Author/s:
Butterly, CR;Wang, X;Armstrong, RD;Chen, D;Tang, C

Title:
Elevated CO2 induced rhizosphere effects on the decomposition and N recovery from crop residues

Date:
2016-11-01

Citation:
Butterly, C. R., Wang, X., Armstrong, R. D., Chen, D. & Tang, C. (2016). Elevated CO2 induced rhizosphere effects on the decomposition and N recovery from crop residues. Plant and Soil, 408 (1-2), pp.55-71. https://doi.org/10.1007/s11104-016-2911-4.

Persistent Link:
https://hdl.handle.net/11343/123474

| | |
|---|---|
| Journal: | Plant and Soil |
| Pages: | 20 (including cover pages) |
| Figures: | 4 |
| Tables: | 4 |
| Supplementary table | 1 |

Title:

# Elevated $CO_2$ induced rhizosphere effects on the decomposition and N recovery from crop residues.

Running title:

**Elevated $CO_2$ and rhizosphere effects on residue decomposition**

Authors:
Clayton R Butterly[a,*], Xiaojuan Wang[a], Roger D Armstrong[a,b], Deli Chen[e], and Caixian Tang[a,*]

Institute/s:
[a]Department of Animal, Plant & Soil Sciences, Centre for AgriBioscience, La Trobe University, Melbourne, Vic 3086, Australia
[b]Department of Economic Development, Jobs, Transport & Resources, Horsham, Vic 3401, Australia
[e]Faculty of Veterinary and Agricultural Science, The University of Melbourne, Melbourne, Vic 3010, Australia

Corresponding authors:

Clayton Butterly
Tel +61 3 9032 7418
C.Butterly@latrobe.edu.au

Caixian Tang
C.Tang@latrobe.edu.au
Tel +61 3 9032 7416

**Keywords**
Atmospheric $CO_2$; Carbon and nitrogen cycling; Dual-labelling; Rhizosphere priming; Stable isotopes; Field pea; Wheat

## Abstract

*Background & Aims* Elevated atmospheric $CO_2$ ($eCO_2$) can affect soil-plant systems via stimulating plant growth, rhizosphere activity and the decomposition of added (crop residues) or existing (priming) soil organic carbon (C). Increases in C inputs via root exudation, rhizodeposition and root turnover are likely to alter the decomposition of crop residues but will ultimately depend on the N content of the residues and the soil.

*Methods* Two soil column experiments were conducted under ambient $CO_2$ ($aCO_2$, 390 ppm) and $eCO_2$ (700 ppm) in a glasshouse using dual-labelled ($^{13}C/^{15}N$) residues of wheat (*Triticum aestivum* cv. Yitpi) and field pea (*Pisum sativum* L. cv. PBA Twilight). The effects of $eCO_2$ and soil N status on wheat rhizosphere activity and residue decomposition and also N recovery from crop residues with different N status (C/N ratio 19.4-115.4) by different plant treatments (wheat, wheat + 25 mg N $kg^{-1}$ and field pea).

*Results* Total belowground $CO_2$ efflux was enhanced under $eCO_2$ despite no increases in root biomass. Plants decreased residue decomposition, indicating a negative rhizosphere effect. For wheat, $eCO_2$ reduced the negative rhizosphere effect, resulting in greater rates of decomposition and recovery of N from field pea residues, but only when N fertiliser was added. For field pea, $eCO_2$ enhanced the negative rhizosphere effect resulting in lower decomposition rates and N recovery from field pea residue.

*Conclusions* The effect of $eCO_2$ on N utilisation varied with the type of residue, enhancing N utilisation of wheat but repressing that of field pea residues, which in turn could alter the amount of N supplied to subsequent crops. Furthermore, reduced decomposition of residues under $eCO_2$ may slow the formation of new soil C and have implications for long-term soil fertility.

## Introduction

The impact of rising atmospheric $CO_2$ concentrations on soil fertility is of critical importance for the future productivity and sustainability of agricultural systems. High concentrations of $CO_2$ in the atmosphere, which have increased by over 42% since the industrial revolution (IPCC 2001), are known to stimulate photosynthesis and enhance net primary productivity of a wide range of plant species (Ainsworth and Long 2005; de Graaff et al. 2006). Comparable increases in the growth of annual crops such as field pea and wheat have been observed in dryland agricultural systems (Jin et al. 2012; Lam et al. 2012a; Butterly et al. 2015). Elevated $CO_2$ concentrations can increase rhizodeposition of root exudates, cellular material from living roots and fine-root turnover, leading to greater concentrations of labile C in the soil (Carrillo et al. 2011). For non-legumes, $eCO_2$ often reduces the N concentrations in plant tissue (Cotrufo et al. 1998; Jensen and Christensen 2004), even when N fertiliser is applied (Butterly et al. 2015). Changes in the amounts and quality (C/N ratio) of organic matter inputs under $eCO_2$ could alter soil C and N cycling.

In addition to higher C/N ratio, crop residues derived from plants grown under $eCO_2$ often contain greater proportion of structural compounds (Pritchard et al. 1999). These changes in litter chemistry under $eCO_2$ are expected to slow their decomposition. However, experimental evidence utilising agricultural plants is inconclusive with most reporting reduced decomposition and N release from residues produced under $eCO_2$ or no effect of $CO_2$ (Torbert et al. 2000; van Vuuren et al. 2000; Norby et al. 2001; Marhan et al. 2008; de Graaff et al. 2011). Furthermore, neither $eCO_2$ nor N fertilisation altered the decomposition of white clover and ryegrass pasture materials (de Graaff et al. 2004). It is likely that these contrasting results in the literature are due to differences in ecosystem N status. Reductions in N concentration of crop residues could have little effect on decomposition if the C/N ratio remains relatively low (<35). Additionally, effects of C/N ratio may be negated in fertile soils if N mineralization can supply microbes with sufficient N for residue decomposition. For cropping systems dominated by cereals, residues remaining after harvest have C/N ratios (>60) much greater than used in many previous studies. The importance of changes in litter chemistry under $eCO_2$ and subsequent impacts on C and N cycling in agricultural systems is unclear. Although slowly, higher C/N ratios of plants under $eCO_2$ can alter that of the soil organic matter (SOM) (Yang et al. 2011). However, Carrillo et al. (2014) suggest that many incubation studies may not accurately predict $eCO_2$ effects because of the absence of active plant roots.

Plant roots and their associated soil microbes in the rhizosphere are known to be fundamental for SOM cycling. Decomposition of SOM may be enhanced (by up to 380%) or inhibited (by 50%) in rhizospheres compared with non-planted soils (Cheng et al. 2014). The relative difference between species appears to be related to the volume of roots and rhizosphere (Paterson et al. 2008) and the quantity and quality of rhizodeposits (Zhu and Cheng 2012; Zhu et al. 2014). The direction and magnitude of priming effects are primarily driven by the availability of soil nutrients, especially N and P (Dijkstra et al. 2013). Positive priming effects occur via microbial mining of SOM for N when supplied with high amounts of labile C substrate (Fontaine et al. 2004; Craine et al. 2007). In contrast, negative priming effects are thought to result from increased competition for N and P in infertile soils or the preferential utilisation of labile rhizodeposits in fertile soils, which ultimately reduce the decomposition of existing SOM (Cheng 1999; Dijkstra et al. 2013).

The effects of $eCO_2$ on rhizosphere priming has gained recent attention. Rhizosphere priming effects, which are mediated by soil microbes, are expected to be intensified under $eCO_2$ due to greater C flow and increased competition for N between plants and microbes (Billings et al. 2010). Besides roots *per se*, C inputs are dominated by root exudates (Shahzad et al. 2015) and their quantity is commonly proportional to root mass (Jones et al. 2009). Trees are known to enhance root exudation under $eCO_2$ (Phillips et al. 2011), but evidence of specific changes in exudation (per unit of root) in agricultural crop species is lacking (Billes et al. 1993; Martens et al. 2009). Greater rhizodeposits under $eCO_2$ increase microbial biomass, predominantly bacteria, and the subsequent immobilisation of nutrients (Jin et al. 2014). Increased competition for N and changes in N availability under $eCO_2$ are likely to alter plant C allocation, stoichiometric constraints to microbial growth and rhizosphere chemistry (Cheng et al. 2014). Furthermore, $eCO_2$-induced changes in root growth and greater water-use efficiency from reduced evapotranspiration could alter the volume, distribution and activity of plant rhizospheres (Allard et al. 2006; Paterson et al. 2008).

Nevertheless, few studies have directly investigated $eCO_2$-induced rhizosphere effects on residue decomposition (Paterson et al. 2008), a critical pathway for the formation of new soil C and N. Little net change in SOM content under $eCO_2$ in agricultural cropping systems (Martens et al. 2009) and pastures (van Groenigen et al. 2003) indicates that additional C inputs, including residues, are offset by increased decomposition (Hopkins et al. 2014; van Groenigen et al. 2014). Similarly, reduced SOM content under $eCO_2$ could be due to enhanced SOM decomposition via rhizosphere priming (Finzi et al. 2015). Greater microbial activity in wheat rhizosphere under free-air $CO_2$ enrichment (FACE) did not enhance residue decomposition, possibly due to the high background soil N content (Lam et al. 2014). Consistent with other studies, N status of the soil-plant system appears critical.

The present study investigated the rhizosphere effect of two key agricultural crop species on the decomposition of crop residues. In particular, the study aimed to quantify the influence of three important soil N components on residue decomposition under $eCO_2$, namely residue C/N ratio, soil N status and legume versus non-legume rhizospheres. We hypothesised that $eCO_2$-induced rhizosphere effects would (a) enhance residue decomposition due to greater availability of labile substrate and subsequent increases in microbial activity and capacity, (b) be greater for field pea (legume) than wheat (non-legume) due to additional N deposition, and (c) be quantitatively smaller for systems with a lower N status (i.e. for residues with high C/N ratio and when fertiliser N was not applied).

## Materials and Methods

### $CO_2$ glasshouse facility

Two experiments were conducted in the $CO_2$-regulated glasshouse facility at Horsham (36°43′S 142°10′E). The facility consisted of four adjoining glasshouse rooms at either ambient $CO_2$ ($aCO_2$, 390 ppm) or $eCO_2$ (700 ppm) (two rooms for each $CO_2$ level). Ambient $CO_2$ was maintained by continual introduction of outside air via a non-recirculating air-conditioner in each room. Elevated $CO_2$ was achieved by injecting pure $CO_2$ into the air-conditioner airstreams from cylinders fitted with solenoid values and controlled using infra-red gas analysers (Guardian SP97301, Edinburgh Instruments).

### Soil and crop residues

Surface soil (0-10 cm) of a Calcisol (WRB 2014) or Calcarosol (Isbell 1996) was collected from Warracknabeal, VIC, Australia (36°14′S 142°31′E) on 6th July 2012. The field was under an annual cropping rotation with lentil in 2010, wheat in 2011 and barley in 2012. The soil was air-dried, passed through a 4-mm sieve and thoroughly mixed. Initial physiochemical properties of the soil were: total C 18 mg $g^{-1}$, total N 1.7 mg $g^{-1}$, pH 6.6 (1:5 in 0.01 M $CaCl_2$), clay 41%.

Dual $^{13}C/^{15}N$ labelled residues of wheat (*Triticum aestivum* cv. Yitpi) and field pea (*Pisum sativum* L. cv. PBA Twilight) were generated under free-air $CO_2$ enrichment (FACE) conditions in 2011 as outlined in Butterly et al. (2015). Briefly, wheat and field pea were grown under $aCO_2$ (390 ppm) or $eCO_2$ (550 ppm) with either 40 (low) or 100 (high) mg N $kg^{-1}$ [$Ca(^{15}NO_3)_2$, 20% atom excess] and pulse-labelled with $^{13}CO_2$ 7 times throughout the growing season. Aboveground biomass was collected at physiological maturity, grain removed, and the remaining residues were ground (<2 mm). The initial $^{13}C$ and $^{15}N$ abundances of residue are presented in Table S1.

