1 **Title:**

2 Crop residue incorporation negates the positive effect of elevated atmospheric carbon
3 dioxide concentration on wheat productivity and fertilizer nitrogen recovery

4

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18

19 **Abstract**

20 *Background and purpose*

21 Rapid increases in atmospheric carbon dioxide concentration ([CO₂]) may increase
22 crop residue production and carbon: nitrogen (C:N) ratio. Whether the incorporation
23 of residues produced under elevated [CO₂] will limit soil N availability and fertilizer
24 N recovery in the plant is unknown. This study investigated the interaction between
25 crop residue incorporation and elevated [CO₂] on the growth, grain yield and the
26 recovery of ¹⁵N-labeled fertilizer by wheat (*Triticum aestivum* L. cv. Yitpi) under
27 controlled environmental conditions.

28 *Methods*

29 Residue for ambient and elevated [CO₂] treatments, obtained from wheat grown
30 previously under ambient and elevated [CO₂], respectively, was incorporated into two
31 soils (from a cereal-legume rotation and a cereal-fallow rotation) one month before
32 the sowing of wheat. At the early vegetative stage ¹⁵N-labeled granular urea (10.22
33 atom%) was applied at 50 kg N ha⁻¹ and the wheat grown to maturity.

34 *Results*

35 When residue was not incorporated into the soil, elevated [CO₂] increased wheat
36 shoot (16%) and root biomass (41%), grain yield (19%), total N uptake (4%) and
37 grain N removal (8%). However, the positive [CO₂] fertilization effect on these

parameters was absent in the soil amended with residue. In the absence of residue, elevated [CO₂] increased fertilizer N recovery in the plant (7%), but when residue was incorporated elevated [CO₂] decreased fertilizer N recovery.

Conclusions

A higher fertilizer application rate will be required under future elevated [CO₂] atmospheres to replenish the extra N removed in grains from cropping systems if no residue is incorporated, or to facilitate the [CO₂] fertilization effect on grain yield by overcoming N immobilization resulting from residue amendment.

Keywords

Elevated [CO₂]; residue incorporation; wheat growth; fertilizer N recovery; ¹⁵N

Introduction

Rapid increases in atmospheric carbon dioxide concentration ($[CO_2]$) since 1950 (IPCC 2007) have generally resulted in increased plant photosynthesis and crop growth, and as a consequence the quantity of crop residues produced has also increased (Drake et al. 1997; Ainsworth and Long 2005). The carbon: nitrogen (C:N) ratio of C_3 non-legume crops is widely reported to increase under elevated $[CO_2]$ (Ball 1997; Kimball et al. 2002). These $[CO_2]$ -induced changes in quantity and quality of residue materials entering the soil can affect crop growth and yield through changes in microbial activities and N immobilization or availability (Hungate et al. 1997; Luo et al. 2004). A meta-analytic review reported that elevated $[CO_2]$ stimulated microbial C (7%) and microbial N contents (6%), and gross N immobilization (22%), but had no effect on gross and net N mineralization rates (de Graaff et al. 2006).

The retention of crop residues is important for improving soil physical, chemical and biological properties (Smil 1999; Mandal et al. 2004), but residues can also lead to increased N immobilization in cropping systems by increasing the amount of C relative to N (e.g. Azam et al. 1985; Jensen 1997; Montoya-González et al. 2009). Crop residues added to soils have been shown to reduce the recovery of fertilizer N in cereals due to either N immobilization (Bijay-Singh et al. 2001) or N input from the

69 residue applied (Kongchum et al. 2007). Fertilizer N recovery can be inversely
70 proportional to residue application rate but proportional to the extent of residue
71 decomposition (Azam et al. 1990).

72 Previous studies of the effect of elevated [CO₂] on fertilizer N recovery in
73 cereals are scanty and contradictory. Martín-Olmedo et al. (2002) indicated that the
74 recovery of fertilizer N in spring barley was increased by elevated [CO₂] (716 μmol
75 mol⁻¹), whereas in grain sorghum (under 715 μmol mol⁻¹; Torbert et al. 2004) and
76 spring wheat (under 550 μmol mol⁻¹; Lam et al. 2012) it was unaffected. In rice
77 elevated [CO₂] resulted in either positive (under 700 μmol mol⁻¹; Weerakoon et al.
78 2005) or neutral effects (under 622 μmol mol⁻¹; Kim et al. 2011). The interaction
79 between elevated [CO₂] and residue ([CO₂]-affected) incorporation on fertilizer N
80 recovery has not been examined to our knowledge. Such information will be critical
81 to developing efficient management strategies for fertilizer N and crop sustainability
82 in future CO₂ atmospheres. The objectives of the present study were to investigate (i)
83 the interaction between elevated [CO₂] and residue incorporation on growth, grain
84 yield and N uptake of wheat; and (ii) the availability of soil and fertilizer N to wheat
85 following residue amendment under elevated [CO₂]. We conducted this study under
86 controlled environments to remove the confounding effects of varying soil moisture
87 during the wetting and drying cycle that was experienced in the field in our earlier

study (Lam et al. 2012).

