



**Early vigour in wheat: could it lead to more severe terminal drought stress under elevated atmospheric [CO<sub>2</sub>] and semi-arid conditions?**

Maryse Bourgault<sup>1,2,3\*</sup>, Heidi A. Webber<sup>4,5</sup>, Karine Chenu<sup>6</sup>, Garry J. O’Leary<sup>7</sup>, Thomas Gaiser<sup>4</sup>, Stefan Siebert<sup>4,8</sup>, Fernanda Dreccer<sup>9</sup>, Neil Huth<sup>10</sup>, Glenn J. Fitzgerald<sup>2,7</sup>, Michael Tausz<sup>11,12</sup>, Frank Ewert<sup>4,5</sup>

1. Northern Agricultural Research Center, Montana State University, 3710 Assiniboine road, Havre, Montana 59501, USA.
2. Faculty of Veterinary and Agricultural Sciences, University of Melbourne, 4 Water street, Creswick, Victoria 3363, Australia.
3. Current address: College of Agriculture and Bioresources, University of Saskatchewan, 51 Campus drive, Saskatoon, Saskatchewan S7N 5A8, Canada
4. Institute of Crop Science and Resource Conservation (INRES), University of Bonn, Katzenburgweg 5, Bonn, North Rhine-Westphalia 53115, Germany.
5. Leibniz Centre for Agricultural Landscape Research (ZALF), Eberswalder Straße 84, Müncheberg, Brandenburg 15374, Germany.
6. Queensland Alliance for Agriculture and Food Innovation (QAAFI), University of Queensland, 203 Tor street, Toowoomba, Queensland 4350, Australia.
7. Agriculture Victoria, Grains Innovation Park, 110 Natimuk road, Horsham, Victoria 3401, Australia.
8. Department of Crop Sciences, University of Göttingen, Von Siebold Straße 8, Göttingen, Lower Saxony 37075, Germany.
9. CSIRO Agriculture and Food, Cooper Laboratory, PO Box 863, University of Queensland, Warrego Highway, Gatton Queensland, 4343, Australia.
10. CSIRO Agriculture and Food, 203 Tor street, Toowoomba, Queensland 4350, Australia.

11. Department of Agriculture, Science and the Environment, CQ University, 114-190 Yaamba road, Norman Gardens QLD, 4701, Australia.

12. Previous address: School of Ecosystem and Forest Sciences, University of Melbourne, 4 Water Street, Creswick VIC, 3363, Australia.

\* Corresponding author: [maryse.bourgault@usask.ca](mailto:maryse.bourgault@usask.ca); (306) 966-4313.

## Abstract

Early vigour in wheat is a trait that has received attention for its benefits reducing evaporation from the soil surface early in the season. However, with the growth enhancement common to crops grown under elevated atmospheric CO<sub>2</sub> concentrations (e[CO<sub>2</sub>]), there is a risk that too much early growth might deplete soil water and lead to more severe terminal drought stress in environments where production relies on stored soil water content. If this is the case, the incorporation of such a trait in wheat breeding programs might have unintended negative consequences in the future, especially in dry years. We used selected data from cultivars with proven expression of high and low early vigour from the Australian Grains Free Air CO<sub>2</sub> Enrichment (AGFACE) facility, and complemented this analysis with simulation results from two crop growth models which differ in the modelling of leaf area development and crop water use. Grain yield responses to e[CO<sub>2</sub>] were lower in the high early vigour group compared to the low early vigour group, and although these differences were not significant, they were corroborated by simulation model results. However, the simulated lower response with high early vigour lines was not caused by an earlier or greater depletion of soil water under e[CO<sub>2</sub>] and the mechanisms responsible appear to be related to an earlier saturation of the radiation intercepted. Whether this is the case in the field needs to be further investigated. In addition, there was some evidence that the timing of the drought stress during crop growth influenced the effect of e[CO<sub>2</sub>] regardless of the early vigour trait. There is a need for FACE investigations of the value of traits for drought adaptation to be conducted under more severe drought conditions and variable timing of drought stress, a risky but necessary endeavour.

Keywords: physiological pre-breeding, climate change adaptation, model intercomparison, *Triticum aestivum*, drought adaptive traits.

Abbreviations: atmospheric carbon dioxide concentration ( $[\text{CO}_2]$ ); elevated atmospheric carbon dioxide concentration ( $e[\text{CO}_2]$ ); environment type (ET); genotype by environment by management interaction (GxExM); harvest index (HI); leaf area index (LAI); specific leaf area (SLA)

## Introduction

Atmospheric CO<sub>2</sub> concentrations ([CO<sub>2</sub>]) have been increasing from about 280 ppm to more than 400 ppm from the pre-industrial era until now. If global greenhouse gas emissions remain at the 2010 level, then atmospheric [CO<sub>2</sub>] will reach 550 ppm by 2050 (IPCC, 2014). For C3 crops like wheat, this considerable increase in the substrate of photosynthesis leads to higher growth rates, better water use efficiency and larger yields in the absence of temperature or rainfall changes (Leakey *et al.*, 2009). Because e[CO<sub>2</sub>] reduces stomatal conductance, and therefore increases transpiration efficiency, the response to elevated CO<sub>2</sub> (e[CO<sub>2</sub>]) has often been reported to be greater under conditions of water stress and/or in semi-arid environments (Fitzgerald *et al.*, 2016; Leakey *et al.*, 2009; Gifford 1979). However, there are also recent reports that seem to indicate the contrary (Gray *et al.*, 2016) or that there are no consistent differences (van der Kooi *et al.*, 2016), leading some to suggest the response to e[CO<sub>2</sub>] might depend on the timing of the drought experienced by the crop (Bourgault *et al.*, 2017). The relationships between timing and extent of drought and trade-offs between biomass stimulation and leaf level water use efficiency gains have been shown to be especially important in highly variable, semi-arid or Mediterranean field conditions (Tausz-Posch *et al.*, 2019).

Early vigour, defined as a larger leaf area early in the season, is a trait that has recently received attention for its benefits under Mediterranean environments where intermittent, low rainfall conditions prevail (Rebetzke and Richards, 1999). The greater cover reduces evaporation from the soil surface and this water is then available for transpiration. Early vigour in wheat is associated with greater early root development, greater early nitrogen uptake and higher photosynthetic nitrogen use efficiency (Pang *et al.*, 2014), and appears to be partially achieved through a larger specific leaf area (SLA) early in the development of the plant (Rebetzke *et al.*, 2004). Multi-regional field trials have shown that the value of the trait is more important in areas that receive frequent small rainfall

events during the growing season, but is not as useful in areas that depend on stored soil water (Wilson *et al.*, 2015).

If crop traits affect the response to  $e[\text{CO}_2]$  in crops, it might be possible to take advantage of rising atmospheric  $[\text{CO}_2]$  by selecting for greater responsiveness in crop breeding programmes either directly or by selecting traits that are associated with a greater response (Ainsworth *et al.*, 2008; Ziska *et al.*, 2012; Tausz *et al.*, 2013). On the other hand, there could also be drought adaptive traits that, by modifying growth patterns in time, might negatively impact the response to  $e[\text{CO}_2]$  (Bourgault *et al.*, 2013). Considering crop breeding takes 10-20 years to release cultivars, it is important to evaluate future environmental conditions, such as increased  $[\text{CO}_2]$  on their relative performance. On the one hand, larger leaf area early in crop development leads to greater photosynthesis and lower evaporation from the soil surface. It is possible therefore that early vigour lines might benefit more than low vigour lines from the effects of  $e[\text{CO}_2]$ , as they may carry an early benefit in both carbon gain and increased transpiration efficiency throughout the season. On the other hand, depending on the result of the competing effects of increased transpiration efficiency and lower evaporation compared to the higher water use of the larger leaf area development, too much growth early in the season could deplete the soil profile of water before grain filling. In environments where crops depend on stored water, this would lead to severe terminal drought stress (Nuttall *et al.*, 2012), analogue to the “haying-off” phenomenon observed in response to excess early N fertilizer application (van Herwaarden *et al.*, 1998). This could therefore negate some of the benefits from  $e[\text{CO}_2]$  under terminal drought but not under well-watered conditions or more moderate types of drought. We would thus expect that the benefits of this trait and its impact on the response to  $e[\text{CO}_2]$  might depend on the seasonal pattern of water availability.

Crop simulation models are particularly useful to investigate genotype by environment (GxE) interactions that are difficult to test experimentally and for climate scenarios that are not yet

realised. Field and controlled environment experiments testing pairs of lines differing in traits such as tillering propensity, early vigour or water soluble carbohydrate accumulation in wheat have rarely been able to detect genotypic differences in the response to  $e[\text{CO}_2]$  (Bourgault *et al.*, 2013; Tausz-Posch *et al.*, 2015). This trait by  $[\text{CO}_2]$  interaction, if it exists, may be masked by large GxE variability related to environmental factors other than changes in  $[\text{CO}_2]$  and by background genetic differences not related to the trait being evaluated. This makes the occurrence of Type II statistical errors highly likely where true differences are not detected as significant. While this risk can be minimised by more elaborate and expensive experimental designs, complementing field data with simulation results can help explore GxE or, in this case,  $\text{Gx}[\text{CO}_2]\text{xE}$  interactions and can offer additional insights regarding likely interactive mechanisms between physiological traits and environmental factors, information that is useful to breeders. They can also deliver information about variables that are not typically observed and highlight further research questions to evaluate in the field. However, comparison against measured data is still fundamental to validate findings and improve crop models (Boote and Sinclair, 2006; Fischer, 2011).

Because drought as an environmental factor can have vastly different effects on crop growth based on its timing and intensity, more refined analyses of the E component of the GxE interaction might be needed. For example, Chenu *et al.* (2013) clustered wheat growing seasons for various locations across Australia into four environment types (ET) based on the ratio of crop water demand met by the soil water supply. The four environment types described four different drought patterns: no stress or negligible stress (ET1), moderate water stress post-anthesis (ET2), moderate to severe water stress beginning before anthesis but relieved during grain filling (ET3), or that continued through to maturity as a severe terminal drought (ET4). Because of the various feedback mechanisms between crop growth and the environment, such characterisation is more useful in exploring GxE interactions than aggregated meteorological data to understand the response to stressful conditions and evaluate the usefulness of adaptive traits (Chenu *et al.*, 2013).

The objective of this study was to evaluate the response to  $e[\text{CO}_2]$  of wheat lines that differed in early vigour under semi-arid conditions, and whether this was influenced by seasonal patterns of water availability. Specifically, we hypothesised that the greater increase in leaf area development in high early vigour lines grown under  $e[\text{CO}_2]$  would lead to more water use pre-anthesis and subsequently greater drought stress post-anthesis, therefore reducing the grain yield response to  $e[\text{CO}_2]$ , but only in severe terminal stress conditions of ET3 and ET4 environments as characterised by Chenu *et al.* (2013). In order to do this, we identified a subset of lines grown in the Australian Grains Free Air  $\text{CO}_2$  Enrichment (AGFACE) research programme which included lines with proven expression of high and low early vigour, and compare their response to  $e[\text{CO}_2]$ . We also extracted from the experimental data variables needed to modify early leaf area development and model the early vigour trait in two crop models: APSIM and SIMPLACE. We were then able to compare the simulated response to  $e[\text{CO}_2]$  between high and low early vigour lines, but also to investigate the water use dynamics between the two groups. We were thus able to answer the following questions:

1. Do simulations give us similar responses to  $e[\text{CO}_2]$  than the experimental data?
2. Does the response to  $e[\text{CO}_2]$  depend on the type of drought experienced by the crop?
3. Does  $e[\text{CO}_2]$  lead to more severe terminal drought stress in high vigour lines?

The experimental and simulation results showed that the response to  $e[\text{CO}_2]$  of high early vigour lines was indeed lower, but greater water use pre-anthesis under  $e[\text{CO}_2]$  was not associated with the early vigour trait. Therefore, we further asked:

4. How else could we explain the lower response to  $e[\text{CO}_2]$  in high early vigour lines?

## **Materials and Methods**

### *AGFACE Experiment*

The Australian Grains Free Air  $\text{CO}_2$  Enrichment (AGFACE) facility was located near Horsham, Victoria (36°45'07"S 142°06'52"E, 127 m above sea level). Long term average (based on 1981-2010 period)

annual rainfall is 435 mm, with approximately 320 mm falling during the winter growing season (from May to November inclusively). Average maximum and minimum temperatures are 17.6°C and 5.3°C respectively during the season, with July being the coldest month (Bureau of Meteorology, 2016). Elevated CO<sub>2</sub> levels (target 550 mmol mol<sup>-1</sup> air) were maintained during daylight hours by injecting pure CO<sub>2</sub> into the air on the upwind side from horizontal stainless-steel tubes so the gas would be carried across the ring. Concentrations were maintained within 90% target (495-605 mmol mol<sup>-1</sup> air) for 93-98% of the time. More details on the site and the CO<sub>2</sub> exposure equipment are given in Mollah *et al.* (2009). Details about the general management of the experiment are also given in O'Leary *et al.* (2015) and Fitzgerald *et al.* (2016).

The experimental design was a randomized complete block split-split-plot design with 4 ambient and 4 elevated CO<sub>2</sub> octagonal wheat plots organised in blocks each containing one ambient (a[CO<sub>2</sub>]) and one e[CO<sub>2</sub>] main plot. In each year, a plus/minus supplemental irrigation treatment was usually nested within each main plot, except in 2007 when time of sowing was nested as sub-plots, and supplemental irrigation treatments were on separate main plots. There were two or more cultivars grown inside each sub-plot. More details are available on the design and treatments in O'Leary *et al.* (2015) and Fitzgerald *et al.* (2016) for 2007 to 2009, from Tausz-Posch *et al.* (2012, 2013, 2015) for 2009 to 2010 and in Houshmandhar *et al.* (2015) for 2013.

Initial soil water data was obtained from field samples collected before sowing in each main plot using a hydraulic soil corer and the gravimetric method at 0-10, 10-20 and in 20 cm increments thereafter up to 1.2 to 1.8 m depending on the year. These samples were averaged over the entire site from 2007 to 2010 as main plots were moved, whereas from 2011 to 2013, these were averaged over irrigation treatments as the main plots were rotated with field pea plots that were also subject to a plus/minus supplemental irrigation treatment. Bulk density was calculated for each layer based on the site average and assumed constant throughout the experiment. Initial soil mineral nitrate

concentrations were also obtained from field samples at depths of 0-10, 10-20, 20-60 cm on separate samples and averaged over the entire site in each year.

Destructive harvests were performed at the first node stage, anthesis and maturity (DC31, DC65 and DC90 according to the scale by Zadoks *et al.* (1974); see Table 1 for dates) on pre-determined areas of 50 to 75 cm in length (depending on year and sampling) across 4 rows excluding edge rows (from 2007 to 2009) or 5 rows including edge rows (from 2010-2013). The inclusion of edge rows is a source of increased experimental error, both in terms of increased variability and overestimation of treatment means (Rebetzke *et al.*, 2013), but we assumed that DC31 data, the calculated specific leaf area data, and mean biomass and yield responses to  $e[CO_2]$  were not affected. At DC31 and DC65, a subsample was taken from the biomass sample and separated into green leaves, dead leaves, stems and heads (if applicable), and leaf area was determined on this subsample (LI-3100C or LI-3000C with conveyor belt, LI-COR Biosciences, Lincoln, NE, USA). Leaf area of the quadrat harvested was extrapolated based on the ratio of the subsample biomass to the total quadrat biomass, and leaf area index (LAI) was calculated by dividing this by the quadrat area. Specific leaf area (SLA) was calculated by dividing the subsample leaf area by the subsample green leaf biomass. At DC90, samples were dried at 40 or 70°C depending on the year, but biomass and grain yield were calculated and presented on a 0% water content basis. Sampling dates are given for each cultivar and each year in Table 1.

