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Elevated CO_2 alters the rhizosphere effect on crop residue decomposition

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Abstract
Background and aims Elevated atmospheric CO_2 (eCO_2) can affect microbial decomposition of native soil organic carbon (SOC) via enhanced root exudation and rhizosphere activity. Few studies have examined the effect of eCO_2 on the decomposition of newly-added crop residues which are important to improve soil fertility and to understand below-ground C changes. A soil microcosm experiment was conducted to examine whether eCO_2 enhanced the rhizosphere effects on the decomposition of crop residues.
Methods White lupin (*Lupinus albus* L. cv. Kiev) was grown for 34 or 62 days under ambient CO_2 (aCO_2, 400 μmol mol^{-1}) or eCO_2 (800 μmol mol^{-1}) in a low-C (2.0 mg g^{-1}) soil which was amended with or without dual ^{13}C and ^{15}N labelled wheat, field pea or canola crop residues. Isotopic tracing technique was adopted to partition residue-derived CO_2 from total below-ground CO_2 efflux. Two independent groups of data were analysed statistically at either Day 34 or 62.
Results The presence of plants increased the decomposition of all residues at Day 34. This positive rhizosphere effect on residue decomposition decreased and was even reversed at Day 62, probably due to depletion of labile C, microbial N limitation and rhizosphere acidification. The

eCO_2-induced decomposition depended on residue type at Day 34. Specifically, when compared to aCO_2, the decomposition of canola residue remained the same, field pea residue higher (13.5%) and wheat straw lower (7.4%) under eCO_2. However, residue decomposition was generally higher (by an average of 13%) under eCO_2 than aCO_2 at Day 62, which was correlated positively with the increase in rhizosphere extractable C ($P < 0.01$).
Conclusions Elevated CO_2 generally increased residue decomposition in the rhizosphere, but this effect is mediated by residue type at Day 34. Enhanced residue decomposition under legumes at eCO_2 may favour the release of residue N.

Keywords ^{13}C, ^{15}N, dual-labelling, *Lupinus albus*, residue type, rhizosphere priming, root exudation.

Introduction

Soil organic carbon (SOC) sequestration has been proposed as an appropriate way to improve soil quality and productivity (Lal 2004; Smith et al. 2012) and to potentially mitigate the increasing CO_2 emissions (Meinshausen et al. 2009). The return of crop residues to the soil is a technically simple and appropriate agricultural management practice to achieve this purpose. A fraction of the incorporated residue is stabilised as soil organic matter by physical, chemical, and biochemical mechanisms (Six et al. 2002), with the remaining fraction being lost to the environment via microbial decomposition. The effectiveness of SOC stabilisation depends on the quality and quantity of residues returned to the soil and soil microbial responses. Elevated CO_2 (eCO_2) could potentially change the rhizosphere environment and therefore microbial parameters, which may further alter decomposition of soil organic carbon including crop residues.

Elevated CO_2 is anticipated to alter rhizosphere processes and affect organic-matter decomposition in three ways. Firstly, eCO_2 could alter both the quantity and quality of root-derived C substances (Phillips et al. 2011; Jia et al. 2014; Butterly et al. 2016; Calvo et al. 2017) which can regulate the microbial processing of other C sources such as crop residues and native SOC. The difference in SOC decomposition induced by plant roots is termed the rhizosphere priming effect (RPE) (Zhu et al. 2014; Nie and Pendall 2016; Wang et al. 2016). Higher priming