Soil column experiments

Experiment 1 (Exp 1) aimed to quantify the effects of $eCO_2$ and soil N status on wheat rhizosphere activity and residue decomposition. PVC columns (7.5 cm ID × 20 cm long) cut lengthways and re-joined with tape and silicon sealant were filled with 600 g dry soil (Bulk density 1.0 g $cm^{-3}$). The soil was supplied with basal nutrients (mg $kg^{-1}$: $KH_2PO_4$, 180; $K_2SO_4$, 120; $CaCl_2.2H_2O$, 180; $MgSO_4.7H_2O$, 50; $MnSO_4.H_2O$, 6; $ZnSO_4.7H_2O$, 8; $CuSO_4.5H_2O$, 6; $CoCl_2.6H_2O$, 0.4; FeEDTA, 1.3; $Na_2MoO_4.2H_2O$, 0.4) and amended with either wheat or field pea residues (0.5% w/w, equivalent to 7.6 t $ha^{-1}$ on surface area basis). The C/N ratio of the wheat and field pea were 59.7 and 20.7, respectively. Two sets of columns, one containing 25 mg N $kg^{-1}$ [$Ca(NO_3^-)_2$] in the topsoil (0-7.5 cm) and one containing no added N, were included. To facilitate the construction of columns, soils were dried at 25°C following nutrient addition, mixed with residues and assembled in an air-dried state. The top of each column was sealed with a plastic lid fitted with a central tube (3 cm ID × 6 cm long, inserted into the soil ~1 cm) to allow plants to be grown while maintaining an airtight soil headspace. Columns were wet to 80% of field capacity ($\theta g = 0.388$ g $g^{-1}$), allowed to equilibrate for 5 h, and three pre-germinated wheat seeds were sown at 1-cm depth in each on the 26th July 2012. After 1 week, plants were thinned to 2 seedlings per column. Planted columns were arranged with 4 replicates (2 in each $CO_2$-regulated room). Overall this experiment consisted of a nested factorial design with 2 $CO_2$ concentrations (main-plots) × 2 residues (sub-plots) × 2 N levels (sub-plots) with 4 replicates (32 columns). Non-planted controls were also included.

Experiment 2 (Exp 2) aimed to examine the relative importance of plant treatments (wheat, wheat + 25 mg N $kg^{-1}$ and field pea) and residue N status (C/N ratio 19.4-115.4) on N recovery from crop residues. PVC columns were constructed as previously outlined, except that 25-cm long columns containing 790 g dry soil were used. In this experiment, four residues (0.5% w/w, equivalent to 10 t $ha^{-1}$ surface area basis) and three plant treatments were investigated. The residues were wheat previously grown under low N (C/N = 115) and high N (C/N = 31) and field pea previously grown under low N (C/N = 21) and high N (C/N = 19) (Section 2.2). The three plant treatments were wheat, wheat + N and field pea. The wheat + N treatment received 25 mg N kg$^{-1}$ [$Ca(NO_3^-)_2$] in the topsoil (0-10 cm). Columns were planted on the 1st August 2012 into moist (80% field capacity) soil as previously described. Field pea was inoculated using commercial Group E peat inoculum (*Rhizobium leguminosarum*). Wheat and field pea were thinned to 2 plants after 7 and 10 days, respectively. For each $CO_2$ treatment, columns were randomly arranged and rotated at least once per week between the two $CO_2$-regulated rooms. Overall the experiment consisted of a randomised block design with 2 $CO_2$ concentrations × 4 residues × 3 plant treatments with 3 replicates. Plant-free controls were also included.

Plants were grown under natural light conditions with glasshouse air-conditioners set to 25°C with no diurnal change. Mean minimum and maximum temperatures over the experimental period were 19.4°C and 26.2°C, respectively. Columns were watered to 80% field capacity. All wheat-plant treatments received a single addition of 494 μg N kg$^{-1}$ week$^{-1}$ in the last 3 weeks for Exp 1 (total 0.89 mg N column$^{-1}$) and in the last 4 weeks for Exp 2 (total 1.56 mg N column$^{-1}$) added as dilute $Ca(NO_3)_2$. solution prior to normal watering.

Total belowground $CO_2$ efflux and residue decomposition

An alkali trapping approach was used to quantify total belowground $CO_2$ efflux in all treatments in Exp 1 and the residue treatments with the most contrasting C/N (low-N-wheat and high-N-field pea) in Exp 2, plus relevant controls. Two vials containing 14 ml NaOH solution (28 ml total; 1 M for Exp 1 and 1.5 M for Exp 2) were placed within the sealed headspace of each column. Solutions were exchanged periodically via two holes in the lids that were sealed using Blu-tack®. Two vials were used to ensure sufficient surface area of the traps (24.8% of soil surface). The remaining columns of Exp 2 without alkali traps (and lids) were covered with a 2-cm layer of white polyethylene beads to minimise evaporation.

Cumulative $CO_2$ release (μg $CO_2$-C g$^{-1}$ soil) was estimated according to Zibilske (1994) with the following modifications. Briefly, 5 ml of each trap and 1.72 M $BaCl_2$ (1:1) were titrated with 0.25 N HCl (Exp 1) or 0.5 N HCl (Exp 2) and phenolphthalein indicator (1% w/v in ethanol) using a digital burette (Brand Titrette, Germany). Precipitates of each trap were formed for $\delta^{13}C$ analysis using Isotope Ratio Mass Spectrometry (IRMS). Specifically, 2 ml of each trap was neutralised with 0.5 M HCl and combined with 2 ml 1 M $SrCl_2$ and then dried in an oven at 60°C for 3 days. The proportion of $CO_2$ derived from residue ($CO_{2\ RES}$) was estimated using an isotopic approach according to the following equation;

$$CO_{2\ RES} = (\delta^{13}C\ residue\text{-}amended\ soil - \delta^{13}C\ soil) / (\delta^{13}C\ residue - \delta^{13}C\ soil) \qquad (1)$$

where $\delta^{13}C$ residue-amended soil and $\delta^{13}C$ soil are the $\delta^{13}C$ of the precipitates formed from residue- and non-amended soil columns, respectively, and $\delta^{13}C$ residue is the $\delta^{13}C$ value of the added residue. The amount of $CO_2$ derived from residue was calculated by multiplying $CO_{2\ RES}$ by the cumulative $CO_2$ released at each sampling time.

## Plant and soil sampling

Columns from Exp 1 and 2 were destructively sampled after 8 weeks (26th September) and 9 weeks (3rd October), respectively. Shoots were cut off at the soil surface, soil columns split and roots were carefully extracted. Shoot and root material was washed with reverse osmosis (RO) water and dried at 70°C for 3 days. The soil was thoroughly mixed, stored at 5°C overnight, and C and N in the microbial biomass and soil was determined the following day using moist soil. The remaining soil was air-dried at 25°C for subsequent analyses. Dried plant samples were ground (<2 mm) using a centrifugal mill to reduce sample volume, and sub-samples of both ground plant material and whole soil were then finely ground using a Retsch MM400 mixer mill.

## Soil and plant analyses

Soil $pH_{CaCl_2}$ was determined using a pH meter (Thermo Orion 720A+, Beverly, MA, USA) following extraction of 5 g air-dried soil with 0.01 M $CaCl_2$ (1:5) by shaking end-over-end for 1 h and centrifugation at 492 *g* for 10 min.  Soil texture was characterised by determining the particle-size distribution using a Laser Particle Size Analyser (Malvern Mastersizer 2000, Worcestershire, UK) following dispersion of soil (~10 g) with 10 ml of 0.164 M $Na_6P_5O_{18}$ in 800 ml of RO water. Total C (TC) and N (TN) as well as the $^{13}C$ ($\delta\ ^{13}C$ Pee Dee Belemnite, PDB) and $^{15}N$ ($\%^{15}N$) content of soil and plant samples was determined using IRMS (Hydra 20-22, SerCon, Crewe, UK). The proportion of N derived from residue ($pN_{DFR}$) was estimated directly according to the following equation;

$$pN_{DFR} = (\%^{15}N\ plant + residue - \%^{15}N\ soil) / (\%^{15}N\ residue - \%^{15}N\ soil) \quad (2)$$

where $\%^{15}N$ plant + residue is the atom% $^{15}N$ of plants growing in residue-amended soil, $\%^{15}N$ soil is the natural $^{15}N$ abundance of the soil (0.368811 atom% $^{15}N$) and $\%^{15}N$ residue is the atom% $^{15}N$ value of the added residue. The amount of N derived from residue ($N_{DFR}$) was calculated by multiplying $pN_{DFR}$ by the total N uptake.

Microbial biomass C (MBC) and N (MBN) were quantified using 24-h fumigation-extraction according to Vance et al. (1987) but with the following modifications. Soil (20 g DW) was extracted with 80 ml of 0.5 M $K_2SO_4$ by shaking end-over-end for 1 h. Extracts were passed through a Whatman

#42 filter and stored at -20°C until analysis. Organic C concentrations in fumigated and non-fumigated extracts were determined using wet-oxidation (Vance et al. 1987) as outlined in Heanes (1984). Briefly, 5 ml of extract, 5 ml of 1 N $K_2Cr_2O_7$ and 10 ml of 98% $H_2SO_4$ were mixed and heated at 130°C for 30 min, allowed to cool, made up to 50 ml with RO water and the C concentration was determined spectrophotometrically at 600 nm. Each sample was analysed in duplicate. Sucrose solutions with known concentrations were included as standards. The C contained within digested non-fumigated samples was denoted extractable organic C (EOC). Microbial biomass C (MBC) was estimated as the difference between fumigated and non-fumigated samples using a $k_{\mathrm{EC}}$ of 0.37 (Sparling and Zhu 1993; Joergensen 1996).

Total N contained within fumigated and non-fumigated extracts was determined using the wet-oxidation method of Cabrera and Beare (1993). Specifically, 2.5 ml of extract and digestion mix (50 g $K_2S_2O_8$ and 30 g $H_3BO_4$ in 100 ml of 3.75 M NaOH adjusted to 1 l with $H_2O$) (1:1) were autoclaved (121°C, 104 kPa) for 30 min and stored at 4°C until analysis. Solutions with known concentrations of urea were included as controls. The N ($NH_4^+$ + $NO_3^-$) concentration of extracts was determined using a flow injection analyser (Lachat QuickChem 8500 Series II, USA). The N contained within digested non-fumigated samples was denoted extractable organic N (EON). Microbial biomass N (MBN) was estimated as the difference between fumigated and non-fumigated samples using a $k_{\mathrm{EN}}$ of 0.54 (Brookes et al. 1985).

### Statistical analyses

For Exp 1, a three-way analysis of variance (ANOVA) was used to test the effects of $CO_2$, residue and N level on soil and plant properties of planted columns. A one-way residual maximum likelihood (REML) analysis was used to test the effects of $CO_2$, residue and N level on soil chemical properties between planted and non-planted columns. For Exp 2, a three-way ANOVA was used to test the effects of $CO_2$, plant treatment and residue on soil and plant properties of planted columns. A one-way REML analysis was used to test the effects of $CO_2$, plant treatment and residue on soil properties between planted and non-planted columns. Differences between means were tested using least significance difference (LSD) test at $P = 0.05$.

## Results

### Plant biomass, N content and $^{15}N$ abundance

In Exp 1, the biomass of wheat plants was significantly affected by residue type and N level but not $CO_2$ concentration (Table 1). Specifically, wheat biomass was ~3 times greater when grown in soil containing field pea than wheat residue. Wheat shoot and root biomass were 78 and 73% greater at the higher N level, respectively. N level significantly affected the N concentration within wheat shoot (*P*=0.012) and root (*P*=0.045). However, added N reduced the N concentration (by 17%) of wheat grown under a$CO_2$ in soil containing wheat residue (Table 1).