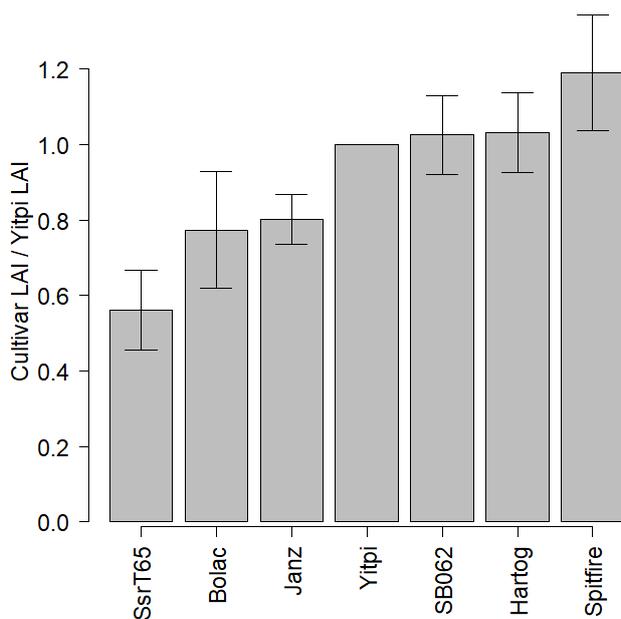
Table 1: Cultivars selected from AGFACE 2007-2013 with planting date, date of first node development (DC31), anthesis (DC65) and final harvest (DC90) samplings.

Cultivar	Vigour classification (see Figure 1)	Planting date	Stem elongation (DC31) sampling date	Anthesis (DC65) sampling date	Final harvest (DC90) sampling date
2007					
Janz	Low	18 Jun	6 Sept	29 Oct	12 Dec
Yitpi	Reference	18 Jun	6 Sept	29 Oct	12 Dec
2008					
Janz	Low	4 Jun	20 Aug	20 Oct	8 Dec
Yitpi	Reference	4 Jun	20 Aug	20 Oct	8 Dec
2009					
Hartog	High	23 Jun	2 Sept	27 Oct	4 Dec
Janz	Low	23 Jun	2 Sept	27 Oct	4 Dec
Yitpi	Reference	23 Jun	2 Sept	27 Oct	4 Dec
2010					
Hartog	High	27 May	5 Aug	18 Oct	9 Dec
Janz	Low	27 May	5 Aug	18 Oct	9 Dec
Yitpi	Reference	27 May	5 Aug	22 Oct	9 Dec
2011					
Janz	Low	25 May	1 Aug	12 Oct	5 Dec
SB062	High	25 May	1 Aug	12 Oct	5 Dec
SsrT65	Low	25 May	1 Aug	7 Oct	5 Dec
Yitpi	Reference	25 May	1 Aug	17 Oct	5 Dec
2012					
Janz	Low	30 May	15 Aug	18 Oct	5 Dec
SB062	High	30 May	15 Aug	15 Oct	5 Dec
SsrT65	Low	30 May	7 Aug	8 Oct	5 Dec
Yitpi	Reference	30 May	15 Aug	18 Oct	5 Dec
2013					
Bolac	Low	29 May	13 Aug	21 Oct	6 Dec
Spitfire	High	29 May	13 Aug	8 Oct	2 Dec
Yitpi	Reference	29 May	13 Aug	16 Oct	6 Dec

We restricted our analysis to include the reference cultivar “Yitpi” which was grown in every year from 2007 to 2013, as well as three cultivars which expressed high early vigour and three cultivars with low early vigour, based on LAI data collected at DC31 in AGFACE. In order to remove the effect of the year on LAI, we calculated the ratio of each cultivar LAI to the reference cultivar “Yitpi” under

a[CO<sub>2</sub>] for each year, and averaged this ratio across years if the cultivar was grown in more than one year (Figure 1). Efforts were made to select cultivar combinations that would span as many years as possible in the experimental data. The list of selected cultivars and years in which they were grown is given in Table 1. The normalized LAI (or ratio to reference cultivar “Yitpi”) at DC31 was found to be significantly different between the two groups ( $p < 0.001$ ).

Figure 1: Ranking of cultivars for early vigour based on comparison with reference cultivar Yitpi leaf area index (LAI) at the first node stage (DC31) in AGFACE 2007-2013 based on subsequent grouping into low (<1), and high (>1) early vigour groups. Bars represent the average ratio of the cultivar leaf area index (LAI) to Yitpi LAI across the years grown and are presented  $\pm$  standard errors.



### Statistical Analyses

Given the design was slightly different across years, analyses were conducted separately for each year with ASREML-R 3 (Butler *et al.*, 2009; R Core Team, 2017). The effects of [CO<sub>2</sub>], plus/minus supplemental irrigation, and early vigour groups (or cultivars) were treated as fixed and Wald tests were performed. Blocks, main plot number (nested within blocks), subplot number (nested within

plots) and sub-subplot number (nested within subplots) were considered random effects. Predicted means were obtained for leaf area index at DC31 with the complete data set and used to select cultivars that expressed high and low early vigour, as described above. Analyses of the response to e[CO<sub>2</sub>] were performed using early vigour groups or cultivars depending on the question being investigated.

### *Simulation models*

#### APSIM-Wheat

The Agricultural Production Systems Simulator (APSIM version 7.8) is a modular modelling simulation framework that employs a daily time step and has been extensively tested for wheat in Australian conditions. APSIM modules consist of plant, soil and management modules that simulate crop growth, soil water and nitrogen dynamics and various management controls (Holzworth *et al.*, 2014).

Growth is conceptualised as biomass accumulation from radiation intercepted, limited by water deficit (if any) and modified by elevated [CO<sub>2</sub>] (if applicable) and stress factors such as temperature and nitrogen deficiency. The [CO<sub>2</sub>] factor is calculated from the atmospheric CO<sub>2</sub> concentration and temperature as published in Reyenga *et al.*, (1999). This growth is partitioned into roots, stems, leaves and reproductive structures according to plant developmental stage. Wheat phenology is based on thermal time accumulation for each of 11 predefined stages of development and modified for cultivar-specific differences with photoperiod sensitivity and vernalisation sensitivity parameters. Plant organ biomass and leaf area are initialised at emergence. The default value of the initial leaf area is set at 200 mm<sup>2</sup> plant<sup>-1</sup>. The daily increase in carbon-limited leaf area is calculated from the increase in leaf dry weight and maximum specific leaf area which varies from 27000 to 22000 mm<sup>2</sup> g<sup>-1</sup> depending on leaf area index. The actual daily increase in leaf area is the minimum between the carbon-limited leaf area and the stressed leaf area, which is a function of the potential increase in

leaf number and leaf size and modified by stress factors related to nitrogen deficiency and soil water deficit effects on canopy expansion. Crop water demand is modelled as a function of the potential daily biomass accumulation divided by the transpiration efficiency, which in turn is a function of the average daylight vapour pressure deficit (VPD) and  $[CO_2]$ , but capped at a factor of 1.5 of the Priestly-Taylor potential evapotranspiration ( $E_o$ ). The  $[CO_2]$  factor is calculated from a linear function and is approximately 1.05 at 400 ppm, and 1.21 at 550 ppm. The VPD is estimated using daily maximum and minimum temperatures according to Tanner and Sinclair (1983). The crop water supply (or potential soil water uptake) is based on the approach by Monteith (1986) as the sum of potential root water uptake from each profile layer where the rate of water extraction is determined by the KL factor which varies by layer with crop species and soil type (Zheng *et al.*, 2015).

#### SIMPLACE

The SIMPLACE modeling framework (Gaiser *et al.*, 2013) was used to bring together the aboveground growth and development aspects of the Lintul-5 crop growth model (Wolf, 2012), the SlimWater3 water balance model based on SLIM (Addiscott and Whitmore, 1991), and modified to use evaporation and crop water extraction functions from the FAO-56 ET<sub>0</sub> manual (Allen *et al.*, 1998), a heat stress reduction function (Gabaldón-Leal *et al.*, 2016) and a canopy temperature model (Webber *et al.*, 2016). The resulting model configuration, SIMPLACE<Lintul5, SlimWater3, CanopyT, HeatStressHourly> is further referred to as SIMPLACE.

In SIMPLACE, intercepted photosynthetically active radiation is converted to crop biomass by multiplication with radiation use efficiency (RUE), which varies with phenological stage, mean temperature, water and nutrient stress as well as atmospheric  $CO_2$  concentration. At 400 ppm, the  $[CO_2]$  factor is approximately 1.04 and 1.18 at 550 ppm. Daily biomass production is allocated to roots, leaves, stems and grain yield with water stress increasing partitioning to roots. Phenological development rates are driven by 24-hour mean temperatures, photoperiod and vernalization

requirements. Leaf area growth is initially exponential and later governed by the product of biomass expansion and specific leaf area, which can vary with the phenological stage. Water stress is conceptualized as the ratio of actual transpiration to potential transpiration, which is estimated as the product of a reference crop evapotranspiration and the transpiration crop coefficient following the FAO-56 dual crop coefficient methodology (Allen *et al.*, 1998). This is denoted TRANRF in SIMPLACE outputs. Transpiration is also reduced linearly by 0.336% per ppm as ambient CO<sub>2</sub> concentrations increases (Zhao *et al.*, 2015). Daily crop water use is calculated as the minimum of crop available water in the soil and potential transpiration.

#### *Cultivar-specific calibration*

In APSIM, cultivar values were available to determine time to anthesis and time to maturity for all but two genotypes (SsrT65 and SB062). SsrT65 was given values of Silverstar based on its genetic background and previous observations of similar phenology within AGFACE and elsewhere (Bourgault *et al.*, 2013; Mitchell *et al.*, 2012). SB062 was given values identical to Yitpi based again on observed values within AGFACE. In addition, the cultivar Bolac had additional parameters associated with thermal time to flower initiation, flowering and grain filling. These were set to the base cultivar values for a better fit with the experimental data. The values for the other genotypes were checked against experimental data and deemed acceptable without modifications (Table 2; Figure S1). The APSIM model was run without further calibration apart from modifications to parameters to define the early vigour groups (see below). In SIMPLACE, thermal time to anthesis and thermal time from anthesis to maturity were estimated from the experimental data (provided in Table 2; see also Figure S1). The default values for biomass partitioning were also checked against experimental data and deemed acceptable without modifications.

Table 2: Crop model phenological parameters for the cultivars used in this study.

	APSIM		SIMPLACE/Lintul5	
	photo_sens*	vern_sens*	TSUM1**	TSUM2**
Yitpi	3.0	1.5	850	750
Janz	2.2	2.9	800	750
SsrT65	1.5	1.5	750	750
Bolac	3.1	3.1	925	750
Hartog	3.0	1.5	850	750
SB062	3.0	1.5	850	750
Spitfire	3.0	1.5	850	750

\* photo\_sens and vern\_sens are parameters in the APSIM wheat module that relate to photoperiod and vernalisation sensitivity, respectively. These modify thermal time parameters to extend or contract thermal time in early phases of crop development.

\*\* TSUM1 and TSUM2 in SIMPLACE-Lintul5 specify the thermal time requirements from emergence to anthesis and from anthesis to maturity, respectively, and are modified by both a response to photoperiod and a small vernalisation response.

#### *Modification of early leaf area development and vigour groups*

Early vigour was modified in APSIM by modifying the relationship between SLA and LAI. To obtain regression coefficients of the maximum SLA as a function of LAI as observed in AGFACE and compare this to APSIM model parameters, we fitted boundary lines on experimental data with quantile regression estimate of 0.95 using the “quantreg” package in R (Koenker, 2017). SIMPLACE uses SLA values as a function of crop development and these were therefore modified according to approximations of experimental data from AGFACE (Table 3). SLA values used by SIMPLACE are for newly expanded leaves and therefore some calibration was needed to arrive at the integrated SLA value at DC65. The differences in SLA at the DC31 and DC65 growth stages were in general well simulated by APSIM and SIMPLACE with the modifications we imposed (Figure S2). The modification of this one parameter in the models was sufficient to bring about the changes in leaf area index at DC31 and at DC65 that we observed in the experimental data (Figures S3 and S4).



- 1 Table 3: Specific Leaf Area (SLA, in  $\text{cm}^2 \text{g}^{-1}$ ) differences between early vigour groups from selected cultivars in AGFACE (2007-2013) and translation of values
- 2 into model inputs.

Cultivar/Group	APSIM			SIMPLACE			
	Calculated slope and intercept for 95 percentile of SLA ( $\text{cm}^2 \text{g}^{-1}$ ) as a function of LAI	max SLA		SLA ( $\text{cm}^2 \text{g}^{-1}$ ) at DC31	SLA ( $\text{cm}^2 \text{g}^{-1}$ ) at DC65	SLATB (in $\text{m}^2 \text{g}^{-1}$ )	
High vigour group	300 – 7x	LAI	Max SLA ( $\text{mm}^2 \text{g}^{-1}$ )	224 ± 14	174 ± 12	DVS	SLA
		0.0	30000			0.0	0.022
		5.0	26000			0.4	0.022
						1.0	0.016
						2.0	0.016
Low vigour group	250 – 5x	LAI	Max SLA ( $\text{mm}^2 \text{g}^{-1}$ )	191 ± 14	152 ± 12	DVS	SLA
		0.0	25000			0.0	0.018
		5.0	22000			0.4	0.018
						1.0	0.011
						2.0	0.011
Yitpi	256 – 5x	LAI	Max SLA ( $\text{mm}^2 \text{g}^{-1}$ )	196 ± 14	155 ± 12	DVS	SLA
		0.0	27000			0.0	0.019
		5.0	22000			0.4	0.019
			(Default values)			1.0	0.012
						2.0	0.012
							(Default values)

- 3
- 4 In APSIM, the maximum SLA parameter value is a function of the LAI, whereas in SIMPLACE, the SLA value (within the SLATB parameter) is a function of the
- 5 developmental stage (DVS), which varies from 0 to 2 (maturity), with 1 being anthesis.

6 *Simulation of AGFACE results*

7 Simulations were performed with all seven cultivars in the seven years investigated (2007-2013)  
8 using weather data obtained on-site (MEA Premium Weather Station, Measurement Engineering  
9 Australia, Magill, SA, Australia). Soil parameters related to the soil water lower limit, drained upper  
10 limit and saturated water content, as well as other soil parameters necessary for running the two  
11 models were taken from O'Leary *et al.* (2015) (Table S1). Four treatments were simulated  
12 (plus/minus supplemental irrigation, a[CO<sub>2</sub>]/e[CO<sub>2</sub>] treatments) using the experimental planting  
13 dates (Table 1), initial soil water content (Table S2), nitrate content (Table S3), and supplemental  
14 irrigation water used in AGFACE (Table S4). For comparisons with experimental data, only  
15 simulations with the cultivars grown in specific years were used. In addition, for DC31 data,  
16 comparisons were conducted based on the date of sampling rather than the simulated growth stage.  
17 To explore differences in simulated water use, all combinations were used to provide a balanced  
18 comparison between high and low early vigour lines.

19

20 *Environmental characterisation*

21 In APSIM, for each simulation, the proportion of the crop water demand satisfied by the soil water  
22 supply was calculated as a daily water stress index. This value was constrained between 0 and 1  
23 where 1 represents 100% of the water demand satisfied by the available soil water content. The  
24 daily water stress index value was aggregated over periods of 100°Cd (thermal time) centred at  
25 anthesis, as presented in Chenu *et al.* (2013). Classification of each year by irrigation treatment by  
26 [CO<sub>2</sub>] by cultivar combination into an environment type was done by minimising the sums of squared  
27 differences between the aggregated water stress index curve for each simulation and the water  
28 stress index curves of the four environment types from Chenu *et al.* (2013)(Figure 2).

