Effect of elevated CO_2 and N level on growth of wheat and field pea

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Abstract
Increases in atmospheric carbon dioxide (CO_2) concentration have been shown to enhance photosynthetic gain and net primary productivity and increase water- and N-use efficiency. However, it is uncertain whether this extends to grain crops in semi-arid agricultural systems, such as southern Australia, which are often N and water limited. We investigated the interactive effect of CO_2 concentration and nitrogen (N) fertiliser application on carbon (C) and N partitioning at the soil free-air CO_2 enrichment (SoilFACE) facility. Wheat and field pea were grown under elevated CO_2 (eCO_2; 550 ppm) or ambient CO_2 (aCO_2; 370 ppm) with two N levels (40 and 100 mg N/kg soil over 4 applications) in soil columns containing a Vertosol. We report greater effects of eCO_2 on shoot biomass than root biomass of wheat and field pea. Wheat produced more roots at 25-50 cm under eCO_2. Conversely, N level only affected wheat shoot growth. Elevated CO_2 increased the C:N ratio of wheat root and shoot regardless of N level and this could affect C cycling.

Key Words
Elevated CO_2, SoilFACE, biomass partitioning, carbon, nitrogen, C:N.

Introduction
Elevated atmospheric CO_2 concentrations have been shown to increase plant productivity and are attributed to direct stimulation of photosynthesis and increased water-use efficiency due to reduced evapotranspiration (Kimball et al., 2002). Studies indicate that crop biomass and yield can increase by up to 30% under eCO_2, with a greater response in legumes than non-legumes due to the stimulation of N fixation, which fulfils the additional N requirement under eCO_2 (Ainsworth and Long, 2005). However, most studies have only examined the effects of eCO_2 on above-ground components, such as shoot development, biomass and yield. Few studies have considered below-ground C and N partitioning within soil-plant systems, particularly under field conditions (Ko et al., 2010; Manderscheid et al., 2009; Mitchell et al., 1993). The response of plants to eCO_2 will depend on abiotic factors such as temperature and availability of water and nutrients. In Australian dryland agricultural systems non-legumes rely almost entirely on N fertiliser to meet their growth requirements. In these systems responses to eCO_2 will be determined by soil N status and are likely to be quantitatively smaller in situations where N is not optimal. This study aimed to quantify the changes in biomass partitioning of wheat and field pea under eCO_2 and different N levels, and to assess the implications for C and N cycling.

Methods
Experimental details and design
We conducted a column experiment at the SoilFACE facility, Department of Primary Industries, Horsham. SoilFACE consists of eight bunkers (3.7 m wide) with either aCO_2 (370 ppm) or eCO_2 (550 ppm) concentrations in a randomised 4 x 2 design. The eCO_2 is achieved using FACE technology as described by Mollah et al. (2009) and the level of elevation is based on the predicted atmospheric CO_2 concentration in the year 2050. A Vertosol soil (Isbell, 1996) was collected from a roadside, sieved (<4 mm), and mixed 1:1 with triple washed white sand. Briefly, the Vertosol had the following properties; pH_{CaCl}, 7.7; total C 14.2 g/kg, total N 0.8 g/kg. Twelve kg (dry weight equivalent) of the soil: sand mix was added to plastic columns (15 cm ID x 60 cm long), which equated to a bulk density of 1.2 g/cm^3. The soil: sand mix contained the following basal nutrients in mg/kg; $CaCl_2$, 186; $CoCl_2$, 0.4; $CuSO_4$, 6; $FeCl_3$, 0.6; K_2SO_4, 103; KH_2PO_4, 70.4; $MgSO_4$, 122; $MnSO_4$, 6; $Na_2B_4O_7$, 1.6; Na_2MoO_4, 0.4 and $ZnSO_4$, 8. Soil columns were adjusted to 80% field capacity and sown with either wheat (*Triticum aestivum* L. cv. Yitpi) or field pea (*Pisum sativum* L. cv. PBA Twilight) on the 14th June 2011. Group E inoculum was used for field pea. After 3 weeks, wheat and field pea plants were thinned to 3 and 5 plants/column, respectively. Two N levels, 40 or 100 mg N/kg soil, were added as $Ca(^{15}NO_3)_2$ split over four equal applications at sowing and at 3 times during the growing season. Columns only received water when N was added (10% Field Capacity) and in the final 3 weeks before sampling (20% FC).