Materials and methods

Glasshouse chambers

This study was conducted between October 2009 and February 2010, in a set of two identical naturally lighted glasshouse chambers (3.1 m long × 2.4 m wide × 2.6 m high) located at the Department of Primary Industries Grains Innovation Park in Horsham (36°43'S, 142°10'E), Victoria, Australia. The atmospheric CO₂ level in one chamber was held at 390 μmol mol⁻¹ (ambient), whereas the other (elevated) was maintained 24 hours a day throughout the experimental period at the predicted CO₂ level (700 μmol mol⁻¹) which will be reached by 2100 according to IPCC emissions scenario A1B (Houghton et al. 2001). The elevated [CO₂] treatment was created by adding pure CO₂ to a mixing fan within the chambers, and the [CO₂] was monitored using an infra-red gas analyser (Guardian SP 97301, Edinburgh Instruments Ltd). Air temperature in each chamber was measured by a temperature sensor (DS1923 Hygrochron iButton, Thermodata Pty Ltd) mounted above the plant canopy. The average day/night temperature of the chambers throughout the experimental period was 27/21°C.

107 *Experimental design*

108 The design of the experiment was 2 CO₂ concentrations (ambient 390 μmol mol⁻¹ and
109 elevated 700 μmol mol⁻¹) × 2 residue incorporation treatments (with and without
110 residue incorporation; residue from wheat grown under ambient [CO₂] conditions was
111 used for the study under ambient [CO₂] conditions and that from wheat grown under
112 elevated [CO₂] was used for the elevated [CO₂] study) × soils from 2 rotation histories
113 (wheat-oats-pea rotation and wheat-fallow rotation), with four replications, totalling
114 32 pots (150 mm diameter, 150 mm high). All pots were grown with wheat plants.
115 The control treatments were ambient [CO₂] and no residue amendment. While a
116 meta-analysis concluded that results from experiments using one growth chamber per
117 CO₂ treatment do not significantly differ from those using replicated chambers
118 (Zvereva and Kozlov 2006), [CO₂] treatments together with the plants were rotated
119 weekly between the chambers to avoid a possible chamber effect in our study.

120

121 *Soil and pot preparation*

122 Two soils (Vertosols, Australian Soil Classification, Isbell 1996) were collected from
123 a long term (since 1916) rotation experimental field at Longerenong (36°40'S,
124 142°18'E), Victoria. One soil was collected from a wheat-oats-pea rotation subplot
125 (38% clay; 52% silt; 10% sand) and the other from a wheat-fallow rotation subplot

(42% clay; 49% silt; 9% sand), here after designated as ‘cereal-legume rotation’ and ‘cereal-fallow rotation’. These rotations are common local practice. No N fertilizer was added to any subplots. Single superphosphate was added to wheat at 115 kg ha⁻¹, and peas and oats at 55 kg ha⁻¹. All the subplots were grazed after harvest but prior to burning of residues. The subplot after fallow was cultivated with a scarifier on an as-needed basis, whereas residues from other subplots were incorporated with a scarifier after residue burning. In 2008, pea (*Pisum sativum* L.) was grown in the wheat-legume subplot while the wheat-fallow subplot was left fallow. In 2009 (the year when the soils were collected for the present study), wheat (*Triticum aestivum* L.) had been grown on both subplots. The soils were collected on 7 September 2009 from the plough layer (top 0.20 m) of each subplot, air dried, crushed into < 10 mm fractions, and mixed thoroughly. Any visible plant residues were picked out manually and discarded. Some general properties of the two soils are presented in Table 1. Note that in 2009, after the long term rotation, the C:N ratio of the soil in the wheat-legume subplot was 14.6 and that in the soil of the wheat-fallow subplot was 16.3.

>>Insert Table 1 near here>>

Sixteen polyethylene lined, sealed pots were filled with 1.6 kg (dry weight) of

the soil from the wheat-legume subplot and another 16 pots were filled with soil from the wheat-fallow subplot. A basal dressing of 21.2 mg P pot⁻¹ (as KH₂PO₄, equivalent to 12 kg P ha⁻¹) was added to each pot and the contents of the pot were thoroughly mixed.