29

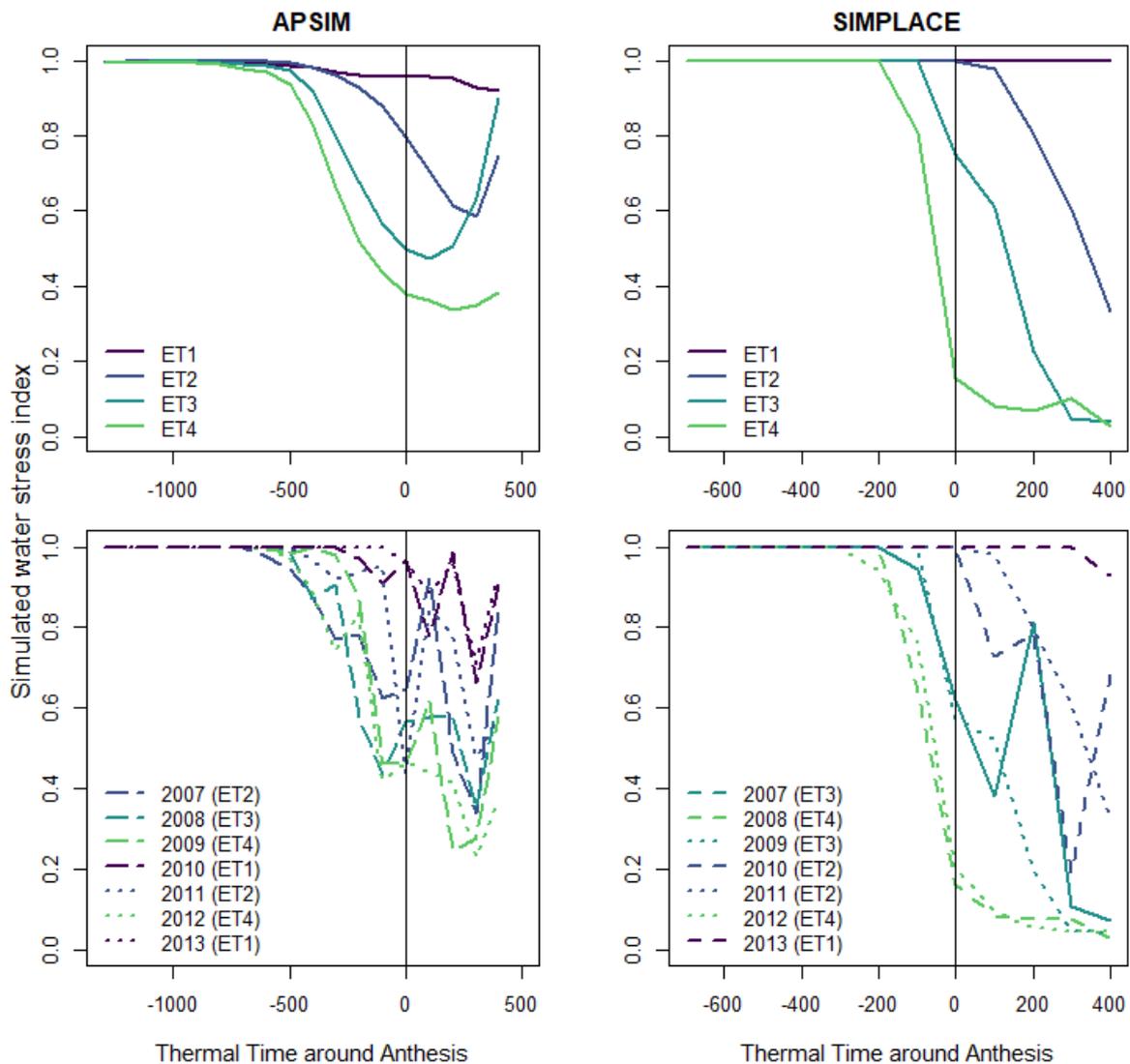
30 In SIMPLACE, the parameter TRANRF, which represents the ratio of actual transpiration to potential  
31 transpiration, was similarly aggregated over 100°Cd and centred at anthesis. Since environmental

32 type curves were not easily transferable between the two models, we used the partitioning  
33 clustering function *pam* from the *cluster* package in R (R Core Team, 2017) to define four  
34 environmental types (adapted from Chenu *et al.*, 2013) based on the full set of simulations (i.e.  
35 combinations of 7 cultivars, 7 years, a[CO<sub>2</sub>]/e[CO<sub>2</sub>] and plus/minus supplemental irrigation for a total  
36 of 198 simulations). The results of the environment type clustering for both models (including curves  
37 reproduced from Chenu *et al.*, 2013) are presented in Figure 2 (top row). Examples of the  
38 environmental characterisation based on the reference cultivar Yitpi under a[CO<sub>2</sub>] and rainfed  
39 conditions are also presented (Figure 2; bottom row).

40

41

42 Figure 2: Environmental characterisation from modelling outputs. Top row refers to clusters  
 43 identifying environmental types, while bottom row shows patterns of simulated water stress for the  
 44 reference cultivar Yitpi under ambient [CO<sub>2</sub>] and rainfed conditions. This water stress index was  
 45 calculated in APSIM (left) from the ratio of the soil water supply to crop water demand (with an  
 46 upper bound of 1), while the water stress index in SIMPLACE (right) is the ratio of actual  
 47 transpiration to potential transpiration (TRANRF parameter). A water stress index of 1 represents no  
 48 stress, while a value of 0 represents a situation where no soil water is available to the crop. Daily  
 49 ratios were aggregated over periods of 100°Cd thermal time centred at anthesis. The top left panel is  
 50 taken from Chenu *et al.*, 2013.



51

52

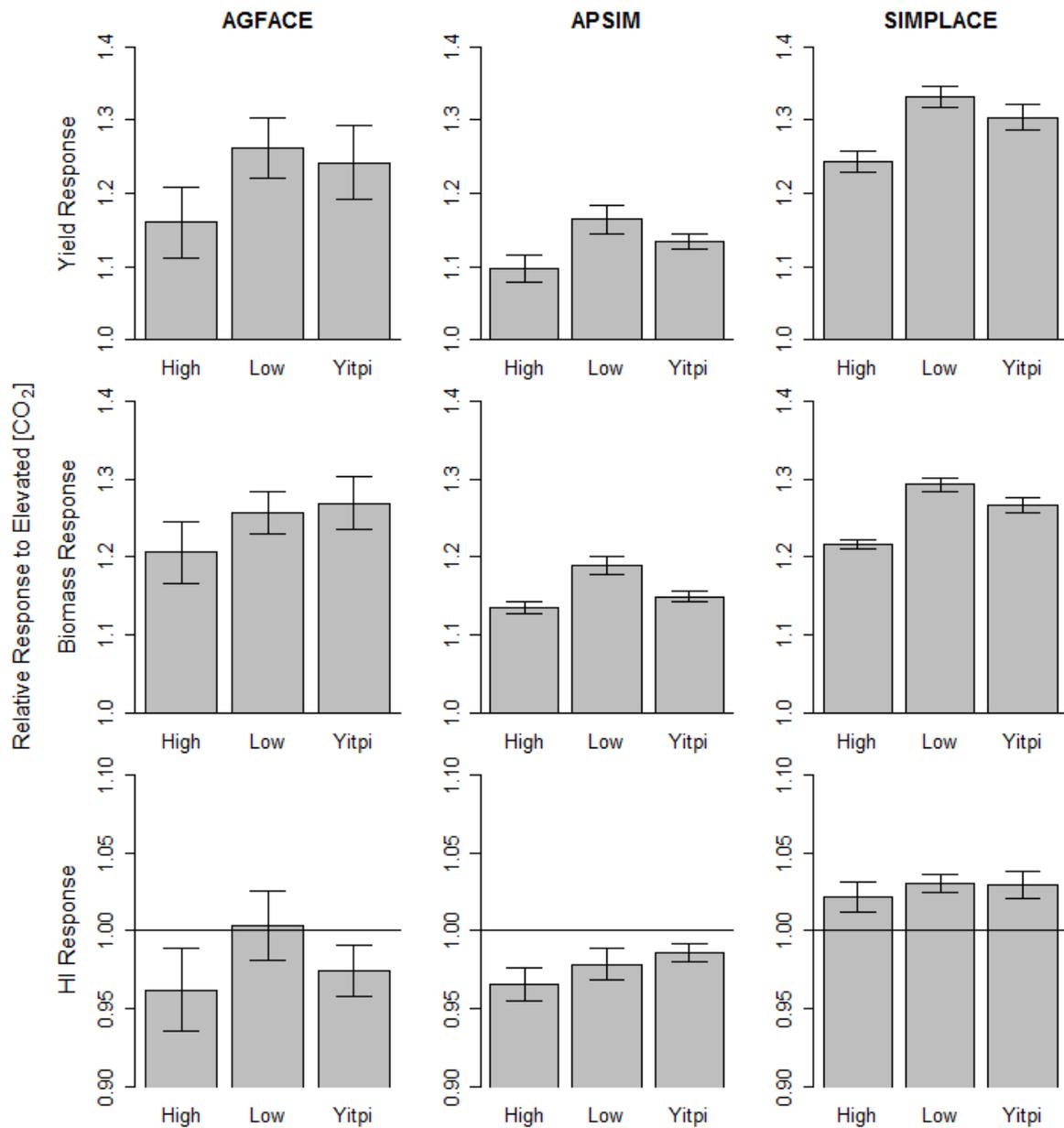
53 **Results**

54 *Do model simulations give us similar responses to e[CO<sub>2</sub>] than the experimental data?*

55 Experimental results suggested that early vigour decreased the average grain yield response to  
56 e[CO<sub>2</sub>] with high early vigour lines showing a 16% mean increase compared to 26% increase for low  
57 early vigour lines, although vigour group effects on the response were not statistically significant  
58 (p=0.2879; Figure 3). The biomass response at maturity was similar to the grain yield response,  
59 although with slightly higher mean values: 30% versus 39% for high and low early vigour lines,  
60 respectively (p=0.4375; Figure 3). For the harvest index (HI), there is a small non-significant decrease  
61 of 4% for high early vigour lines, but no change in low vigour lines (p=0.1644; Figure 3). Simulated  
62 results from both APSIM and SIMPLACE showed similar trends to the experimental data. In APSIM,  
63 the response to e[CO<sub>2</sub>] was 10 and 16% for grain yield, and 13 and 19% for biomass at maturity for  
64 high and low vigour, respectively. Results from SIMPLACE showed slightly higher values but similar  
65 differences: 24 and 33% for grain yield, 22 and 29% for biomass at maturity (Figure 3). Although  
66 SIMPLACE generally showed a slight increase in HI as a response to e[CO<sub>2</sub>], rather than the slight  
67 decrease observed in the experimental data and in APSIM simulation results, both models simulated  
68 the small differences in the response of HI between high and low early vigour lines accurately (Figure  
69 3).

70

71 Figure 3: Responses to elevated [CO<sub>2</sub>] according to early vigour group (high, low, and the cultivar  
 72 Yitpi as reference) for yield, above-ground biomass at maturity (DC90) and harvest index (HI) in the  
 73 Australian Grains Free Air CO<sub>2</sub> Enrichment (AGFACE) facility in 2007-2013 (experimental data) and as  
 74 simulated by APSIM and SIMPLACE. Results presented are response means ± standard error of  
 75 means.



76

77

78 *Does the response to e[CO<sub>2</sub>] depend on the type of drought experienced by the crop?*

79 As mentioned previously, the clustering of environment types was slightly different between the two  
80 models (Figure 2), and therefore the environmental characterization also varied between the two  
81 models. In general, however, both models followed the same trends, with SIMPLACE indicating more  
82 stress than APSIM in 29 instances out of 84 year-cultivar-irrigation-[CO<sub>2</sub>] combinations (Table 4).

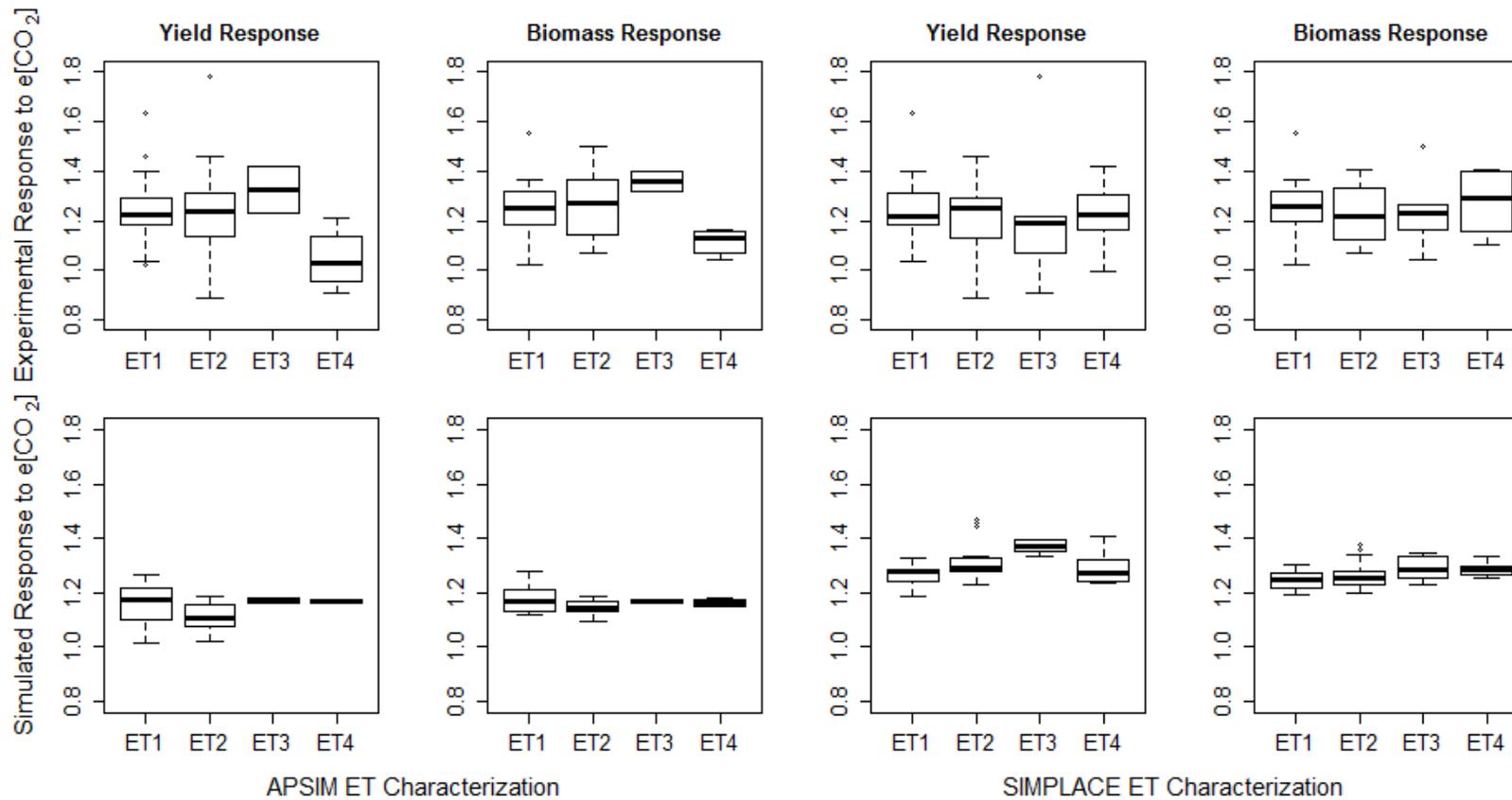
83 Using the ET characterization from APSIM (under a[CO<sub>2</sub>]), the experimental response to e[CO<sub>2</sub>] for  
84 grain yield and biomass at maturity was smaller in the most severe terminal stress conditions (ET4;  
85 Figure 4, top left). This was not significant with the response in grain yield ( $p=0.1485$ ), but was  
86 significant with the biomass response ( $p=0.0296$ ). The vigour group by ET interactions for these two  
87 parameters were not statistically significant, but according to APSIM, the low stress environment  
88 types ET1 and ET2 represented the large majority of instances, while ET3 and ET4 were only  
89 experienced by Yitpi or high vigour lines (Table 4). Therefore, there were no experimental data for  
90 low vigour lines in ET3 and ET4 to compare to high early vigour lines in these same environments. By  
91 contrast, the ET characterization of experimental data with SIMPLACE did show instances of low  
92 early vigour lines experiencing ET3 and ET4 environment types in the experimental data set (Table  
93 4). However, the responses to e[CO<sub>2</sub>] in grain yield and final biomass showed no differences  
94 between the environment types (Figure 4 top right;  $p=0.9710$  and  $p=0.7992$  respectively). Similarly,  
95 there were no early vigour group by ET interactions, so there were no significant differences in  
96 response between high and low vigour lines in the more stressful environments.

