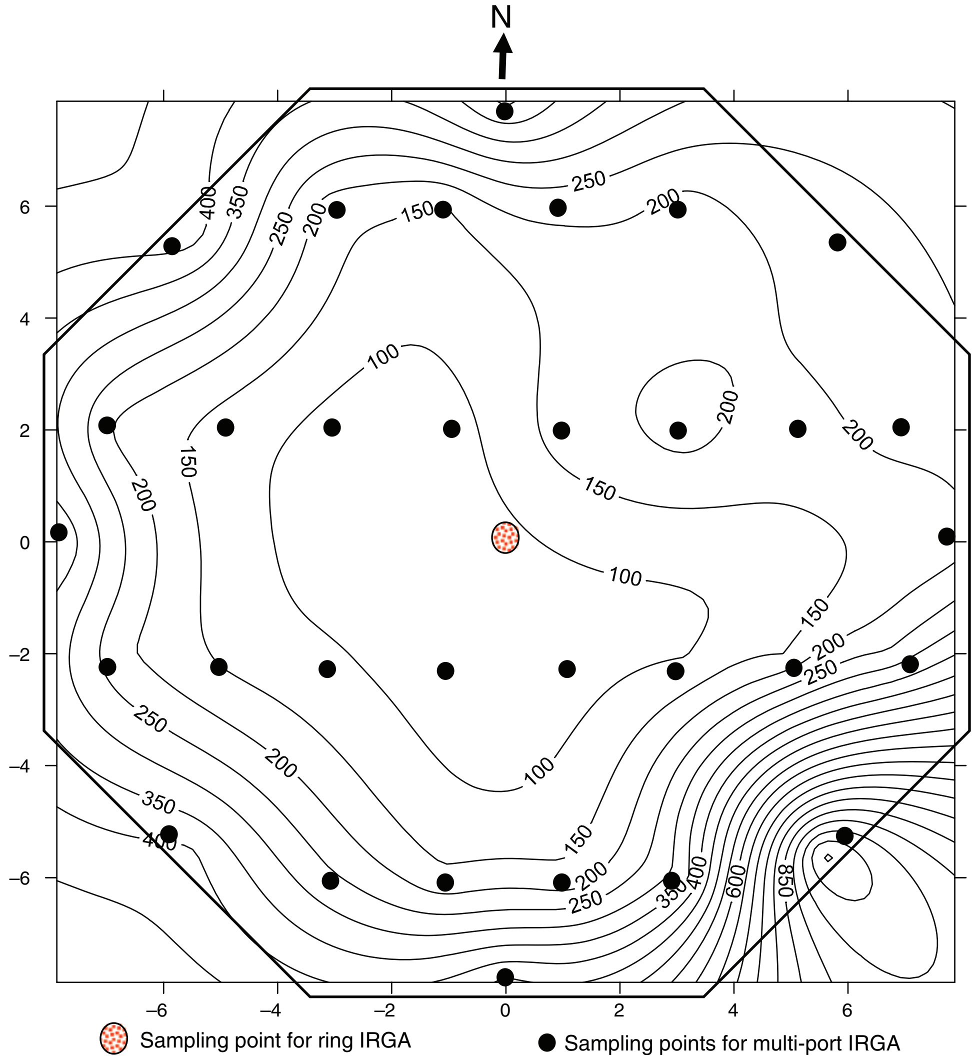

Fig. 2. The standard deviations of $[CO_2]$ concentrations at different locations inside a 16-m Australian grains free-air carbon dioxide enrichment ring.

(Fig. 3). During 5–10 November 2009 (Expt 1), 75% of the time, wind blew between 0 and 182°. The AGFACE system is designed for fumigation from the upwind facing sectors and the $[CO_2]$ was reflected in the results. The upwind facing sectors S1, S4 and S5 of the octagonal ring were the prominent sectors for fumigations as the average median of $[CO_2]$ at these locations were very high (Fig. 3).

Wind speeds affect variability in fumigations but not spatial variation

The regression analysis showed that spatial variations in 1-min average $[CO_2]$ inside AGFACE rings were not affected by wind speed. This corroborates earlier findings by the AGFACE team (Mollah *et al*. 2009). However, wind speeds affected the variability in the amount of CO_2 injected from fumigation tubes. The $[CO_2]$ near fumigation tubes at low wind speeds were highly variable as the mid-95% quartile range (difference between 97.5 and 2.5% quartiles) was relatively larger at low wind speeds (Fig. 4). The highest (97.5% quartile) $[CO_2]$ measured near the fumigation tubes decreased logarithmically ($r^2 = 0.91$) with increased wind speeds (Fig. 4).