effects are linked to increased root exudation under eCO_2 (Bengtson et al. 2012; Nie and Pendall 2016). Secondly, plants growing under eCO_2 often exhibit larger root systems and hence large rhizosphere volumes (Dijkstra et al. 2009; Nie et al. 2013), which indicates more organic matter would be susceptible to microbial decomposition. Lastly, eCO_2 could change the soil environment. For example, eCO_2 could induce rhizosphere acidification especially in soils with low pH buffer capacity, via enhanced efflux of carboxylates and/or unbalanced cation-anion uptake by plant roots (Haynes 1990; Guo et al. 2012). Low pH can directly inhibit microbial activity, microbial community size and structure and/or substrate availability (Andersson et al. 2000; Kemmitt et al. 2006; Rousk et al. 2009), leading to decreased decomposition of SOC (Wang et al. 2016; Wang and Tang 2018). Soil moisture could also be altered by eCO_2 due to improvement in plant water-use efficiency (Cruz et al. 2016). Although several studies have examined the effect of eCO_2 on SOC decomposition (Cheng and Johnson 1998, Nie and Pendall 2016; Xu et al. 2017, 2018), the eCO_2-induced changes in the rhizosphere on residue decomposition received less attention. Apart from rhizosphere responses, distinctive types of crop residues differ intrinsically in properties like N concentration and C complexity which may affect the decomposition under eCO_2. High N could potentially ease microbial N constraints (Drake et al. 2013) and labile C is easier to decompose than chemically-complex compounds (Ruiz-Dueñas and Martínez 2009).

Many incubation experiments have been performed to examine the decomposition of residues and associated mechanisms from perspectives of residue chemistry, soil N availability as well as soil microbial activity and function (van Groenigen et al. 2005; Marx et al. 2007; Grosso et al. 2016). However, such experiments may not reflect the actual decomposition of residues in the field because plants that can affect the physical, chemical and biological environments of soil (Cheng and Kuzyakov 2005; Pregitzer et al. 2007; Wang et al. 2016) have been excluded. Due to this, there is a need to study residue decomposition in the presence of plants-i.e. the rhizosphere effect on residue decomposition.

Plants actively secrete root exudates into rhizosphere soil, which could affect soil C dynamics. Approximately 11-17% of photo-assimilates are distributed below-ground as root exudates (Nguyen 2003; Jones et al. 2009). Soil microbes utilise such C substrates for respiration and

biomass production, and the subsequent turnover of microbial biomass helps to build up SOC (Lloyd et al. 2016). Moreover, root exudates can also fuel soil microbes to decompose other C sources (e.g. crop residues and native SOC). Previous studies have demonstrated that the RPE on soil organic matter decomposition can be stimulated by up to 380% and repressed by 50% (Zhu et al. 2014), depending on plant species, soil properties and environmental parameters (Huo et al. 2017; Xu et al. 2017, 2018). The positive RPEs are mostly explained by 'co-metabolism' (Kuzyakov et al. 2000) and 'microbial N mining' (Kuzykov and Xu 2013), while 'preferential substrate utilization' and 'nutrient competition' (Cheng and Kuzyakov 2005) attribute to the negative RPEs. However, it is still largely unknown whether the presence of rhizosphere of growing plants affects decomposition of crop residues and how eCO_2 influences the decomposition processes.

The aim of this study was to examine the eCO_2-induced changes in rhizosphere effect on the decomposition of different crop residues using a stable ^{13}C isotopic tracing technique. White lupin was selected as the test plant for its strong capability of root exudation (Weisskopf et al. 2008). Dual ^{13}C and ^{15}N-labelled wheat, field pea and canola residues were mixed with a Tenosol before sowing. Below-ground CO_2 efflux was measured and residue-derived CO_2-C was partitioned at 34 and 62 days after sowing. We hypothesized that the rhizosphere effect on residue decomposition would be greater for low than high C:N ratio residues and that eCO_2 would further enhance the rhizosphere effect on the decomposition of residues.

Materials and methods

Soil description

Subsurface soil (10-30 cm) of a Tenosol (Isbell and NCST 2016) was collected from a grass pasture. The soil was air-dried, sieved to pass a 2-mm sieve with plant roots and gravels removed, and thoroughly mixed. The soil was selected for its low SOC and N content, and a similar ^{13}C signature to the roots of white lupin. A preliminary experiment showed that a low amount of CO_2 (< 2.7 μg C g^{-1} soil d^{-1}, which was only 6% of total below-ground CO_2 efflux) had been released from this soil when amended with crop residues. Therefore, the plant- and soil-derived CO_2 could be integrated as one pool with the same ^{13}C signature, which could further be discriminated from CO_2 derived from ^{13}C-enriched residues. The soil is a sandy loam