In Exp 2, plant shoot biomass increased in the order of wheat, wheat +N then field pea (Table 2). Furthermore, shoot biomass greatly differed between low-N-wheat and high-N-wheat residues (56 times) and less between high-N-wheat and both low-N-field pea and high-N-field pea residues (14 times) (Table 2). Elevated $CO_2$ altered the N concentration of shoots (*P*<0.001) but not roots (*P*=0.107). For shoots, N concentration was 5.1-18.5% lower under e$CO_2$, except for field pea with high-N-wheat residue, where N concentration increased (by 5.2 %). However, the relative magnitude of the $CO_2$ treatment effect on N concentration was much lower than that of residue and plant treatments. The effects of plant and residue treatments on N concentrations in roots were the same as shoots, but the N concentration was lower in roots than shoots for the wheat and wheat +N treatments.

Total N uptake, N derived from residue and % residue N recovered

In Exp 1, e$CO_2$ tended to increase total N uptake from field pea residue (*P*=0.062) (Figure 1a). Total N uptake increased with residue N concentration (field pea > wheat) and with N addition. Notably, total N uptake increased by 6 mg N column$^{-1}$ when N was added with wheat residue, and by 13.5 mg N column$^{-1}$ when N was added with field pea residue. The addition of N fertiliser decreased the amount of N derived from wheat residue for the a$CO_2$ treatment but not the e$CO_2$ treatment (Figure 1b). In contrast, adding fertiliser N reduced the amount of N derived from field pea residue by ~6% for both $CO_2$ treatments. The %N recovered in the plant from residues, which accounts for the differences in N content between residues and the control, was significantly (*P*=0.028) greater under e$CO_2$ (Figure 1c). The recovery of N from field pea residue was greater than from wheat residue, and increased further when fertiliser N was added, although only main effects of residue (*P*<0.001) and N level (*P*<0.001) were significant.

In Exp 2, e$CO_2$ increased total N uptake (*P*=0.002) with the magnitude of the increase being greatest for the field pea, and least for the wheat plant treatment (Fig 2a). A reduction in total N uptake by wheat was observed for the high-N-field pea residue treatment. Adding fertiliser N (9.9 mg N column$^{-1}$) increased total N uptake by 5.9 - 15.7 mg N column$^{-1}$ under e$CO_2$, and by 4.8 - 8.3 mg N column$^{-1}$ under a$CO_2$. For field pea, %$N_{DFR}$ decreased when fertiliser N was added and also with increased N content in the residues (Fig 2b). Interestingly, e$CO_2$ had a positive effect on %$N_{DFR}$ in the wheat and wheat +N plant treatments but a negative effect in the field pea plant treatment. Also, %$N_{DFR}$ was greater for the high-N than low-N field pea residue, despite these having similar C/N ratios. Adding fertiliser N increased the amount of N recovered from residues by wheat and facilitated the positive effect of $CO_2$ concentration (Figure 2c). Without fertiliser N, there was generally no e$CO_2$

response, except that $eCO_2$ decreased N recovery from high-N-field pea residue was reduced under $eCO_2$. For field pea, $eCO_2$ reduced the N recovered from high-N-wheat and high-N-field pea residues.

## Total belowground $CO_2$ efflux and $CO_2$-C partitioning

In Exp 1, total belowground $CO_2$ efflux did not differ between non-planted columns, except in the wheat +N treatment where total $CO_2$ efflux was greater than the other treatments (Fig 3a). Furthermore, total $CO_2$ efflux was greater for field pea than wheat residue, and N fertiliser increased total $CO_2$ compared to when wheat residue only was applied. For columns planted with wheat, total $CO_2$ efflux was 22% and 52% greater than non-planted columns for soils containing wheat and field pea residues, respectively. Importantly, $eCO_2$ significantly ($P$=0.004) increased total $CO_2$ efflux by 8.6% for wheat grown in soil containing field pea residue. $CO_2$ derived from wheat residue was greater under $eCO_2$ when no fertiliser N was added but lower than $aCO_2$ and plant-free columns in the +N treatment (Fig 3b). Between 31-35% of wheat residue C and 31% of the field pea residue C was recovered in the alkali traps over the 8-week experiment.

In Exp 2, there was a trend for total belowground $CO_2$ efflux from non-planted columns to be greater for low-N-wheat than high-N-field pea residues ($P$=0.066) (Fig 4a). Elevated $CO_2$ increased ($P$=0.001) total $CO_2$ efflux by up to 12%, with the largest effects for wheat growing in soil containing high-N-field pea residue and for field pea growing with low-N-wheat residue. There was no effect of plant treatment on total $CO_2$ produced ($P$>0.05). The amount of $CO_2$ derived from low-N-wheat residue was the same for wheat and wheat +N plant treatments, but this was lower than the non-planted control for the field pea plant treatment (Fig 4b). Similarly, all plant treatments reduced the $CO_2$ derived from high-N-field pea residue compared with the non-planted controls. Over the 9-week experiment, approximately 42% of the residue-C were recovered in the alkali traps of non-planted controls and the recovery of residues was lower with higher residue and plant N status (Fig 4c).

## Soil C and N pools

For Exp 1, total C was reduced compared with the non-amended soil (18 g C $kg^{-1}$) with the greatest reductions occurring for field pea residue with no N (-27%) and wheat residue with +N (-20%) (Table 3). Total N was also reduced by up to 33% compared with the original soil. The abundance of $^{13}C$ in soil ($\delta$ $^{13}C$ PDB) was significantly reduced under $eCO_2$ representing loss of $^{13}C$ from residues. However, the $^{13}C$ abundance of the non-planted controls was the same or lower than under $eCO_2$ treatments, indicating similar loss of residue $^{13}C$ without plants compared to the $aCO_2$ treatment. MBC was lower under $eCO_2$ and this reduction was greater for the +N treatment. MBN was reduced under $aCO_2$ for the field pea +N treatment, increasing the MBC-to-MBN ratio. The MBC-to-MBN ratio was also lower under $eCO_2$ for wheat +N treatment. Overall, EOC was lower for wheat than field pea amended treatments. EON was reduced compared with non-planted controls but was not affected by the treatments.

In Exp 2, no effect of $CO_2$ on total C, total N or C/N ratio was observed. In contrast to Exp 1, total C and total N did not differ from the non-amended soil, except that total C was 34% higher for the wheat +N plants with the low-N-wheat residues (Table 4). Total N ranged from 17.7-22.9 g N $kg^{-1}$ soil and C/N ratio from 10.5-12 (data not shown). The abundance of $^{13}C$ in soil was lower under $eCO_2$ for wheat plants with low-N-wheat residues and lower under $aCO_2$ for the field pea plants with low-N-wheat residues. Elevated $CO_2$ greatly decreased MBC (up to 178 mg $kg^{-1}$ soil) for low-N-field pea and high-N-wheat residues; however $CO_2$ had a much smaller effect for high-N-field pea and low-N-wheat residues. In contrast, MBN was lower in all planted treatments compared with non-planted controls and consequently MBC-to-MBN ratio was increased. The field pea plant treatment and N rich residues (high-N-field pea, low-N-field pea and high-N-wheat) had much greater reductions in MBN than the low-N-wheat residue treatment. Furthermore, EON was reduced compared with non-planted controls, with the magnitude of the reduction in the order of low-N-wheat, high-N-wheat, low-N-field pea and high-N-field pea residue. For the field pea plant treatment, EON was ~30% greater with low-N-field pea and ~200% for high-N-field pea residues but did not differ between other treatments. Generally, EOC was similar across all treatments including non-planted controls, except for high-N-field pea residue where $aCO_2$ reduced EOC in the wheat-planted treatment and $eCO_2$ increased EOC in the field pea-planted treatment. Wheat plants with low-N-wheat residues had lower EOC than the non-planted controls for both $CO_2$ levels.

## Discussion

### Residue decomposition

Enhanced residue decomposition in the presence of plants (a positive rhizosphere effect) was anticipated. In particular, higher amounts of labile C substrates in the form of rhizodeposits were expected to stimulate microbial activity and induce greater mineralisation of residues for N, consistent with theories of rhizosphere priming effects on soil organic matter decomposition (Fontaine et al. 2004; Craine et al. 2007). However, we observed little or no change in residue decomposition in the presence of plant compared with residues alone. Therefore, the 16-52% increase in $CO_2$ efflux between planted and non-planted treatments likely occurred via plant-derived substrate, although distinction between plant and soil C sources cannot be made in this study. Around 32-34% and 42% of residues were decomposed in Exp 1 and 2, respectively. The greater decomposition in the later experiment probably reflected the larger column size and longer duration of the study. Reduced residue decomposition that was observed between planted and non-planted treatments could have been due to a negative rhizosphere effect via increased competition for N and P (Cheng 1999; Dijkstra et al.

2013). However, since a negative rhizosphere effect also occurred under field pea, preferential mineralization of labile rhizodeposits by microbes could have reduced the decomposition of other more recalcitrant sources such as crop residues (Cheng 1999; Dijkstra et al. 2013).

Elevated $CO_2$ had both positive and negative effects on residue decomposition, compared with $aCO_2$. However, changes in residue decomposition under $eCO_2$ were not proportional to total belowground $CO_2$ efflux. Enhanced turnover of rhizodeposits under $eCO_2$ may not increase rhizosphere effects since these labile compounds are primarily degraded by intracellular enzymes and more recalcitrant components like crop residues require an array of extracellular enzymes (Kuzyakov 2010). Instead, the three main observations appeared to be related with N availability. Firstly, $eCO_2$ decreased wheat decomposition under wheat with added N in Exp 1 but not Exp 2. In this case, microbial competition for N with plants under $eCO_2$ most likely occurred, which was overcome in Exp 2 by additional N from both N fertiliser and high-N-field pea residues. This competition for N under $eCO_2$ can also have a negative impact on wheat growth (Lam et al. 2013b) and hence rhizodeposition. Secondly, the reduction in field pea decomposition under wheat was less under $eCO_2$ than $aCO_2$ and adding additional N had no effect (Exp 2). In this case, the N-mining hypothesis could explain enhanced decomposition under $aCO_2$ triggered by low N availability. Elevated $CO_2$ enables microbes to access more recalcitrant SOM pools, including residues (Carney et al. 2007; de Graaff et al. 2009). Although the high-N-field pea residue had a low C/N ratio, residue derived N was greater under $eCO_2$ and was reduced by fertiliser N (discussed later). Thirdly, decomposition of the high-N-field pea residue was significantly reduced by $eCO_2$ under field pea. In this case, preferential mineralization of labile substrates was likely to have occurred given the high N content of the residue and the likely N deposition by the legume. The current study showed a much greater influence of residue N content and plant species than fertiliser N on enhancing residue decomposition. However, the effect of $eCO_2$ on field pea and wheat decomposition was minimal in a cropping soil with high soil N content (Lam et al. 2014). Importantly, the relative importance of different mechanisms on residue decomposition was supported by differences in the N balance within each plant-soil system. Future studies should examine the links between $eCO_2$, the quantity and quality of root exudates and changes in rhizosphere microbial community composition and C and N functional capacity.

## Total belowground $CO_2$ efflux

The relative differences in $CO_2$ efflux between planted and non-planted soils amended with residues (10-48%) were smaller than expected. High amounts of $CO_2$ were released by microbes in the non-planted treatments. The additional $CO_2$ released in planted columns was mainly from root-derived $CO_2$ such as root exudates and root respiration, as residue-derived $CO_2$ efflux was reduced or not affected by the presence of plants. However, the design of this study cannot discriminate between microbial decomposition of soil organic matter and root exudates and root respiration. Total belowground $CO_2$

efflux was greatest for wheat grown with field pea residue. However, considering wheat plants had 3-4 times more root mass than field pea (Exp 2), total belowground $CO_2$ efflux per unit root length was proportionally smaller under wheat than field pea, consistent with other studies (Wang et al. 2016). de Graaff et al. (2006) also observed that microbial biomass was a poor indicator of total belowground $CO_2$ efflux. Since only a small component of the microbial biomass is active (<2 %) (Blagodatskaya and Kuzyakov 2013), increases in the proportion of active organisms and the rate of microbial turnover could have been more important than overall biomass (Blagodatskaya et al. 2010). However, we expected that total $CO_2$ efflux would be proportional to root and/or shoot biomass, which mediate photosynthetic capacity and rhizosphere volume (van Veen et al. 1991; Rogers et al. 1994). This was not the case in this study.