Sampling and analyses
Columns were removed from SoilFACE and destructively sampled at peak biomass (2nd November 2011). Plant shoots were removed, rinsed with reverse osmosis (RO) water and dried at 70°C for 3 days. The soil was separated into depths of 0-10 cm, 10-25 cm and 25-50 cm. Roots were carefully removed, washed with RO water, and root length and surface area were determined on fresh samples using WinRHIZO Pro 2003b (Regent Instruments, Quebec, Canada). Roots were then dried at 70°C for 3 days, ground (<0.5 mm) using a Retsch ZM200 centrifugal mill, thoroughly mixed and subsamples were ground further using a Retsch MM400 mixer mill (Retsch GmbH, Haan, Germany) for analyses. Total C and N, and ^{15}N content were determined using an isotope-ratio mass spectrometer (IRMS) (Hydra 20-20, SerCon, Crewe, UK).

Statistical analyses

For each species, a two-way analysis of variance (ANOVA) in a randomised complete block split-plot design was used to test the effects of CO_2 concentration (main-plots) and N level (sub-plots) on shoot and root variables. Root variables were analysed separately for each depth to improve the accuracy and interpretation. Significant (P = 0.05) differences between means were established using least significance difference (LSD) test.

Results

Shoot growth

Nitrogen supply and eCO_2 concentration significantly increased shoot growth (P = 0.05), and altered N variables of wheat and field pea. For wheat, plant height was greater under eCO_2 but lower at higher N levels (Table 1). In contrast, the height of field pea was not affected by either CO_2 concentration or N supply. A significant (P = 0.05) positive interaction between CO_2 concentration and N supply was observed for wheat shoot growth. Similarly, greater dry matter production occurred under eCO_2 for field pea, but shoot growth was not affected by N supply, presumably because it is a legume. The shoot N concentration and total N uptake of wheat were greater at 100 mg N/kg than 40 mg N/kg, and this resulted in a significant decrease in the C:N ratio. Furthermore, eCO_2 decreased the N concentration in wheat shoot and this decrease was greater at the higher rate of N addition. The C:N of wheat shoot grown under eCO_2 increased by 6.3 and 5.7 for the low N level and high N level, respectively. In contrast to wheat, N concentration in field pea shoot, total N uptake and C:N were not significantly different (P = 0.05) between 40 mg N/ kg and 100 mg N/kg (Table 1). For field pea, N concentration within the shoot was not altered by CO_2 concentration. Hence, the significant increase in N uptake by field pea at eCO_2 occurred due to greater biomass accumulation. A decrease in the C:N of field pea shoot occurred when the plants were grown under eCO_2, but only for plants grown at the low N level.

Table 1. Effect of eCO_2 and N supply on shoot variables.

CO_2 conc. (ppm)	N rate (mg/kg soil)	Height (cm)	Dry Matter (g/column)	N conc. (g/kg plant)	Total N uptake (mg/column)	C:N
Wheat						
370	40	51.9	27.7	10.3	286	39.9
550	40	58.3	37.0	8.9	329	46.2
370	100	45.4	28.9	20.1	561	21.3
550	100	53.2	43.2	15.5	669	27.0
	LSD (P=0.05)	*4.8*	*2.9*	*2.0*	*55*	*3.4*
	CO_2	*n.s.*	**	*	*n.s.*	*
	N	*	*	***	***	***
	CO_2 x N	*n.s.*	*	*n.s.*	*n.s.*	*n.s.*
Field Pea						
370	40	47.0	26.0	23.2	603	17.8
550	40	56.0	32.3	26.5	859	15.7
370	100	50.0	25.9	23.7	614	17.4
550	100	53.7	35.2	23.0	812	17.5
	LSD (P=0.05)	-	*3.7*	-	*163*	*0.6*
	CO_2	*n.s.*	*	*n.s.*	*	*
	N	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*
	CO_2 x N	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*