Residue incorporation

The residue for the ambient and elevated [CO₂] treatments was obtained from wheat (cv. Yitpi) grown under ambient and elevated CO₂ concentrations, respectively, in the Australian Grains Free-Air Carbon dioxide Enrichment (AGFACE) experiment in 2008 (Table 2). The details of the AGFACE exposure system and the experimental design were described in detail by Mollah et al. (2009). Wheat residue was collected from irrigated plots fertilized with N because these treatments resemble the present experimental conditions (viz. added N and water).

>>Insert Table 2 near here>>

The residue was cut into segments of around 30 mm length and homogenized before mixing with the soils. The amount of residue added to each pot for the ambient and elevated [CO₂] treatments was based on the wheat biomass and harvest index

previously obtained under ambient and elevated [CO₂] conditions, respectively, in the 2008 AGFACE experiment (Lam et al. 2012). This corresponded to 6.0 g pot⁻¹ (3.9 t ha⁻¹) of ambient [CO₂]-residue and 9.5 g pot⁻¹ (6.2 t ha⁻¹) of elevated [CO₂]-residue. These amounts of residue (dead leaf and stem) added are at levels commonly added to the field according to local agronomic practice. It was not possible to partition the specific response due to residue quantity from that due to residue quality. However, the overall effect of residue incorporation (encompassing the effects of [CO₂] on residue quantity and quality, which happens in reality) on fertilizer N recovery could be investigated. The amount of C added through residue incorporation was 2.5 and 3.9 g C pot⁻¹, respectively, under ambient and elevated [CO₂]. The corresponding values for N were 55.2 and 73.2 mg N pot⁻¹. Reverse osmosis water was added to each pot to constant weight (80% of field capacity) once a week for one month before sowing. This simulated the period of residue decomposition between ploughing and the sowing of a subsequent crop.

Plant cultivation and fertilizer ¹⁵N application

After the wetting and drying cycle of one month, eight seeds of wheat (cv. Yitpi) were sown on 27 November to all pots. Ten days after emergence, plant seedlings were thinned to four per pot. All the pots were watered with reverse osmosis water to

183 constant weight (80% of field capacity) every two to three days.

184 At the 3-4-leaf growth stage of wheat ^{15}N -enriched (10.22 atom%) granular urea
185 was topdressed onto the soil surface of each of the 32 pots of the incorporation study
186 at the rate of $0.167 \text{ g N pot}^{-1}$ (equivalent to 50 kg N ha^{-1}). Non-labeled granular urea
187 was applied to eight extra pots (one for each of the eight treatments) for the
188 determination of natural ^{15}N enrichment. The rate and method of urea application
189 were based on local agronomic practice where N fertilizer is normally topdressed
190 during early vegetative stages of the crop.

191

192 *Sample collection and preparation*

193 All plants in each pot were harvested when leaves were completely senesced and
194 grains fully developed and ripened (90 days after emergence). The plants were cut at
195 ground level to separate the above-ground parts from the bulk soil, and the
196 above-ground parts were separated into grain and shoot (stem and leaf). The soil left
197 in each pot was removed and crushed to particles less than 2 mm in diameter. Coarse
198 root material was separated manually, and was taken for subsequent analysis. The fine
199 roots were mixed thoroughly with the soil, and a sample of the mixture retained for
200 analysis. This sample was separated into two portions, one for the analysis of mineral
201 N content and the other for total N and ^{15}N contents. The plant parts (grain, shoot and

root) and soil samples in the whole pot were dried at 70°C and 40°C, respectively, for 48 h and weighed. Subsamples of the plant and soil samples for total N and ^{15}N analysis were finely ground in a ball mill (TissueLyser II, QIAGEN) to yield a powder of ~100 μm . The soil samples (< 2 mm) for mineral N analysis were not finely-ground. To determine moisture content, soil samples were dried at 105°C for 48 h.

Chemical analysis and calculations

The finely-ground plant and soil samples were analyzed for total C, total N and ^{15}N enrichment by isotope ratio mass spectrometry (Hydra 20-20, SerCon). The percentages of ^{15}N applied that were recovered in crop and soil were calculated according to Hauck and Bremner (1976). Fifty grams of the dried soil (< 2 mm) were extracted with 250 mL 2 M KCl (Keeney and Nelson 1982). The concentrations of ammonium (NH_4^+) and nitrate (NO_3^-) in the filtered extract were determined colorimetrically by a segmented flow analyser (San⁺⁺, Skalar). As there was less than 1 mg mineral N kg^{-1} in these soil samples, mineral ^{15}N was not determined. Total soil organic N was determined by subtracting mineral N from total soil N.

