

# Can additional N fertiliser ameliorate the elevated CO<sub>2</sub>-induced depression in grain and tissue N concentrations of wheat on a high soil N background?

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1 **Can additional N fertiliser ameliorate the elevated CO<sub>2</sub>-induced depression in**  
2 **grain and tissue N concentrations of wheat on a high soil N background?**

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25 change, dryland agriculture

26 Contains 1 Table and 3 Figures

27 **Abstract**

28 Elevated CO<sub>2</sub> stimulates crop yields but leads to lower tissue and grain nitrogen  
29 concentrations [N], raising concerns about grain quality in cereals. To test whether  
30 N fertiliser application above optimum growth requirements can alleviate the  
31 decline in tissue [N], wheat was grown in a Free Air CO<sub>2</sub> Enrichment facility in a low  
32 rainfall cropping system on high soil N. Crops were grown with and without  
33 addition of 50-60 kg N ha<sup>-1</sup> in 12 growing environments created by supplemental  
34 irrigation and two sowing dates over three years.

35 Elevated CO<sub>2</sub> increased yield and biomass (on average by 25%), and decreased  
36 biomass [N] (3-9%) and grain [N] (5%). Nitrogen uptake was greater (20%) in  
37 elevated CO<sub>2</sub> grown crops. Additional N supply had no effect on yield and biomass,  
38 confirming high soil N. Small increases in [N] with N addition were insufficient to  
39 offset declines in grain [N] under elevated CO<sub>2</sub>. Instead, N application increased the  
40 [N] in straw and decreased N harvest index.

41 The results suggest that conventional addition of N does not mitigate grain [N]  
42 depression under elevated CO<sub>2</sub>, and lend support to hypotheses that link decreases  
43 in crop [N] with biochemical limitations rather than N supply.

44

## 45 **Introduction**

46 Atmospheric CO<sub>2</sub> concentration ([CO<sub>2</sub>]) has been increasing since the industrial  
47 revolution and is predicted to reach 550 μL L<sup>-1</sup> or more by 2050, that is a 35%  
48 change from the current (400 μL L<sup>-1</sup> in 2016) concentration (Stocker et al. 2013).

49 Because CO<sub>2</sub> is the main substrate for photosynthesis, such a large increase will  
50 affect all plants and ecosystems (Ziska 2008). Many studies demonstrated that, at  
51 least in C3 plants, elevated [CO<sub>2</sub>] (e[CO<sub>2</sub>]) stimulates photosynthesis and  
52 subsequently growth and yield (Kimball et al. 2002, Ainsworth and Long 2005)  
53 through the so-called 'CO<sub>2</sub> fertilisation effect'.

54 While the 'CO<sub>2</sub> fertilisation effect' may result in greater crop yields and help offset  
55 some of the negative effects of climate change on food production (Hatfield et al.  
56 2011), concerns have been raised about reductions in mineral nutrients and grain  
57 quality (Högy and Fangmeier 2008, Myers et al. 2014). It is well established that  
58 growth under e[CO<sub>2</sub>] changes the stoichiometry of plants, whereby the  
59 concentration of many minerals, especially nitrogen (N), in plant tissues decreases  
60 (Loladze 2002). Because photosynthetic N use efficiency (the photosynthetic carbon  
61 fixation rate per g leaf N) increases under e[CO<sub>2</sub>], the critical tissue N concentration,  
62 i. e. the leaf N concentration ([N]) that is necessary for optimum growth,  
63 consequently decreases under e[CO<sub>2</sub>] (Conroy and Hocking 1993, Seneweera and  
64 Norton 2011, Tausz-Posch et al. 2014). Despite lower tissue [N], the greater  
65 biomass reported under e[CO<sub>2</sub>] may contain more N per ground surface area than  
66 biomass under ambient [CO<sub>2</sub>], hence N uptake of the crop may be greater (Tausz-  
67 Posch et al. 2014, Lam et al. 2012b). In natural ecosystems, where N is often

68 limiting, the CO<sub>2</sub>-stimulation on growth often decreases over time because  
69 available N in the soil becomes depleted, a phenomenon termed 'progressive N  
70 limitation' (Oren et al. 2001, Luo et al. 2004). In N-managed agro-ecosystems  
71 progressive N limitation may not be relevant or immediately apparent, but some  
72 experiments have shown that growth stimulation by e[CO<sub>2</sub>] is less under low than  
73 high N supply (Stitt and Krapp 1999).

74 For non-legume food and fodder crops, decreases in tissue [N] are particularly  
75 concerning because they translate to lower protein concentrations, thus lowering  
76 food and feed quality as shown in wheat (Högy et al. 2013; Wroblewitz et al. 2013).  
77 Grain protein concentration is also an important determinant of baking quality and  
78 market value of wheat. Dough and baking quality of wheat was shown to  
79 deteriorate under e[CO<sub>2</sub>] (Panozzo et al. 2014). Synthesis papers report around 5-  
80 10% reduction in grain protein concentration in wheat (Högy and Fangmeier 2008;  
81 Lam et al. 2012b, Wang et al. 2013), and about 10% for leaf [N] (Ainsworth and  
82 Long 2005; Wang et al. 2013).

83 The exact mechanism for the decrease in [N] is unclear, and a number of not  
84 mutually exclusive hypotheses have been proposed (Tausz-Posch et al. 2014). The  
85 most straightforward one contends that soil N supply does not keep up with  
86 increased demand by e[CO<sub>2</sub>]-stimulated biomass growth, leading to a 'dilution' of N  
87 in tissue biomass and N-limitation to biochemistry and growth (Taub and Wang  
88 2008). Evidence for this hypothesis comes from experiments where leaf [N]  
89 decreased upon e[CO<sub>2</sub>] exposure at low, but not at high soil N supply (Stitt and  
90 Krapp 1999, Sinclair et al. 2000). If limited N availability leads to decreases in tissue

91 [N], it could be hypothesised that reductions in biomass tissue [N] in managed agro-  
92 ecosystems could be reversed by additional soil N inputs.

93 Alternative hypotheses for the decline in tissue [N] under e[CO<sub>2</sub>], such as nutrient  
94 uptake limitations by reduced transpiration flow (Conroy and Hocking 1993,  
95 McGrath and Lobell 2013), or decreased rates of nitrate reduction (Bloom et al.  
96 2010; Bloom et al. 2014), do not suggest that an increase in soil N supply would  
97 restore tissue [N]. For example, in one study conducted in a high yielding, irrigated  
98 wheat cropping system under ample N supply, the deleterious effect of e[CO<sub>2</sub>] on  
99 grain protein concentration was partially alleviated but was still present, even if  
100 very small (Kimball et al. 2001). This would imply that insufficient soil N supply is  
101 not the only mechanism contributing to decreased [N]. Recent meta-analyses  
102 suggested that high soil N supply cannot fully restore, but at best only moderates  
103 the negative effect of e[CO<sub>2</sub>] on tissue and grain [N] (Lam et al 2012b, Wang et al.  
104 2013). It is not clear under what conditions, and to which extent, additional N  
105 application can restore leaf and grain [N] (and protein) under e[CO<sub>2</sub>].

106 In cereals such as wheat, N requirement for growth and grain yield is generally  
107 satisfied before that of increased grain protein, so that grain protein concentration  
108 can be increased by N application above the level needed for growth and yield  
109 responses (Fowler 2003; Hooper et al. 2015). To understand whether N supply  
110 beyond the demand for growth and yield can restore grain protein under e[CO<sub>2</sub>] to  
111 that achieved under ambient CO<sub>2</sub>, it would be important to investigate an agro-  
112 ecosystem that has adequate N supply for growth and yield. Under these  
113 conditions, added N would not promote additional growth or yield, but the

114 additional N may meet the protein synthesis demands in the grain. Previous e[CO<sub>2</sub>]  
115 studies (Sinclair et al. 2000, Kimball et al. 2001) have compared adequate with  
116 deficient soil N-supply and were conducted in high rainfall or well irrigated agro-  
117 ecosystems where growth and yield was most likely limited without fertiliser N  
118 application. Conversely, in some rainfed Mediterranean and semiarid agro-  
119 ecosystems yield and growth is primarily limited by water availability. These are  
120 also relatively low yielding systems, so that N demand of crops is low by global  
121 standards, and in some cases crop N requirements can even be met by soil supply  
122 without the need for fertilisation (Angus 2001).