***, **, * and n.s. represent P<0.001, P<0.01, P<0.5, P>0.5, respectively.

Root growth

CO_2 concentration and N supply affected roots less than shoots (Table 2). No significant effect of CO_2 or N levels on any root variable of field pea was observed. Wheat root growth was increased under eCO_2 but was only significant (P = 0.05) in the 25-50 cm layer. Furthermore, there was no effect of N level on root mass in any layer. CO_2 concentration and N supply altered the morphology of roots in the 0-10 cm and 25-50 cm layers but had no effect on the middle (10-25 cm) layer. In the low N treatment a decrease in wheat root length occurred in the 0-10 cm layer under eCO_2. In most cases, wheat root length was greater in the 0-10 cm and 25-50 cm layers at 100 mg N/kg than 40 mg N/kg, however this was not significant (P = 0.05) for aCO_2 in the 0-10 cm layer. A similar response to N level was observed for specific root length. Furthermore, specific root length in the 25-50 cm layer was significantly greater under aCO_2 and 100 mg N/kg than all of the other treatments. The surface area of wheat roots grown under eCO_2 was lower in the soil surface layer. In contrast, root surface area was greater at 100 mg N/kg than 40 mg N/kg in both 0-10 cm and 25-50 cm layers. Root N concentration and total N uptake were greater at 100 mg N/kg than 40 mg N/kg and this resulted in lower C:N ratios. Conversely, eCO_2 did not significantly alter root N concentration and total N uptake, although in most cases lower values were observed under eCO_2. Subsequently, the C:N ratio was significantly higher under eCO_2, although this was not significant (P = 0.05) in the 0-10 cm layer.

Table 2. Effect of eCO_2 and N supply on root variables.

CO_2 conc. (ppm)	N rate (mg/kg soil)	Root mass (g)	Root length (cm)	SR[a] length (cm/g)	Surface area (cm^2 /column)	N conc. (g/kg plant)	Total N uptake (mg /column)	C:N
Wheat 0-10 cm								
370	40	6.36	3521	552	316	4.9	31	76.2
550	40	7.02	2851	404	269	3.7	26	104.7
370	100	6.55	3737	572	343	9.1	59	38.2
550	100	7.09	3508	494	314	8.0	57	45.0
	LSD (P=0.05)	-	*281*	*53*	*26*	*1.5*	*8*	*15.4*
	CO_2	*n.s.*	*	*n.s.*	*	*n.s.*	*n.s.*	*n.s.*
	N	*n.s.*	*	*	*	**	***	***
	CO_2 x N	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*
Wheat 10-25 cm								
370	40	5.08	4601	913	375	8.0	41	49.6
550	40	5.22	4775	917	385	6.3	33	63.0
370	100	5.22	4436	849	378	13.5	70	29.5
550	100	5.53	4857	880	418	10.3	57	37.7
	LSD (P=0.05)	-	-	-	-	*1.0*	*7*	*3.0*
	CO_2	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*
	N	*n.s.*	*n.s.*	*n.s.*	*n.s.*	***	***	***
	CO_2 x N	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*
Wheat 25-50 cm								
370	40	7.57	4755	629	418	8.5	64	46.0
550	40	8.75	4831	552	428	7.0	62	54.6
370	100	6.82	5581	820	487	14.0	95	28.7
550	100	8.46	5392	637	473	12.0	102	32.0
	LSD (P=0.05)	*0.65*	*527*	*72*	*24*	*1.2*	*12*	*4.8*
	CO_2	*	*n.s.*	*	*n.s.*	*n.s.*	*n.s.*	*
	N	*n.s.*	*	**	*	***	**	***
	CO_2 x N	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*
Field Pea 0-10 cm								
370	40	4.67	2250	482	224	24.0	112	16.7
550	40	4.73	2267	478	251	25.6	121	16.3
370	100	4.69	2518	533	259	24.7	116	17.1
550	100	4.83	3004	619	336	25.4	123	16.6
	CO_2, N, CO_2 x N	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*
Field Pea 10-25 cm								
370	40	4.57	3706	807	421	23.9	109	17.4
550	40	4.72	4060	857	498	24.5	115	17.2
370	100	4.59	3665	798	389	23.3	107	18.1
550	100	4.57	3047	667	351	23.6	108	17.3
	CO_2, N, CO_2 x N	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*
Field Pea 25-50cm								
370	40	5.18	2622	505	312	24.1	125	17.4
550	40	5.46	3672	680	422	24.1	131	18.0
370	100	5.21	3086	595	353	24.1	126	17.7
550	100	5.49	2782	507	331	25.5	140	16.5
	CO_2, N, CO_2 x N	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*

