Soil type influences N_2 fixation in fieldpeas more than elevated CO_2

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Little is known about how elevated atmospheric carbon dioxide (eCO_2) affects pulse growth and nitrogen (N) fixation in medium rainfall dryland cropping systems. We examined the growth and N_2 fixation of field-peas (Pisum sativa cv. OZ0601) in the SoilFACE array at Horsham (annual rainfall 420 mm). Fieldpeas were grown under either ambient (ca. 390 ppm) or elevated CO_2 (550 ppm) in large (30 cm width x 100 cm long) intact soil cores placed in replicated bunkers in the ground. The intact cores, which maintain the physical and chemical integrity of the soil profile, were collected from 3 soil types: a Calcarosol, a Vertosol and a Chromosol. Soil N concentration of the 3 soils was Chromosol > Vertosol > Calcarosol. The fieldpeas were grown in rotation with wheat (*Triticum aestivum* cv. Yipti) over 2 seasons and N fixation assessed using the natural abundance method. In 2010, the growth and grain yield of fieldpea was increased by eCO_2 and affected by soil type. Neither factor however affected the proportion of plant N derived from N_2 fixation (%Ndfa), although there was a significant interaction between soil type and eCO_2 on the amount of N fixed. In 2011, soil type significantly affected growth, yield and %Ndfa of field pea but eCO_2 had no effect. %Ndfa was strongly related to the amount of NO_3-N in the profile at sowing. N_2 fixation in fieldpeas is, at least in the initial phase of CO_2 treatment, influenced more by soil type (soil N availability) than eCO_2.

Key words
SoilFACE, elevated CO2, N_2 fixation

Introduction
Future increases in atmospheric CO_2 offer the chance to greatly increase plant productivity via effects on a range of plant physiological processes including photosynthesis and water relations (Kimball *et al*. 2002). These increases however are contingent on adequate supplies of available nitrogen (N) (Newton *et al*. 2007). The productivity of Australian cropping systems is heavily reliant on soil N supply, with a large proportion of this N traditionally coming from biological fixed N including that from grain legumes (Angus 2001, Evans et al. 2001). The rate of N_2 fixed, and the value of the N derived from legumes to subsequent crops, can vary markedly with seasonal conditions and site/soil (Kirkegaard *et al*. 2008).

Growth stimulation by elevated CO_2 (eCO_2) is generally greater in legumes than non-legumes, probably as a result of maintaining high leaf N status (Kimball *et al*. 2002). FACE (Free Air Carbon Dioxide Enrichment) studies have revealed many factors operating in the open field situation that cannot be identified in other types of studies (Leakey et al. 2009). There have been very few studies however (other than for soybeans) of the response of grain legumes to eCO_2 in the field (Rogers et al. 2009). In this paper we report an experiment that examined the effect of eCO_2 on the growth and N_2 fixation of a pulse (field pea) grown in rotation with wheat in three different soil types in the SoilFACE array.

Methods
SoilFACE, located near Horsham (36°45'S, 142°06'E; 127m elevation) is a FACE array based on the use large intact cores (30 cm diameter x 100 cm deep cased in a PVC sleeve), that maintain the physicochemical integrity of the soil profile, to assess interactions between soil type and eCO_2 on crop growth. Cores were collected from the Victorian Mallee (Calcarosol), Wimmera (Vertosol) and High Rainfall Zone (Chromosol) (Table 1) with experimentation commencing in 2009. The cores were placed in 8 bunkers sunk into the ground (the top of the cores are at ground level). Four bunkers are maintained at ambient CO_2 (ca. 390 ppm) whilst four are maintained at 550 ppm atmospheric CO_2 (eCO_2) as per Mollar et al. (2009). The experimental design consisted of field pea (cv. OZ00601) grown in rotation with wheat (cv. Yipti) x 3 soil types x eCO_2/ambient CO_2 x 4 reps in a split plot design.