123 The relationship between plant demand for N and its supply from soil and fertiliser  
124 is a function of interactions between a range of plant and soil processes and the  
125 environment (Angus 2001). Consequently, it is important to investigate crop-level  
126 system responses to e[CO<sub>2</sub>] in a realistic field setting, where these environmental  
127 and physiological interactions are present. Free Air CO<sub>2</sub> Enrichment (FACE)  
128 technology provides a platform to investigate crop growth under e[CO<sub>2</sub>] without  
129 potential large artefacts on irradiance and canopy and root microclimate common  
130 to chamber systems (Ainsworth et al. 2008). Importantly, undisturbed soil  
131 processes are present in such a system. The Australian Grains Free Air CO<sub>2</sub>  
132 Enrichment (AGFACE) facility is globally unique in that it operates in a water-limited  
133 wheat cropping agro-ecosystem and is on a site where the soil N supply is generally  
134 high and adequate to meet demand for crop yield (Fitzgerald et al. 2016). This  
135 enabled the present study to address the question whether N application above the  
136 level normally recommended for growth and yield response in current CO<sub>2</sub>

137 environments can ameliorate the e[CO<sub>2</sub>]-related decline in biomass and grain [N] of  
138 wheat.

## 139 **Materials and Methods**

### 140 *Site*

141 The Australian Grains Free Air CO<sub>2</sub> Enrichment (AGFACE) facility is located on an  
142 experimental farm managed by the Victorian State Government, near Horsham,  
143 Victoria, Australia (36°45'07"S, 142°06'52"E, 127 m above sea level), and described  
144 in detail in previous papers (Mollah et al. 2009, Fitzgerald et al. 2016). In brief: The  
145 experimental plots are on a 7.5 ha field on heavy Vertosol clay soil (~35% clay at  
146 the surface and 60% in 1.4 m depth). Long term (30-year) average annual rainfall is  
147 435 mm with 274 mm typically falling during the growing season of winter wheat  
148 (May – Nov). Typical commercial wheat yields under rainfed conditions and local  
149 agronomic practice are 3-4 t ha<sup>-1</sup>, but range from 1 to 6 t ha<sup>-1</sup>. Mean annual growing  
150 season temperature is 16.5 °C and the mean annual evaporation rate is around  
151 1500 mm. Detailed weather data for the seasons in question (2007-2009 growing  
152 seasons) were recorded by an on-site automatic weather station and are given in  
153 Fitzgerald et al. (2016).

### 154 *Plant Material and Experimental Design*

155 All measurements were done on a popular local bread wheat cultivar (*Triticum*  
156 *aestivum* L. cv. 'Yitpi'). The experiment comprised a factorial combination of two  
157 levels of [CO<sub>2</sub>] (elevated e[CO<sub>2</sub>] viz. target 550 μmol mol<sup>-1</sup> air and ambient a[CO<sub>2</sub>]  
158 viz. approximately 370 μmol mol<sup>-1</sup> air; daytime medians for 2007-2009) that were



159 each split for two levels of N application (N-sufficient N<sub>0</sub> and N addition N<sub>+</sub>) in four  
160 replicates (plots or 'rings'), fully repeated in 12 different growing environments  
161 created by various combinations of water supply (rainfed or supplemented  
162 irrigation), and sowing times. The irrigation treatments were not designed to create  
163 non-limiting conditions but to create conditions within the site that are typical of  
164 the multiseason rainfall variability. This approach provides a range of crop yields  
165 that are realistic in the region. Two sowing times per year (TOS1 according to local  
166 practice and TOS2 late sowing) were used so that the later sowing moved the crop  
167 growing season towards hotter and drier conditions. These treatments were  
168 repeated over three growing seasons (2007, 2008, 2009). Sowing dates and an  
169 overview over the growing environments investigated in this study is given in Table  
170 1.

171 Plots were re-established each season, so that wheat was not grown consecutively  
172 to avoid soil-borne disease carry over and residual treatment effects from the  
173 previous season. In 2007, plots were split in half and each (East or West) half  
174 randomly assigned to one of two time of sowing (TOS1 and TOS2) treatments, while  
175 in 2008 and 2009, each plot was randomly split to irrigation. A plastic barrier buried  
176 to 0.8 m depth ensured hydraulic separation between half-plots. In 2007, the  
177 experiment was replicated for water supply treatment (separate plots), in 2008 and  
178 2009 for time of sowing. In 2007 and 2008 the plots ('rings') were 12 m in diameter  
179 and in 2009, 16 m diameter. Details on the FACE system and its performance are  
180 given in (Mollah et al. 2009), and more details on experimental design, agronomic  
181 treatments, and weather data in (Fitzgerald et al. 2016).

182 Within each half-plot, two N-treatments were allocated to sub-plots, each 1.4 m x 4  
183 m with 8 rows of wheat sown in a north-south direction. Rows were spaced either  
184 0.214 m (2007, 2008) or 0.195 m (2009) and samples were collected from middle  
185 rows, leaving the outside rows as buffers. Plant counts about three weeks after  
186 emergence reported an average 120 plants m<sup>-2</sup>.

187 Pre-sowing soil test results from the sites showed a total soil N of 0.14% (0-10 cm)  
188 and mineral N in 0-50 cm depth of 145±50 kg N ha<sup>-1</sup> in 2007, 233±114 kg ha<sup>-1</sup> in  
189 2008, and 164±98 kg ha<sup>-1</sup> in 2009. The N sufficient treatment (N0) did not receive  
190 any N fertiliser, and the N+ treatment received 50-60 kg N ha<sup>-1</sup> as urea top dressing:  
191 50 kg ha<sup>-1</sup> before growth stage DC30 (decimal code according to Zadoks et al. 1974)  
192 in 2008 and 2009, and split in two times 30 kg ha<sup>-1</sup> between after sowing and DC31  
193 in 2007.

#### 194 *Biomass and N measurements*

195 Biomass samples were taken at stem elongation (DC31), anthesis (DC65) and  
196 maturity (DC90) from three pre-determined sample areas in 2008 and 2009, and  
197 from sub-plot random row lengths in 2007. The areas sampled were 0.43 m<sup>2</sup> in  
198 2007 and 2008 for DC31 and DC65, 1.28 m<sup>2</sup> for DC90 in 2007, 0.86 m<sup>2</sup> for DC90 in  
199 2008 and, in 2009 0.4 m<sup>2</sup> for DC31 and DC65, and 0.78 m<sup>2</sup> for DC90. At DC31,  
200 samples were separated into leaf blades (cut off at the ligule) and stems (including  
201 leaf sheaths) and at DC65 samples were separated into leaves (cut off at the ligule),  
202 stems (including leaf sheaths) and heads and then oven dried at 70°C. At DC90, dry  
203 samples were separated into heads and straw (stems and leaves together), the

204 heads threshed to separate grains and chaff, and the chaff combined with the  
205 straw. All biomass and grain yield are expressed on a dry weight basis.

206 Biomass [N] was analysed on dried and ground tissue aliquots by Dumas  
207 combustion in an elemental analyser (LECO, TruMac, MI), and grain [N] by near-  
208 infrared (NIR) spectrometry calibrated against the elemental analyser method. N  
209 content of biomass fractions (leaves, stems, heads) was calculated as: [N] in the  
210 fraction x biomass of that fraction expressed on a m<sup>2</sup> ground area basis. N content  
211 of biomass fractions were summed for total biomass N content at each sampled  
212 growth stage. Nitrogen uptake was calculated as the difference in biomass N  
213 content between two sampled growth stages. Post-anthesis N remobilisation from  
214 stems and leaves was calculated as the difference between maturity (DC90) and  
215 anthesis (DC65) of the products of average [N] in vegetative biomass and that  
216 vegetative biomass. As straw samples at maturity (DC90) were not separated into  
217 leaves and stems, 'vegetative biomass' refers to stems and leaves taken together.