On average $[CO_2]$ remains the same in vertical profile

When readings from different locations were averaged for a particular height, there were no significant ($P = 0.05$) differences in median $[CO_2]$ at various heights up to the fumigation height (750 mm, ref. Fig. 1). The average median $[CO_2]$ was significantly ($P = 0.05$) lower at a height above the fumigation tube compared with heights below fumigation tube (Table 2).

The interaction of location and heights revealed that the median $[CO_2]$ above the fumigation height was not affected by the location, it was virtually the same all over the ring (Fig. 5 – representing half ring). Inside the ring, $[CO_2]$ was very similar throughout the vertical profile with the exception of the location near the fumigation tube, i.e. ring-edge. As expected, the $[CO_2]$

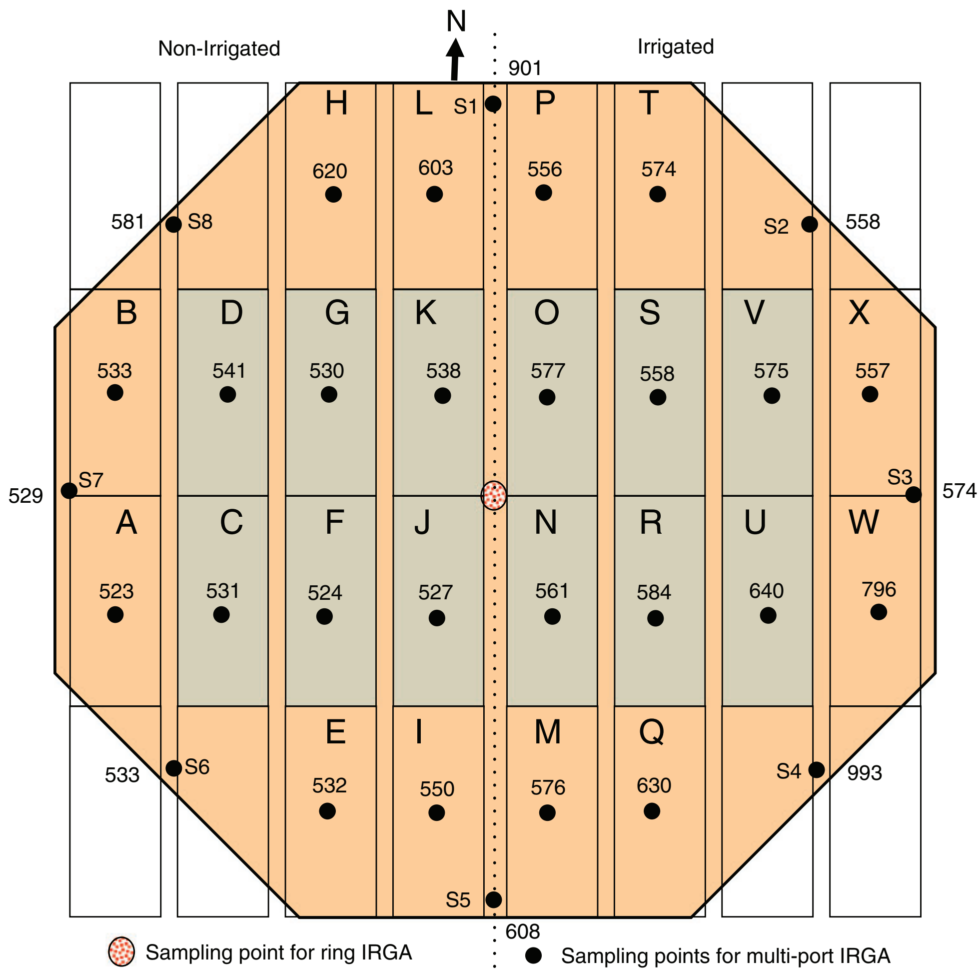

Fig. 3. The median [CO_2] in a horizontal plane at different locations in a 16-m Australian grains free-air carbon dioxide enrichment ring.