Statistical analysis

Data were analyzed with MINITAB 16 statistical package using a General Linear Model analysis of variance with a level of significance of $p < 0.05$ unless otherwise stated. Data were tested for normality and $\log_e$ -transformed where necessary to equalize variances between treatments. The least significant difference (LSD) at $p = 0.05$ was used to compare the means between treatments. The effect of elevated $[\text{CO}_2]$ on the quality of crop residue used for amendment was analyzed using a Student's unpaired t -test.

Results

Wheat biomass accumulation and tissue quality

When wheat was grown in soils that had not been amended with residue, increasing the atmospheric $[\text{CO}_2]$ resulted in increased grain yield (by 19%, $p < 0.001$), shoot biomass (by 16%, $p < 0.001$) and root biomass (by 41%, $p < 0.001$). However, when residue was incorporated into these soils changing the atmospheric $[\text{CO}_2]$ had no significant effect on these parameters (Table 3). When averaged across $[\text{CO}_2]$ and residue amendment treatments, wheat grain yield, and shoot and root biomass were 13% ($p < 0.01$), 19% ($p < 0.001$), and 16% ($p < 0.05$), respectively, greater in soil under the long-term cereal-legume rotation than in soil under cereal-fallow rotation (Table 3).

In the unamended soil, increasing [CO₂] also increased ($p < 0.01$) the C:N ratio of the shoots and roots by 34% and 18%, respectively, but when residue was incorporated into the soil increasing [CO₂] had no effect on the shoot C:N ratio. Irrespective of residue incorporation and rotation history, increasing [CO₂] reduced the grain N concentration by 7% ($p < 0.01$; Table 3).

>>Insert Table 3 near here>>

N uptake and grain N removal

In the unamended soils, elevated [CO₂] increased whole plant N uptake by 4% ($p < 0.1$) regardless of rotation history, but when the soils were amended with residue, elevated [CO₂] reduced whole plant N uptake by 13% ($p < 0.001$; Table 4). In soils without residue addition, grain N removal was greater ($p < 0.01$) under elevated [CO₂] (63.6 mg N pot⁻¹) than under ambient [CO₂] (58.9 mg N pot⁻¹). In contrast, when residue was incorporated into the soils, grain N removal under elevated [CO₂] (41.1 mg N pot⁻¹) was less than that observed ($p < 0.01$) under ambient [CO₂] (47.4 mg N pot⁻¹; Table 4).

When averaged across [CO₂] and residue amendment treatments, whole plant N uptake and grain N removal were 15% ($p < 0.001$) and 14% ($p < 0.001$) greater in soil

under the long-term cereal-legume rotation than in soil under cereal-fallow rotation (Table 4).

>>Insert Table 4 near here>>

¹⁵N balance study

The recovery of fertilizer N in plant tissue during the experimental period was in the order of grain > shoot > root (Table 5). In the unamended soils the recovery of fertilizer N in the whole plant in the elevated [CO₂] treatments was 7% greater ($p < 0.001$) than that in the ambient [CO₂] treatments, irrespective of rotation history. In contrast, when residue was incorporated, the recovery of fertilizer N in the whole plant was lower under elevated [CO₂] (by 8%; $p < 0.001$) than that under ambient [CO₂].

When averaged across treatments of residue incorporation and rotation history, elevated [CO₂] increased fertilizer N recovery in soil by 21% ($p < 0.01$; Table 5).

The total recovery of N in the plant-soil system ranged from 61–73%. In the unamended soils, elevated [CO₂] increased the total recovery of N in the plant-soil system by 8% ($p < 0.05$; Table 5). However, when residue was incorporated the total recovery of N in the plant-soil system was not affected by elevated [CO₂].

278

279 >>Insert Table 5 near here>>

280

281 *Mineral and organic N in soil at harvest*

282 In all treatments after the harvest of wheat there was less than 1 mg mineral N kg⁻¹ in
283 the soil (< 2.5 mg mineral N per pot) which means that most of the soil N (> 99.7%)
284 was present in organic form (Table 4). On average residue incorporation increased the
285 organic N in the soil by 6% (range 4.2–8.9%; Table 4). Soil organic N under the
286 cereal-legume rotation was 42% higher than that in the cereal-fallow rotation (Table
287 4).