97

98 Table 4: Environmental characterisation of AGFACE experimental data with APSIM and SIMPLACE simulations outputs, according to methodology by Chenu  
 99 *et al.* (2013). The four environment types describe four different drought patterns: no stress or negligible stress (ET1), moderate water stress post-anthesis  
 100 (ET2), moderate to severe water stress beginning before anthesis but relieved during grain filling (ET3), or that continued through to maturity as a severe  
 101 terminal drought (ET4).

Year	Cultivar	APSIM Outputs				SIMPLACE Outputs			
		Rainfed		Supplemental		Rainfed		Supplemental	
		a[CO <sub>2</sub> ]	e [CO <sub>2</sub> ]	a[CO <sub>2</sub> ]	e [CO <sub>2</sub> ]	a[CO <sub>2</sub> ]	e [CO <sub>2</sub> ]	a[CO <sub>2</sub> ]	e [CO <sub>2</sub> ]
2007	Janz	ET2	ET2	ET2	ET2	ET2	ET2	ET2	ET2
	Yitpi	ET2	ET3	ET2	ET2	ET3	ET3	ET2	ET2
	2008	Janz	ET2	ET2	ET2	ET2	ET4	ET4	ET3
Yitpi		ET3	ET3	ET3	ET3	ET4	ET4	ET4	ET4
2009		Hartog	ET4	ET4	ET2	ET3	ET3	ET3	ET2
	Janz	ET2	ET2	ET2	ET2	ET3	ET3	ET2	ET2
	Yitpi	ET4	ET4	ET2	ET2	ET3	ET3	ET2	ET2
2010	Hartog	ET2	ET2	ET1	ET1	ET2	ET2	ET1	ET1
	Janz	ET1	ET1	ET1	ET1	ET2	ET2	ET1	ET1
	Yitpi	ET1	ET1	ET1	ET1	ET2	ET2	ET1	ET1
2011	Janz	ET2	ET2	ET1	ET1	ET2	ET2	ET1	ET1
	SB062	ET2	ET2	ET2	ET1	ET2	ET2	ET1	ET1
	SsrT65	ET1	ET1	ET1	ET1	ET1	ET1	ET1	ET1
2012	Yitpi	ET2	ET2	ET1	ET1	ET2	ET2	ET1	ET1
	Janz	ET2	ET2	ET1	ET2	ET4	ET3	ET2	ET2
	SB062	ET4	ET4	ET2	ET2	ET4	ET4	ET2	ET2
2013	SsrT65	ET1	ET1	ET1	ET1	ET3	ET3	ET1	ET1
	Yitpi	ET4	ET4	ET2	ET2	ET4	ET4	ET2	ET2
	Bolac	ET1	ET1	ET1	ET1	ET2	ET2	ET2	ET2
2013	Spitfire	ET1	ET1	ET1	ET1	ET1	ET1	ET1	ET1
	Yitpi	ET1	ET1	ET1	ET1	ET1	ET1	ET1	ET1

102 Figure 4: Experimental (top row) and simulated (bottom row) responses to elevated  $[\text{CO}_2]$  according to environment type (ET) for grain yield and above-  
 103 ground biomass at maturity (DC90) in the Australian Grains Free Air  $\text{CO}_2$  Enrichment (AGFACE) facility in 2007-2013 (experimental data) and as simulated by  
 104 APSIM and SIMPLACE (with their respective ET characterization). Results are presented as boxplots, with the central line as the median, and the edges of  
 105 the box as first and third quartiles.



106

107 The lower experimental response under ET4 with the APSIM ET characterization was not captured in  
108 simulated yield results (Figure 4 bottom row). Using the full set of simulations (i.e. all 7 cultivars in all  
109 7 years, with a[CO<sub>2</sub>]/e[CO<sub>2</sub>], and plus/minus supplemental irrigation (196 instances)), we were able  
110 to simulate ET3 and ET4 environments experienced by low vigour lines (mostly with the longer-  
111 season cultivar Bolac). Simulated yield and biomass accumulation at maturity with APSIM did not  
112 show the lower response in ET4 (Figures S5 and S6 for yield and biomass response respectively)  
113 observed in the experimental data. On the other hand, in this balanced set of simulated data, both  
114 models simulated larger responses for low vigour lines compared to high vigour lines under ET1 and  
115 ET2 environments (Figures S5 and S6). Differences in response were quite consistent between the  
116 two models, despite using distinct clustering analyses.

117

118 *Does e[CO<sub>2</sub>] lead to more severe terminal drought stress in high early vigour lines?*

119 Simulation results with both models suggested that in most cases, the savings from greater  
120 transpiration efficiency and lower soil evaporation were more than enough to compensate for the  
121 greater transpiration from the larger leaf area, so that pre-anthesis water use was generally lower  
122 under e[CO<sub>2</sub>]. When pre-anthesis water use was greater under e[CO<sub>2</sub>], this was not necessarily  
123 associated with high early vigour lines or with greater water stress post-anthesis. In APSIM, the only  
124 instances (14 out of 98) when e[CO<sub>2</sub>] led to greater water use pre-anthesis compared to growth at  
125 a[CO<sub>2</sub>] involved low vigour lines (Janz and SsrT65). This led to greater water stress post-anthesis in  
126 the majority of cases (10/14 instances), but considering these all occurred in ET1 and ET2  
127 environments, the stress was not important. For high vigour lines, e[CO<sub>2</sub>] consistently led to more  
128 soil water availability in the profile at anthesis: although transpiration pre-anthesis was consistently  
129 greater with e[CO<sub>2</sub>] due to the greater leaf area index, water savings from the lower soil surface  
130 evaporation and greater transpiration efficiency were more than enough to compensate. In  
131 SIMPLACE, pre-anthesis water use was greater in 16 instances in all vigour groups, but only 5 of  
132 these led to greater post-anthesis water stress: one with a low vigour line (Janz), one with Yitpi and

133 three with high vigour lines (Hartog, SB062 and Spitfire). All these instances were characterized with  
134 SIMPLACE as ET4 environment types. This could indicate a greater propensity, in some cases, for  
135 e[CO<sub>2</sub>] conditions to exacerbate drought stress in dry years, but the grain yield response to e[CO<sub>2</sub>]  
136 did not seem affected (Figure 4).

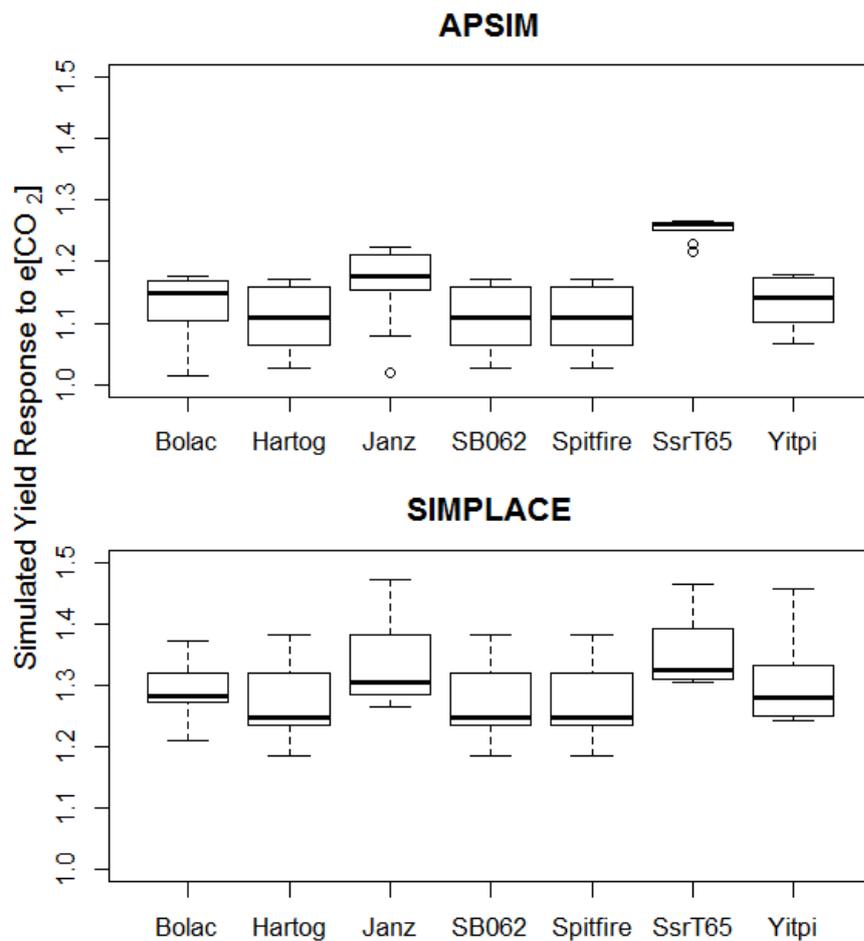
137

138 *How else could we explain the lower response to e[CO<sub>2</sub>] in high early vigour lines?*

139 Cultivar differences in simulations for this study were limited to phenology and early vigour. Out of  
140 the three selected low vigour cultivars in this study, SsrT65 and Janz are shorter-season than Yitpi  
141 and high vigour lines, and phenology could therefore be a confounding factor: pre-anthesis and post-  
142 anthesis drought stress would occur under different conditions in the same season depending on  
143 when anthesis occurs. Based on the comparison between the three low vigour lines SsrT65, Janz and  
144 Bolac (which only differed in phenology in the models), time to anthesis did have a substantial effect  
145 on the simulated response to e[CO<sub>2</sub>] (Figure 5). Both models were consistent in showing a higher  
146 response with a shorter time to anthesis. The comparison between high vigour lines and Yitpi (which  
147 all have the same phenology) confirmed that early vigour also reduced the response to e[CO<sub>2</sub>]. In  
148 addition, the cultivar Bolac is a low vigour long-season cultivar which showed a larger grain yield  
149 response than high vigour lines despite its long phenology. Clearly, both traits have effects on the  
150 response to e[CO<sub>2</sub>] according to simulated results.

151

152 Figure 5: Simulated Responses to elevated [CO<sub>2</sub>] according to cultivars for grain yield in the  
153 Australian Grains Free Air CO<sub>2</sub> Enrichment (AGFACE) facility in 2007-2013, including 7 cultivars across  
154 7 years, plus/minus supplemental irrigation (therefore including combinations that did not occur  
155 experimentally, but providing a balanced data set). Results are presented as boxplots, with the  
156 central line as the median, and the edges of the box as first and third quartiles.



157

158

159 We hypothesised high early vigour lines under e[CO<sub>2</sub>] would respond with a greater increase in leaf  
160 area early in their development, which would lead to more water use pre-anthesis and greater  
161 drought stress post-anthesis, which would reduce the grain yield response to e[CO<sub>2</sub>]. Models showed  
162 that a greater response in leaf area led to a greater increase in pre-anthesis water use and this led to  
163 more post-anthesis water stress with e[CO<sub>2</sub>] in APSIM, but not in SIMPLACE (Figure S7). More post-

164 anthesis water stress with e[CO<sub>2</sub>] led to larger grain yield responses with APSIM, but smaller yield  
165 responses in SIMPLACE (Figure S8). In addition, models showed that low vigour lines, and not high  
166 vigour lines, responded with a larger pre-anthesis leaf area stimulation (Figure S7) and larger  
167 increases in radiation intercepted, and this was positively, rather than negatively, correlated with  
168 the grain yield response (Figure S8). The pre-anthesis response in radiation intercepted explained  
169 63% of the response in grain yield in APSIM and 50% in SIMPLACE. The simulated higher grain yield  
170 responses in low vigour lines in ET1 and ET2 environments (Figures S5 and S6) further suggested a  
171 larger potential for growth increases in these lines under favourable conditions. This is consistent  
172 with a scenario where high vigour lines closed the canopy earlier in the crop development compared  
173 to low vigour lines and radiation becomes saturated and limiting growth.

174

175 **Discussion**

176 We hypothesised there might be a three-way interaction between vigour, [CO<sub>2</sub>] and water  
177 availability post-anthesis, with high vigour lines under e[CO<sub>2</sub>] being more prone to deplete soil water  
178 before grain filling, which would negate some of the benefits of e[CO<sub>2</sub>]. Experimental data from  
179 AGFACE with a subset of cultivars with proven expression of high and low early vigour suggested a  
180 lower response from high early vigour lines but was not statistically significant. While simulations  
181 with both APSIM and SIMPLACE also suggested high vigour lines responded less to e[CO<sub>2</sub>] than low  
182 vigour lines, this did not appear to be directly related to the scenario envisaged in our hypothesis.  
183 Instead, pre-anthesis growth stimulation, and in particular larger increases in radiation intercepted  
184 in low early vigour lines, explained differences in grain yield responses. The importance of radiation  
185 intercepted as illustrated by the models is in sharp contrast with the current literature: among  
186 environmental factors hypothesized to influence the response to e[CO<sub>2</sub>], the scientific focus has  
187 rather been on investigating water and nutrient availabilities, sometimes with varying temperatures  
188 (see for example recent reviews by Kimball, 2016; Tausz-Posch *et al.*, 2019; van der Kooi *et al.*, 2016)  
189 while differences in radiation intercepted are not typically among the primary factors being  
190 considered. A notable connection, however, is the work by Shimono (2011; and Shimono *et al.* 2014,  
191 2019) who suggested using the response to low density planting (in rice) as a surrogate for grain  
192 yield response to e[CO<sub>2</sub>], although they also argue that the two responses would likely occur through  
193 different physiological mechanisms (Shimono *et al.*, 2019). To properly test the effect of radiation  
194 intercepted on the response to e[CO<sub>2</sub>] in an experimental setting, data on the rate of canopy  
195 development and timing of canopy closure in addition to daily radiation would need to be collected.