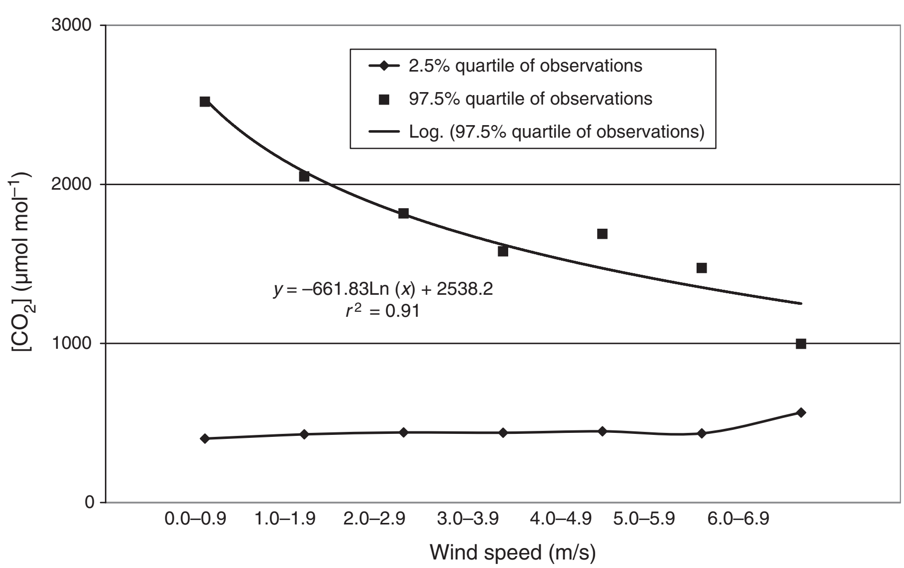

Fig. 4. 2.5 and 97.5% quartiles of observations of average CO_2 concentrations near (250 mm inward) fumigation tubes of a 16-m Australian grains free-air carbon dioxide enrichment ring.

Table 2. Average median of $[CO_2]$ at different heights within a 16-m Australian grains free-air carbon dioxide enrichment ring

Note: medians followed by the same letter are not significantly different at $P = 0.05$. The height of the fumigation tubes is 750 mm above the ground

Height (mm)	250	500	700	750	1000
Average median $[CO_2]$ (μmol mol^{-1})	585a	595a	580a	575ab	554b

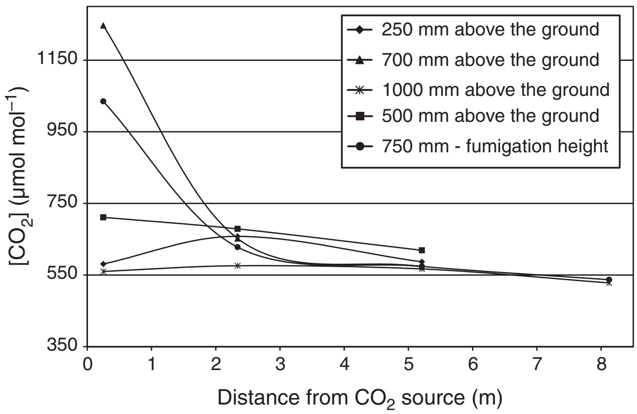

Fig. 5. Median $[CO_2]$ concentrations at different heights and locations – shown for half of 16-m ring.

Table 3. One-minute average $[CO_2]$ at the ring-centres

$[CO_2]$ at ring-centres (μmol mol^{-1})	Occurrence in each ring (% time)		
	4-m ring	12-m ring	16-m ring
>495 (90% of the target)	83	89.5	96.5
495–605 (±10% of the target)	68.5	79.0	91.7
Mid-95 percentile range (μmol mol^{-1})	512–580	520–576	534–566

Table 4. Effects of gap between fumigation and canopy heights on spatial variation in $[CO_2]$ in 4- and 12-m Australian grains free-air carbon dioxide enrichment (AGFACE) rings

Gap	$\text{Log}[CO_2] = a + b\text{Log}$(distance downwind of CO_2 source)			
	4-m AGFACE ring		12-m AGFACE ring	
	a	b	a	b
≤50 mm	3.00	–0.43	3.14	–0.47
50–100 mm	3.00	–0.41	3.03	–0.33
100–150 mm	2.97	–0.38	3.02	–0.32
>150 mm	2.89	–0.31	2.89	–0.19

was very high at or near fumigation height close to the ring-edge (Fig. 5).