288

289 **Discussion**

290 *Residue incorporation reduces [CO₂] fertilization effect on wheat productivity*

291 When wheat was grown in soils that had not been amended by residue addition, the
292 increase in plant biomass and grain yield under elevated [CO₂] was consistent with
293 that observed by others e.g. for wheat biomass (14–48%; Wechsung et al. 1999, Wall
294 et al. 2006, Ma et al. 2007) and grain yield (11–31%; Kimball 1983, Cure and Acock
295 1986, Amthor 2001, Long et al. 2006). Although growing plants at high density
296 (small pots) may alter root growth and yield response to elevated [CO₂], the

consistency of wheat biomass and yield response between our study and that of others indicates that N was not deficient when 50 kg N ha⁻¹ (the local practice) was applied. Despite N application, residue incorporation reduced the '[CO₂] fertilization effect' on wheat biomass and grain yield in our study. This could be attributed to an increase in N immobilization following residue incorporation (as shown by Azam et al. 1985; Jensen 1997; Torbert et al. 1998; and Montoya-González et al. 2009) and is evidenced by (i) the reduction in total N uptake by wheat in the residue-amended soils, (ii) the negligible soil mineral N content at harvest, and that (iii) organic N was the dominant form of soil N at harvest in our study. Indeed, the C:N ratio (46–54) of the incorporated residue materials in the present study was higher than the critical residue C:N ratio of 30–45 for net immobilization to occur (Alexander 1977; Vigil and Kissel 1991; Nourbakhsh and Dick 2005).

In the present study, shoot C:N ratio was increased under elevated [CO₂] when no residue was added (Table 3). This indicates that N uptake might not be able to keep up with demand by [CO₂]-increased growth even without amendment of straw. However, the C:N ratio was not increased significantly by elevated [CO₂] when residue was incorporated. This suggests that processes in addition to N immobilization may account for the reduced growth under elevated [CO₂] after residue amendment. Residue incorporation has been shown to increase

316 root-colonizing pseudomonads which could inhibit plant growth, including wheat,
317 under ambient [CO₂] (Elliott and Lynch 1984; Fredrickson and Elliott 1985;
318 Schippers et al. 1987). Under elevated [CO₂], Melloy et al. (2010) found that biomass
319 of residue-borne fungal pathogen *Fusarium pseudograminearum* (which causes crown
320 rot of wheat) per unit of wheat tissue was generally higher. The study by Melloy et al.
321 (2010) was conducted at the AGFACE facility from where the wheat residues used in
322 the present study were collected. This could be a possible reason for the lack of a
323 '[CO₂] fertilization effect' on growth under residue incorporation in the present study
324 although no disease symptoms were noted in any of the treatments. Nonetheless, the
325 deleterious growth effect of residue related pathogens, and the competition of the
326 residue degrading microbes with crops for N, might have been exacerbated in the pots
327 compared to the field situation.

328 In contrast, soybean yield was increased by elevated [CO₂] (516 $\mu\text{mol mol}^{-1}$)
329 under a range of residue incorporation rates (Shivashankar and Vlassak 1978). This
330 yield increase was accounted for by the stimulation in the growth and activity of
331 nodules due to soil N immobilization (Shivashankar and Vlassak 1978). Residue was
332 incorporated one month prior to sowing in both Shivashankar and Vlassak (1978)'s
333 and our study. These results imply that residue incorporation shortly before sowing
334 might be more beneficial to the grain yield of legumes (which can potentially supply a

significant proportion of their N requires via N₂ fixation) than cereals under future elevated [CO₂] environments. Nonetheless, it is not known whether this would have been so if more time had been allowed for the decomposition of the incorporated residue or for N remineralization. This is important because incorporation of residue over time (5–12 years) under ambient [CO₂] has been shown to improve soil fertility and biomass production, grain yield and N uptake of cereals, which was not apparent when residue was incorporated immediately before sowing (Verma and Bhagat 1992; Takajashi et al. 2003).

Residue incorporation reduces fertilizer N recovery under elevated [CO₂]

When residue was not incorporated, elevated [CO₂] increased the recovery of fertilizer N by wheat following an increase in plant biomass and N demand. This is consistent with the results of Martín-Olmedo et al. (2002), who found that fertilizer N recovery by spring barley was increased by elevated [CO₂]. However, this positive [CO₂] effect on fertilizer N recovery was not apparent when wheat was grown under open-air (FACE) conditions (Lam et al. 2012). The contrasting responses could be attributed to different types, and/or application methods of fertilizers. Nitrogen was applied as NH₄NO₃ solution in the study of Martín-Olmedo et al. (2002), and as granular urea under regular watering in the present study. Nitrogen availability in

these two studies was likely higher than that in the study of Lam et al. (2012) (topdressed as granular urea without regular watering). Alternatively, the contrasting responses could be due to different proportions of fertilizer N in the mineral N pool. The ratio of fertilizer N to total mineral N (excluding N mineralization during the growing season) was 0.99 in the study of Martín-Olmedo et al. (2002), 0.93 in the present study and 0.25 in the study of Lam et al. (2012). When soil N availability is limited, the [CO₂]-induced increase in plant biomass and N demand will likely result in a detectable increase in fertilizer N recovery in the plant.