196

197 The experimental yield and biomass response to e[CO<sub>2</sub>], when characterized by drought type with  
198 APSIM, suggested that the response increased slightly with moderate water stress (in ET2 and ET3  
199 environments), but decreased sharply in severe terminal drought stress (ET4 environment). These  
200 results are consistent with the study of Gray *et al.* (2016) with 8-year of soybean experimentation,

201 which showed that severe drought led to a complete lack of response to e[CO<sub>2</sub>]. This might also  
202 explain why the response to e[CO<sub>2</sub>] is more variable with water stress as shown in a recent meta-  
203 analysis by van de Kooi *et al.* (2016). This study highlighted a gap in knowledge of the response to  
204 e[CO<sub>2</sub>] under severe water stress scenarios. While the environmental characterisation with APSIM  
205 showed a tendency towards a lower response in the strongest terminal stress environment (ET4)  
206 with the experimental data (Figure 4), we still do not have many replicates of each ETs even with 7  
207 years of data. In fact, by adding vigour as an additional factor, we are completely missing  
208 representations of low early vigour lines in ET3 and ET4 seasons, partly because of confounding  
209 effects of short phenology in 2 of the 3 low early vigour lines. While advanced statistical models such  
210 as mixed models used here can handle some missing data (Smith *et al.*, 2005), the absence of  
211 treatment combinations limits our confidence in results. In particular, the environmental  
212 characterization with SIMPLACE did not lead to the same pattern of effects on the response (Figure  
213 4), which might be partially explained by some cultivar-season combinations being characterized  
214 differently based on the model used. However, the clustering analysis for SIMPLACE was also based  
215 on a much smaller data set than the APSIM clustering.

216

217 The lack of response to e[CO<sub>2</sub>] under severe water stress as reported here and by Gray *et al.*, (2016)  
218 suggests there are still real gaps in knowledge about interactions between [CO<sub>2</sub>] and water stress  
219 above and beyond the relationships with primary productivity and stomatal conductance. As  
220 simulated responses did not show this pattern, such knowledge has not been incorporated in  
221 simulation models. For example, Gray *et al.* (2016) showed that soybean plants grown under e[CO<sub>2</sub>]  
222 were more responsive to water stress than those grown under a[CO<sub>2</sub>], showing a lower stomatal  
223 conductance for the same leaf-level ABA concentration. Whether, or to what extent, this might also  
224 be true for wheat has not been investigated. In addition, Tausz-Posch *et al.* (2013) observed that  
225 differences in stomatal conductance in wheat depended on the time of day or the measurement  
226 day, so there may be other factors that interact with [CO<sub>2</sub>] on an hourly or minute scale that are not

227 captured in the single TE modifier used by models. Other research pointed out differences in the  
228 root-to-shoot ratio under e[CO<sub>2</sub>]: for example, Pandey *et al.* (2018) showed increased partitioning to  
229 roots with e[CO<sub>2</sub>] and low phosphorus conditions for hydroponically grown wheat plants. These  
230 findings remain to be confirmed in field settings. Nie *et al.* (2013) in a meta-analysis with heavy  
231 reliance on perennial systems showed e[CO<sub>2</sub>] not only led to increased root-to-shoot ratio, but also  
232 increased proportion of roots at depth and increased proportion of fine roots. Such effects of e[CO<sub>2</sub>],  
233 if also present in wheat, would modify the ratios of water demand and water supply in the models,  
234 and therefore the water stress indices from simulation models, potentially leading to different  
235 environmental characterisations under e[CO<sub>2</sub>] and/or better relationships between water stress  
236 indices and the grain response to e[CO<sub>2</sub>].

237

238 The simulation of leaf area curves assumes a bell-shaped growth, peaking shortly before anthesis,  
239 and simulation of early vigour therefore leads to a larger maximum leaf area. However, there are  
240 indications that in some populations bred for high early vigour this might not be the case: for  
241 example, Bourgault *et al.* (2013) demonstrated high early vigour lines had a greater leaf area for the  
242 first three leaves, but had fewer leaves, so their leaf area at anthesis was less than for the low early  
243 vigour parent. If we were to explore this in greater detail with such lines, the simulation of leaf area  
244 would need to be conceptualized in more detail and possibly with less reliance on SLA, which is  
245 assumed by models to be either fixed for a specific growth stage (SIMPLACE) or vary with LAI  
246 (APSIM), even though we know it is also sensitive to environmental factors and therefore a source of  
247 uncertainty in these models (Tardieu *et al.*, 1999; Setiyono *et al.*, 2008).

248

249 Then, how confident are we about the partitioning of ET into soil evaporation and transpiration in  
250 models? Although the LAI was increased approximately 30% with e[CO<sub>2</sub>], cumulative differences  
251 between ambient and e[CO<sub>2</sub>] simulations in both evaporation and transpiration were below 9 and 16  
252 mm respectively. While it is expected that the higher transpiration demand from a greater LAI under

253 e[CO<sub>2</sub>] might be offset by savings through improved transpiration efficiency, are such small  
254 differences realistic for differences in soil evaporation? The time period during which differences in  
255 soil evaporation between high and low vigour lines is arguably quite short, and usually lies during the  
256 time of the year for which potential ET is low. Then perhaps the value of the early vigour trait in  
257 reducing evaporation might be secondary to other benefits, such as a more vigorous root system,  
258 which would allow for quicker uptake of soil water and nutrients early in the season (Palta *et al.*,  
259 2011).

260

261 Finally, increasing atmospheric [CO<sub>2</sub>] will also cause increasing temperatures in most regions of the  
262 world. The net effect of the water savings from e[CO<sub>2</sub>] combined with the advanced crop  
263 development and higher water use from higher chronic temperatures and/or the effects of potential  
264 heat waves during grain filling with regards to biomass development and grain yield are highly  
265 uncertain. Although the inclusion of this fourth factor is not possible with this study, a proper  
266 assessment of the implications of genetic traits for climate change adaptation would require the  
267 evaluation of several scenarios in regards to temperature pattern changes. Previous research has  
268 shown that considering temperature and [CO<sub>2</sub>] effects separately resulted in very different findings  
269 compared to studies where the effects were combined, in particular with regards to crop water  
270 requirement and heat stress (Zhao *et al.*, 2015).

271

## 272 **Conclusion**

273 Experimental data from AGFACE gave some indications that lines expressing high early vigour might  
274 respond less to e[CO<sub>2</sub>] than low vigour lines. Although the effect was not found to be statistically  
275 significant in the experimental data, two crop growth models supported this notion. We had  
276 hypothesized these observations could be explained by that high early vigour lines depleting  
277 available soil water earlier in the season when grown under e[CO<sub>2</sub>], and thus suffering from more  
278 severe terminal drought stress. Environmental characterizations of the seasonal pattern of drought

279 stress in the experimental data and patterns of water use in the simulations however did not  
280 support this hypothesis. Rather, both simulation models suggested early canopy closure in high early  
281 vigour lines would limit the potential for increased growth and yield, especially in more favourable  
282 environments. Under less favourable conditions, the timing of the drought stress appears to  
283 influence the effect of  $e[CO_2]$ . This study highlighted the need to resolve whether there are  
284 differences in the response to  $e[CO_2]$  under drought types that differ in intensity and timing.

285

### 286 **Acknowledgements**

287 Research at the Australian Grains Free Air Carbon dioxide Enrichment (AGFACE) facility was jointly  
288 run by the Victorian Government and the University of Melbourne and received substantial  
289 additional funding from the Australian Commonwealth Department of Agriculture and Water  
290 Resources and the Grains Research and Development Corporation. We wish to acknowledge the  
291 crucial contributions of Mahabubur Mollah and Russel Argall and their team in running and  
292 maintaining the AGFACE facility, as well as Peter Howie and Samuel Henty from the University of  
293 Melbourne for technical help. M. Bourgault also acknowledges an Endeavour Research Fellowship  
294 from the Australian government that enabled the visit to Prof. Ewert's laboratory at the University of  
295 Bonn in Germany. Acknowledgements are also due to Andreas Enders and Gunther Krauss from the  
296 University of Bonn for technical help with SIMPLACE and to Greg McLean for questions related to  
297 APSIM outputs.

298

299 The authors declare no conflict of interest.

300

301 The data that support the findings of this study are available from the corresponding author upon  
302 reasonable request and approval from the former AGFACE program leaders (M. Tausz and G.  
303 Fitzgerald).

304

305 **References**

- 306 Addiscott TM, Whitmore AP (1991) Simulation of solute leaching in soils with different  
307 permeabilities. *Soil Use and Management* **7**, 94-102.
- 308 Ainsworth E, Beier C, Calafapietra C, Ceulemans R, Durand-Tardif M, Farquhar GD, Godbold DL,  
309 Hendry GR, Hickler T, Kaduk J, Karnosky DF, Kimball B, Körner C, Koornneef M, Lafarge T,  
310 Leakey AD, Lewin KF, Long SP, Manderscheid R, McNeil DL, Mies TA, Miglieta F, Morgan JA,  
311 Nagy J, Norby RJ, Norton RM, Percy KE, Rogers A, Soussana J-F, Stitt M, Weigel H-J, White  
312 JW (2008) Next generation of elevated [CO<sub>2</sub>] experiments with crops: a critical investment  
313 for feeding the future world. *Plant, Cell and Environment*, **31**, 1317-1324.
- 314 Allen RG, Pereira LS, Raes D, Smith M (1998) Crop Evapotranspiration – Guidelines for computing  
315 crop water requirements. FAO Irrigation and Drainage paper 56. FAO, Rome.
- 316 Boote KJ, Sinclair TR (2006) Crop physiology: Significant Discoveries and Our Changing Perspective on  
317 Research. *Crop Science*, **46**, 2270-2277.
- 318 Bourgault M, Dreccer MF, James AT, Chapman SC (2013) Genotypic variability in the response to  
319 elevated CO<sub>2</sub> of wheat lines differing in adaptive traits. *Functional Plant Biology*, **40**, 172-  
320 184.
- 321 Bourgault M, Brand J, Tausz-Posch S, Armstrong RD, O’Leary GL, Fitzgerald GJ, Tausz M (2017) Yield,  
322 growth and grain nitrogen response to elevated CO<sub>2</sub> in six lentil (*Lens culinaris*) cultivars  
323 grown under Free Air CO<sub>2</sub> Enrichment (FACE) in a semi-arid environment. *European Journal*  
324 *of Agronomy* **87**, 50-58.
- 325 Bureau of Meteorology (Australian Government), 2016. Climate Data Online database (Horsham  
326 Polkemmet Rd VIC). URL: <http://www.bom.gov.au/climate/data/>. Last accessed July 2016.
- 327 Butler DG, Cullis BR, Gilmour AR, Gogel BJ (2009) Mixed models for S language environments:  
328 ASReml-R reference manual. Queensland Department Primary Industries and Fisheries,  
329 Toowoomba, QLD, Australia. 149 pp.

330 Chenu K, Deihimfard R, Chapman SC (2013) Large-scale characterisation of drought patterns: a  
331 continent-wide modelling approach applied to the Australian wheat-belt – spatial and  
332 temporal trends. *New Phytologist*, **198**: 801-820.

333 Deryng, D., Elliott, J., Folberth, C., Müller, C., Pugh, T.A.M., Boote, K.J., Conway, D., Ruane, A.C.,  
334 Gerten, D., Jones, J.W., Khabarov, N., Olin, S., Schaphoff, S., Schmid, E., Yang, H.,  
335 Rosenzweig, C., 2016. Regional disparities in the beneficial effects of rising CO<sub>2</sub>  
336 concentrations on crop water productivity. *Nature Climate Change* **6** (8): 786-790.  
337 doi:10.1038/nclimate2995

338 Fischer, R.A., 2011. Wheat Physiology: a review of recent developments. *Crop & Pasture Science*, **62**,  
339 95-114.

340 Fitzgerald GJ, Tausz M, O’Leary G, Mollah MR, Tausz-Posch S, Seneweera S, Mock I, Löw M,  
341 Partington DL, McNeil D, Norton RM (2016) Elevated atmospheric [CO<sub>2</sub>] can dramatically  
342 increase wheat yields in semi-arid environments and buffer against heat waves. *Global*  
343 *Change Biology*, **22**, 2269-2284.

344 Gabaldón-Leal C, Webber H, Otegui ME, Slafer GA, Ordóñez RA, Gaiser T, Lorite IJ, Ruiz-Ramos M,  
345 Ewert F (2016) Modelling the impact of heat stress on maize yield formation. *Field Crops*  
346 *Research* **198**, 226-237.

347 Gaiser T, Perkons U, Küpper PM, Kautz T, Uteau-Puschmann D, Ewert F, Enders A, Krauss G (2013)  
348 Modeling biopore effects on root growth and biomass production on soils with pronounced  
349 sub-soil clay accumulation. *Ecological Modelling* **265**, 6-15.

350 Gifford RM (1979) Growth and yield of CO<sub>2</sub>-enriched wheat under water-limited conditions.  
351 *Australian Journal of Plant Physiology* **6**, 367-378.

352 Gray SB, Dermody O, Klein SP, Locke AM, McGrath JM, Paul RE, Rosenthal DM, Ruiz-Vera UM,  
353 Siebers MH, Strellner R, Ainsworth EA, Bernacchi CJ, Long SP, Ort DR, Leakey ADB (2016)  
354 Intensifying drought eliminates the expected benefits of elevated carbon dioxide for  
355 soybean. *Nature Plants* **2** (9), article 16132.

356 Holzworth DP, Huth NI, deVoil PG, Zurcher EG, Herrmann NI, McLean G, Chenu K, van Oosterom EJ,  
357 Snow V, Murphy C, Moore AD, Brown H, Whish JPM, Verrall S, Fainges J, Bell LW, Peake AS,  
358 Poulton PL, Hochman Z, Thorburn PJ, Gaydon DS, Dalgliesh NP, Rodriguez D, Cox H,  
359 Chapman S, Doherty A, Teixeira E, Sharp J, Cichota R, Vogeler I, Li FY, Wang E, Hammer GL,  
360 Robertson MJ, Dimes JP, Whitbread AM, Hunt J, van Rees H, McClelland T, Carberry PS,  
361 Hargreaves JNG, MacLeod N, McDonald C, Harsdorf J, Wedgwood S, Keating BA (2014)  
362 APSIM – Evolution towards a new generation of agricultural systems simulation.  
363 *Environmental Modelling & Software* **62**, 327-350.

364 Houshmandfar A, Fitzgerald GJ, Tausz M (2015) Elevated CO<sub>2</sub> decreases both transpiration flow and  
365 concentrations of Ca and Mg in the xylem sap of wheat. *Journal of Plant Physiology*, **174**,  
366 157-160.

367 Intergovernmental Panel on Climate Change (IPCC) (2014) *Climate Change 2014: Synthesis Report*.  
368 *Contribution of Working Groups I, II and III to the Fifth Assessment Report of the*  
369 *Intergovernmental Panel on Climate Change* [Core Writing Team, R.K. Pachauri and L.A.  
370 Meyer (eds.)]. IPCC, Geneva, Switzerland, 151 pp.

371 Kimball BA (2016) Crop responses to elevated CO<sub>2</sub> and interactions with H<sub>2</sub>O, N and temperature.  
372 *Current Opinion in Plant Biology* **31**, 36-43.

373 Koenker R (2017). Package 'quantreg': Quantile Regression. Available on [www.r-project.org](http://www.r-project.org). Last  
374 accessed August 2017.

375 Leakey ADB, Ainsworth EA, Bernacchi CJ, Rogers A, Long SP, Ort DR (2009) Elevated CO<sub>2</sub> effects on  
376 plant carbon, nitrogen, and water relations: six important lessons from FACE. *Journal of*  
377 *Experimental Botany*, **60**, 2859-2876.

378 Mitchell JH, Chapman SC, Rebetzke GJ, Bonnett DG, Fukai S (2012) Evaluation of a reduced-tillering  
379 (tin) gene in wheat lines grown across different production environments. *Crop & Pasture*  
380 *Science* **63**, 128-141.

381 Mollah M, Norton R, Huzzey J (2009) Australian grains free-air carbon dioxide enrichment (AGFACE)  
382 facility: design and performance. *Crop and Pasture Science*, **60**, 697–707.

383 Monteith JL (1986) Significance of the coupling between saturation vapour-pressure deficit and  
384 rainfall in monsoon climates. *Experimental Agriculture* **22**, 329-338.

385 Nie M, Lu M, Bell J, Raut S, Pendall E (2013) Altered root traits due to elevated CO<sub>2</sub>: a meta-analysis.  
386 *Global Ecology and Biogeography* **22**, 1095-1105.