CO_2 distribution depends on ring size

In the AGFACE system the performance goal was set as having at least 80% of the ring area with a $[CO_2]$ at or above 90% of the target concentration at the ring-centre for 80% of the time. Monitoring showed that while engineering performance criteria were met in all sized rings, variation in performance between different sized rings existed. The mid-95 percentile ranges of $[CO_2]$ at the ring-centres were smaller for large rings, implying that larger rings maintained the target $[CO_2]$ of 550 μmol mol^{-1} at the ring-centres better than the smaller rings (Table 3). More importantly, the spatial variations of $[CO_2]$ in the inner 80% of the rings were found to be lower in larger rings compared with smaller rings.

Gap between fumigation and canopy heights affect CO_2 distribution

The variation in $[CO_2]$ inside 12-m rings decreased with increasing gap between fumigation and canopy heights. This was evidenced by gradual decrease in intercepts ('a' values, Table 4) and slopes ('b' values, Table 4) with increasing gaps for distribution lines (linear relationship) of $[CO_2]$. This reduction was significantly low at gaps >150 mm compared with other gaps (Table 4). The trend was followed by 4-m rings but not to the extent of 12-m rings (Table 4). No data are available for 16-m ring.

Discussion

For a population with a normal distribution in a measured variable, the mean is the most efficient estimate of the population. However, the median is a more accurate indicator of an experiment because it is a closer reflection of the sample set as it is less sensitive to outliers than the mean. The statistical measures of Skewness and Kurtosis of our data showed that they were skewed (Skewness value = 3) to the right, towards values greater than the median and had a relatively long tail (Kurtosis value = 19). This meant that most of the observations were around the median value. Consequently, we have used the median for the analysis, because our data were skewed.

The $[CO_2]$ was always higher on the upwind side of the FACE ring than the downwind side but was usually compensated as wind directions change (swap between upwind and downwind) during the season (Mollah *et al.* 2009; Miglietta *et al.* 2001). However, on average the plots close to the ring-edge receive higher concentrations of CO_2 compared with their counterparts close to the ring-centre.

The $[CO_2]$ in a particular location within a FACE ring oscillates symmetrically and it happens within a few seconds, which might have some biological implications. This may only be true around the edge of the FACE rings where mean variation (in all directions) was very high. In 2001, Environmental Sciences Division, Oak Ridge National Laboratory, USA reviewed 156 experiments and reported that with ample water, nutrients and favourable temperature, $[CO_2]$ up to ~2000 μmol mol^{-1} increased wheat yield, with a maximum effect (+37%) at ~890 μmol mol^{-1} (Amthor 2001). Above 350 μmol mol^{-1} the relationship between $[CO_2]$ and wheat yield (response to photosynthesis) was non-linear (Amthor 2001).

In 1997 researchers identified that a symmetrical oscillation of 225 μmol mol^{-1} about a fixed target concentration of 575 μmol mol^{-1} or 650 μmol mol^{-1} enduring less than 1-min had no effect on net carbon gain in wheat (Hendrey and Miglietta 2006). Most FACE systems report the 1-min average data and this has been adopted as a benchmark across several systems for the assessment of the system performance and reporting results (Lewin *et al.* 1994; Nagy *et al.* 1994;

Hendrey *et al*. 1999; Okada *et al*. 2001; Stokes *et al*. 2005; Hovenden *et al*. 2006).

The AGFACE and other similar FACE systems have a gradient in [CO_2] from the edge towards the centre (Miglietta *et al*. 1997, 2001; Okada *et al*. 2001; Mollah *et al*. 2009). Our results showed that the 1-min average [CO_2] in plots close to the fumigation tubes were markedly higher (e.g. 822 µmol mol^{-1}) and oscillated more broadly compared with the [CO_2] in plots close to the ring-centre (Table 1, Fig. 6). Although, the spatial variations of [CO_2] in the inner 80% of the rings were found to be lower in larger rings compared with smaller rings, still the median concentration over 80% of 16-m ring varied between 550 and 800 µmol mol^{-1} during 5–10 November 2009 (Fig. 7).

The net effect of the broader variations in [CO_2] and non-linear response to photosynthesis may balance the yield close to the ring-edge or may have biological implications mentioned earlier. A few examples of the implications are: the elevated CO_2 stimulates photosynthetic carbon gain and net primary production, improves nitrogen-use efficiency, increases water-use efficiency, and stimulates dark respiration via transcriptional reprogramming of metabolism (Leakey *et al*. 2009; Dijkstra *et al*. 2010; Kirkham 2011) Therefore, understanding the distribution patterns of CO_2 inside AGFACE rings is crucial for the interpretation of the agronomical and physiological data.