Total belowground $CO_2$ efflux under $eCO_2$ was not related to root mass. However, $eCO_2$ did not increase plant biomass as expected (Ainsworth and Long 2005; de Graaff et al. 2006; Madhu and Hatfield 2013) even for the same species and soil types (Jin et al. 2012; Lam et al. 2012a; Butterly et al. 2015). Despite the lack of change in root biomass, root respiration was generally less affected or constrained by $eCO_2$ (Kou et al. 2007). Therefore, greater C flow from root exudation and rhizodeposition under $eCO_2$ due to enhanced photosynthetic activity was the likely source of additional $CO_2$-C in our study, although microbial decomposition of root exudates and root respiration could not be separated. Higher rhizodeposition of wheat under FACE (Martens et al. 2009) and greater efflux of labile C substrate from the plant and subsequent mineralisation by soil microbes (Reinsch et al. 2013) support this theory. However, direct evidence of higher specific exudation (per unit of root) for crop species under $eCO_2$ is limited (Cheng et al. 1993). Enhanced C release of wheat under $eCO_2$ has been associated with both greater root biomass (Billes et al. 1993) and increases in specific root activity (Cheng and Johnson 1998). Furthermore, $^{13}CO_2$-pulse-labelling of wheat and field pea and greater belowground $^{13}C$ abundance were associated with increased root biomass (Jin et al. 2014; Butterly et al. 2015).

Increased competition for N between microbes and plant roots can be an important bottleneck which limits rhizodeposit mineralisation under $eCO_2$ (Paterson et al. 1996). Increased total belowground $CO_2$ efflux under $eCO_2$ occurred for wheat in field pea-amended soil but there was no effect of N fertiliser (Exp 1 and 2). Hence, greater N status of the residues enhanced the $CO_2$ effect for wheat (non-legume). In contrast, Martin-Olmedo et al. (2002) showed greater difference in $CO_2$ efflux under barley between $aCO_2$ and $eCO_2$ at low N than high N supply via stimulation of root biomass. It is likely that wheat plants were more competitive for fertiliser N in our study and rhizosphere microbes were only stimulated in the presence of N-rich residues. For field pea, $eCO_2$ increased total belowground $CO_2$ efflux only in soil amended with low-N-wheat residue (Exp 2), and the relative effect of $eCO_2$ on total belowground $CO_2$ efflux decreased with increasing C/N ratio. Rhizosphere effects are known to depend on soil nutrient status, particularly N and P (Dijkstra et al. 2013). Cheng and Johnson (1998) showed that rhizosphere effects were positive with added N but negative without fertiliser N, highlighting that non-legumes require N to be above a critical level in order to have a functioning rhizosphere. Our study highlights that the C/N ratio of residues has opposite effects on total belowground $CO_2$ efflux under cereals and legumes.

## N uptake and recovery from residues

Greater N uptake of wheat (de Graaff et al. 2009; Lam et al. 2012b; Butterly et al. 2015) and field pea (Jin et al. 2012; Lam et al. 2013b; Butterly et al. 2016a) under $eCO_2$ is commonly observed, primarily via increased plant biomass. In the current study, $eCO_2$ only increased total N uptake for field pea (Exp 2). For legumes, $N_2$ fixation provides an important source of additional N under $eCO_2$ (Butterly et al. 2016a). Greater total N uptake under $eCO_2$ primarily occurs via enhanced productivity, despite small decreases in N concentration of cereals (Jensen and Christensen 2004; Madhu and Hatfield 2013). However, reduced N concentration under $eCO_2$ with no change in biomass can reduce total N uptake such as that for wheat growing in high-N-field pea amended soil. $CO_2$ concentration had a comparatively smaller effect on total N uptake than C/N ratio and soil N status, consistent with previous reports (Martin-Olmedo et al. 2002; Lam et al. 2013b; Butterly et al. 2015).

Overall, plants obtained a small component of their N derived from residues. As expected the greatest levels of $N_{DFR}$ occurred under wheat (max 39%), were lower when fertiliser N was added (max 33%) and were the least under field pea (max 15%). Although temporal changes in N availability were not quantified, plants are expected to preferentially utilise other N sources before residue N. Microbial mineralisation of residues and the availability of residue N is likely to occur only once other N sources were exhausted. Interestingly, the N content of field pea residues was a poor indicator of their contribution to plant N nutrition. Specifically, the high-N-field pea residue contributed a significantly greater amount of N to plants than low-N-field pea for all plant treatments, despite similar N concentrations (C/N of 20.5 for low-N-field pea and 19.4 for high-N-field pea). Furthermore, N concentrations in plant tissues were greater for soils amended with the residue of the same plant species. The differences in decomposition and N release from field pea residues could be due to the types of N present within the residues or differences in the decomposability (i.e. structural C, protein content, soluble N concentration) (Pritchard et al. 1999).

The effect of $eCO_2$ on $N_{DFR}$ and the residue N recovery depended on the plant type (cereal v legume). Consistent with overall residue decomposition, $eCO_2$ enhanced $N_{DFR}$ and residue N recovery for wheat but decreased these parameters for field-pea-planted treatments. Generally, the effects of $eCO_2$ on the $N_{DFR}$ were not significant given their small contribution to overall N fertility. However, the $^{15}N$ approach revealed clear effects of $CO_2$ concentration on residue N recovery. Residue recovered in wheat plant were greater at $eCO_2$ than $aCO_2$ when fertiliser N was added, highlighting that residue N alone was insufficient to promote a positive rhizosphere effect under $eCO_2$. The negative effect of $eCO_2$ on N recovery from residues in the field-pea-planted treatment was likely preferential utilisation of other N sources, particularly $N_2$ fixation, as discussed previously. Nevertheless, enhanced decomposition of SOM (priming) can be an important mechanism for increased N supply to wheat under $eCO_2$ (de Graaff et al. 2009) and the results presented here indicate greater N-use efficiency of wheat under $eCO_2$. This is consistent with a 21% increase in N recovery by wheat from barley residues under $eCO_2$ (Lam et al. 2013a). For field pea, reduced decomposition and utilisation of N from residues during a legume crop could mean a greater carryover of N to subsequent cropping phases. However, Lam et al. (2013a) showed that the contribution of field pea residue to the proceeding wheat crop could be significantly reduced (~8.6%) under $eCO_2$ if the C/N ratio of legume residue was increased (C/N from 44 to 52). Therefore, rhizosphere effects on residue decomposition and replenishment of soil N and C pools under $eCO_2$ are likely to alter the C and N balance in cropping systems (Butterly et al. 2016b).

## Conclusion

Understanding $eCO_2$-induced rhizosphere effects on residue decomposition is critical for predicting changes in soil fertility of future agricultural productions systems. This study showed that residue decomposition was generally reduced in the presence of plant roots, due to enhanced competition for N between plants and microbes. Elevated $CO_2$ both increased and decreased this negative rhizosphere effect. These changes were largely controlled by plant treatment, residue C/N ratio, less so by fertiliser N, and were not related to root mass nor microbial biomass. Importantly, $eCO_2$ lessened the negative rhizosphere effect of $aCO_2$-grown wheat but exacerbated that of field pea. However, temporal changes in the contribution of rhizosphere effects are likely to occur within and between growing seasons. Although residues only contributed a small component of overall plant N uptake, wheat utilised a greater amount of N from residues under $eCO_2$, and this stimulation of rhizosphere N-recovery only occurred when N fertiliser was added. Hence, residues with high N (low C/N ratio) alone did not induce a positive rhizosphere effect. Our results indicate that reduced decomposition of residues under $eCO_2$-grown field pea could potentially increase the N available to subsequent crops. Consistent with our previous study, the C content in the rhizosphere soil of wheat appeared to decrease under $eCO_2$. A reduction in soil C could indicate that reduced residue decomposition in the presence of plants and the subsequent replenishment of soil C are interrupted under $eCO_2$. These mechanisms need to be investigated over an extended period of growth as rhizosphere effects are likely to amplify during later stages of growth.

## Acknowledgements

This research was supported by an Australian Research Council *Linkage Project* (LP100200757), and was conducted at the $CO_2$ glasshouse facility of the Department of Economic Development, Jobs, Transport and Resources (DEDJTR), Victoria at Horsham. We thank Dr Shu Kee Lam for his useful comments on the manuscript, Kaien Ra for her excellent technical support, Leanne Lisle for performing the IRMS analyses and Nicholas Sitlington Hansen who worked on this project as part of his Honours project.

## References

Ainsworth EA and Long SP (2005) What have we learned from 15 years of free-air $CO_2$ enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising $CO_2$. New Phytologist 165: 351-371

Allard V, Robin C, Newton PCD, Lieffering M and Soussana JF (2006) Short and long-term effects of elevated $CO_2$ on *Lolium perenne* rhizodeposition and its consequences on soil organic matter turnover and plant N yield. Soil Biology & Biochemistry 38: 1178-1187

Billes G, Rouhier H and Bottner P (1993) Modifications of the carbon and nitrogen allocations in the plant (*Triticum aestivum* L.) soil system in response to increased atmospheric $CO_2$ concentration. Plant and Soil 157: 215-225

Billings SA, Lichter J, Ziegler SE, Hungate BA and Richter DD (2010) A call to investigate drivers of soil organic matter retention vs. mineralization in a high $CO_2$ world. Soil Biology & Biochemistry 42: 665-668

Blagodatskaya E, Blagodatsky S, Dorodnikov M and Kuzyakov Y (2010) Elevated atmospheric $CO_2$ increases microbial growth rates in soil: results of three $CO_2$ enrichment experiments. Global Change Biology 16: 836-848

Blagodatskaya E and Kuzyakov Y (2013) Active microorganisms in soil: Critical review of estimation criteria and approaches. Soil Biology & Biochemistry 67: 192-211

Brookes PC, Landman A, Pruden G and Jenkinson DS (1985) Chloroform fumigation and the release of soil nitrogen: A rapid direct extraction method to measure microbial biomass nitrogen in soil. Soil Biology & Biochemistry 17: 837-842

Butterly CR, Armstrong R, Chen D and Tang C (2015) Carbon and nitrogen partitioning of wheat and field pea grown with two nitrogen levels under elevated $CO_2$. Plant and Soil 391: 367-382

Butterly CR, Armstrong RD, Chen D and Tang C (2016a) Free-air $CO_2$ enrichment (FACE) reduces the inhibitory effect of soil nitrate on $N_2$ fixation of *Pisum sativum* L. Annals of Botany 117: 177-185

Butterly CR, Phillips LA, Wiltshire JL, Franks AE, Armstrong RD, Chen D, Mele PM and Tang C (2016b) Long-term effects of elevated $CO_2$ on carbon and nitrogen functional capacity of microbial communities in three contrasting soils Soil Biology & Biochemistry 97: 157-167

Cabrera ML and Beare MH (1993) Alkaline persulfate oxidation for determining total nitrogen in microbial biomass extracts. Soil Science Society of America Journal 57: 1007-1012

Carney KM, Hungate BA, Drake BG and Megonigal JP (2007) Altered soil microbial community at elevated $CO_2$ leads to loss of soil carbon. Proceedings of the National Academy of Sciences 104: 4990-4995

Carrillo Y, Dijkstra F, Pendall E, LeCain D and Tucker C (2014) Plant rhizosphere influence on microbial C metabolism: the role of elevated $CO_2$, N availability and root stoichiometry. Biogeochemistry 117: 229-240