218 Nitrogen utilisation efficiency (NutE) was defined as the ratio of grain yield over  
219 total N in biomass (at DC90), and N harvest index (NHI) as the proportion of N  
220 content in grains in total N in biomass at DC90.

### 221 *Statistical evaluation*

222 This present study addresses potential interactions of N and [CO<sub>2</sub>]. Growing year,  
223 time of sowing, and water supply were therefore combined into a factor  
224 'environment', resulting in 12 different environments (Table 1). Data analysis was  
225 performed in the software R (version 3.13, R Core Team 2015). The statistical  
226 evaluation was done with a linear mixed-effect model using the default REML

227 method (R package nlme, version 3.1-120, Pinheiro et al. 2016) with [CO<sub>2</sub>] and  
228 environment as main plots, and N-treatment as split-plot.

## 229 **Results**

230 The grain yield of wheat under a[CO<sub>2</sub>] in each environment (combination of  
231 irrigation x TOS x season) ranged from just over 1.0 t ha<sup>-1</sup> to just below 3.5 t ha<sup>-1</sup>  
232 (Table 1). Yields were relatively low, but are typical of the district averages for those  
233 years. Not surprisingly across such a wide range of yields, the factor environment  
234 had a significant effect on most investigated variables. Because environmental  
235 effects on wheat growth, grain yield and grain protein are well studied and data on  
236 the N0 treatment only were included in analyses in previous papers (yield and yield  
237 components in Fitzgerald et al. 2016, and grain protein in Fernando et al. 2014),  
238 environment effects will only be considered in this present study where there were  
239 significant interactions with N or CO<sub>2</sub> treatments. Across all environments, e[CO<sub>2</sub>]  
240 stimulated biomass at maturity and grain yield by about 25%: Biomass at maturity  
241 increased from 6.89 (±0.25) to 8.70 (±0.34) t ha<sup>-1</sup> and grain yield increased from  
242 2.30 (±0.09) to 2.87 (±0.13) t ha<sup>-1</sup> (means (SE) of n = 96 from 24 N x environment  
243 combinations). Addition of 50-60 kg ha<sup>-1</sup> fertiliser N had no significant effect on  
244 either biomass or grain yield.

245 Elevated [CO<sub>2</sub>] depressed [N] in grains on average by 5%. Nitrogen concentrations  
246 in the leaves decreased on average by only 3% at stem elongation, but by 9% at  
247 anthesis. The [CO<sub>2</sub>] effect was similar for stems at anthesis (9% decrease), but not  
248 significant for stems at stem elongation, or for heads at anthesis. Elevated [CO<sub>2</sub>] did  
249 not significantly affect N concentrations in straw and chaff at maturity (Figure 1).

250 Despite these decreases in tissue [N], N uptake into aboveground biomass was  
251 significantly greater under e[CO<sub>2</sub>]. By the time of stem elongation, e[CO<sub>2</sub>] grown  
252 crops had accumulated about 20% more N per unit area than a[CO<sub>2</sub>] grown ones, by  
253 anthesis 17% more, and by harvest 20% more (Figure 2). Because all crops were on  
254 similar soil and had similar N availability, the increase in N uptake increased N  
255 uptake efficiency by the same proportion. N utilisation efficiency was however not  
256 significantly affected by e[CO<sub>2</sub>] (Figure 3).

257 On average, only a very small fraction of the total N content was taken up after  
258 anthesis. Up to 50% of total N was taken up during the early vegetative phase (up  
259 to stem elongation; DC31; Figure 2), and about 90% by anthesis (DC65; Figure 2).  
260 Even under the assumption that all N taken up after anthesis went into the grains,  
261 only about 10% of N recovered in the grain could have come from post-anthesis  
262 uptake. These fractions were not significantly affected by either [CO<sub>2</sub>] or N  
263 treatments.

264 There was no significant increase under e[CO<sub>2</sub>] in the amount of N remobilised from  
265 stems and leaves post-anthesis (Figure 3), and the proportion of this remobilised N  
266 in grain N remained unchanged under e[CO<sub>2</sub>] (between 60-65%).

267 Compared to N-sufficient (N0) treatment, additional N had little effect on leaf or  
268 stem [N] at stem elongation, but significantly increased [N] in leaves at anthesis,  
269 and more so under e[CO<sub>2</sub>] (significant interaction). The N treatment also  
270 significantly increased grain [N], but this was independent of the [CO<sub>2</sub>]-treatment  
271 (no significant interaction) and not sufficient to completely restore grain [N] to  
272 a[CO<sub>2</sub>] values. However, N treatment increased [N] by about 9% in straw, where [N]

273 was not significantly affected by e[CO<sub>2</sub>] (all Figure 1). This led to a significantly lower  
274 N harvest index (NHI) when N fertiliser was applied (by on average about 5%), and a  
275 significant negative effect of N application on N utilisation efficiency, independently  
276 of [CO<sub>2</sub>]-treatments (Figure 3). N remobilisation remained unaffected by N  
277 application (Figure 3).

278 Despite small increases in [N] in some tissues upon N treatment, total N uptake was  
279 on average not significantly increased by the additional fertiliser application (Figure  
280 2).

## 281 **Discussion**

282 The lack of yield response to additional application of 50-60 kg ha<sup>-1</sup> N confirmed  
283 that the experimental plots had sufficient soil N for the prevailing growing  
284 conditions, especially the range of soil water supply. Yields in water-limited  
285 Australian wheat crops are low by global standards (Angus 2001) so that N  
286 demands are modest compared to higher yielding production regions in Australia  
287 and internationally. However, unlike many other cropping lands, the site used in  
288 this present study has high organic N and mineral N concentrations, probably a  
289 consequence of prior land use of growing lucerne for 5 years, a faba bean crop in  
290 2006, and years of irrigation with communal effluent.

291 Tuohey and Robson (1980), working on the same soils as our site, proposed that  
292 grain yield was not increased by N fertiliser in any season where total soil N (0-15  
293 cm) was greater than 0.11%, compared to 0.14% (albeit for 0-10 cm) at our site.

294 Adequate mineral N concentration in the top 60 cm for a 5 t ha<sup>-1</sup> crop yield

295 potential has been reported at 110 kg N ha<sup>-1</sup> (Bell et al. 2013), compared to a  
296 minimum of 145 kg N ha<sup>-1</sup> (in 2007) at our site. Using both metrics, the site was  
297 more than adequately supplied with N.

298 Average grain [N] in the present study was high, translating to between 14 and 15%  
299 average grain protein, thereby apparently exceeding most standards. For example,  
300 in Australian wheat classification protein concentrations above 13% represent the  
301 highest wheat quality classes (Blakeney et al. 2009). The high grain N result reflects  
302 that half of the growing environments were achieved by delaying sowing of the  
303 crops (Table 1), effectively reducing the length of the growing season and moving  
304 the grain filling phase towards hotter and drier conditions. Grain yields, already  
305 comparatively low in the three seasons investigated here, were even lower under  
306 these conditions (Table 1, TOS2 environments 3, 4, 7, 8, 11, 12). For wheat grain,  
307 conditions that lower maximum yield often lead to greater protein concentrations  
308 (Blumenthal et al. 1993; Fowler 2003), because of the typical inverse relationship  
309 between yield and grain protein (Simmonds 1995). Significant interactions with the  
310 growing environment of [CO<sub>2</sub>] and N were only found for two parameters (leaf [N]  
311 at DC65 and stem [N] at DC31); all other reported average trends remain therefore  
312 valid across all investigated environments.