When residue was incorporated under elevated [CO₂], the recovery of fertilizer ¹⁵N in the soil was greater than that in all the other treatments under current experimental conditions. As very little mineral N remained in the soil at harvest, the ¹⁵N recovered in the soil would have been mainly organic. This greater recovery of fertilizer N in the soil of these treatments further suggests that the greatest amount of N immobilization occurs under residue incorporation and elevated [CO₂]. As a result, fertilizer N recovery by the plant was reduced under these conditions. This result occurred regardless of whether the soils originated from cropping rotations where legumes were part of the rotation or were absent, although the soil under a cereal-legume rotation was more beneficial to wheat growth and N uptake than the soil under a cereal-fallow rotation. The increase in N recovery in soil under elevated

373 [CO₂] (21%) and residue incorporation in the present study associated with
374 immobilization of N is comparable to the [CO₂]-induced increase in N immobilization
375 (22%) reported by de Graaff et al. (2006).

376 The total fertilizer N recovery in the soil-plant system (63–68%) under ambient
377 [CO₂] in our study was comparable to the 62% reported in a meta-analysis of fertilizer
378 ¹⁵N recovery in grain cropping systems (Gardner and Drinkwater 2009). Under
379 elevated [CO₂] when residue was not incorporated, we found a higher fertilizer N
380 recovery in the plant-soil system (72–73%). However, residue incorporation offset
381 this positive [CO₂] effect on N recovery in the plant-soil system, i.e. there was a
382 greater loss to the environment. Nitrogen can be lost to the environment via leaching,
383 ammonia volatilization and denitrification. In our study, leaching of N was prevented
384 by controlled water supply to the soil in the polyethylene lined, sealed pots. Ammonia
385 volatilization was unlikely to have been differentially affected by the experimental
386 treatments, and should have been small because the frequent application of water
387 would have washed the urea below the soil surface (Angus 2001). Therefore,
388 denitrification was the most likely pathway responsible for the increased N loss in the
389 plant-soil system in our study (pots were watered to 80% of field capacity). This was
390 probably because residue incorporation increased C substrate availability and
391 stimulated denitrification (Baggs et al. 2003).

These findings have two major implications for fertilizer N management in future [CO₂] environments. First, if residue is not incorporated, more fertilizer N will be required to replenish the additional N removed in grains. Second, if residue is incorporated, a higher N application rate can ease the negative effect of N immobilization, and optimize the potential of [CO₂] fertilization effect on wheat yield. While it is difficult to extrapolate the results of pot experiments from controlled environments to the field, this study demonstrates the likely impact of residue incorporation and elevated [CO₂] on the cropping industry. A consequence of the continued increase in atmospheric [CO₂] is an increase in air temperature. Kim et al. (2011) found that the 'CO₂ fertilization effect' on rice grain yield and fertilizer N allocation to the grain was reduced by an atmospheric temperature increase of 2–3°C. Further studies are required to confirm the interaction among elevated temperatures, elevated [CO₂] and residue incorporation on the underlying mechanisms influencing crop N use and N recovery in association with soil and microbial processes, properties and N fractionation.

Conclusions

Under the conditions imposed in our study, in soils without residue amendment,

elevated [CO₂] increased wheat growth, grain yield and N uptake, resulting in higher fertilizer N recovery in the plant. A higher rate of fertilizer N application rate may be necessary to compensate for the extra grain N removal under elevated [CO₂]. Residue incorporation, however, retarded the [CO₂] fertilization effect on wheat growth, grain yield and N uptake due to N immobilization, and consequently reduced the recovery of fertilizer N in the plant in our study. In this regard, a higher rate of fertilizer N should be applied to boost crop growth and yield and realize the [CO₂] fertilization effect in this wheat production region, whether or not a legume rotation is incorporated into the cropping system.

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566

567 **Table 1** Some properties of the two Vertosols collected from the long term rotation experiment for
568 this study. Values are means ($\pm$ SE) of three replicates

Properties	cereal-legume rotation	cereal-fallow rotation	
pH (1:5 water)	8.5 $\pm$ 0.0	8.7 $\pm$ 0.0	***
Organic carbon (%)	1.06 $\pm$ 0.20	0.74 $\pm$ 0.10	NS
Total nitrogen (%)	0.073 $\pm$ 0.004	0.045 $\pm$ 0.004	**
Carbon: nitrogen ratio	14.6 $\pm$ 3.2	16.3 $\pm$ 3.46	NS
Ammonium (mg kg ⁻¹)	2.40 $\pm$ 0.07	2.91 $\pm$ 0.27	NS
Nitrate (mg kg ⁻¹)	1.63 $\pm$ 0.02	1.19 $\pm$ 0.01	***
Colwell phosphorus (mg kg ⁻¹)	33.7 $\pm$ 0.8	43.7 $\pm$ 0.4	***
Available potassium (mg kg ⁻¹)	643 $\pm$ 8	687 $\pm$ 10	*