(sand 81%, silt 6%, clay 13%, Butterly et al. 2013). Other basic properties were: pH 6.2 (1:5 w/v in 0.01 *M* $CaCl_2$), pH buffer capacity 6.0 $mmol_c\ kg^{-1}\ pH^{-1}$, SOC 1.8 mg g^{-1}, total N 0.28 mg g^{-1}, K_2SO_4-extractable inorganic N 10.5 μg g^{-1} and $\delta^{13}C$ -25.6‰PDB. Soil was supplied with the following basal nutrients before the experiment (μg g^{-1}): KH_2PO_4, 180; K_2SO_4, 120; $CaCl_2.2H_2O$, 180; $MgSO_4.7H_2O$, 50; $MnSO_4.H_2O$, 15; $ZnSO_4.7H_2O$, 8; $CuSO_4.5H_2O$, 6; FeEDTA, 1.3; $CoCl_2.6H_2O$, 0.4; $Na_2MoO_4.2H_2O$, 0.4.

Crop residues

Dual ^{13}C and ^{15}N-labelled plant materials (wheat, field pea and canola shoot residues) were generated as described in Butterly et al. (2015). Briefly, wheat (*Triticum aestivum* L.), field pea (*Pisum sativum* L.) and canola (*Brassica napus* L.) plants were fertilized with ^{15}N-labelled $Ca(^{15}NO_3)_2$ (20% atom excess) and pulse-labelled 7 times with $^{13}CO_2$ by injecting 12 ml of 9.2 *M* H_2SO_4 into 90 ml of 1.23 *M* $Na_2{}^{13}CO_3$ (98% atom excess) throughout the growing season. At maturity, plant shoots were collected, oven-dried at 70 ºC and finely ground (< 2 mm). Basic chemical properties of the residues are given in Table 1.

Experimental design

Plants of white lupin (*Lupinus albus* L. cv. Kiev) were grown in bottom-capped polyvinyl chloride (PVC) columns (height 40 cm, diameter 7.5 cm). Each of the columns had an air-inlet and air-outlet tubing at the top and bottom of the column. Wheat, field pea or canola residues were mixed with 1.5 kg soil at a rate of 5 mg g^{-1} and packed into soil columns. Two hundred grams of plastic beads were enclosed in nylon mesh (diameter 45 μm) and placed at the bottom of each column before adding the soil and residue mixture to facilitate CO_2 trapping and prevent anaerobic condition. The soil was re-wetted to 80% field capacity with reverse osmosis water and allowed to equilibrate overnight.

Four pre-germinated seeds of white lupin were inoculated with a lupin rhizobial inoculant (EasyRhiz, New-Edge Microbials, Albury, Australia) and sown to 2-cm depth in a line into each column. The planted columns were then transferred into four growth cabinets (SGC 120, Fitotron, Loughborough, UK) with two receiving elevated CO_2 (eCO_2, 800 ± 30 μmol mol^{-1}, within the range of published studies) and the other two receiving ambient CO_2 (aCO_2, 400 ± 15

μmol mol^{-1}). All growth cabinets were set at temperature regimes of 18 °C night (10 h) and 22 °C day (14 h) and relative humidity of 60%. The photosynthetic active photon flux density at plant canopy was approximately 350 μmol m^{-2} s^{-1}. The columns were weighed and kept at 80% field capacity by adding reverse osmosis water daily. Plants were thinned to two seedlings per column two weeks from sowing. The columns were randomly reallocated within the two replicated growth cabinets weekly to ensure homogenous growing conditions. No additional fertiliser was applied throughout the experiment.

Overall this experiment consisted of two CO_2 concentrations, three residues and six replicates being separated into two harvests with the first one at 34 days after sowing (Day 34) and the second one at 62 days after sowing (Day 62). To ensure four replicates for below-ground CO_2 collection at each harvest, only two replicate columns were destructively harvested for soil and plant measurements at Day 34. Additionally, three sets of controls were included: two columns without residue amendment or plant growth were included at each CO_2 concentration as the control; no-residue but planted soil columns were set as the no-residue control with two replicates for each CO_2 concentration at Day 34 and four at Day 62; and unplanted columns with residue amendments in duplicate were also included for each CO_2 concentration as the no-plant control.