313 Studies undertaken in more humid temperate or continuously irrigated  
314 environments have reported greater yield stimulation by e[CO<sub>2</sub>] under high than  
315 under low N conditions (Stitt and Krapp 1999). Other FACE studies showed no such  
316 interaction between [CO<sub>2</sub>] and N supply, suggesting that growth and yield  
317 responses were of similar magnitudes under low and high N (Weigel and

318 Manderscheid 2012). Those studies were designed to address N limitation and  
319 therefore compared sufficient with inadequate N supply levels. In our study, N  
320 supply was non-limiting for growth and yield in all treatments and the e[CO<sub>2</sub>]-  
321 stimulation of growth and yield was similar under both N treatments.

322 In previous FACE experiments, e[CO<sub>2</sub>] decreased wheat grain protein by an average  
323 of about 5-10% (Högy and Fangmeier 2008, Taub et al. 2008, Lam et al. 2012b), and  
324 results from AGFACE were of similar magnitude (Fernando et al. 2014). Interactions  
325 (or lack of interactions) of e[CO<sub>2</sub>] with environmental conditions on grain protein  
326 concentrations were reported elsewhere for AGFACE (Fernando et al. 2014).

327 In the present study we focused on the question whether additional N application  
328 mitigates the deleterious effect of e[CO<sub>2</sub>] on grain [N]. The observations from most  
329 FACE studies show a decline in grain protein concentration under e[CO<sub>2</sub>], but the  
330 depression seen varies possibly due to the relative soil and fertiliser N supply and  
331 the demand by the crop. Where N supply was relatively low, e[CO<sub>2</sub>] reduced grain  
332 protein, but this reduction was very small with adequate N fertiliser (Kimball et al.  
333 2001). In other experiments, grain protein concentrations were significantly  
334 depressed by both e[CO<sub>2</sub>] and low N, and no interaction between N supply and  
335 eCO<sub>2</sub> was reported (Erbs et al. 2010, Wroblewitz et al. 2013). The “high N” rates in  
336 those studies were considered sufficient according to local agronomic practice,  
337 although it was not specifically demonstrated that N was not limiting for yield. The  
338 “low N” rates referred to half the normal fertiliser application, and growth and  
339 yields were lower than under “high N”. Furthermore, these studies were conducted  
340 in high yielding, high input agro-ecosystems, either under continuous irrigation



341 (Kimball et al. 2001), or in high rainfall temperate climates (Erbs et al. 2010;  
342 Wroblewitz et al. 2013). In contrast, in our study, N was added to a cropping system  
343 with adequate soil N for yield that was largely limited by water availability (Table 1).  
344 N application above the requirement for growth and yield generally increases grain  
345 protein concentrations further (Fowler 2003). Such an effect of N treatment on  
346 grain [N], albeit small, was observed in our study, even though N application that  
347 targets grain protein would ideally be applied later in the season (Hooper et al.  
348 2015). However, there was no interaction between N application and [CO<sub>2</sub>] effect  
349 on grain [N], indicating that crops grown under e[CO<sub>2</sub>] suffered a grain protein  
350 penalty compared to those grown under a[CO<sub>2</sub>] irrespective of N supply.  
351 Furthermore, additional N was not able to compensate for this decline under  
352 e[CO<sub>2</sub>].

353 Grain N in cereals is supplied by root uptake during grain filling or by translocation  
354 of N previously accumulated in the biomass. In agro-ecosystems where cereals  
355 ripen under terminal drought conditions that largely inhibit further N uptake, N  
356 remobilisation from vegetative biomass (stems and leaves) contributes a large  
357 proportion to grain N (Palta et al. 1994; Buchner et al. 2015), placing particular  
358 importance on leaf [N]. In our study, N uptake after anthesis (calculated as the  
359 difference between maturity and anthesis of the products of N concentration in  
360 biomass and biomass; cf. Figure 2) could contribute only about 10% of grain N, and  
361 post-anthesis N remobilisation from stems and leaves contributed around 60-65%  
362 of grain N (the rest was already in heads at anthesis). These figures are broadly

363 consistent with earlier reports in environments with terminal drought (Palta et al.  
364 1994).

365 Whilst this underlines the importance of post-anthesis remobilisation of nitrogen  
366 from vegetative biomass into grains under the prevailing environmental conditions,  
367 there was no indication that  $e[\text{CO}_2]$  changed the extent of that remobilisation.

368 Nitrogen harvest index (NHI), the proportion of grain N in total amount of N in  
369 biomass at maturity, results from the sum of N translocation from vegetative  
370 biomass, pre-anthesis N uptake into developing heads, and post-anthesis N uptake  
371 into grains, and remained unaffected by  $e[\text{CO}_2]$  (Figure 3). Similarly, post-anthesis N  
372 remobilisation from vegetative organs was not affected by elevated  $e[\text{CO}_2]$  (Figure  
373 3).

374 Decreases in [N] in vegetative plant parts, which are well documented under  $e[\text{CO}_2]$   
375 (Stitt and Krapp 1999, Tausz-Posch et al. 2014), could therefore be directly related  
376 to decreases in grain [N], because proportionally less N is available for  
377 remobilisation per each g grain yield. At anthesis, leaf [N] in our study averaged 9%  
378 lower under  $e[\text{CO}_2]$ . This is comparable with Buchner et al. (2015) who reported  
379 that N concentrations were reduced by about 8% in flag leaves or 9% in second  
380 leaves under  $e[\text{CO}_2]$  as compared to  $a[\text{CO}_2]$ . Additionally, Wang et al. (2013)  
381 reported an average 9% decrease in their meta-analysis for wheat. Averages for  
382 multiple species under FACE conditions were of similar magnitude (Ainsworth and  
383 Long 2005, Tausz-Posch et al. 2014). The relative decrease in leaf [N] under  $e[\text{CO}_2]$   
384 was less, albeit still significant, at the vegetative growth stage.

385 Some previous FACE investigations on wheat found that decreases in leaf [N] under  
386 e[CO<sub>2</sub>] were less pronounced with adequate N nutrition than under N deficit  
387 (Sinclair et al. 2000, Weigel and Manderscheid 2012), and photosynthetic  
388 downward acclimation, a response to e[CO<sub>2</sub>] commonly linked to decreases in leaf  
389 [N], was less pronounced under high N (Stitt and Krapp 1999). At the earlier  
390 vegetative growth stage in our study, the additional N application had no effect on  
391 leaf [N], and leaf [N] was decreased by e[CO<sub>2</sub>] regardless of N application. This is in  
392 agreement with the FACE results reported by (Sinclair et al. 2000) who found no  
393 effect of soil fertility (*viz.* soil N supply) on leaf [N] early in the season, but e[CO<sub>2</sub>]  
394 decreased leaf [N] regardless of N supply at that stage. At anthesis, our results did  
395 indicate some attenuation of the decrease in leaf [N] by additional N supply, as  
396 shown by the interaction between [CO<sub>2</sub>] x N (Figure 1). However, supplying  
397 additional N during the vegetative growth phase did not restore leaf [N] under  
398 e[CO<sub>2</sub>]. In contrast, in Sinclair et al. (2000), leaf [N] depression by e[CO<sub>2</sub>] at anthesis  
399 was only evident in plants where insufficient N was supplied but not in those  
400 adequately supplied with N. In contrast, but in agreement with Wang et al. (2013),  
401 our results suggest that increased N supply can moderate the effect of e[CO<sub>2</sub>] on  
402 leaf [N] to some extent, but not restore leaf [N] under e[CO<sub>2</sub>]. Insufficient N supply  
403 will amplify the effect of e[CO<sub>2</sub>] on leaf [N], but is not the sole cause for decreased  
404 leaf [N]. The attenuation effect of the additional N application on leaf [N] at  
405 anthesis could represent the mitigation of a short-term supply deficit, because in  
406 dryland agro-ecosystems (such as the one investigated here) even soils with high N  
407 status can leave the crop with insufficient mineral N supply during certain stages,  
408 because mineralisation rates and crop demand can be temporarily mismatched

409 (Angus 2001). This effect does however not explain the full extent of leaf [N]  
410 depression under e[CO<sub>2</sub>], and alternative mechanisms, such as a direct limitation to  
411 nitrate assimilation (Bloom et al. 2014, Bloom 2015), decreased N allocation to the  
412 photosynthetic machinery due to downward acclimation of photosynthesis or  
413 increased leaf area index (leading to denser canopies), or changes in N mass flow  
414 related to changes in transpiration, are very likely (for review see Tausz-Posch et al.  
415 2014).