569

Table 2 Composition of residue, for the ambient and elevated [CO₂] treatments, obtained from wheat (cv. Yitpi) grown under ambient and elevated CO₂ concentrations. Values are means (± SE) of three replicates

	%C	%N	C:N
Ambient [CO ₂]	42.0 ± 0.1	0.92 ± 0.01	45.7 ± 0.4
Elevated [CO ₂]	41.5 ± 0.2	0.77 ± 0.02	53.7 ± 1.8
	NS	*	*

Significant effects are indicated as * $p < 0.05$ according to *t*-test. NS, not significant

Table 3 The effects of residue incorporation and elevated [CO₂] on the biomass, tissue C:N ratio and grain N concentration of wheat. Values are means of the four replicates for each treatment

	Biomass (g pot ⁻¹)				C:N ratio		Grain N
	grain	shoot	root	total	shoot	root	(%)
<hr/>							
	Soil under cereal-legume rotation						
<hr/>							
No residue							
Ambient [CO ₂]	2.44±0.09	3.69±0.17	0.424±0.030	6.55±0.28	90.1±3.9	66.2±2.7	2.56±0.03
Elevated [CO ₂]	2.77±0.10	4.32±0.18	0.580±0.054	7.67±0.15	117.1±5.3	77.6±3.9	2.37±0.08
<hr/>							
With residue							
Ambient [CO ₂]	1.93±0.04	2.98±0.03	0.291±0.007	5.20±0.03	89.0±4.3	61.6±4.8	2.70±0.05
Elevated [CO ₂]	1.81±0.15	2.79±0.14	0.249±0.022	4.85±0.29	83.0±6.5	77.5±4.8	2.53±0.10
<hr/>							
	Soil under cereal-fallow rotation						
<hr/>							
No residue							
Ambient [CO ₂]	2.16±0.07	3.16±0.28	0.361±0.026	5.68±0.34	94.9±4.9	66.8±4.2	2.57±0.05
Elevated [CO ₂]	2.71±0.14	3.62±0.10	0.526±0.039	6.86±0.18	130.3±2.9	79.5±3.0	2.30±0.09
<hr/>							
With residue							
Ambient [CO ₂]	1.62±0.13	2.57±0.05	0.236±0.011	4.42±0.18	75.4±8.6	63.3±5.1	2.66±0.09
Elevated [CO ₂]	1.42±0.05	2.22±0.09	0.203±0.018	3.83±0.15	78.8±2.7	79.2±3.8	2.61±0.02
<hr/>							
[CO ₂]	NS	NS	** (0.043)	* (0.32)	*** (7.6)	*** (6.0)	** (0.10)
Residue	*** (0.15)	*** (0.22)	*** (0.043)	*** (0.32)	*** (7.6)	NS	** (0.10)
Rotation history	** (0.15)	*** (0.22)	* (0.043)	*** (0.32)	NS	NS	NS
[CO ₂] × Residue	*** (0.21)	*** (0.31)	*** (0.061)	*** (0.46)	*** (10.8)	NS	NS
[CO ₂] × Rotation history	NS	NS	NS	NS	NS	NS	NS
Residue × Rotation history	NS	NS	NS	NS	* (10.8)	NS	NS
[CO ₂] × Residue × Rotation history	NS	NS	NS	NS	NS	NS	NS

Significant effects are indicated as *** $p < 0.001$, ** $p < 0.01$ and * $p < 0.05$. NS, not significant.

Values in parentheses indicate LSD at $p = 0.05$

Table 4 The effects of residue incorporation and elevated [CO₂] on plant N uptake and soil N content. Values are means of the four replicates for each treatment