Because the residues might not be uniformly labelled and microbial discrimination might occur (Zhu and Cheng 2011), the ^{13}C abundances of residues may thus change over the decomposing processes, leading to variations in the ^{13}C abundances of residues and the residue-derived CO_2. To minimise such an effect when partitioning residue-derived CO_2 from total below-ground CO_2, we used the ^{13}C abundances of residue-derived CO_2 from a concurrent incubation experiment other than the original ^{13}C abundances of the residues. The incubation experiment was conducted under the same conditions except that there is no headspace CO_2 treatment. Briefly, 40 g sands were firstly mixed with pre-incubated Tenosol at a rate of 50 mg g^{-1} for microbial inoculation and then amended with one of the three residues. After adjusting the water content to 80% field capacity, the sand and residue mixtures were placed into 1-L Mason jars. To maintain the moisture, a vial with 8 ml of Milli-Q water was included. The CO_2 released during one were trapped in 8 ml of 1 *M* NaOH solution, the traps were replaced every week for 9 weeks. Two ml

of the trapped solution at the end of the fourth and ninth week was added with $SrCl_2$ to form precipitate for the quantification of ^{13}C abundance of the respired CO_2, which was used to represent the ^{13}C abundance of crop residues at each time point (Table 2).

Below-ground CO_2 trapping

At Days 34 and 62 (representing the early vegetative and early flowering stage respectively), the tops of the columns were enclosed with two clear PVC plates around plant stems, and the open spaces were sealed with Blu-tack (Bostik, Thomastown, Australia) (Wang et al. 2016, Fig. S1). The seal was checked by vacuuming CO_2-free air through each column into a 150-ml NaOH solution (1 *M*). No air leak was indicated if the bubbles formed in the solution were stable and consistent before and after pressing the adhered area.

Before trapping, the initial CO_2 inside soil pores was removed by pumping CO_2-free air through all soil columns for 30 min. The CO_2 generated inside the column during a 48-h period was then trapped into 150 ml of 0.5 *M* NaOH solution by pumping and vaccuming CO_2-free air through each column. Total below-ground CO_2 was trapped 30 min at each time and three times per day with a 6-h interval between 9:00 am and 11:00 pm. To determine the total CO_2 trapped, a subsample of the NaOH trap was firstly added with 0.5 *M* $BaCl_2$ standard solution to precipitate the carbonate and the excessive NaOH was back-titrated with 0.25 *M* HCl using the phenolphthalein indicator. Another subsample of the trapping solution was mixed with excessive 0.25 *M* $SrCl_2$ to form $SrCO_3$ precipitates (Cheng and Johnson 1998) at a pH of 7.0 to prevent the formation of $Sr(OH)_2$. The precipitates were rinsed and centrifuged three times with Milli-Q water before being oven-dried at 70 °C. The ^{13}C abundance of the $SrCO_3$ precipitate was analysed by an Isotope Ratio Mass Spectrometer ('IRMS', SerCon Hydra 20-22, Crewe, UK).

Plant and soil analyses

After each CO_2 trapping, plants were harvested and rhizosphere soils were sampled. The rhizosphere is of particular interest as it has high microbial abundance, diversity and growth rate due to root exudation (Blagodatskaya et al. 2014). Plant shoots were cut at the soil surface and roots were collected on a 2-mm soil sieve after collecting the rhizosphere soil. Roots were then washed and scanned with an EPSON EU-35 scanner (Seiko Epson Corp, Suwa, Japan) with root

length being generated from a WinRHIZO STD 1600+ image analysis system (Regent Instruments, Quebec City, Canada). Shoots and root materials were oven-dried at 70 °C for 48 h to determine dry mass.