387 Nuttall JG, O’Leary GJ, Khimashia N, Asseng S, Fitzgerald G, Norton R (2012) ‘Haying-off’ in wheat is  
388 predicted to increase under a future climate in south-eastern Australia. *Crop and Pasture*  
389 *Science*, **63**, 593-605.

390 O’Leary GJ, Christy B, Nuttall J, Huth N, Cammarano D, Stockle C, Basso B, Shcherbak I, Fitzgerald G,  
391 Luo Q, Farre-Condina I, Palta J, Asseng S (2015) Response of wheat growth, grain yield and  
392 water use to elevated CO<sub>2</sub> under Free-Air CO<sub>2</sub> Enrichment (FACE) experiment and modelling  
393 in a semi-arid environment. *Global Change Biology*, **21**, 2670-2686.

394 Palta JA, Chen X, Milroy SP, Rebetzke GJ, Dreccer MF, Watt M (2011) Large root systems: are they  
395 useful in adapting wheat to dry environments? *Functional Plant Biology* **38**, 347-354.

396 Pandey R, Lal MK, Vengavasi K (2018). Differential response of hexaploid and tetraploid wheat to  
397 interactive effects of elevated [CO<sub>2</sub>] and low phosphorus. *Plant Cell Reports*, **37**, 1231-1244.

398 Pang J, Palta JA, Rebetzke GJ, Milroy SP (2014). Wheat genotypes with high early vigour accumulate  
399 more nitrogen and have higher photosynthetic nitrogen use efficiency during early growth.  
400 *Functional Plant Biology*, **41**, 215-222.

401 R Core Team (2017) R: A language and environment for statistical computing. R Foundation for  
402 Statistical Computing, Vienna, Austria. URL <http://www.R-project.org/>.

403 Rebetzke GJ, Botwright TL, Moore CS, Richards RA, Condon AG (2004) Genotypic variation in specific  
404 leaf area for genetic improvement of early vigour in wheat. *Field Crops Research* **88**, 179-  
405 189.

406 Rebetzke GJ, Fisher RA, van der Herwaarden AF, Bonnett DG, Chenu K, Rattey AR, Fettell NA (2013).  
407 Plot size matters: interference from intergenotypic competition in plant phenotyping  
408 studies. *Functional Plant Biology* **41**, 107-118.

409 Rebetzke GJ, Richards RA (1999). Genetic improvement of early vigour in wheat. *Australian Journal*  
410 *of Agricultural Research*, **50**, 291-301.

411 Setiyono TD, Weiss A, Specht JE, Cassman KG, Dobermann A (2008) Leaf area index simulation in  
412 soybean grown under near-optimal conditions. *Field Crops Research* **108**, 82-92.

413 Shimono, H (2011) Rice genotypes that respond strongly to elevated CO<sub>2</sub> also respond strongly to  
414 low planting density. *Agriculture, Ecosystems & Environment* **141**, 240-243.

415 Shimono H, Ozaki Y, Jagadish KSV, Sakai H., Usui Y, Hasewaga T, Kumagai E, Nakano H, Yoshinaga S  
416 (2014) Planting geometry as a pre-screening technique for identifying CO<sub>2</sub> responsive rice  
417 genotypes: a case study of panicle number. *Physiologia Plantarum* **152**, 520-528.

418 Shimono H, Farquhar G, Brookhouse M., Busch FA, O'Grady A, Tausz M, Pinkard EA (2019)  
419 Prescreening in large populations as a tool for identifying elevated CO<sub>2</sub>-responsive  
420 genotypes in plants. *Functional Plant Biology* **46**, 1-14.

421 Smith AB, Cullis BR, Thomson R (2005) The analysis of crop cultivar breeding and evaluation trials: an  
422 overview of current mixed model approaches. *Journal of Agricultural Science* **143**, 449-462.

423 Tanner CB, Sinclair TR (1983) Efficient water use in crop production: research or re-research. In Taylor  
424 HM, Jordan WR, Sinclair TR (eds) *Limitations to efficient water use in crop production*.  
425 American Society of Agronomy, Madison, WI, pp. 1-27.

426 Tardieu F, Granier C, Muller B (1999) Modeling leaf expansion in a fluctuating environment: are  
427 changes in specific leaf area a consequence of changes in expansion rate? *New Phytologist*  
428 **143**, 33-43.

429 Tausz M, Tausz-Posch S, Norton RM, Fitzgerald GJ, Nicolas ME, Seneweera S (2013) Understanding  
430 crop physiology to select breeding targets and improve crop management under increasing  
431 atmospheric CO<sub>2</sub> concentrations. *Environmental and Experimental Botany*, **88**, 71-80.

432 Tausz-Posch S, Seneweera S, Norton RM, Fitzgerald GJ, Tausz M (2012) Can a wheat cultivar with  
433 high transpiration efficiency maintain its yield advantage over a near-isogenic cultivar under  
434 elevated CO<sub>2</sub>? *Field Crops Research*, **133**, 160-166.

435 Tausz-Posch S, Norton RM, Seneweera S, Fitzgerald GJ, Tausz M (2013) Will intra-specific differences  
436 in transpiration efficiency be maintained in a high CO<sub>2</sub> world? A FACE study. *Physiologia*  
437 *Plantarum*, **148**, 232-245.

438 Tausz-Posch S, Dempsey RW, Seneweera S, Norton RM, Fitzgerald GJ, Tausz M (2015) Does a free  
439 tillering wheat cultivar benefit more from elevated CO<sub>2</sub> than a restricted tillering cultivar in a  
440 water-limited environment? *European Journal of Agronomy*, **64**, 21-18.

441 Tausz-Posch S, Tausz M, Bourgault M (2019) Elevated [CO<sub>2</sub>] effects on crops: Advances in  
442 understanding acclimation, nitrogen dynamics and interactions with drought and other  
443 organisms. *Plant Biology* [Early online View/In press] doi:10.1111/plb.12994

444 van der Kooi CJ, Reich M, Löw M, De Kok LJ, Tausz M (2016) Growth and yield stimulation under  
445 elevated CO<sub>2</sub> and drought: A meta-analysis on crops. *Environmental and Experimental*  
446 *Botany* **122**, 150-157.

447 van Herwaarden AF, Farquhar GD, Angus JF, Richards RA, Howe GN (1998) 'Haying-off', the negative  
448 grain yield response of dryland wheat to nitrogen fertiliser – I. Biomass, grain yield, and  
449 water use. *Australian Journal of Agricultural Research* **49**: 1067-1081.

450 Webber H, Ewert F, Kimball BA, Siebert S, White JW, Wall GW, Ottman MJ, Trawally DNA, Gaiser T  
451 (2016) Simulating canopy temperature for modelling heat stress in cereals. *Environmental*  
452 *Modelling & Software* **77**, 143-155.

453 Wilson PB, Rebetzke GJ, Condon AG (2015) Of growing importance: combining greater early vigour  
454 and transpiration efficiency for wheat in variable rainfed environments. *Functional Plant*  
455 *Biology*, **42**, 1107-1115.

456 Wolf J (2012) User Guide for LINTUL5: Simple generic model for simulation of crop growth under  
457 potential, water limited and nitrogen, phosphorus and potassium limited conditions. Plant  
458 Production Systems Group. Wageningen University, Wageningen, Netherlands.

459 Zadoks JC, Chang TT, Konzak CF (1974) A decimal code for the growth stage of cereals. *Weed*  
460 *Research*, **14**, 415-421.

461 Zhao G, Webber H, Hoffmann H, Wolf J, Siebert S, Ewert F (2015) The implication of irrigation in  
462 climate change impact assessment: a European-wide study. *Global Change Biology* **21**, 4031-  
463 4048.

464 Zheng B, Chenu K, Doherty A, Chapman S (2015) The APSIM-Wheat Module (7.5 R3008).  
465 <http://www.apsim.info/Portals/0/Documentation/Crops/WheatDocumentation.pdf>. Last  
466 accessed October 2 2019.

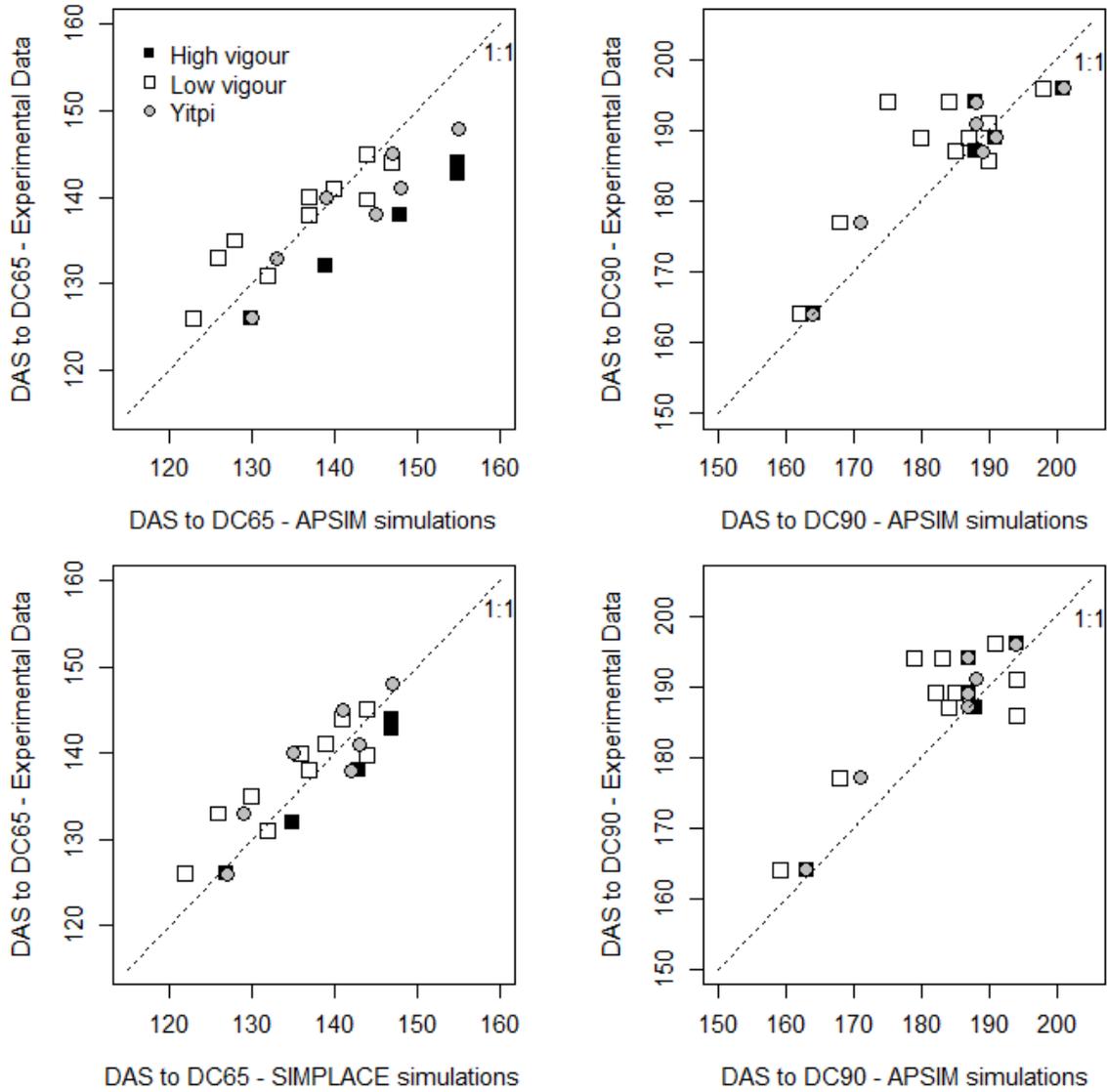
467 Ziska LH, Bunce JA, Shimono H, Gealy DR, Baker JT, Newton PCD, Reynolds M, Jagadish KSV, Zhu C,  
468 Howden M, Wilson LT (2012) Food security and climate change: on the potential to adapt  
469 global crop production by active selection to rising atmospheric carbon dioxide. *Proceedings*  
470 *of the Royal Society B*, **279**, 4097-4105.