416 Despite these evident decreases in grain and biomass [N], overall N uptake of the  
417 crops averaged 20% (or around 30 kg ha<sup>-1</sup>) greater under e[CO<sub>2</sub>]. Whilst N supply by  
418 mineralisation was sufficient to meet additional crop demand at this high N  
419 experimental site in the short term, this may not be sustainable and in the mid to  
420 long-term this N will have to be provided by additional inputs. Where biomass and  
421 yield stimulation are relatively greater than the decrease in biomass [N], crops will  
422 have greater N demands under CO<sub>2</sub>-enrichment. This seems to be the case in many,  
423 but not all reported analyses (Lam et al. 2012a, b, Chen et al. 2012, Wang et al.  
424 2013; Tausz-Posch et al. 2014).

425 The N source and management methods to meet additional crop demands for N in  
426 a high [CO<sub>2</sub>] atmosphere need to be carefully considered (Carlisle et al. 2012, Bloom  
427 2015), because N fertiliser can have large negative impacts on the environment  
428 (Robertson and Vitousek 2009) and already constitutes a relatively costly and risky  
429 farm input in these cropping systems primarily limited by low and unreliable rainfall  
430 (Angus 2001). Probably even more important than such quantitative considerations  
431 are qualitative aspects of nitrogen management (Bloom 2015), such as selection of

432 nitrogen form to promote uptake of reduced nitrogen so that biochemical  
433 limitations to nitrate assimilation can be circumvented (Carlisle et al. 2012, Bloom  
434 et al. 2014), or appropriate timing of N applications to target grain N (Hooper et al.  
435 2015).

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#### 445 **References**

- 446 Ainsworth, E. A., C. Beier, C. Calfapietra, R. Ceulemans, M. Durand-Tardif, G. D.  
447 Farquhar, D. L. Godbold, G. R. Hendrey, T. Hickler, J. Kaduk, D. F. Karnosky,  
448 B. A. Kimball, C. Koerner, M. Koornneef, T. Lafarge, A. D. B. Leakey, K. F.  
449 Lewin, S. P. Long, R. Manderscheid, D. L. McNeil, T. A. Mies, F. Miglietta, J. A.  
450 Morgan, J. Nagy, R. J. Norby, R. M. Norton, K. E. Percy, A. Rogers, J. F.  
451 Soussana, M. Stitt, H. J. Weigel and J. W. White, 2008: Next generation of  
452 elevated [CO<sub>2</sub>] experiments with crops: a critical investment for feeding the  
453 future world. *Plant Cell Environ.* **31**, 1317-1324.
- 454 Ainsworth, E. A. and S. P. Long, 2005: What have we learned from 15 years of free-  
455 air CO<sub>2</sub> enrichment (FACE)? A meta-analytic review of the responses of  
456 photosynthesis, canopy. *New Phytol.* **165**, 351-371.

457 Angus, J. F., 2001: Nitrogen supply and demand in Australian agriculture. Aust. J.  
458 Exp. Agric. **41**, 277-288.

459 Bell, M. J., W. Strong, D. Elliot and C. Walker, 2013: Soil nitrogen-crop response  
460 calibration relationships and criteria for winter cereal crops grown in  
461 Australia. Crop Past. Sci. **64**.

462 Blakeney, A. B., R. L. Cracknell, G. B. Crosbie, S. P. Jefferies, D. M. Miskelly, L.  
463 O'Brien, J. F. Panozzo, D. A. I. Suter, V. Solah, T. Watts, T. Westcott and R. M.  
464 Williams, 2009: Understanding Australian wheat quality. A basic  
465 introduction to Australian wheat quality. Grains Research and Development  
466 Corporation, Kingston, Australia.

467 Bloom, A. J., 2015: The increasing importance of distinguishing among plant  
468 nitrogen sources. Current Opinion in Plant Biology **25**, 10-16.

469 Bloom, A. J., M. Burger, B. A. Kimball and P. J. Pinter, 2014: Nitrate assimilation is  
470 inhibited by elevated CO<sub>2</sub> in field-grown wheat. Nature Climate Change **4**,  
471 477-480.

472 Bloom, A. J., M. Burger, J. S. Rubio-Asensio and A. B. Cousins, 2010: Carbon dioxide  
473 enrichment inhibits nitrate assimilation in wheat and *Arabidopsis*. Science  
474 **328**, 899-903.

475 Blumenthal, C. S., E. W. R. Barlow and C. W. Wrigley, 1993: Growth Environment  
476 and Wheat Quality: the Effect of Heat Stress on Dough Properties and  
477 Gluten Proteins. J. Cereal Sci. **18**, 3-21.

478 Buchner, P., M. Tausz, R. Ford, A. Leo, G. J. Fitzgerald, M. J. Hawkesford and S.  
479 Tausz-Posch, 2015: Expression patterns of C- and N-metabolism related  
480 genes in wheat are changed during senescence under elevated CO<sub>2</sub> in dry-  
481 land agriculture. Plant Sci. **236**, 239-249.

482 Carlisle, E., S. Myers, V. Raboy and A. Bloom, 2012: The effects of inorganic nitrogen  
483 form and CO<sub>2</sub> concentration on wheat yield and nutrient accumulation and  
484 distribution. Front. Plant Sci. **3**, 195.

485 Conroy, J. and P. Hocking, 1993: Nitrogen nutrition of C-3 plants at elevated  
486 atmospheric CO<sub>2</sub> concentrations. Physiol. Plant. **89**, 570-576.

487 Erbs, M., J. Franzaring, P. Högy and A. Fangmeier, 2009: Free-air CO<sub>2</sub> enrichment in  
488 a wheat-weed assembly - effects on water relations. *Basic Appl. Ecol.* **10**,  
489 358-367.

490 Erbs, M., R. Manderscheid, G. Jansen, S. Seddig, A. Pacholski and H. J. Weigel, 2010:  
491 Effects of free-air CO<sub>2</sub> enrichment and nitrogen supply on grain quality  
492 parameters and elemental composition of wheat and barley grown in a crop  
493 rotation. *Agric. Ecosyst. Environ.* **136**, 59-68.

494 Fernando, N., J. Panozzo, M. Tausz, R. M. Norton, N. Neumann, G. J. Fitzgerald and  
495 S. Seneweera, 2014: Elevated CO<sub>2</sub> alters grain quality of two bread wheat  
496 cultivars grown under different environmental conditions. *Agric. Ecosyst.*  
497 *Environ.* **185**, 24-33.

498 Fitzgerald, G. J., M. Tausz, G. O'Leary, M. R. Mollah, S. Tausz-Posch, S. Seneweera, I.  
499 Mock, M. Löw, D. L. Partington, D. McNeil and R. M. Norton, 2016: Elevated  
500 atmospheric [CO<sub>2</sub>] can dramatically increase wheat yields in semi-arid  
501 environments and buffer against heat waves. *Glob. Change Biol.* **22**, 2269-  
502 2284.

503 Fowler, D. B., 2003: Crop nitrogen demand and grain protein concentration of  
504 spring and winter wheat. *Agron. J.* **95**, 260-265.