[a]Specific root (SR) length is the root length per unit dry root mass (cm/g).
***, **, * and n.s. represent $P<0.001$, $P<0.01$, $P<0.5$, $P>0.5$, respectively.

Discussion and conclusions

Our study showed that eCO_2 increased the above-ground biomass of wheat and field pea. A 24-35% increase in the shoot biomass of field pea was observed but was unaffected by N supply. Therefore, N fixation was able to meet the greater N demand under eCO_2 and field pea was able to maintain similar tissue N concentrations under aCO_2 and eCO_2. In contrast, the increase in wheat shoot biomass under eCO_2 was greater at high levels of applied N (49%) than at low N levels (34%). Other studies have recorded increases in wheat shoot biomass under eCO_2 even at low N levels, although smaller in magnitude (Mitchell et al., 1993). The effect of soil N status on above-ground biomass of wheat was greater than the CO_2 effect and is consistent with other studies (Ko et al., 2010). Total N uptake under eCO_2 was greater for both field pea and wheat. For wheat, this indicated greater access to soil N, however this was not sufficient to meet the N demand under eCO_2 and wheat tissue N concentration decreased considerably. The contribution of N fertiliser and N fixation are currently being assessed (Armstrong, pers comm). This study showed that eCO_2 resulted in an increase in shoot: root ratio of both species. In fact, eCO_2 only increased root biomass for wheat and only the deeper (25-50 cm) soil layer. The effect of elevated CO_2 concentration on root growth is inconsistent but is generally positive if water is not limiting (Wechsung et al., 1999; Li et al., 2003) and/or if soil N status is low (Mitchell et al., 1993), although there are reports of decreases (Li et al., 2003). The lack of an effect of eCO_2 on below-ground biomass allocation may indicate that the plants within the current study were drought stressed during the growing season. An increase in root: shoot ratio to overcome the decrease in tissue N concentration was expected; however this was not observed. Reduced mass flow and N uptake under drought may also explain the lower C:N ratio of wheat shoot at the high soil N level. eCO_2 tended ($P = 0.062$) to decrease wheat root N concentration and significantly ($P = 0.05$) increased the C:N ratio. These roots could decompose slower than roots grown under aCO_2, particularly those additional roots accumulated at lower soil depths. Further, specific root length of wheat decreased under eCO_2 indicating coarser roots. Martens et al., (2009) indicated that despite no effect of eCO_2 on root biomass, significant increases in soil organic ^{14}C under eCO_2 were revealed using repeated $^{14}CO_2$ pulse-labelling. This requires further investigation. This study confirmed the dependence of wheat on soil N status to respond to eCO_2. Field pea appears to take advantage of the positive effect of eCO_2 better than wheat, provided conditions for efficient symbiosis and N fixation are met.

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