Often, it is not cost-effective and practical to leave a large boundary around the perimeter of FACE rings. Therefore, in the FACE experiments, stratified treatment allocation and analysis should be used to overcome this problem. Also, AGFACE research team suggests that plots nearest the perimeter of a FACE ring should be used for destructive sampling during the growth season, while the final harvest data and non-destructive measurements are made within the more central plots where the measured [CO_2] is close to the target [CO_2].

Measuring the time-and-non-linear response weighted exposure to CO_2 of a particular plot (knowing the plot position) is critical for understanding the effect of [CO_2] on the crop performance within the plot. The variation in crop response within plot of similar treatment could in part be due to exposure at different levels of [CO_2]. This is important for data interpretation especially when ANOVA or crop modelling of responses is part of the analysis. Thus, plot-level [CO_2] estimates could be used as a covariate to help explain any variation in the biological response due to position inside the FACE rings.

Immediately after the release of pure CO_2 from the fumigation tubes, it mixes with the air and becomes diluted. The extent of dilution depended on the wind speeds. At higher wind speeds, the [CO_2] were relatively less, i.e. more diluted. It implied that more CO_2 is used at high wind. The opposite happens with low wind speeds.

It was expected that inside the AGFACE ring, close to the ground level, [CO_2] would be significantly higher as CO_2 is heavier than air; but that was not found. At and just below the fumigation height, the curve of [CO_2] looked like a basin (Fig. 5). Apart from the edge, there were no vertical gradients of [CO_2] inside the 16-m AGFACE ring, indicating the CO_2 is well mixed within the canopy.

The results on the effects of ring size were conclusive, despite the fact that the data for all ring sizes did not come from the same location and same season. The 4- and 12-m rings just failed to meet ±10% of the target protocol, which can be attributed to limited number of data available for analysis (Table 3). Monitoring in 2007 showed that [CO_2] at the ring-centre was maintained within ±10% of the target across eight 12-m rings between 86 and 94% of the time (Mollah *et al*. 2009). Therefore, it is expected that analysing the data for the whole season would improve the figures for 4- and 12-m rings but the trend shown in Table 3 will persist. Larger rings are relatively cheap to run in terms of the number of experimental plots that can be accommodated. In 2009, by increasing the ring size from 12- to 16-m we accommodated twice as many experimental plots for a 33% increase in gas use. The extra capital cost was insignificant.

The gap between the fumigation tubes and the canopy plays a role in the uniformity of the distributions of [CO_2] inside FACE rings. The earlier research by the AGFACE team has shown that

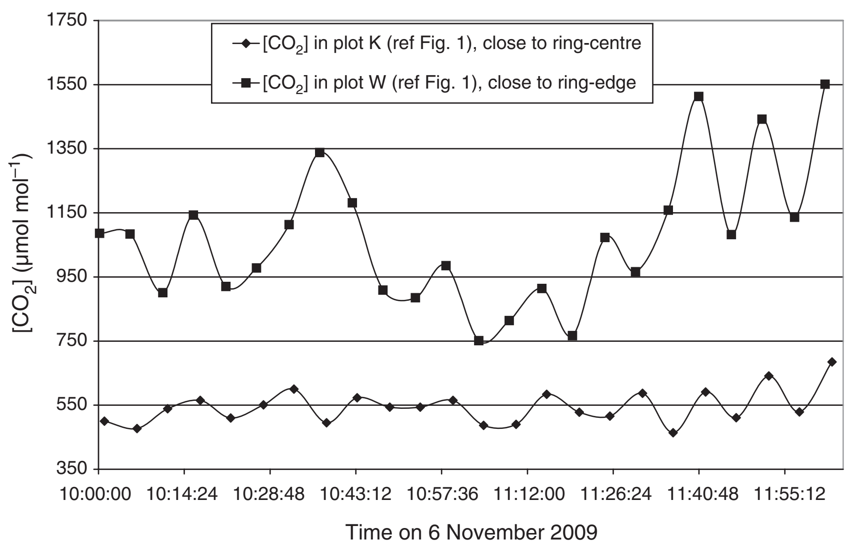

Fig. 6. Example of 1-min average [CO_2] in plots close to and far from the ring-edge.