505 Hatfield, J. L., K. J. Boote, B. A. Kimball, L. H. Ziska, R. C. Izaurralde, D. Ort, A. M.  
506 Thomson and D. Wolfe, 2011: Climate impacts on agriculture: Implications  
507 for crop production. *Agron. J.* **103**, 351-370.

508 Högy, P., M. Brunnbauer, P. Koehler, K. Schwadorf, J. Breuer, J. Franzaring, D.  
509 Zhunusbayeva and A. Fangmeier, 2013: Grain quality characteristics of  
510 spring wheat (*Triticum aestivum*) as affected by free-air CO<sub>2</sub> enrichment.  
511 *Environ. Exp. Bot.* **88**, 11-18.

512 Högy, P. and A. Fangmeier, 2008: Effects of elevated atmospheric CO<sub>2</sub> on grain  
513 quality of wheat. *J. Cereal Sci.* **48**, 580-591.

514 Hooper, P., Y. Zhou, D. R. Coventry and G. K. McDonald, 2015: Use of nitrogen  
515 fertilizer in a targeted way to improve grain yield, quality, and nitrogen use  
516 efficiency. *Agron. J.* **107**, 903-915.

517 Kimball, B. A., K. Kobayashi and M. Bindi, 2002: Responses of agricultural crops to  
518 free-air CO<sub>2</sub> enrichment *Advances in Agronomy, Vol 77., pp.* 293-368.

519 Kimball, B. A., C. F. Morris, P. J. Pinter, G. W. Wall, D. J. Hunsaker, F. J. Adamsen, R.  
520 L. LaMorte, S. W. Leavitt, T. L. Thompson, A. D. Matthias and T. J. Brooks,  
521 2001: Elevated CO<sub>2</sub>, drought and soil nitrogen effects on wheat grain quality.  
522 *New Phytol.* **150**, 295-303.

523 Lam, S. K., X. Han, E. Lin, R. Norton and D. Chen, 2012a: Does elevated atmospheric  
524 carbon dioxide concentration increase wheat nitrogen demand and recovery  
525 of nitrogen applied at stem elongation? *Agric. Ecosyst. Environ.* **155**, 142-  
526 146.

527 Lam, S. K., D. Chen, R. M. Norton, R. Armstrong and A. R. Mosier, 2012b. Nitrogen  
528 dynamics in grain crop and legume pasture systems under elevated CO<sub>2</sub>: A  
529 meta-analysis. *Global Change Biology* **18**, 2853-2859.

530 Loladze, I., 2002: Rising atmospheric CO<sub>2</sub> and human nutrition: toward globally  
531 imbalanced plant stoichiometry? *Trends Ecol. Evol.* **17**, 457-461.

532 Luo, Y., B. Su, W. S. Currie, J. S. Dukes, A. C. Finzi, U. Hartwig, B. Hungate, R. E.  
533 McMurtrie, R. Oren, W. J. Parton, D. E. Pataki, M. R. Shaw, D. R. Zak and C. B.  
534 Field, 2004: Progressive nitrogen limitation of ecosystem responses to rising  
535 atmospheric carbon dioxide. *Bioscience* **54**, 731-739.

536 McGrath, J. M. and D. B. Lobell, 2013: Reduction of transpiration and altered  
537 nutrient allocation contribute to nutrient decline of crops grown in elevated  
538 CO<sub>2</sub> concentrations. *Plant Cell Environ.* **36**, 697-705.

539 Mollah, M., R. Norton and J. Huzzey, 2009: Australian Grains Free-Air Carbon  
540 dioxide Enrichment (AGFACE) facility: design and performance. *Crop Past.*  
541 *Sci.* **60**, 697-707.

542 Myers, S. S., A. Zanobetti, I. Kloog, P. Huybers, A. D. B. Leakey, A. J. Bloom, E.  
543 Carlisle, L. H. Dietterich, G. Fitzgerald, T. Hasegawa, N. M. Holbrook, R. L.  
544 Nelson, M. J. Ottman, V. Raboy, H. Sakai, K. A. Sartor, J. Schwartz, S.  
545 Seneweera, M. Tausz and Y. Usui, 2014: Increasing CO<sub>2</sub> threatens human  
546 nutrition. *Nature* **510**, 139-142.

547 Oren, R., D. S. Ellsworth, K. H. Johnsen, N. Phillips, B. E. Ewers, C. Maier, K. V. R.  
548 Schafer, H. McCarthy, G. Hendrey, S. G. McNulty and G. G. Katul, 2001: Soil  
549 fertility limits carbon sequestration by forest ecosystems in a CO<sub>2</sub>-enriched  
550 atmosphere. *Nature* **411**, 469-472.



551 Palta, J. A., T. Kobata, N. C. Turner and I. R. Fillery, 1994: Remobilization of carbon  
552 and nitrogen in wheat as influenced by postanthesis water deficits. *Crop Sci.*  
553 **34**, 118-124.

554 Panozzo, J. F., C. K. Walker, D. L. Partington, N. C. Neumann, M. Tausz, S. Seneweera  
555 and G. J. Fitzgerald, 2014: Elevated carbon dioxide changes grain protein  
556 concentration and composition and compromises baking quality. A FACE  
557 study. *J. Cereal Sci.* **60**, 461-470.

558 Pinheiro, J., D. Bates, S. DebRoy, D. Sarkar and R. C. Team, 2016: nlme: Linear and  
559 Nonlinear Mixed Effects Models. R package version 3.1-128. [http://CRAN.R-](http://CRAN.R-project.org/package=nlme)  
560 [project.org/package=nlme](http://CRAN.R-project.org/package=nlme).

561 R Core Team, 2015: R: A language and environment for statistical computing. R  
562 Foundation for Statistical Computing Vienna, Austria.

563 Robertson, G. P. and P. M. Vitousek, 2009: Nitrogen in Agriculture: Balancing the  
564 Cost of an Essential Resource *Annual Review of Environment and Resources*,  
565 *pp.* 97-125. Annual Reviews, Palo Alto.

566 Seneweera, S. and R. M. Norton RM, 2011. Plant Response to increased carbon  
567 dioxide. In: S. S. Yadav, B. Redden, J. Hatfield, H. Lotze-Campen, eds. *Crop*  
568 *Adaptation to Climate Change pp.* 198-211. John Wiley & Sons Inc. Iowa  
569 USA.

570 Sinclair, T. R., P. J. Pinter, B. A. Kimball, F. J. Adamsen, R. L. LaMorte, G. W. Wall, D.  
571 J. Hunsaker, N. Adam, T. J. Brooks, R. L. Garcia, T. Thompson, S. Leavitt and  
572 A. Matthias, 2000: Leaf nitrogen concentration of wheat subjected to  
573 elevated CO<sub>2</sub> and either water or N deficits. *Agric. Ecosyst. Environ.* **79**, 53-  
574 60.

575 Stitt, M. and A. Krapp, 1999: The interaction between elevated carbon dioxide and  
576 nitrogen nutrition: the physiological and molecular background. *Plant Cell*  
577 *Environ.* **22**, 583-621.

578 Stocker, T. F., D. Qin, G.-K. Plattner, L.V. Alexander, S.K. Allen, N.L. Bindoff, F.-M.  
579 Bréon, J.A. Church, U. Cubasch, S. Emori, P. Forster, P. Friedlingstein, N.  
580 Gillett, J.M. Gregory, D.L. Hartmann, E. Jansen, B. Kirtman, R. Knutti, K.  
581 Krishna Kumar, P. Lemke, J. Marotzke, V. Masson-Delmotte, G.A. Meehl, I.I.  
582 Mokhov, S. Piao, V. Ramaswamy, D. Randall, M. Rhein, M. Rojas, C. Sabine,

583 D. Shindell, L.D. Talley, D.G. Vaughan and S.-P. Xie 2013: Technical Summary.  
584 In: T. F. Stocker, D. Qin, G.-K. Plattner, M. Tignor, S.K. Allen, J. Boschung, A.  
585 Nauels, Y. Xia, V. Bex and P.M. Midgley ed. *Climate Change 2013: The*  
586 *Physical Science Basis. Contribution of Working Group I to the Fifth*  
587 *Assessment Report of the Intergovernmental Panel on Climate Change.*  
588 Cambridge University Press, Cambridge, United Kingdom and New York, NY,  
589 USA.