Carrillo Y, Pendall E, Dijkstra FA, Morgan JA and Newcomb JM (2011) Response of soil organic matter pools to elevated $CO_2$ and warming in a semi-arid grassland. Plant and Soil 347: 339-350

Cheng WX (1999) Rhizosphere feedbacks in elevated $CO_2$. Tree Physiology 19: 313-320

Cheng WX, Coleman DC, Carroll CR and Hoffman CA (1993) In-situ measurement of root respiration and soluble C concentrations in the rhizosphere. Soil Biology & Biochemistry 25: 1189-1196

Cheng WX and Johnson DW (1998) Elevated $CO_2$, rhizosphere processes, and soil organic matter decomposition. Plant and Soil 202: 167-174

Cheng WX, Parton WJ, Gonzalez-Meler MA, Phillips R, Asao S, McNickle GG, Brzostek E and Jastrow JD (2014) Synthesis and modeling perspectives of rhizosphere priming. New Phytologist 201: 31-44

Cotrufo MF, Ineson P and Scott A (1998) Elevated $CO_2$ reduces the nitrogen concentration of plant tissues. Global Change Biology 4: 43-54

Craine JM, Morrow C and Fierer N (2007) Microbial nitrogen limitation increases decomposition. Ecology 88: 2105-2113

de Graaff M-A, Schadt CW, Rula K, Six J, Schweitzer JA and Classen AT (2011) Elevated $CO_2$ and plant species diversity interact to slow root decomposition. Soil Biology & Biochemistry 43: 2347-2354

de Graaff M-A, van Groenigen K-J, Six J, Hungate B and van Kessel C (2006) Interactions between plant growth and soil nutrient cycling under elevated $CO_2$: a meta-analysis. Global Change Biology 12: 2077-2091

de Graaff MA, Six J, Harris D, Blum H and van Kessel C (2004) Decomposition of soil and plant carbon from pasture systems after 9 years of exposure to elevated $CO_2$: impact on C cycling and modeling. Global Change Biology 10: 1922-1935

de Graaff MA, Van Kessel C and Six J (2009) Rhizodeposition-induced decomposition increases N availability to wild and cultivated wheat genotypes under elevated $CO_2$. Soil Biology & Biochemistry 41: 1094-1103

Dijkstra FA, Carrillo Y, Pendall E and Morgan JA (2013) Rhizosphere priming: a nutrient perspective. Frontiers in Microbiology 4

Finzi AC, Abramoff RZ, Spiller KS, Brzostek ER, Darby BA, Kramer MA and Phillips RP (2015) Rhizosphere processes are quantitatively important components of terrestrial carbon and nutrient cycles. Global Change Biology 21: 2082-2094

Fontaine S, Bardoux G, Abbadie L and Mariotti A (2004) Carbon input to soil may decrease soil carbon content. Ecology Letters 7: 314-320

Heanes DL (1984) Determination of total organic-C in soils by an improved chromic acid digestion and spectrophotometric procedure. Communications in Soil Science and Plant Analysis 15: 1191-1213

Hopkins FM, Filley TR, Gleixner G, Lange M, Top SM and Trumbore SE (2014) Increased belowground carbon inputs and warming promote loss of soil organic carbon through complementary microbial responses. Soil Biology & Biochemistry 76: 57-69

IPCC (2001) Climate Change 2001: Impacts, adaptation and vulnerability. Cambridge University Press, New York

Isbell RF (1996) The Australian Soil Classification. CSIRO Publishing, Melbourne

Jensen B and Christensen BT (2004) Interactions between elevated $CO_2$ and added N: Effects on water use, biomass, and soil $^{15}N$ uptake in wheat. Acta Agriculturae Scandinavica Section B-Soil and Plant Science 54: 175-184

Jin J, Tang C, Robertson A, Franks AE, Armstrong R and Sale P (2014) Increased microbial activity contributes to phosphorus immobilization in the rhizosphere of wheat under elevated $CO_2$. Soil Biology & Biochemistry 75: 292-299

Jin J, Tang CX, Armstrong R and Sale P (2012) Phosphorus supply enhances the response of legumes to elevated $CO_2$ (FACE) in a phosphorus-deficient vertisol. Plant and Soil 358: 86-99

Joergensen RG (1996) The fumigation-extraction method to estimate soil microbial biomass: Calibration of the kEC value. Soil Biology & Biochemistry 28: 25-31

Jones DL, Nguyen C and Finlay RD (2009) Carbon flow in the rhizosphere: carbon trading at the soil-root interface. Plant and Soil 321: 5-33

Kou T, Zhu J, Xie Z, Hasegawa T and Heiduk K (2007) Effect of elevated atmospheric $CO_2$ concentration on soil and root respiration in winter wheat by using a respiration partitioning chamber. Plant and Soil 299: 237-249

Kuzyakov Y (2010) Priming effects: Interactions between living and dead organic matter. Soil Biology & Biochemistry 42: 1363-1371

Lam SK, Chen D, Norton R and Armstrong R (2012a) Does phosphorus stimulate the effect of elevated [$CO_2$] on growth and symbiotic nitrogen fixation of grain and pasture legumes? Crop & Pasture Science 63: 53-62

Lam SK, Chen D, Norton R and Armstrong R (2013a) The effect of elevated atmospheric carbon dioxide concentration on the contribution of residual legume and fertilizer nitrogen to a subsequent wheat crop. Plant and Soil 364: 81-91

Lam SK, Chen DL, Norton R and Armstrong R (2012b) Nitrogen demand and the recovery of $^{15}N$-labelled fertilizer in wheat grown under elevated carbon dioxide in southern Australia. Nutrient Cycling in Agroecosystems 92: 133-144

Lam SK, Chen DL, Norton R and Armstrong R (2013b) Crop residue incorporation negates the positive effect of elevated atmospheric carbon dioxide concentration on wheat productivity and fertilizer nitrogen recovery. Plant and Soil 366: 551-561

Lam SK, Norton R, Armstrong R and Chen D (2014) Increased microbial activity under elevated [$CO_2$] does not enhance residue decomposition in a semi-arid cropping system in Australia. Soil Biology & Biochemistry 72: 97-99

Madhu M and Hatfield JL (2013) Dynamics of plant root growth under increased atmospheric carbon dioxide. Agronomy Journal 105: 657-669

Marhan S, Derain D, Erbs M, Kuzyakov Y, Fangmeier A and Kandeler E (2008) Soil organic matter mineralization and residue decomposition of spring wheat grown under elevated $CO_2$ atmosphere. Agriculture Ecosystems & Environment 123: 63-68

Martens R, Heiduk K, Pacholski A and Weigel HJ (2009) Repeated $^{14}CO_2$ pulse-labelling reveals an additional net gain of soil carbon during growth of spring wheat under free air carbon dioxide enrichment (FACE). Soil Biology & Biochemistry 41: 2422-2429

Martin-Olmedo P, Rees RM and Grace J (2002) The influence of plants grown under elevated $CO_2$ and N fertilization on soil nitrogen dynamics. Global Change Biology 8: 643-657

Norby RJ, Cotrufo MF, Ineson P, O'Neill EG and Canadell JG (2001) Elevated $CO_2$, litter chemistry, and decomposition: a synthesis. Oecologia 127: 153-165

Paterson E, Rattray EAS and Killham K (1996) Effect of elevated atmospheric $CO_2$ concentration on C-partitioning and rhizosphere C-flow for three plant species. Soil Biology & Biochemistry 28: 195-201

Paterson E, Thornton B, Midwood AJ, Osborne SM, Sim A and Millard P (2008) Atmospheric $CO_2$ enrichment and nutrient additions to planted soil increase mineralisation of soil organic matter, but do not alter microbial utilisation of plant- and soil C-sources. Soil Biology & Biochemistry 40: 2434-2440

Phillips RP, Finzi AC and Bernhardt ES (2011) Enhanced root exudation induces microbial feedbacks to N cycling in a pine forest under long-term $CO_2$ fumigation. Ecology Letters 14: 187-194

Pritchard SG, Rogers HH, Prior SA and Peterson CM (1999) Elevated $CO_2$ and plant structure: a review. Global Change Biology 5: 807-837

Reinsch S, Ambus P, Thornton B and Paterson E (2013) Impact of future climatic conditions on the potential for soil organic matter priming. Soil Biology & Biochemistry 65: 133-140

Rogers HH, Runion GB and Krupa SV (1994) Plant responses to atmospheric $CO_2$ enrichment with emphasis on roots and the rhizosphere. Environmental Pollution 83: 155-189

Shahzad T, Chenu C, Genet P, Barot S, Perveen N, Mougin C and Fontaine S (2015) Contribution of exudates, arbuscular mycorrhizal fungi and litter depositions to the rhizosphere priming effect induced by grassland species. Soil Biology & Biochemistry 80: 146-155

Sparling G and Zhu C (1993) Evaluation and calibration of biochemical methods to measure microbial biomass C and N in soils from Western Australia. Soil Biology & Biochemistry 25: 1793-1801

Torbert HA, Prior SA, Rogers HH and Wood CW (2000) Review of elevated atmospheric $CO_2$ effects on agro-ecosystems: residue decomposition processes and soil C storage. Plant and Soil 224: 59-73

van Groenigen K-J, Six J, Harris D, Blum H and van Kessel C (2003) Soil $^{13}C$–$^{15}N$ dynamics in an $N_2$-fixing clover system under long-term exposure to elevated atmospheric $CO_2$. Global Change Biology 9: 1751-1762

van Groenigen KJ, Qi X, Osenberg CW, Luo YQ and Hungate BA (2014) Faster decomposition under increased atmospheric $CO_2$ limits soil carbon storage. Science 344: 508-509

van Veen JA, Liljeroth E, Lekkerkerk LJA and Vandegeijn SC (1991) Carbon fluxes in plant-soil systems at elevated atmospheric $CO_2$ levels. Ecological Applications 1: 175-181

van Vuuren MMI, Robinson D, Scrimgeour CM, Raven JA and Fitter AH (2000) Decomposition of $^{13}C$-labelled wheat root systems following growth at different $CO_2$ concentrations. Soil Biology & Biochemistry 32: 403-413

Vance ED, Brookes PC and Jenkinson DS (1987) An extraction method for measuring soil microbial biomass C. Soil Biology & Biochemistry 19: 703-707

Wang X, Tang C, Severi J, Butterly CR and Baldock JA (2016) Rhizosphere priming effect on soil organic carbon decomposition under plant species differing in soil acidification and root exudation New Phytologist DOI: 10.1111/nph.13966

WRB IWG (2014) World Reference Base for Soil Resources. International soil classification system for naming soils and creating legends for soil maps. FAO, Rome

Yang Y, Luo Y, Lu M, Schaedel C and Han W (2011) Terrestrial C:N stoichiometry in response to elevated $CO_2$ and N addition: a synthesis of two meta-analyses. Plant and Soil 343: 393-400

Zhu B and Cheng W (2012) Nodulated soybean enhances rhizosphere priming effects on soil organic matter decomposition more than non-nodulated soybean. Soil Biology & Biochemistry 51: 56-65

Zhu B, Gutknecht JLM, Herman DJ, Keck DC, Firestone MK and Cheng W (2014) Rhizosphere priming effects on soil carbon and nitrogen mineralization. Soil Biology & Biochemistry 76: 183-192

Zibilske LM (1994) Carbon mineralization. In Methods of soil analysis, Part 2. Microbial and biochemical properties. pp 835-863. Soil Science Society of America, Madison, Wisconsin