Immediately after sampling, rhizosphere soil respiration was determined to reflect rhizosphere soil microbial activity (Wang et al. 2016) by incubating 10 g of fresh rhizosphere soil at 25 °C for 12 h and measuring the microbial respiration at the end of the period using a Servomex 4210 Industrial Gas Analyser (Servomex, Crowborough, UK). The fumigation-extraction method (Brookes et al. 1985) was adopted to determine microbial biomass C and N (MBC and MBN) in fresh rhizosphere soil. Briefly, 8 g of soil was extracted with 40 ml of 0.5 *M* K_2SO_4 solution and the extract was filtered with Whatman no. 42 filter paper and stored at -20 °C for further analysis. Another 8 g of soil was fumigated in dark with chloroform for 24 h, and then extracted and filtered with the same procedure. The soil extractants were analysed for extractable organic carbon (EOC) using a TOC analyser (GE Sievers InnovOx, Boulder, USA). The MBC was calculated as the differences in EOC concentrations between the fumigated and non-fumigated samples with a conversion factor of 0.45 (Vance et al. 1987). The soil extractants were also analysed for NH_4^+ and NO_3^- using a flow-injection analysis system ('FIA', Lachat's QuickChem 8500, Loveland, Colorado, USA) and the sum of NH_4^+ and NO_3^- was defined as extractable inorganic nitrogen (EIN). The soil extractants were further added with $K_2S_2O_8$ and oxidised in an autoclave at 120 °C for 30 min (Cabrera and Beare 1993) and measured for NO_3^- using the FIA for MBN determination. Microbial biomass N was calculated as the differences in NO_3^- concentrations between fumigated and non-fumigated soil extractants with a conversion factor of 0.54 (Brookes et al. 1985).

Air-dried rhizosphere soil was extracted with 0.01 *M* $CaCl_2$ for pH measurement and the pH obtained was termed rhizosphere soil pH. Rhizosphere soil with higher microbial abundance and diversity due to supply of available of substrates. The soil and oven-dried shoots and roots were finely ground and analysed for C and N concentrations using a CHNS/O analyser (PerkinElmer EA2400, Branford, USA). Isotopic ^{13}C and ^{15}N abundances in plant shoots were quantified using IRMS.

Calculation

Residue-derived CO_2-C

The amount of CO_2-C derived from residue was calculated by multiplying total below-ground CO_2 efflux (total CO_2 efflux) by the proportion of residue-derived CO_2 (f_{RES}) based on the following equation:

$$\textit{Residue-derived } CO_2\textit{-C} = \textit{total } CO_2 \textit{ efflux} \times f_{RES}$$

The f_{RES} was calculated according to the equation:

$$f_{RES} = (\delta^{13}C_{residue\text{-}amended\ soil} - \delta^{13}C_{no\text{-}residue\ control}) / (\delta^{13}C_{residue} - \delta^{13}C_{no\text{-}residue\ control})$$

where '$\delta^{13}C_{residue\text{-}amended\ soil}$' and '$\delta^{13}C_{no\text{-}residue\ control}$' are the $\delta^{13}C$ values of CO_2 derived from planted columns with and without residue amendment, respectively (Fig. S2); '$\delta^{13}C_{residue}$' is the $\delta^{13}C$ value of CO_2 released from residues when incubated with sands (Table 2).

Statistical analysis

The effect of CO_2 concentration, residue type and their interaction were tested at two sampling times separately for all measurements using a two-way analysis of variance (ANOVA). Significant differences ($P < 0.05$) among means were identified using the Duncan's multiple range test. Pearson's correlation analysis was performed to examine the effects of microbial biomass C-to-N ratio and rhizosphere soil pH on residue decomposition across the two growth stages and the effect of rhizosphere extractable C induced by eCO_2 on residue decomposition at Day 62. The tests were performed with Genstat (v17; VSN International, Hemel Hempstead, UK). All figures were plotted in Excel 2013 (Microsoft, Redmond, USA).

Results

Plant growth, C, N content and ^{13}C, ^{15}N abundance

The effect of eCO_2 on plant growth are different at two growth stages. There was no CO_2 treatment or residue type effect on plant biomass, root length, shoot C and N concentrations as well as shoot ^{13}C abundance at Day 34 (Table 2). However, eCO_2 decreased the ^{15}N atom% of plant shoot when field pea residue was amended, leading to a significant CO_2 × residue interaction (Table S1). Residue amendments increased shoot ^{15}N abundance when compared to non-amended controls with the highest increase being 5.3% in soil amended with field pea residue (Table S1).