590 Taub, D. R., B. Miller and H. Allen, 2008: Effects of elevated CO<sub>2</sub> on the protein  
591 concentration of food crops: a meta-analysis. *Glob. Change Biol.* **14**.

592 Taub, D. R. and X. Wang, 2008: Why are nitrogen concentrations in plant tissues  
593 lower under elevated CO<sub>2</sub>? A critical examination of the hypotheses. *J.*  
594 *Integr. Biol.* **50**, 1365–1374.

595 Tausz-Posch, S., R. Armstrong and M. Tausz, 2014: Nutrient use and nutrient use  
596 efficiency of crops in a high CO<sub>2</sub> atmosphere. In: M. J. Hawkesford, L. J. De  
597 Kok and S. Kopriva eds. *Nutrient Use Efficiency in Plants. Series; Plant*  
598 *Ecophysiology pp.* 229-252. Springer International Publishing Switzerland.

599 Tuohey, C. L. and A. D. Robson, 1980: The effect of cropping after medic and non-  
600 medic pastures on total soil nitrogen, and on the grain yield and nitrogen  
601 content of wheat. *Aust. J. Exp. Agric. Animal Husb.* **20**, 220-228.

602 Wang, L., Z. Z. Feng and J. K. Schjoerring, 2013: Effects of elevated atmospheric CO<sub>2</sub>  
603 on physiology and yield of wheat (*Triticum aestivum* L.): A meta-analytic test  
604 of current hypotheses. *Agric. Ecosyst. Environ.* **178**, 57-63.

605 Weigel, H.-J. and R. Manderscheid, 2012: Crop growth responses to free air CO<sub>2</sub>  
606 enrichment and nitrogen fertilization: Rotating barley, ryegrass, sugar beet  
607 and wheat. *Eur. J. Agron.* **43**, 97-107.

608 Wroblewitz, S., L. Huther, R. Manderscheid, H. J. Weigel, H. Watzig and S. Danicke,  
609 2013: The effect of free air carbon dioxide enrichment and nitrogen  
610 fertilisation on the chemical composition and nutritional value of wheat and  
611 barley grain. *Arch. Anim. Nutr.* **67**, 263-278.

612 Zadoks, J. C., T. T. Chang and C. F. Konzak, 1974: Decimal code for growth stages of  
613 cereals. *Weed Res.* **14**, 415-421.

614 Ziska, L. H., 2008: Rising atmospheric carbon dioxide and plant biology: The  
615 overlooked paradigm. *DNA and Cell Biol.* **27**, 165-172.

616 Table 1. Summary of growing conditions and wheat (*Triticum aestivum* L. cv Yitpi) grain yields and grain [N] across the 12 growing  
617 environments (numbered in the first column) investigated in this study. Grain yields are averages for ambient [CO<sub>2</sub>] and two N regimes (N0 and  
618 N+), because N treatment had no significant effect on yield (n=8 in each environment). Grain [N] data are averages for ambient [CO<sub>2</sub>] and N0  
619 (without additional N), because N treatment significantly affected grain [N]. Time of sowing (TOS) at local practice (TOS1), late (TOS2), in-  
620 season water rainfall only (Rain) or rainfall plus supplemental irrigation (Sup) treatments, replicated four times (n=4) in each environment  
621 (Env). Temperatures are reported as seasonal means, mean daily minima and maxima for the growing season from sowing to harvest for each  
622 year. Sowing dates, water inputs, temperatures and days to harvest were previously reported in Fitzgerald et al. (2016).

Env	Year	Sowing date	Days to harvest	In-season water [mm]	Air Temperatures (mean, min, max) [°C]	Grain yield [t grain ha <sup>-1</sup> ]	Grain Protein [%]
1	2007	Local practice (18 Jun), TOS1	177	219 (Rain)	12.2, 5.2, 19.3	2.8	14.0
2	2007	Local practice (18 Jun), TOS1	177	267 (Sup)	12.2, 5.2, 19.3	3.4	13.2
3	2007	Late (23 Aug), TOS2	123	178 <sup>1</sup> (Rain)	15.0, 6.9, 23.1	2.1	14.1
4	2007	Late (23 Aug), TOS2	123	226 <sup>2</sup> (Sup)	15.0, 6.9, 23.1	2.2	14.0
5	2008	Local practice (4 Jun), TOS1	187	178 (Rain)	11.1, 4.5, 17.7	3.0	14.8
6	2008	Local practice (4 Jun), TOS1	187	208 (Sup)	11.1, 4.5, 17.7	3.3	16.3
7	2008	Late (5 Aug), TOS2	132	109 (Rain)	12.5, 5.0, 19.9	1.5	15.0
8	2008	Late (5 Aug), TOS2	132	164 (Sup)	12.5, 5.0, 19.9	1.8	15.5
9	2009	Local practice (23 Jun), TOS1	164	223 (Rain)	12.8, 6.2, 19.4	2.6	15.2
10	2009	Local practice (23 Jun), TOS1	164	293 (Sup)	12.8, 6.2, 19.4	2.5	15.3
11	2009	Late (19 Aug), TOS2	116	170 (Rain)	14.8, 7.3, 22.2	1.1	17.3
12	2009	Late (19 Aug), TOS2	116	230 (Sup)	14.8, 7.3, 22.2	1.3	17.1

623 <sup>1,2</sup> These data were reported as 159 and 207 mm of in-season rainfall, respectively in Fitzgerald et al. (2016) but are corrected here.

624 **Figure Legends**

625 Figure 1. Tissue concentrations of N [% of plant dry weight] of wheat grown in the  
626 Australian Grains Free Air CO<sub>2</sub> Enrichment (AGFACE) facility. White columns N0; no N  
627 addition. Black columns N+; 50-60 kg ha<sup>-1</sup> N added during vegetative growth (before  
628 DC30). Each data point represents the mean and SE of n=48 samples (4 replicates in  
629 each of 12 growing environments – Table 1). Ambient [CO<sub>2</sub>] at 370 μmol mol<sup>-1</sup> air<sup>-1</sup>;  
630 Elevated [CO<sub>2</sub>] at 550 μmol mol<sup>-1</sup> air<sup>-1</sup>. P-values for effects of CO<sub>2</sub>, N and CO<sub>2</sub> x N. ns  
631 P≥0.100.

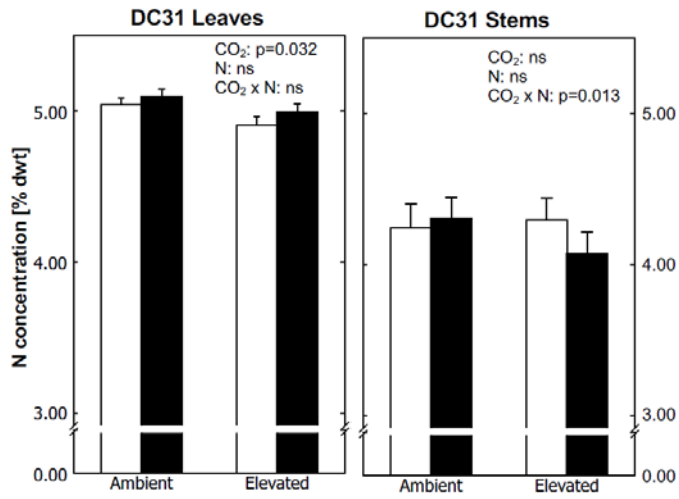
632 Figure 2. N content in aboveground biomass [g N m<sup>-2</sup> ground area] of wheat grown in  
633 the Australian Grains Free Air CO<sub>2</sub> Enrichment (AGFACE) facility. White columns N0; no  
634 N addition. Black columns N+; 50-60 kg ha<sup>-1</sup> N added during vegetative growth (before  
635 DC30). Each data point represents the mean and SE of n=48 samples (4 replicates in  
636 each of 12 growing environments – Table 1). Ambient [CO<sub>2</sub>] at 370 μmol mol<sup>-1</sup> air<sup>-1</sup>;  
637 Elevated [CO<sub>2</sub>] at 550 μmol mol<sup>-1</sup> air<sup>-1</sup>. P-values for effects of CO<sub>2</sub>, N and CO<sub>2</sub> x N. ns  
638 P≥0.100.