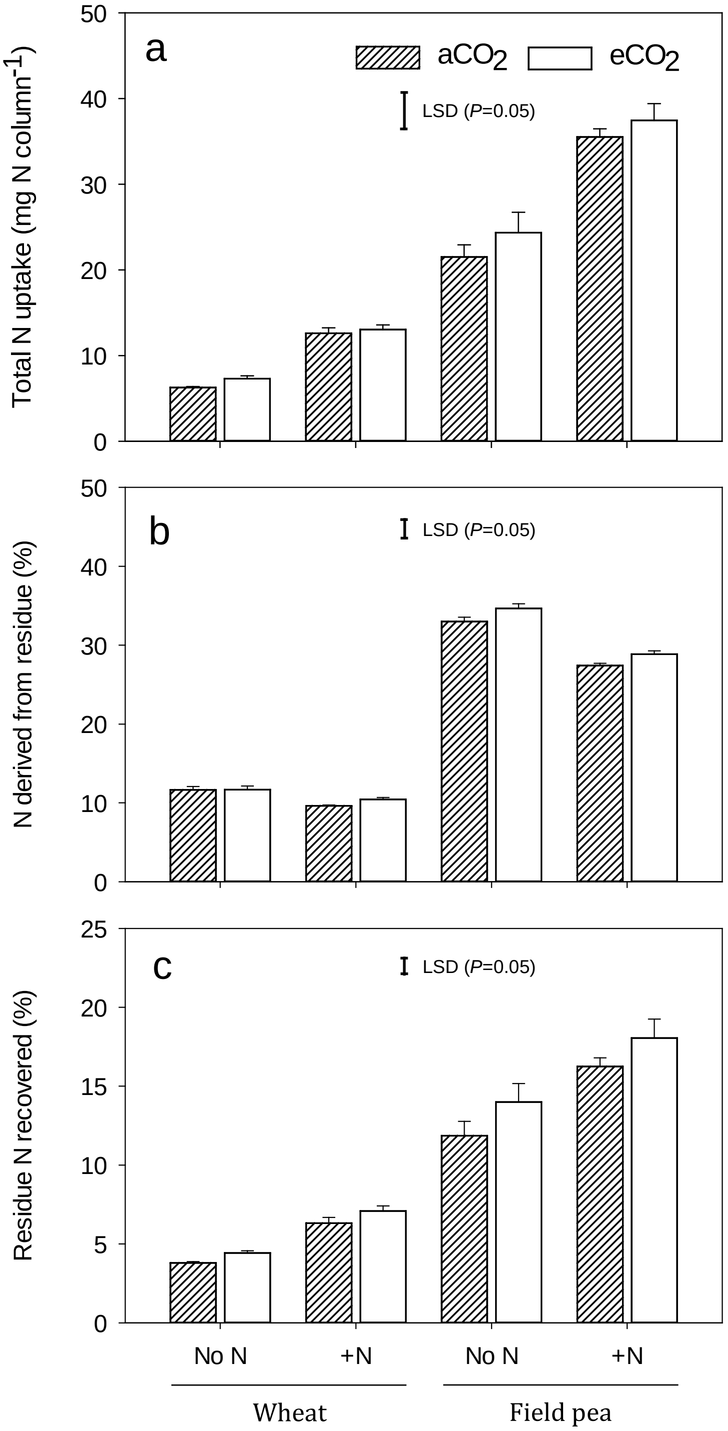

**Fig. 1** Total N uptake (a), % N derived from residue (b) and % residue N recovered (c) by wheat grown under ambient $CO_2$ ($aCO_2$, 390 ppm) and elevated $CO_2$ ($eCO_2$, 700 ppm) in soil containing wheat or field pea residues and either no added N (No N) or 25 mg N $kg^{-1}$ soil (+N). Standard errors of the mean of 4 replicates. Bars indicate least significant difference (LSD) (*P*=0.05) (Exp 1).

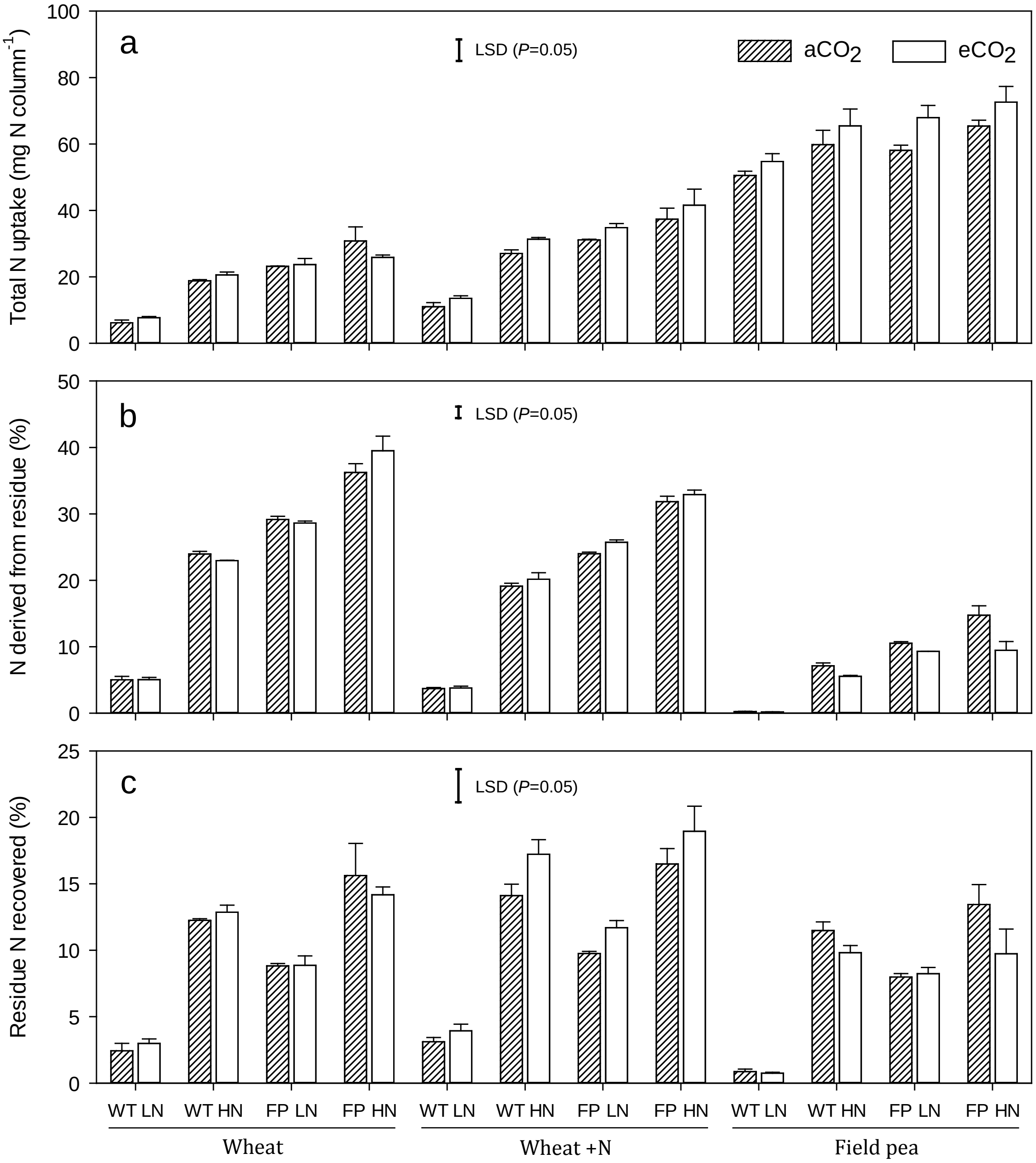

**Fig. 2** Total N uptake (a), % N derived from residue (b) and % residue N recovered (c) by three plant treatments (wheat, left; wheat + 25 mg N kg$^{-1}$, middle and field pea, right) under ambient CO$_2$ (aCO$_2$, 390 ppm) and elevated CO$_2$ (eCO$_2$, 700 ppm) in soil containing residues of wheat (WT) or field pea (FP) previously grown with low N (HN) or high N (HN). Standard errors of the mean of 3 replicates. Bars indicate least significant difference (LSD) (*P*=0.05) (Exp 2).

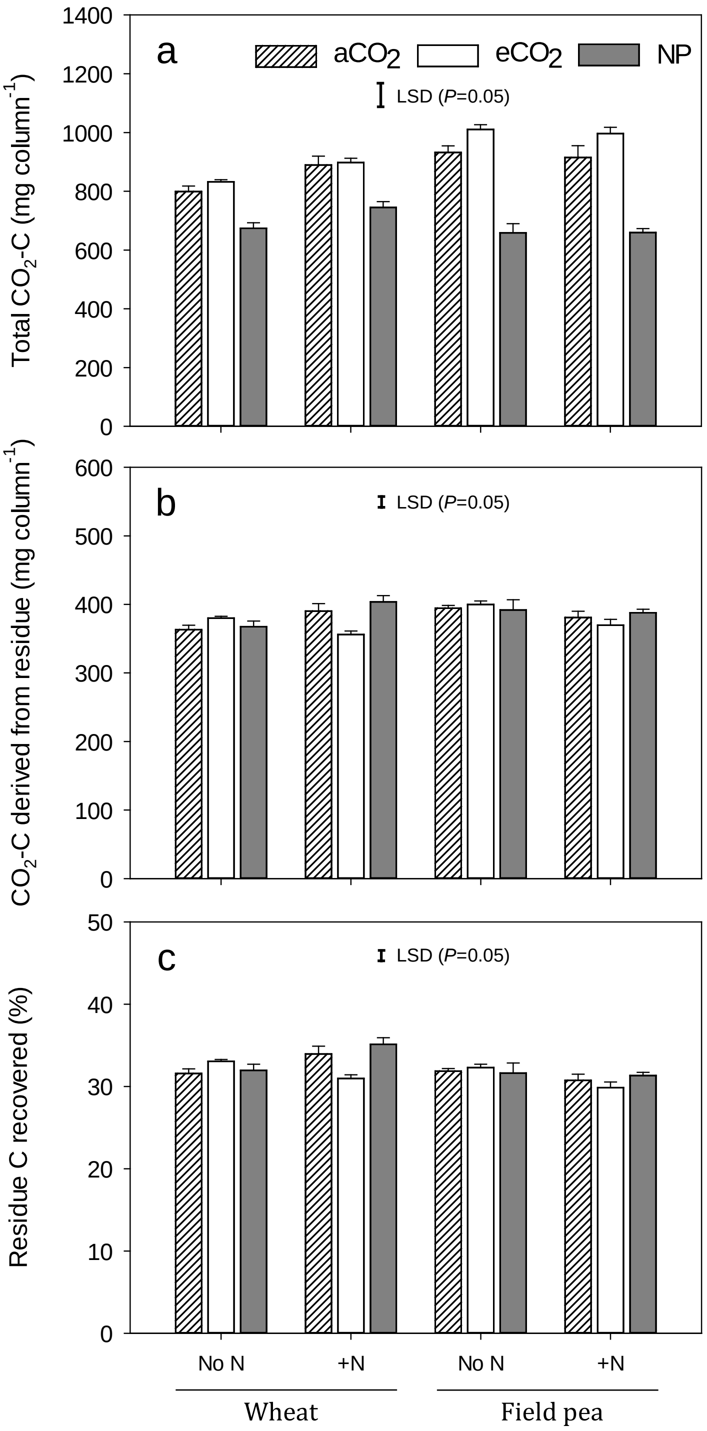

**Fig. 3** Total belowground $CO_2$-C (a), $CO_2$-C derived from residue (b) and % residue C recovered (c) in alkali traps of soil columns planted with wheat grown under ambient $CO_2$ ($aCO_2$, 390 ppm) and elevated $CO_2$ ($eCO_2$, 700 ppm) in soil containing wheat and field pea residues and either no added N (No N) or 25 mg N $kg^{-1}$ soil (+N) and non-planted (NP) controls. Standard errors of the mean of 4 replicates. Bars indicate least significant difference (LSD) (*P*=0.05) (Exp 1).