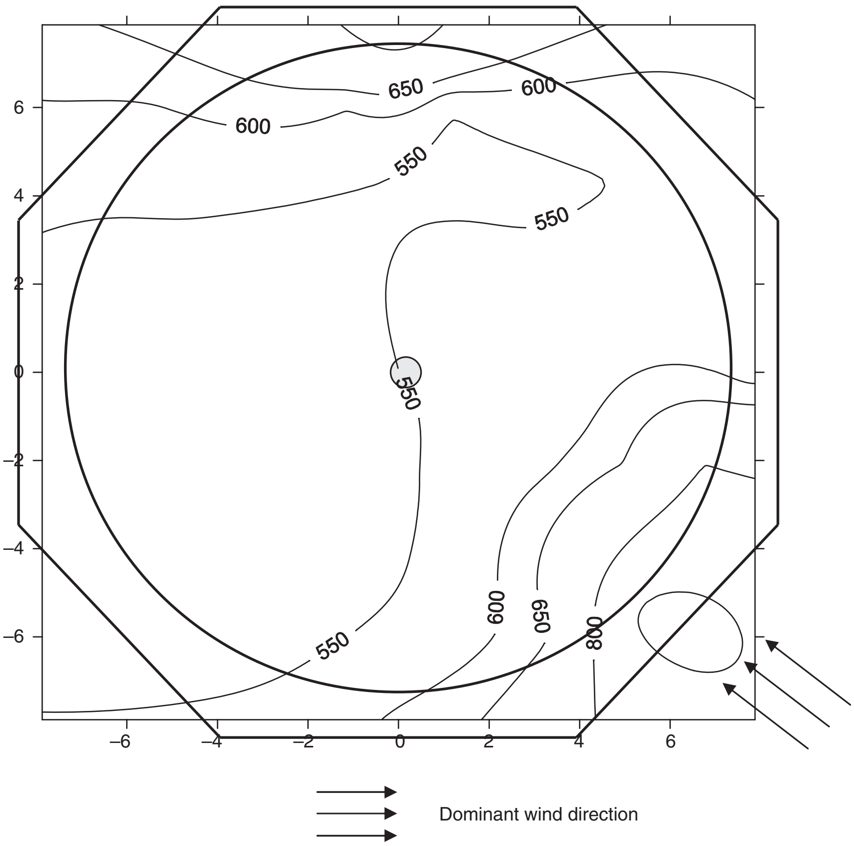

Fig. 7. Median [CO_2] concentrations over 80% (enclosed by the circle) of 16-m ring.

there was a rapid decline in [CO_2] downwind of the CO_2 source inside both 4- and 12-m rings if the gap between fumigation and canopy heights was less than 150 mm. The decline in [CO_2] downwind of the CO_2 source became less rapid, in other words, more uniform when the gap between fumigation and canopy height was over 150 mm (Mollah *et al*. 2009). During our experiments, the rapid growth of late sown wheats in November quickly reduced the gap between fumigation and canopy height below 150 mm. Consequently, we observed a rapid decline in median [CO_2] downwind of the CO_2 source inside the 16-m ring as the wind transported the pure CO_2 away from the source (ring-edge) confirming our earlier findings (Fig. 5).

Conclusions

The [CO_2] measured at different locations inside AGFACE rings showed that position within the ring creates different [CO_2] environments for individual plots. This could have implications to growth, yield and other biological responses. If possible, it would be advisable to establish an appropriate boundary between fumigation tubes and areas where experimental measurements are made to avoid high and variable [CO_2]. Otherwise, stratify the allocation of treatments and sampling within a FACE ring and use the time-non-linear-photosynthesis response weighted exposure to CO_2 of a particular plot for data analysis.

Despite engineering performance criteria being met in all sized rings the larger rings maintained the target [CO_2] of 550 μmol mol^{-1} at the ring-centres better than the smaller rings. More importantly, the spatial variations of [CO_2] in the inner 80% of the rings were found to be higher in smaller rings compared with larger rings. This means that the larger rings had more areas of relatively uniform [CO_2] in which to conduct experiments. Larger rings are also more economical to run on the basis of CO_2 cost per experimental plot. The spatial variation of [CO_2] depends on ring size and the gap between fumigation and canopy heights but not on wind speeds. The highest [CO_2] measured near the fumigation tubes decreased logarithmically with increased wind speeds. Apart from the edge, there were no vertical gradients of [CO_2] inside the 16-m AGFACE ring and at or just below the fumigation height, indicating good mixing of CO_2 within the canopy.

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