At peak flowering one intact core in each treatment was cut at ground level, the material dried at 70°C, weighed and ground. Total N and ^{15}N enrichment was determined by isotope ratio mass spectrometry (IRMS) (continuous flow Isotope Cube (Elementar, Germany) coupled with a continuous flow mass spectrometer (Isoprime, United Kingdom) utilizing Dumas flash combustion. The proportion of N derived from fixation was determined using the natural abundance technique (Peoples *et al*. 1989) using wheat collected from cores of the same soil type as a reference plant. At grain maturity plants were cut at ground level prior to drying, grain separated by threshing and total N content of the ground material determined by Leco™ analyser (St Joseph, MI, USA). A basal application of P (single superphosphate equivalent to 15 kg P/ha) was sown with the seed. Prior to sowing each year, soil was collected at 0-10, 10-20, 20-30, 30-50, 50-70, 80-95 cm using a thin walled tube (42 mm cutting tip) and gravimetric soil water (following drying at 105^{o}C) and NO_3-N measured by extraction (1:10) in 2 M KCl followed by colorimetric analysis (Searle 1984) on a Flow Injection Analyser. Annual and growing season rainfall (April – November: GSR) at the site was 559 and 330 mm in 2010 and 507 and 250 mm in 2011, respectively.

Table 1: Characteristics of 3 soils used in SoilFACE

Soil	pH ($CaCl_2$) 0-10 cm	EC(1:5) 80-100 cm (dS/m)	ESP 80-100 cm (%)	Total N 0-10 cm (%)	Total C 0-10 cm (%)
Chromosol	4.5	0.16	4.3	0.40	4.66
Vertosol	7.7	1.85	20.0	0.08	1.10
Calcarosol	5.9	0.53	7.5	0.05	0.64

Results

In 2010 (second season after CO_2 treatment commenced), eCO_2 stimulated the growth at flowering and maturity, early N content and grain yield of field pea (Table 2). Dry mater production and grain yield were strong affected by soil type in the general order Vertosol > Chromosol > Calcarosol. The proportion of plant N derived from N_2 fixation at flowering was significantly affected by soil type (lowest on the Chromosol and highest on the Vertosol and Calcarosol soils) but CO_2 treatment had no effect. As a consequence, there was a significant interaction between the CO_2 and soil type on the amount of N fixed (kg/ha) at flowering in the eCO_2 Vertosol cores being greater than ambient CO_2 cores, which in turn were higher than that in the Chromosol soil, regardless of CO_2 treatment. In 2011, the relative growth and grain yield of fieldpeas in the Calcarosol cores increased especially compared to the Chromosol cores, to be no different than the Vertosol. CO_2 treatment however had no effect on the growth, yield or rate of N_2 fixation of fieldpeas.

The large differences in background soil N and C at the commencement of SoilFACE (Table 1) were reflected in the amount of soil NO_3-N (0-90 cm) measured at sowing in 2010 with the Chromosol cores having much higher NO_3-N (146 kg/ha) than the Vertosol (64 kg/ha), which in turn was higher than the Calcarosol soil (24 kg/ha) (data not presented). Neither CO_2 nor previous crop had any significant effect on NO_3-N. In 2011, (3rd season of experimentation), this effect on NO_3-N at sowing persisted with Chromosol > Vertosol > Calcarosol and generally no effect of CO_2 or previous crop: the exception was that the large amount of NO_3-N recorded in eCO_2 Chromosol cores following wheat in 2010. These differences in soil NO_3-N were strongly related (R^2 = 0.73) to the %Ndfa of the field peas (Figure 1).

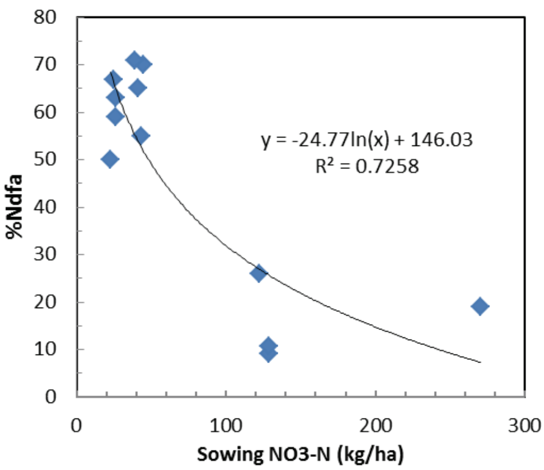

Figure 1: Relationship between profile NO_3-N at sowing and the proportion of legume N derived from N_2 fixation (%Ndfa) of field peas (pooled across years, soil and CO_2 treatments).