471

472

Supplemental information

473 Figure S1: Fit of phenological stages for APSIM and SIMPLACE against experimental data.

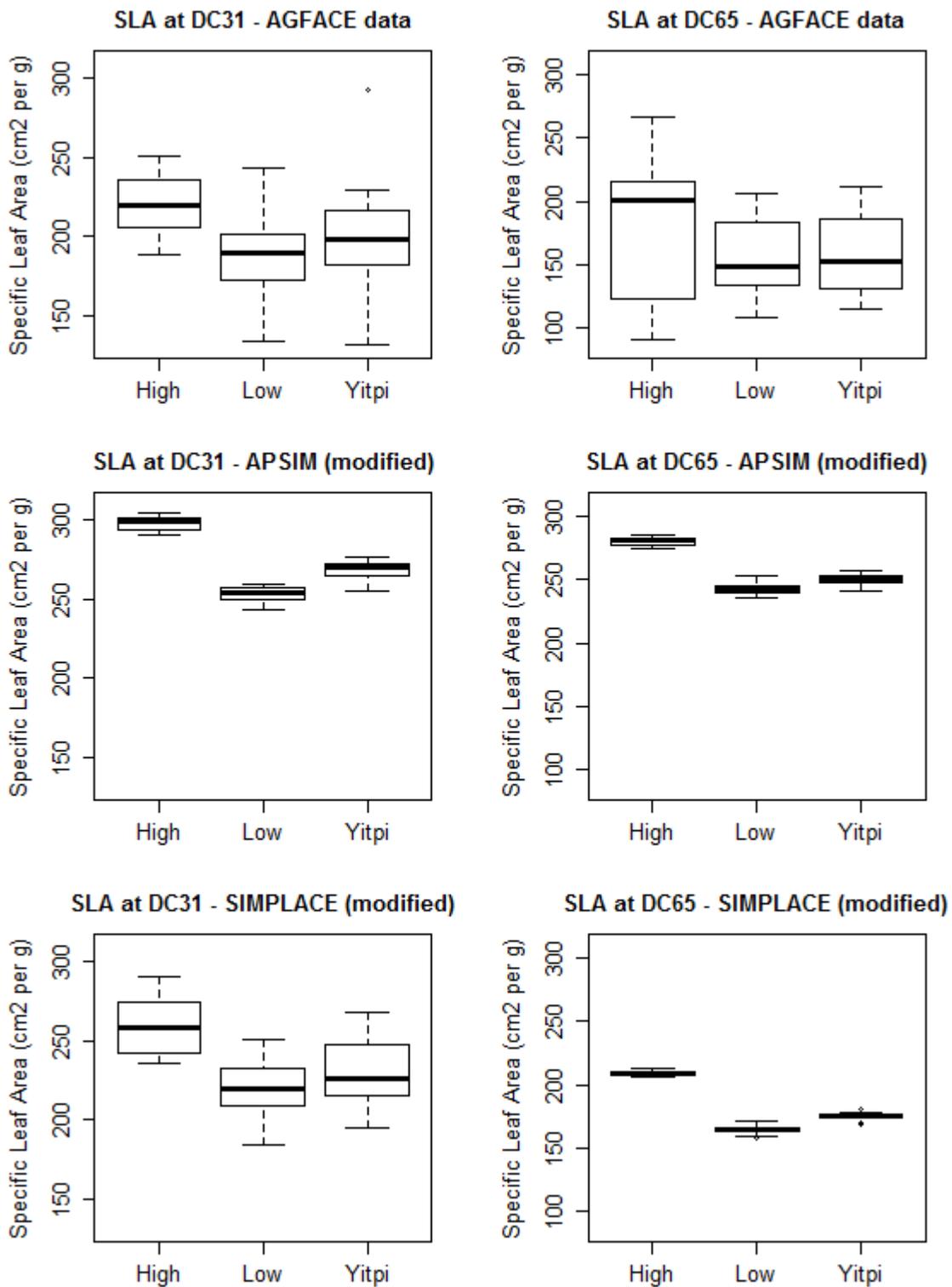


474

475

476

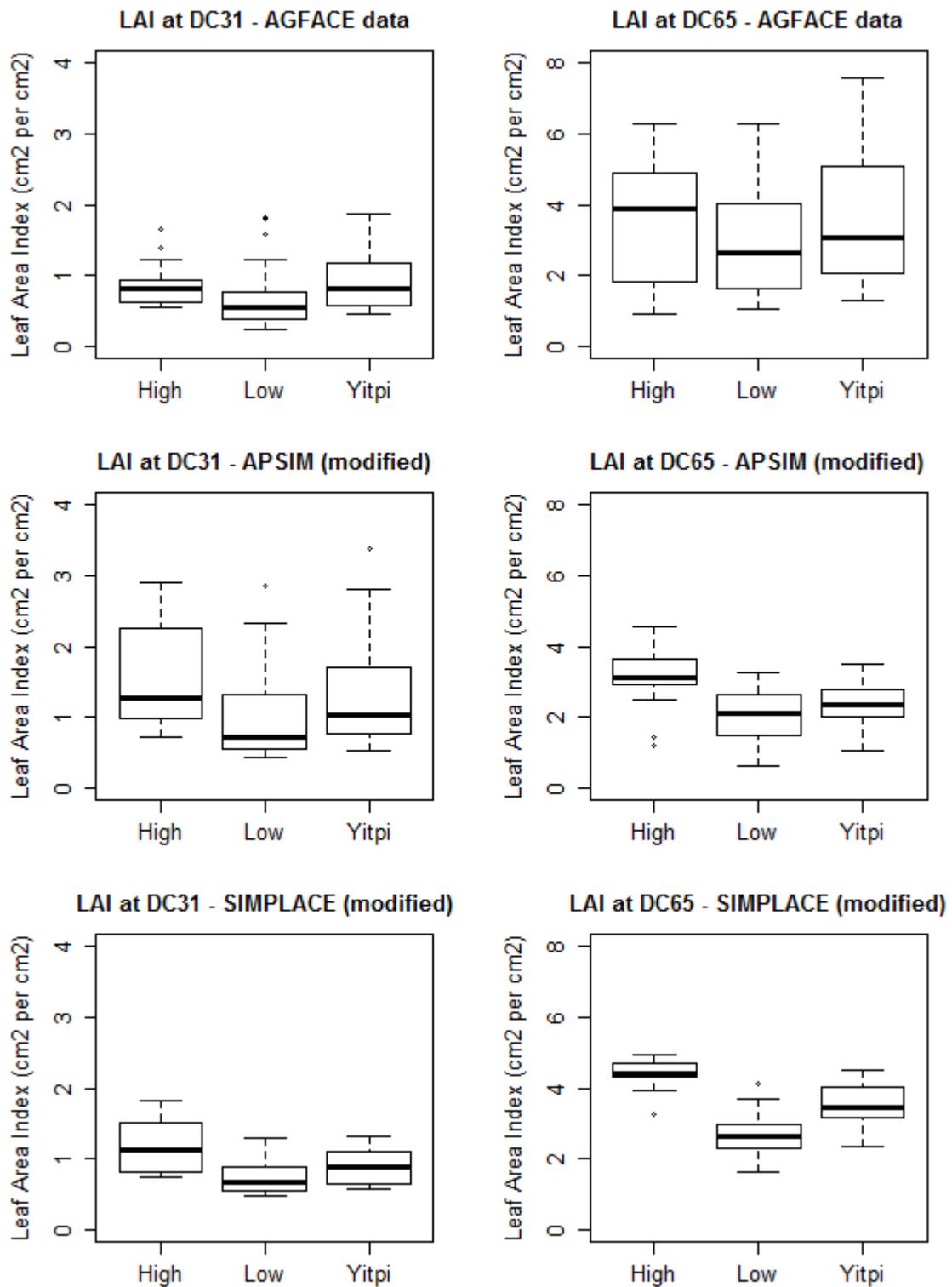
477 Figure S2: Range of specific leaf area (SLA) values of experimental data (AGFACE) and simulated data  
 478 for both models with modifications for early vigour in the high and low vigour lines as described in  
 479 Table 3.



480

481

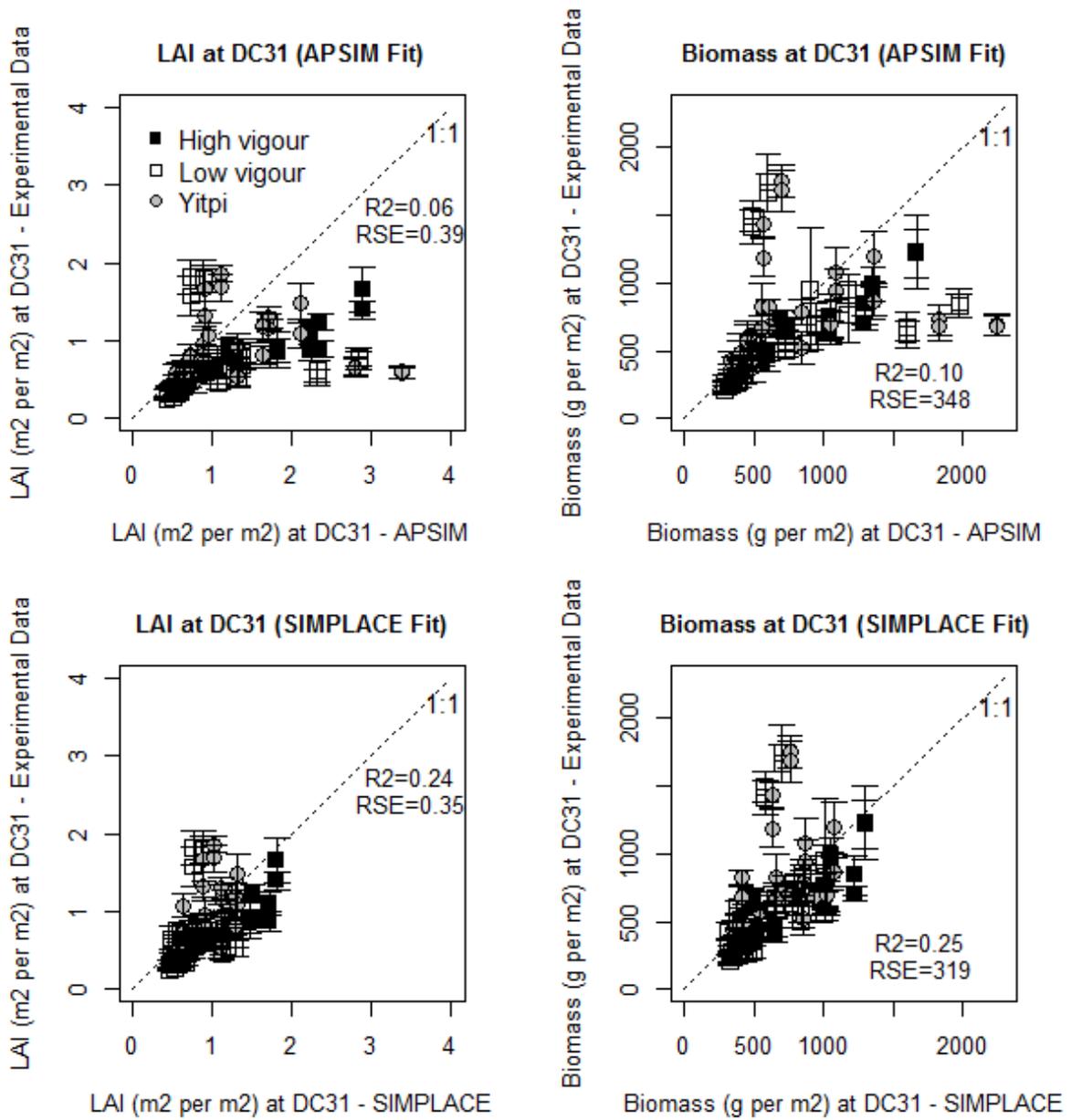
482 Figure S3: Range of leaf area index values (LAI) values of experimental data (AGFACE) and simulated  
 483 data for both models with modifications for early vigour in the high and low vigour lines as described  
 484 in Table 3.



485

486

487 Figure S4: Fit of experimental AGFACE data to simulated results for leaf area index and above-  
 488 ground biomass at stem elongation (DC31) for APSIM and SIMPLACE based on date of sampling. The  
 489 group on the left higher than the 1:1 line is data from 2008, while the diffuse group underneath the  
 490 1:1 line with APSIM simulations is 2007 and 2009 (both planted late).

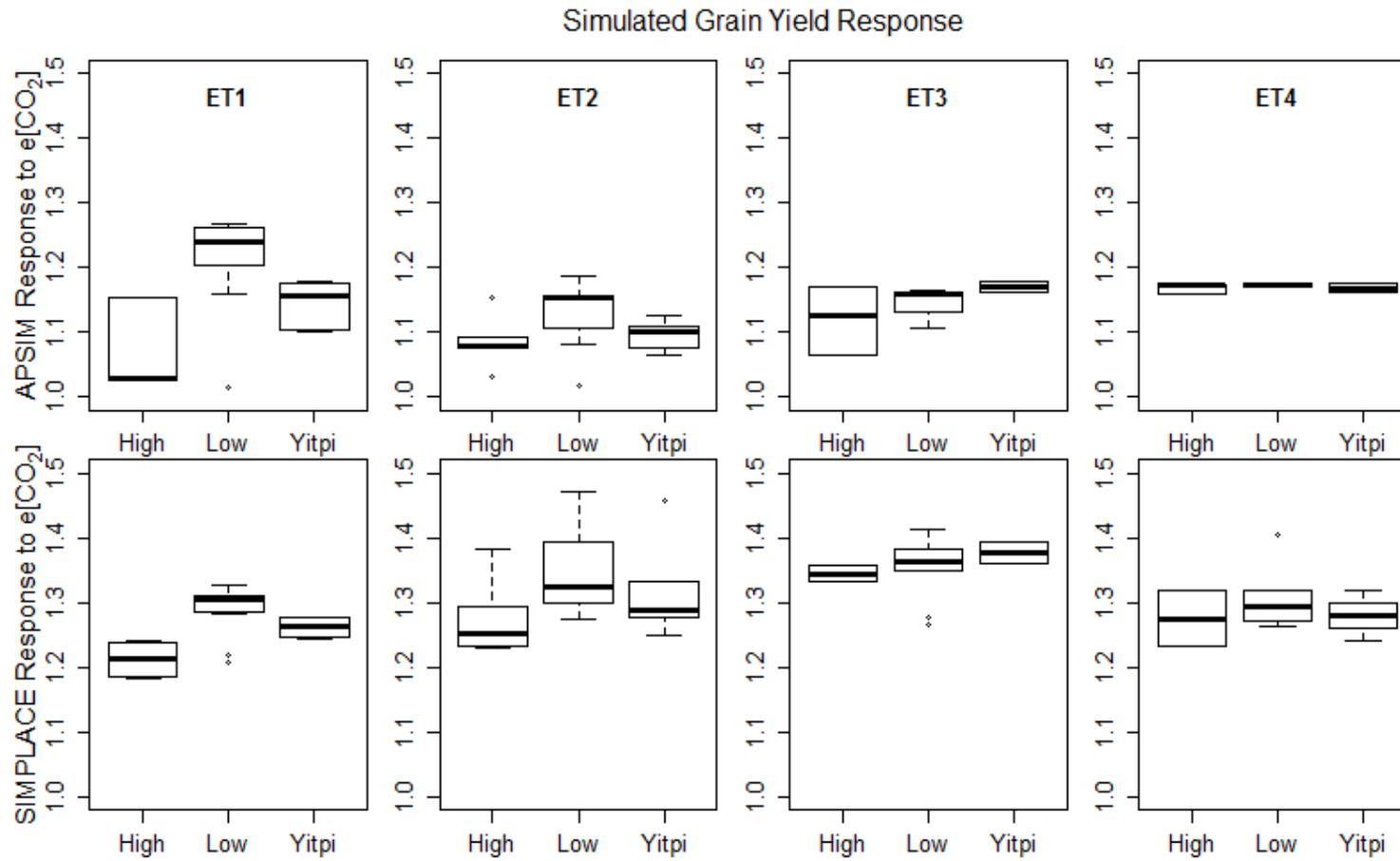


491

492

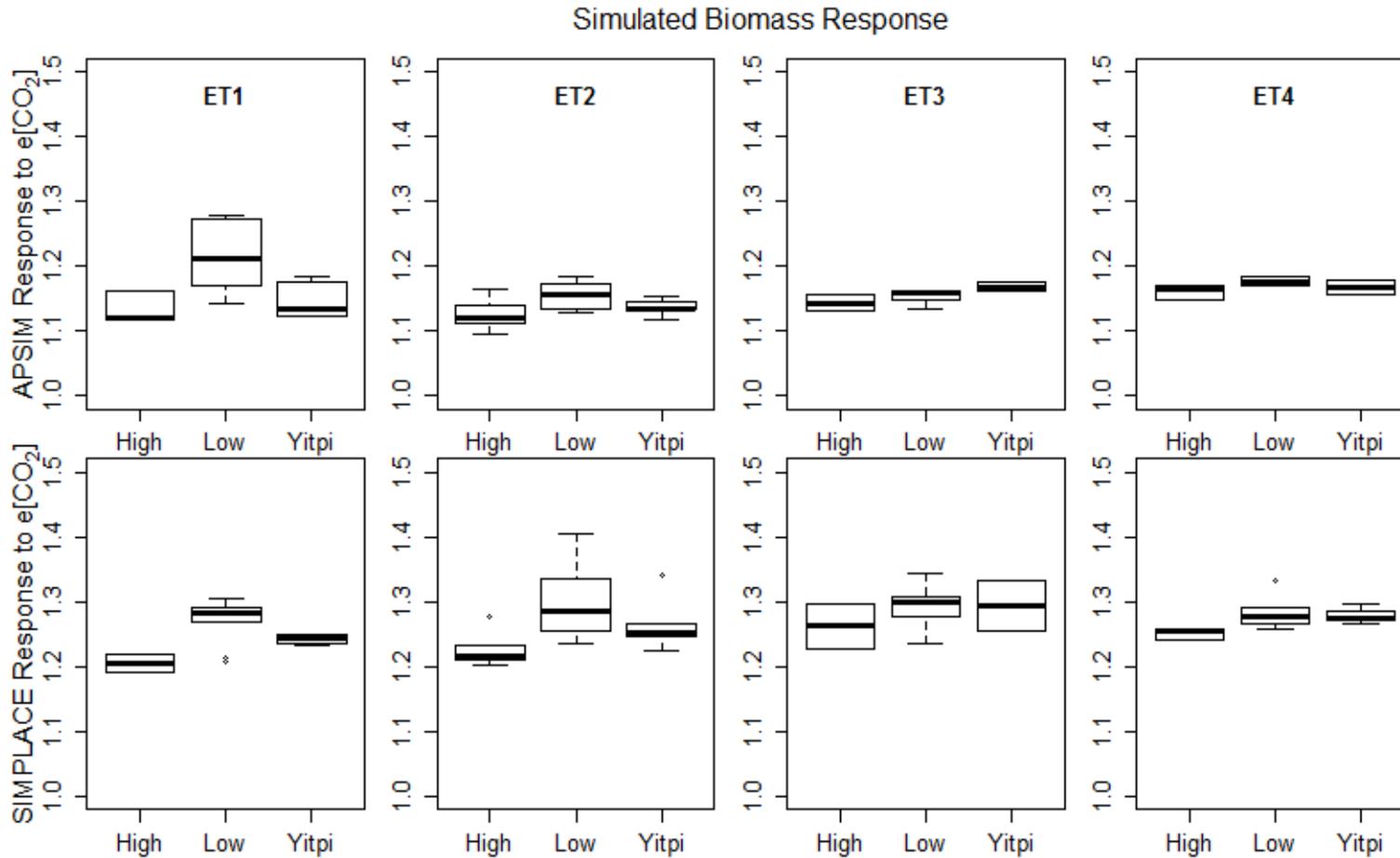
493

494 Figure S5: Simulated grain yield response to elevated  $[\text{CO}_2]$  according to vigour groups in different environment types (with characterization done with  
495 respective model) for the Australian Grains Free Air  $\text{CO}_2$  Enrichment (AGFACE) facility in 2007 to 2013. Simulated data includes all 7 cultivars in all 7 years,  
496 plus/minus irrigation (therefore including combinations that did not occur experimentally).