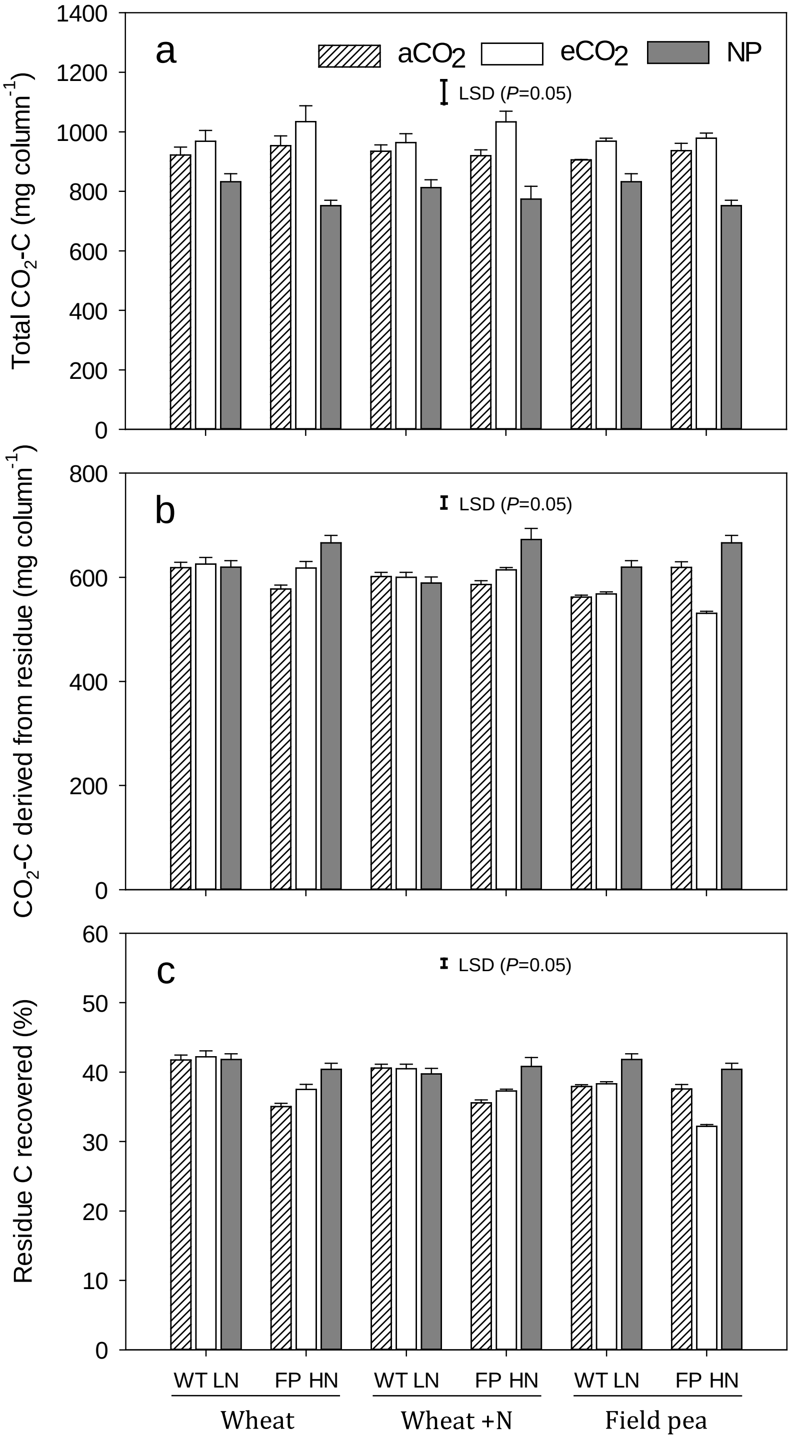

**Fig. 4** Total belowground $CO_2$-C (a), $CO_2$-C derived from residue (b) and % residue C recovered (c) in alkali traps of three plant treatments (wheat, left; wheat + 25 mg N $kg^{-1}$, middle and field pea, right) under ambient $CO_2$ (a$CO_2$, 390 ppm) and elevated $CO_2$ (e$CO_2$, 700 ppm) in soil containing wheat (WT) or field pea (FP) residues grown with low N (LN) or high N (HN) and non-planted (NP) controls. Standard errors of the mean of 3 replicates. Bars indicate least significant difference (LSD) (*P*=0.05) (Exp 2).

**Table 1** Shoot and root dry weight (DW), root-to-shoot ratio, shoot and root N concentration and $^{15}N$ content of wheat grown under ambient $CO_2$ ($aCO_2$, 390 ppm) and elevated $CO_2$ ($eCO_2$, 700 ppm) in soil containing field pea or wheat residues and either no added N (No N) or 25 mg N $kg^{-1}$ soil (+N) (Exp 1).

| $CO_2$ | Residue | N level | Shoot DW | Root DW | Root:Shoot | Shoot N | Root N | Shoot $^{15}N$ | Root $^{15}N$ |
|---|---|---|---|---|---|---|---|---|---|
| | | | (g $column^{-1}$) | | (ratio) | (g N $kg^{-1}$) | | (atom %) | |
| $aCO_2$ | Wheat | No N | 0.37 | 0.154 | 0.450 | 10.9 | 6.65 | 1.87 | 1.78 |
| | | +N | 0.84 | 0.297 | 0.358 | 9.0 | 6.04 | 1.58 | 1.59 |
| | Field pea | No N | 1.26 | 0.456 | 0.351 | 10.7 | 6.31 | 2.11 | 1.94 |
| | | +N | 2.20 | 0.632 | 0.289 | 10.3 | 5.89 | 1.84 | 1.74 |
| $eCO_2$ | Wheat | No N | 0.40 | 0.133 | 0.345 | 10.8 | 7.56 | 1.91 | 1.78 |
| | | +N | 0.81 | 0.239 | 0.299 | 10.0 | 6.20 | 1.71 | 1.64 |
| | Field pea | No N | 1.47 | 0.375 | 0.264 | 10.3 | 6.64 | 2.26 | 2.06 |
| | | +N | 2.40 | 0.770 | 0.320 | 9.6 | 5.95 | 1.94 | 1.79 |
| | | *LSD* | *0.23* | *0.185* | *0.150* | *1.4* | *1.51* | *0.12* | *0.15* |
| | | ***Significance level*** | | | | | | | |
| | | *$CO_2$* | *ns* | *ns* | *ns* | *ns* | *ns* | ** | *ns* |
| | | *Residue* | *** | *** | *ns* | *ns* | *ns* | *** | *** |
| | | *N level* | *** | *** | *ns* | * | * | *** | *** |
| | | *$CO_2$ × Residue* | *ns* | *ns* | *ns* | *ns* | *ns* | *ns* | *ns* |
| | | *$CO_2$ × N level* | *ns* | *ns* | *ns* | *ns* | *ns* | *ns* | *ns* |
| | | *Residue × N level* | *** | *ns* | *ns* | *ns* | *ns* | *ns* | *ns* |
| | | *$CO_2$ × Residue × N level* | *ns* | *ns* | *ns* | *ns* | *ns* | *ns* | *ns* |

Least significant difference (LSD) and significance levels are for three-way analysis of variance (ANOVA)
ns, *, ** and *** indicate, $P>0.05$, $P<0.05$, $P<0.01$ and $P<0.001$, respectively.

**Table 2** Shoot and root dry weight (DW), root-to-shoot ratio, shoot and root N concentration and $^{15}N$ content of three plant treatments (wheat, wheat + 25 mg N $kg^{-1}$ soil and field pea) under ambient $CO_2$ ($aCO_2$, 390 ppm) and elevated $CO_2$ ($eCO_2$, 700 ppm) in soil containing residues of wheat or field pea (Pea) previously grown with low N (40 mg $kg^{-1}$) or high N (100 mg $kg^{-1}$) (Exp 2).

| $CO_2$ | Plant treatment | Residue | Shoot DW | Root DW | Root:Shoot | Shoot N | Root N | Shoot $^{15}N$ | Root $^{15}N$ |
|---|---|---|---|---|---|---|---|---|---|
| | | | (g $column^{-1}$) | | (ratio) | (g N $kg^{-1}$) | | (atom %) | |
| $aCO_2$ | Wheat | Wheat-low N | 0.53 | 0.21 | 0.39 | 9.0 | 6.9 | 0.95 | 0.92 |
| | | Wheat-high N | 1.48 | 0.53 | 0.36 | 10.4 | 6.4 | 3.67 | 3.61 |
| | | Pea-low N | 1.98 | 0.70 | 0.36 | 9.4 | 6.5 | 1.70 | 1.65 |
| | | Pea-high N | 2.22 | 0.81 | 0.37 | 11.3 | 6.9 | 3.13 | 2.94 |
| | Wheat +N | Wheat-low N | 1.02 | 0.48 | 0.47 | 8.3 | 5.3 | 0.76 | 0.90 |
| | | Wheat-high N | 2.25 | 0.53 | 0.23 | 10.5 | 6.5 | 2.98 | 3.06 |
| | | Pea-low N | 2.51 | 0.60 | 0.24 | 10.8 | 6.9 | 1.47 | 1.38 |
| | | Pea-high N | 2.49 | 0.81 | 0.33 | 12.6 | 7.6 | 2.79 | 2.66 |
| | Field pea | Wheat-low N | 2.35 | 0.17 | 0.07 | 19.9 | 21.4 | 0.39 | 0.46 |
| | | Wheat-high N | 2.60 | 0.19 | 0.07 | 21.3 | 24.1 | 1.32 | 1.59 |
| | | Pea-low N | 2.36 | 0.18 | 0.08 | 22.9 | 23.6 | 0.84 | 0.94 |
| | | Pea-high N | 2.69 | 0.18 | 0.07 | 22.8 | 22.9 | 1.48 | 1.45 |
| $eCO_2$ | Wheat | Wheat-low N | 0.74 | 0.23 | 0.32 | 8.6 | 5.8 | 0.93 | 0.99 |
| | | Wheat-high N | 1.82 | 0.64 | 0.36 | 9.0 | 5.9 | 3.61 | 3.49 |
| | | Pea-low N | 2.22 | 0.70 | 0.32 | 8.9 | 5.8 | 1.69 | 1.53 |
| | | Pea-high N | 2.28 | 0.74 | 0.33 | 9.4 | 6.0 | 3.38 | 3.17 |
| | Wheat +N | Wheat-low N | 1.46 | 0.43 | 0.29 | 7.4 | 6.4 | 0.79 | 0.82 |
| | | Wheat-high N | 2.85 | 0.97 | 0.34 | 8.9 | 6.4 | 3.13 | 3.16 |
| | | Pea-low N | 3.19 | 1.10 | 0.34 | 9.0 | 5.6 | 1.54 | 1.50 |
| | | Pea-high N | 3.31 | 1.01 | 0.30 | 10.3 | 7.3 | 2.93 | 2.52 |
| | Field pea | Wheat-low N | 2.69 | 0.18 | 0.07 | 18.8 | 22.3 | 0.39 | 0.44 |
| | | Wheat-high N | 2.76 | 0.15 | 0.06 | 22.4 | 21.6 | 1.11 | 1.43 |
| | | Pea-low N | 3.13 | 0.18 | 0.06 | 20.4 | 22.9 | 0.78 | 0.89 |
| | | Pea-high N | 3.22 | 0.18 | 0.05 | 21.3 | 23.4 | 1.07 | 1.29 |
| | | *LSD (P=0.05)* | *0.28* | *0.27* | *0.15* | *1.6* | *1.8* | *0.19* | *0.28* |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| ***Significance level*** | | | | | | | |
| $CO_2$ | *ns* | *ns* | *ns* | *** | *ns* | *ns* | *ns* |
| *Plant treatment* | *ns* | *ns* | *ns* | *** | *** | *** | *** |
| *Residue* | *ns* | *ns* | *ns* | *** | *ns* | *** | *** |
| $CO_2$ × *Plant treatment* | *ns* | *ns* | *ns* | *ns* | *ns* | *** | *ns* |
| $CO_2$ × *Residue* | *ns* | *ns* | *ns* | *ns* | *ns* | *ns* | *ns* |
| *Plant treatment* × *Residue* | ** | *ns* | * | *ns* | *ns* | *** | *** |
| $CO_2$ × *Plant treatment* × *Residue* | *ns* | *ns* | *ns* | *ns* | *ns* | * | *ns* |

ns, *, ** and *** indicate, $P>0.05$, $P<0.05$, $P<0.01$ and $P<0.001$, respectively.