Table 2: Influence of CO_2 (ambient and eCO_2) and soil (Calcarosol, Vertosol and Chromosol) on the proportion of N derived from N_2 fixation and the amount of N fixed at flowering and the total dry matter, grain yield and N uptake of field pea at maturity in SoilFACE (2010 and 2011)

CO_2	Flower DM (g/core)	Flower N Content (g/core)	%Ndfa	N fixed (g/ core)	Maturity DM (g/core)	Grain yield (g/core)	Maturity N (mg/ core)
2010							
Calcarosol							
Amb	32.6	710	49.9	379	46.9	25.3	nd
eCO_2	28.1	580	58.9	334	49.7	28.5	nd
Vertosol							
Amb	60.1	1330	55.0	451	75.2	39.5	nd
eCO_2	96.0	1950	64.6	995	91.0	48.7	nd
Chromosol							
Amb	47.1	1020	9.1	81	51.0	23.4	nd
eCO_2	57.7	960	10.7	63	66.2	32.6	nd
l.s.d. (5%)	Soil x CO_2 = 21.1	CO_2 ns Soil = 339	Soil = 29.7	Soil x CO_2 = 347	CO_2 = 7.8 Soil = 9.5	CO_2=3.95 Soil = 4.9	nd
2011							
Calcarosol							
Amb	52.9	1112	69.9	780	58.2	30.3	1256
eCO_2	51.9	1001	71.4	716	73.6	37.8	1334
Vertosol							
Amb	60.6	1388	62.6	858	70.9	35.9	1392
eCO_2	50.3	1075	67.0	717	80.9	43.7	1802
Chromosol							
Amb	34.0	699	25.6	138	53.3	26.2	973
eCO_2	26.2	555	18.6	108	60.2	29.2	1068
l.s.d. (5%)	Soil = 14.6	Soil = 307	Soil = 27.2	Soil = 343	Soil = 10.1	Soil = 3.9	Soil = 387

Discussion

Any stimulatory effect of eCO_2 on plant production is strongly related to adequate N supplies (Leakey et al. 2009). This relationship between N supply and eCO_2 will most likely be even more important in the dryland cropping systems of southern Australia as N inputs form a significant proportion of grain grower's variable cost inputs, and N derived from N_2 fixation provides a major proportion of N supplies in many cases. In this study we found that effect of eCO_2 on the dry matter and yield of field peas varied with season, being more important in 2010 when soil water availability was much above long-term average than in 2011.

However eCO_2 had no effect on %Ndfa in either year, although it did increase the amount of N fixed in 2010 as a result of the greater dry matter produced. In contrast, soil type had a much greater effect on growth and yield as well as %Ndfa in both 2010 and 2011. This result is in general agreement with other studies of N dynamics of legumes under eCO_2 that found that whereas the amount of N fixed increases by an average of 38% under eCO_2, the change in %Ndfa is non-significant (Lam *et al*. 2012). Legume N_2 fixation is very sensitive to soil nitrate concentration (Herridge et al. 1998) and in our study we found a strong negative relationship between NO_3-N at sowing and %Ndfa, that appeared to override eCO_2 treatment (Figure 1).

There is a general conclusion that, at least in grasslands, the availability of N for plant growth declines with time under eCO_2 (Progressive Nitrogen Limitation) (Newton *et al*. 2010). Our study found that eCO_2 had little consistent measurably effect on %Ndfa whereas soil type (and especially background N content) had a major impact during the first 3 years after CO_2 treatment commenced. In soils like the Wimmera Vertosol where the background C and N content is relatively low after decades of continuous cropping, even small changes in N_2 fixation rates of pulses may have significant impacts on productivity as the relative value of N derived from legumes to subsequent crops may be reduced in future environments with eCO_2 (Lam et al. 2013).

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