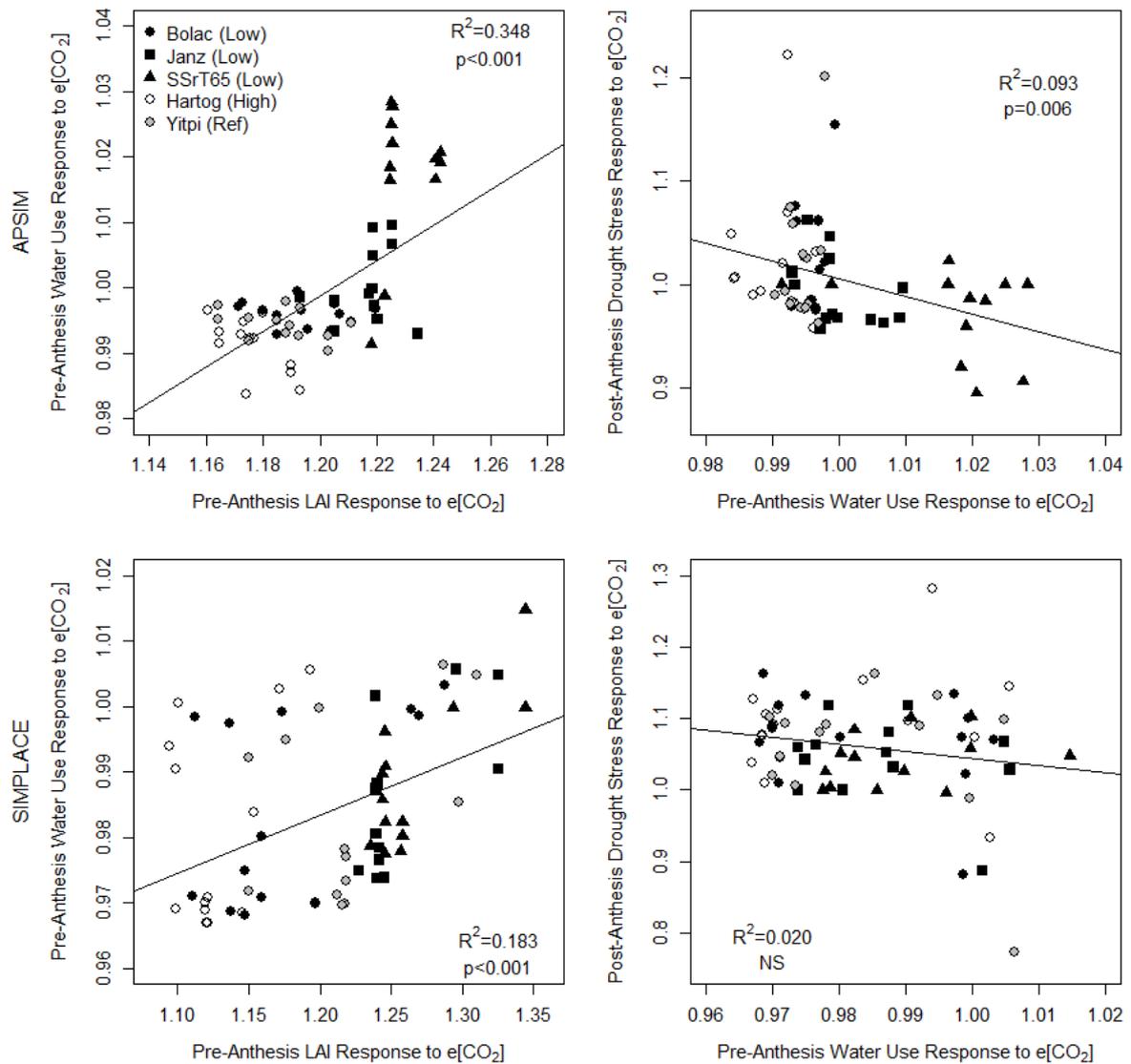
497

498 Figure S6: Simulated biomass response to elevated  $[CO_2]$  according to vigour groups in different environment types (with characterization done with  
 499 respective model) for the Australian Grains Free Air  $CO_2$  Enrichment (AGFACE) facility in 2007 to 2013. Simulated data includes all 7 cultivars in all 7 years,  
 500 plus/minus irrigation (therefore including combinations that did not occur experimentally).



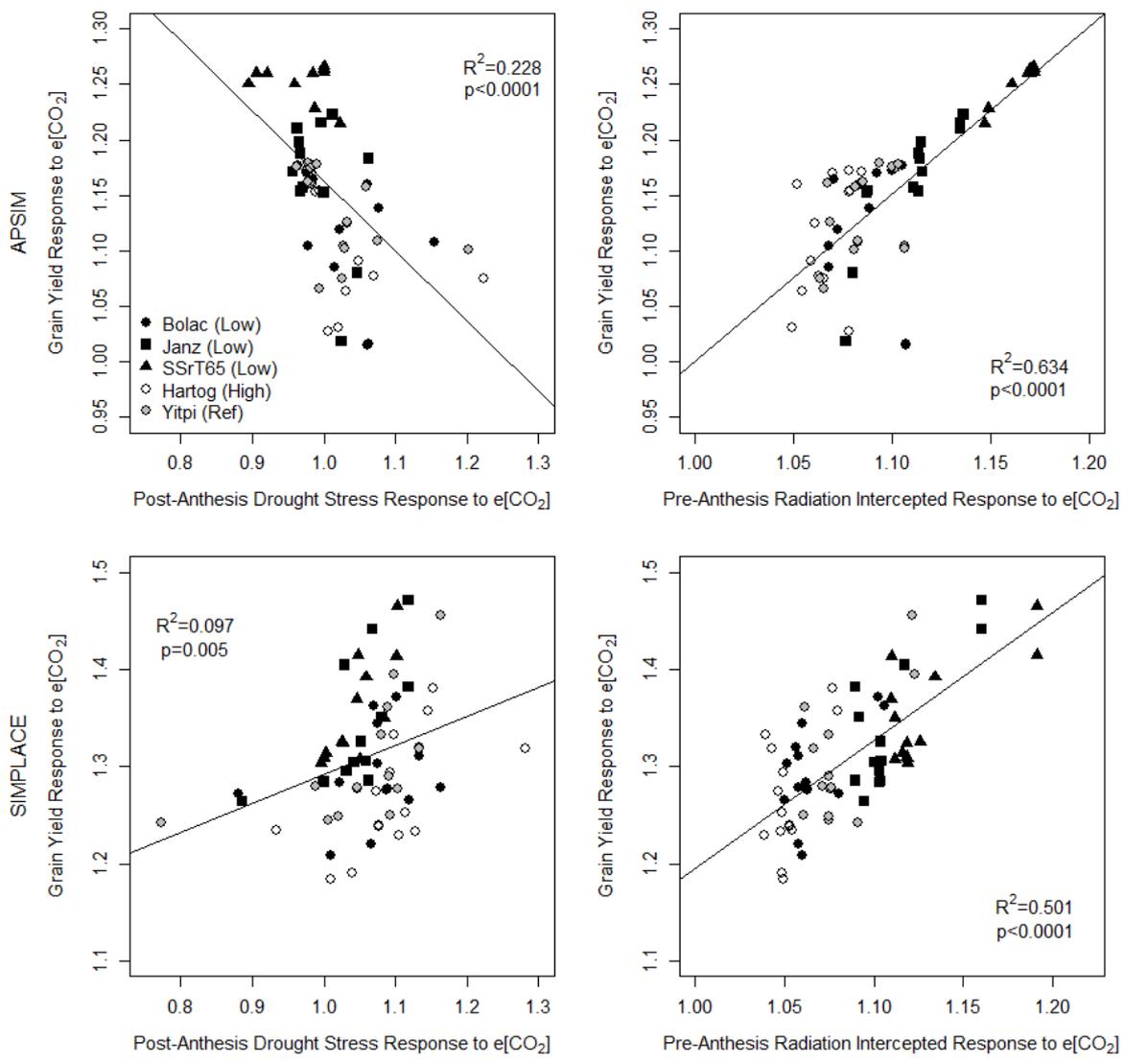
501

502 Figure S7: Correlations between relative simulated responses to  $e[\text{CO}_2]$  for pre-anthesis leaf area  
 503 index and cumulative pre-anthesis water use (evaporation + transpiration), and cumulative pre-  
 504 anthesis water use and average post-anthesis water stress index. For the water stress index, higher  
 505 values refer to less stress with  $e[\text{CO}_2]$  compared to  $a[\text{CO}_2]$ . All high vigour lines are represented by  
 506 the cultivar 'Hartog' as they did not differ in phenology or any other model parameters.



507

508 Figure S8: Correlations between relative simulated responses to e[CO<sub>2</sub>] for average post-anthesis  
 509 water stress index and cumulative pre-anthesis radiation intercepted with the simulated relative  
 510 response in grain yield. For the water stress index, higher values refer to less stress with e[CO<sub>2</sub>]  
 511 compared to a[CO<sub>2</sub>]. All high vigour lines are represented by the cultivar 'Hartog' as they did not  
 512 differ in phenology or any other model parameters.  
 513



514

515 Table S1: Soil profile parameters describing air dry, crop lower limit (LL), drained upper limit (DUL), saturation (SAT), bulk density (BD), pH, and organic  
 516 carbon concentration (OC) of the experimental site used by both models. The wheat module in APSIM also uses additional water extraction coefficients (KL  
 517 and XF), and SIMPLACE uses van Genuchten parameters related to water retention in unsaturated soils (alfa and n).

Depth (cm)	Air dry (g/cm <sup>3</sup> )	LL (g/cm <sup>3</sup> )	DUL (g/cm <sup>3</sup> )	SAT (g/cm <sup>3</sup> )	BD (g/cm <sup>3</sup> )	pH	OC (%)	APSIM KL	APSIM XF	SIMPLACE ALFA	SIMPLACE N
0-10	0.15	0.20	0.39	0.46	1.14	8.4	1.248	0.06	1.00	1	1.23
10-20	0.18	0.23	0.40	0.47	1.30	8.4	0.708	0.06	1.00	1	1.23
20-40	0.25	0.27	0.42	0.48	1.37	8.9	0.354	0.04	1.00	1	1.23
40-60	0.27	0.30	0.43	0.47	1.40	9.0	0.177	0.02	0.80	1	1.23
60-80	0.28	0.33	0.45	0.47	1.40	9.0	0.089	0.02	0.80	1	1.23
80-100	0.30	0.35	0.45	0.47	1.40	9.0	0.044	0.02	0.60	1	1.23
100-120	0.32	0.36	0.45	0.47	1.40	9.0	0.022	0.02	0.60	1	1.23
120-140	0.33	0.37	0.45	0.47	1.40	9.1	0.011	0.02	0.20	1	1.23
140-160	0.34	0.37	0.45	0.47	1.40	9.1	0.011	0.02	0.20	1	1.23
160-180	0.34	0.37	0.45	0.47	1.40	9.1	0.011	0.02	0.20	1	1.23

518

519 Table S2: Starting soil nitrate content used by both models for simulation of AGFACE experiments  
520 (2007-2013).

Depth (cm)	2007	2008	2009	2010	2011	2012	2013
0-10	42	34.8	13.3	38.4	47.2	25.7	19.3
10-20	25	26.6	26.1	23.8	23.3	14.6	8.4
20-40	15	21.4	27.5	24.3	12.7	11.2	11.4
40-60	13	21.4	27.5	22.8	12.7	11.2	11.4
60-80	6	11.8	10.5	22.8	20.4	20.1	15.2
80-100	2.5	11.8	10.5	11.7	20.4	20.1	15.2
100-120	2	2	2	11.7	2	2	2
120-140	1	1	1	1	1	1	1
140-160	1	1	1	1	1	1	1
160-180	0	0	0	0	0	0	0

521

522

523 Table S3: Starting soil water content used by both models for simulation of AGFACE experiments  
 524 (2007-2013).

	2007	2008	2009	2010	2011		2012		2013	
Depth (cm)					Rain	Suppl	Rain	Suppl	Rain	Suppl
0-10	0.363	0.340	0.406	0.269	0.303	0.311	0.165	0.181	0.187	0.181
10-20	0.398	0.278	0.419	0.287	0.392	0.381	0.219	0.234	0.179	0.179
20-40	0.411	0.324	0.383	0.311	0.447	0.423	0.285	0.309	0.265	0.258
40-60	0.419	0.373	0.341	0.347	0.451	0.432	0.340	0.355	0.333	0.337
60-80	0.429	0.396	0.351	0.384	0.450	0.434	0.381	0.385	0.372	0.383
80-100	0.443	0.423	0.376	0.406	0.448	0.451	0.403	0.401	0.411	0.415
100-120	0.441	0.453	0.424	0.414	0.469	0.469	0.418	0.421	0.438	0.431
120-140	0.444	0.443	0.385	0.414	0.469	0.469	0.418	0.421	0.438	0.431
140-160	0.429	0.411	0.390	0.414	0.469	0.469	0.418	0.421	0.438	0.431
160-180	0.415	0.388	0.379	0.414	0.469	0.469	0.418	0.421	0.438	0.431

525

526

527 Table S4: Irrigation amounts and dates for the supplemental irrigation treatment used by both  
 528 models for simulation of AGFACE experiments (2007-2013).

Year	Dates	Amount (mm)
2007	17 September	10
	24 September	10
	2 October	10
	8 October	10
	16 October	28
	14 November	28
2008	8 September	20
	25 September	20
2009	6 October	10
	22 October	30
	3 November	30
2010	6 October	30
	11 October	20
	22 October	30
2011	6 September	10
	12 September	20
	22 September	20
	10 October	20
	18 October	30
2012	11 September	30
	25 September	30
	10 October	30
	29 October	30
2013	No supplemental irrigation applied	

529

530

531

532 S5: Grain yield, above-ground biomass at maturity (DC90) and harvest index (HI) according to vigour  
 533 group (high, low and the reference cultivar Yitpi) in the Australian Grains Free Air CO<sub>2</sub> Enrichment  
 534 (AGFACE) facility in 2007-2013.

	High early vigour		Low early vigour		Yitpi	
	a[CO <sub>2</sub> ]	e[CO <sub>2</sub> ]	a[CO <sub>2</sub> ]	e[CO <sub>2</sub> ]	a[CO <sub>2</sub> ]	e[CO <sub>2</sub> ]
Grain yield (t ha <sup>-1</sup> )	4.30	5.25	4.12	5.23	4.66	5.72
Biomass (t ha <sup>-1</sup> )	11.7	14.6	11.1	14.0	12.5	15.9
HI (%)	36.2	35.0	37.0	36.9	36.8	35.8

535