639 Figure 3. Left panel: N harvest index (NHI; proportion of grain N in total above ground  
640 biomass N content at maturity). Mid panel: N utilisation efficiency (NutE; grain yield  
641 divided by total N in biomass at maturity). Right panel: Post-anthesis N remobilisation  
642 from stems and leaves (per m<sup>2</sup> ground area) of wheat grown in the Australian Grains  
643 Free Air CO<sub>2</sub> Enrichment (AGFACE) facility. Open symbols and columns N0; no N  
644 addition. Black symbols and columns N+; 50-60 kg ha<sup>-1</sup> N added during vegetative  
645 growth. Each data point represents the mean and SE of n=48 samples (4 replicates in  
646 each of 12 growing environments – Table 1). Ambient [CO<sub>2</sub>] at 370 μmol mol<sup>-1</sup> air<sup>-1</sup>;

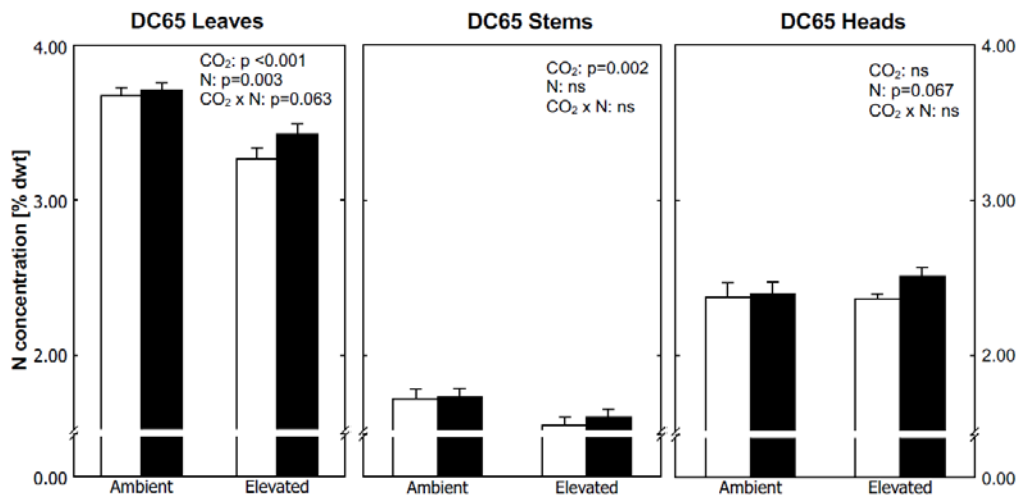
647 Elevated [CO<sub>2</sub>] at 550 μmol mol<sup>-1</sup> air<sup>-1</sup>. P-values for effects of CO<sub>2</sub>, N and CO<sub>2</sub> x N. ns

648 P≥0.100.

649



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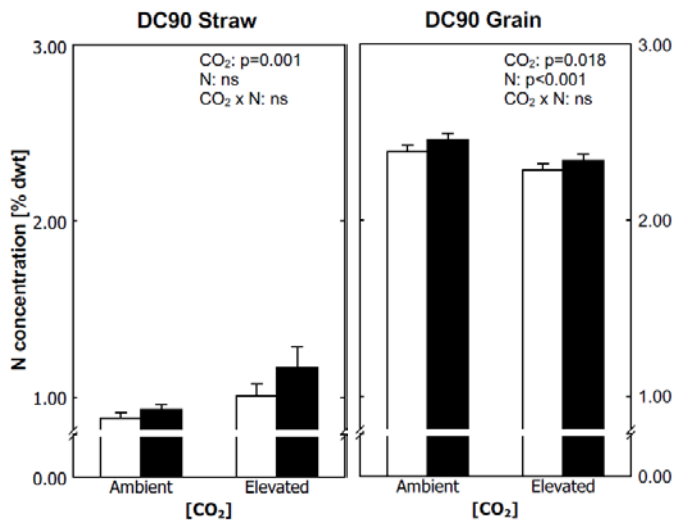


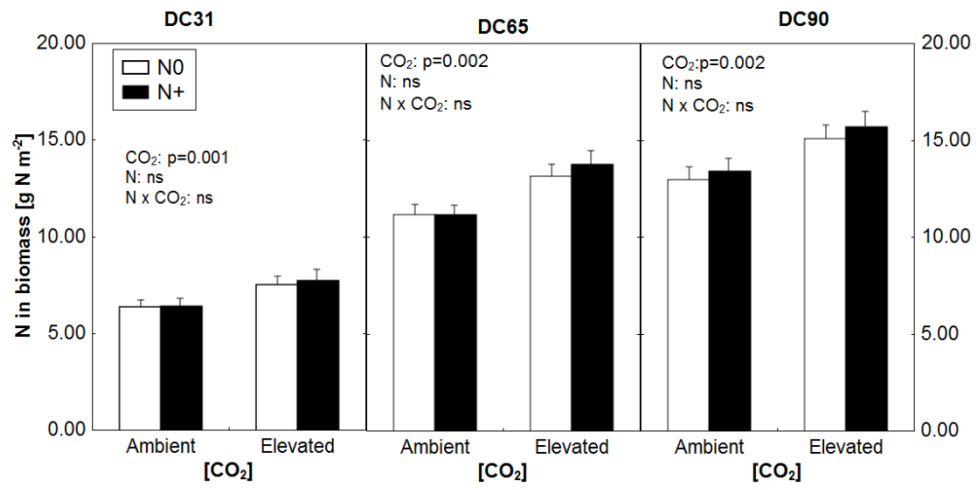
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652

653 Figure 1.

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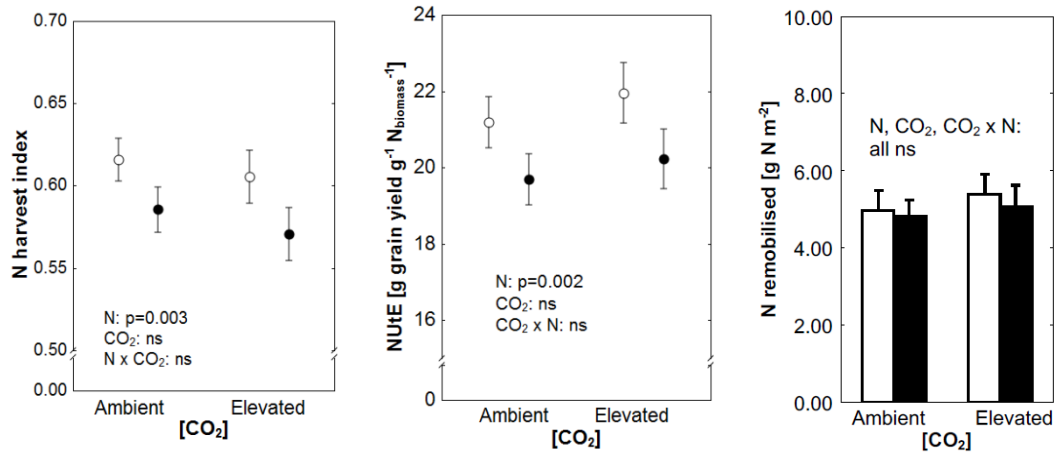




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656 Figure 2.





657

658 Figure 3.

659