Least significant difference (LSD).

**Table 3** Total C (TC) and N (TN), C-to-N ratio (C:N), $^{13}$C and $^{15}$N content, microbial biomass C (MBC) and N (MBN), MBC-to-MBN ratio (MBC:N), extractable organic C (EOC) and N (EON) of soil with wheat grown under ambient $CO_2$ (a$CO_2$, 390 ppm) and elevated $CO_2$ (e$CO_2$, 700 ppm) containing field pea or wheat residues and no added N (No N) or 25 mg N kg$^{-1}$ soil (+N) and non-planted controls (Exp 1).

| $CO_2$ | Residue | N level | TC | TN | C:N | $^{13}$C | $^{15}$N | MBC | MBN | MBC:N | EOC | EON |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | (g kg$^{-1}$ soil) | | (ratio) | (δPDB) | (atom %) | (mg kg$^{-1}$ soil) | | (ratio) | (mg kg$^{-1}$ soil) | |
| a$CO_2$ | Wheat | No N | 15.0 | 1.25 | 12.1 | -2.85 | 0.719 | 595 | 66.3 | 9.0 | 17.4 | 11.2 |
| | | +N | 15.6 | 1.27 | 12.4 | 1.07 | 0.728 | 618 | 68.0 | 9.1 | 30.6 | 10.8 |
| | Field pea | No N | 13.1 | 1.15 | 11.7 | -8.41 | 0.706 | 626 | 72.5 | 8.7 | 38.1 | 10.9 |
| | | +N | 13.6 | 1.15 | 12.0 | -6.44 | 0.750 | 648 | 46.4 | 14.0 | 41.9 | 10.5 |
| e$CO_2$ | Wheat | No N | 15.2 | 1.20 | 12.7 | -3.66 | 0.710 | 535 | 67.3 | 8.0 | 17.8 | 11.4 |
| | | +N | 14.4 | 1.13 | 12.8 | -4.45 | 0.700 | 414 | 69.5 | 5.9 | 28.8 | 10.5 |
| | Field pea | No N | 16.2 | 1.32 | 12.3 | -9.61 | 0.719 | 643 | 76.4 | 8.4 | 30.4 | 10.2 |
| | | +N | 15.1 | 1.23 | 12.3 | -7.83 | 0.743 | 518 | 70.9 | 7.4 | 29.4 | 10.8 |
| Non-planted | Wheat | No N | 18.9 | 1.62 | 11.6 | -3.54 | 0.742 | 553 | 62.8 | 7.8 | 35.9 | 40.0 |
| | | +N | 19.4 | 1.71 | 11.4 | -5.20 | 0.726 | 609 | 63.2 | 9.0 | 30.5 | 52.1 |
| | Field pea | No N | 15.1 | 1.28 | 11.8 | -9.26 | 0.765 | 487 | 64.1 | 8.6 | 29.6 | 16.0 |
| | | +N | 17.5 | 1.56 | 11.3 | -9.94 | 0.731 | 571 | 60.6 | 10.0 | 29.6 | 24.4 |
| | | *LSD (P=0.05)* | *1.9* | *0.18* | *0.8* | *3.70* | *0.036* | *145* | *6.4* | *2.3* | *16.9* | *1.5* |
| | | ***Significance level*** | | | | | | | | | | |
| | | *$CO_2$* | *ns* | *ns* | ** | * | *ns* | * | *** | *** | *ns* | *ns* |
| | | *Residue* | *ns* | *ns* | ** | *** | *ns* | *ns* | *ns* | ** | ** | * |
| | | *N level* | *ns* | *ns* | *ns* | *ns* | *ns* | *ns* | *** | *ns* | *ns* | *ns* |
| | | *$CO_2$ × Residue* | *** | *** | *ns* | *ns* | *ns* | *ns* | *** | *ns* | *ns* | *ns* |
| | | *$CO_2$ × N level* | * | *ns* | *ns* | *ns* | *ns* | * | *** | ** | *ns* | *ns* |
| | | *Residue × N level* | *ns* | *ns* | *ns* | *ns* | *ns* | *ns* | *** | * | *ns* | * |
| | | *$CO_2$ × Residue × N level* | *ns* | *ns* | *ns* | *ns* | *ns* | *ns* | *** | *ns* | *ns* | * |

Least significant difference (LSD) for one-way Residual Maximum Likelihood (REML) analyses.

Significance levels are for three-way Analyses of Variance (ANOVA), excluding non-planted controls.

ns, *, ** and *** indicate, $P>0.05$, $P<0.05$, $P<0.01$ and $P<0.001$, respectively.

**Table 4** Total C (TC), $^{13}C$ and $^{15}N$ content, microbial biomass C (MBC) and N (MBN), MBC-to-MBN ratio (MBC:N), extractable organic C (EOC) and N (EON) of soil with three plant treatments (wheat, wheat + 25 mg N kg$^{-1}$ soil and field pea) under ambient $CO_2$ (a$CO_2$, 390 ppm) and elevated $CO_2$ (e$CO_2$, 700 ppm) containing residues of wheat or field pea (Pea) previously grown with low N (40 mg kg$^{-1}$) or high N (100 mg kg$^{-1}$) and non-planted controls (Exp 2).

| $CO_2$ | Plant treatment | Residue | TC | $^{13}C$ | $^{15}N$ | MBC | MBN | MBC:N | EOC | EON |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | (mg kg$^{-1}$) | (δPDB) | (atom %) | (mg kg$^{-1}$ soil) | | (ratio) | (mg kg$^{-1}$ soil) | |
| a$CO_2$ | Wheat | Wheat-low N | 19.2 | -0.4 | 0.55 | 499 | 50.4 | 10.2 | 23.7 | 14.7 |
| | | Wheat-high N | 19.3 | -4.0 | 1.09 | 547 | 34.6 | 15.8 | 29.9 | 15.3 |
| | | Pea-low N | 20.9 | -12.2 | 0.63 | 609 | 26.8 | 23.3 | 28.6 | 15.5 |
| | | Pea-high N | 17.7 | -4.5 | 0.99 | 648 | 33.1 | 19.7 | 38.8 | 16.9 |
| | Wheat +N | Wheat-low N | 20.6 | -4.0 | 0.53 | 564 | 50.4 | 11.3 | 29.2 | 14.5 |
| | | Wheat-high N | 20.2 | -5.4 | 1.05 | 665 | 26.9 | 24.7 | 28.7 | 15.3 |
| | | Pea-low N | 18.5 | -11.9 | 0.96 | 629 | 27.3 | 23.0 | 36.0 | 15.1 |
| | | Pea-high N | 20.4 | -6.0 | 0.90 | 661 | 31.9 | 20.8 | 37.0 | 15.9 |
| | Field pea | Wheat-low N | 18.4 | -0.5 | 0.53 | 528 | 33.1 | 16.1 | 27.8 | 15.1 |
| | | Wheat-high N | 21.0 | -3.6 | 1.10 | 503 | 23.7 | 21.3 | 28.1 | 18.0 |
| | | Pea-low N | 19.6 | -11.6 | 0.65 | 494 | 27.7 | 18.2 | 28.7 | 19.5 |
| | | Pea-high N | 19.4 | -7.5 | 0.93 | 549 | 38.3 | 15.4 | 30.8 | 28.0 |
| e$CO_2$ | Wheat | Wheat-low N | 18.7 | -4.8 | 0.54 | 509 | 46.5 | 11.0 | 25.3 | 14.5 |
| | | Wheat-high N | 19.2 | -4.4 | 1.10 | 568 | 27.8 | 20.8 | 26.3 | 14.4 |
| | | Pea-low N | 19.8 | -12.6 | 0.64 | 496 | 27.4 | 18.1 | 29.9 | 14.0 |
| | | Pea-high N | 19.2 | -7.7 | 0.92 | 618 | 42.9 | 14.6 | 29.6 | 15.2 |
| | Wheat +N | Wheat-low N | 22.9 | -4.7 | 0.52 | 514 | 45.6 | 11.4 | 27.5 | 14.1 |
| | | Wheat-high N | 20.0 | -4.8 | 1.08 | 486 | 30.1 | 16.6 | 27.6 | 14.9 |
| | | Pea-low N | 19.2 | -10.4 | 0.68 | 528 | 35.3 | 15.2 | 30.1 | 14.8 |
| | | Pea-high N | 19.0 | -5.6 | 0.98 | 691 | 33.6 | 21.0 | 36.8 | 16.1 |
| | Field pea | Wheat-low N | 21.6 | 4.3 | 0.54 | 530 | 37.4 | 15.0 | 29.1 | 16.2 |
| | | Wheat-high N | 20.1 | -3.7 | 1.10 | 444 | 28.5 | 15.8 | 30.3 | 19.2 |
| | | Pea-low N | 19.7 | -11.0 | 0.67 | 451 | 30.1 | 15.2 | 29.7 | 19.3 |
| | | Pea-high N | 19.1 | -6.5 | 0.98 | 510 | 27.6 | 18.8 | 36.8 | 36.2 |
| | Non-planted | Wheat-low N | 19.3 | -1.6 | 0.55 | 485 | 55.1 | 8.8 | 30.8 | 20.4 |
| | Non-planted +N | Wheat-high N | 19.7 | -8.4 | 0.60 | 503 | 55.8 | 9.0 | 28.2 | 31.0 |
| | Non-planted | Pea-low N | 19.1 | -5.6 | 1.01 | 483 | 61.9 | 8.3 | 27.5 | 63.7 |
| | Non-planted +N | Pea-high N | 17.8 | -6.4 | 1.05 | 512 | 59.2 | 8.7 | 29.4 | 92.7 |
| | | *LSD (P=0.05)* | *1.1* | *3.7* | *0.17* | *54* | *10.5* | *4.2* | *4.2* | *5.4* |
| | | ***Significance level*** | | | | | | | | |
| | | *$CO_2$* | *ns* | *ns* | *ns* | ***** | *ns* | ***** | *ns* | *ns* |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *Plant treatment* | *ns* | * | *ns* | *** | ** | *ns* | ** | *** |
| *Residue* | *ns* | *** | *** | *** | *** | *** | *** | *** |
| *$CO_2$ × Plant treatment* | *ns* | * | * | * | *ns* | *ns* | ** | * |
| *$CO_2$ × Residue* | *ns* | *ns* | *ns* | *** | *ns* | * | *ns* | *ns* |
| *Plant treatment × Residue* | * | ** | *** | *** | * | *** | *ns* | *** |
| *$CO_2$ × Plant treatment × Residue* | *ns* | *ns* | *ns* | *** | * | *** | ** | *ns* |

Least significant difference (LSD) for one-way Residual Maximum Likelihood (REML) analyses.
Significance levels are for three-way Analyses of Variance (ANOVA), excluding non-planted controls.
ns, *, ** and *** indicate, $P>0.05$, $P<0.05$, $P<0.01$ and $P<0.001$, respectively.

**Table S1** Initial 13C and 15N abundance of residues used in the study.

| Experiment | Residue | $^{13}$C ($\delta^{13}$C PDB) | $^{15}$N (atom %) |
|---|---|---|---|
| Exp1 | Wheat | 450 | 13 |
| | Field pea | 287 | 6 |
| | | | |
| Exp2 | Low-N-Wheat | 437 | 12 |
| | High-N-Wheat | 321 | 14 |
| | Low-N-Field pea | 234 | 5 |
| | High-N- Field pea | 228 | 8 |