	N uptake (mg N pot ⁻¹)				Soil N (mg N pot ⁻¹)		
	grain	shoot	root	total	NH ₄ ⁺	NO ₃ ⁻	organic N
Soil under cereal-legume rotation							
No residue							
Ambient [CO ₂]	62.3±1.9	19.3±0.7	2.65±0.12	84.3±2.4	1.22±0.15	0.40±0.03	1058.8±16.0
Elevated [CO ₂]	65.4±1.4	17.3±1.0	3.16±0.43	85.8±1.6	1.48±0.19	0.40±0.11	1044.3±18.9
With residue							
Ambient [CO ₂]	52.0±1.1	15.4±0.6	1.94±0.16	69.3±1.1	1.59±0.11	0.58±0.05	1103.5±7.9
Elevated [CO ₂]	45.3±1.8	15.6±0.7	1.33±0.19	62.2±1.3	1.64±0.19	0.86±0.13	1136.9±12.3
Soil under cereal-fallow rotation							
No residue							
Ambient [CO ₂]	55.5±1.3	15.5±1.3	2.19±0.07	73.2±1.6	0.87±0.27	0.35±0.08	748.6±5.4
Elevated [CO ₂]	61.9±1.3	12.8±0.3	2.68±0.28	77.4±1.2	0.97±0.08	0.22±0.02	742.1±5.2
With residue							
Ambient [CO ₂]	42.7±2.0	16.5±2.0	1.57±0.08	60.8±2.1	1.52±0.11	0.62±0.12	795.4±6.4
Elevated [CO ₂]	36.9±1.2	13.0±0.8	1.06±0.10	51.0±1.9	1.49±0.25	0.79±0.22	778.0±5.0
[CO ₂]	NS	* (1.6)	NS	* (2.5)	NS	NS	NS
Residue	*** (2.2)	NS	*** (0.31)	*** (2.5)	** (0.26)	*** (0.17)	*** (15.9)
Rotation history	*** (2.2)	** (1.6)	* (0.31)	*** (2.5)	* (0.26)	NS	*** (15.9)
[CO ₂] × Residue	*** (3.1)	NS	** (0.44)	*** (3.5)	NS	NS	NS
[CO ₂] × Rotation history	NS	NS	NS	NS	NS	NS	NS
Residue × Rotation history	NS	* (2.2)	NS	NS	NS	NS	NS
[CO ₂] × Residue × Rotation history	NS	NS	NS	NS	NS	NS	NS

Significant effects are indicated as *** $p < 0.001$, ** $p < 0.01$ and * $p < 0.05$. NS, not significant.

Values in parentheses indicate LSD at $p = 0.05$

Table 5 The effects of residue incorporation and elevated [CO₂] on fertilizer ¹⁵N recovery in plant and soil, and total loss. Values are means of the four replicates for each treatment

	Recovery in plant (%)				Recovery in	Total recovery	Total loss
	grain	Shoot	Root	Total	soil (%)	(%)	(%)
	Soil under cereal-legume rotation						
No residue							
Ambient [CO ₂]	41.3±1.4	12.1±0.4	1.34±0.05	54.7±1.7	12.8±1.7	67.5±2.6	32.5±2.6
Elevated [CO ₂]	45.7±1.5	10.2±0.6	1.48±0.19	57.4±1.2	15.5±1.2	72.9±1.7	27.1±1.7
With residue							
Ambient [CO ₂]	39.3±0.9	11.4±0.4	1.16±0.08	51.8±0.8	13.1±0.6	65.0±1.1	35.0±1.1
Elevated [CO ₂]	37.5±1.7	11.9±0.5	0.76±0.10	50.2±1.3	16.0±2.3	66.2±2.8	33.8±2.8
	Soil under cereal-fallow rotation						
No residue							
Ambient [CO ₂]	41.8±1.0	10.4±1.0	1.23±0.04	53.4±0.8	13.0±0.3	66.4±0.9	33.6±0.9
Elevated [CO ₂]	48.6±1.1	8.7±0.2	1.44±0.17	58.8±1.0	13.2±0.5	72.0±0.6	28.0±0.6
With residue							
Ambient [CO ₂]	35.1±1.2	13.2±1.8	1.05±0.08	49.3±1.9	13.4±0.6	62.8±1.6	37.2±1.6
Elevated [CO ₂]	32.0±0.9	10.5±0.6	0.66±0.07	43.2±1.5	18.2±0.4	61.4±1.1	38.6±1.1
[CO ₂]	NS	* (1.2)	NS	NS	** (1.7)	* (2.5)	* (2.5)
Residue	*** (1.8)	* (1.2)	*** (0.16)	*** (1.9)	NS	*** (2.5)	*** (2.5)
Rotation history	NS	NS	NS	* (1.9)	NS	NS	NS
[CO ₂] × Residue	*** (2.6)	NS	*** (0.23)	*** (2.7)	NS	* (3.5)	* (3.5)
[CO ₂] × Rotation history	NS	NS	NS	NS	NS	NS	NS
Residue × Rotation history	*** (2.6)	NS	NS	* (2.7)	NS	NS	NS
[CO ₂] × Residue × Rotation history	NS	NS	NS	NS	NS	NS	NS

Significant effects are indicated as *** $p < 0.001$, ** $p < 0.01$ and * $p < 0.05$. NS, not significant.

Values in parentheses indicate LSD at $p = 0.05$

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