At Day 62, eCO_2 increased shoot and root biomass by 23-36% and 40-48%, respectively (Table 2), indicating white lupin distributed more photosynthesized C below-ground under eCO_2. Elevated CO_2 increased the root length by 16%, 19% and 33% in the wheat, field pea and non-residue-amended soils, respectively (Table 2). Residue amendments abated the eCO_2 effect on root length, particularly when canola residue was added. Elevated CO_2 did not affect shoot N concentration or C:N ratio but decreased the $\delta^{13}C$ value of shoots generally, with the largest reduction being 2.0‰PDB when the field pea residue was incorporated and the smallest being 0.8‰PDB in the wheat straw-amended soil (Table 2). Elevated CO_2 reduced the shoot ^{15}N (atom%) by 0.5% in the wheat straw-amended soil, but increased it by 1.6% when field pea residue was amended (Table S1).

Residue decomposition

The rhizosphere effect accelerated residue decomposition when compared to the no-plant control. A positive rhizosphere effect on residue decomposition was found at Day 34. Specifically, the residue-derived CO_2 was 7.0-8.8 μg C g^{-1} soil d^{-1} from planted columns which was higher than the amount of CO_2 (2.7-4.8 μg C g^{-1} soil d^{-1}) evolved from their corresponding unplanted columns (Fig. 1A). When compared to aCO_2, the decompositon of canola residue remained the same, wheat straw lower (7.4%) and field pea residue higher (13.5%) under eCO_2, leading to a CO_2 × residue interaction (Fig. 1A).

The positive rhizosphere effect on residue decomposition decreased and was even reversed at Day 62 (Fig. 1B). On average, the decomposition rates of wheat and field pea residues were both 3.9 μg C g^{-1} soil d^{-1} in unplanted columns (the arrow-ended dash lines on Fig. 1B), while their corresponding decomposition rates were only 2.6 and 2.4 μg C g^{-1} soil d^{-1} in planted soils. The decomposition rate of canola residue was 2.0 μg C g^{-1} soil d^{-1} in the unplanted column which was still lower than that (2.9 μg C g^{-1} soil d^{-1}) in the planted column (Fig. 1B). When compared to aCO_2, the decomposition of residue was 13%, 15% and 11% higher under eCO_2 for the wheat, field pea and canola residue, respectively (Fig. 1B). Besides, canola residue exhibited the highest decomposition rate at this stage under both CO_2 concentrations.

Total below-ground CO_2 efflux and its ^{13}C abundance

The presence of white lupin increased the total below-ground CO_2 efflux (Fig. 2). For example, at Day 34, soil respiration from the unplanted columns (the arrow-ended dash lines on Fig. 2A) was only 5.3-5.7 μg C g^{-1} soil d^{-1} when residues were amended, however, the respiration rate amounted up to 33.2-45.3 μg C g^{-1} soil d^{-1} in the correspnding planted columns. This positive rhizosphere effect on below-ground CO_2 efflux was further increased by 19-36% by eCO_2 when compared to aCO_2 (Fig. 2A). At Day 62, the total below-ground CO_2 efflux increased to 48.4-64.1 μg C g^{-1} soil d^{-1} in the planted columns (Fig. 2B). Elevated CO_2 increased the total below-ground CO_2 efflux by 33% and 13% in field pea and canola residue-amended soils, respectively (Fig. 2B).

The $\delta^{13}C$ value of CO_2 from no-residue control was similar between the two CO_2 levels and was constant throughout the experiment (-23.3 to -25.6‰PDB) (Fig. S2). The amendment of ^{13}C-labelled residue yielded a much higher $\delta^{13}C$ value of below-ground CO_2 and the value decreased with time. At Day 34, the field pea residue treatment showed the highest ^{13}C abundance of total below-ground CO_2 (55.2‰PDB), followed by the wheat straw (43.2‰PDB) and the canola residue treatment (14.5‰PDB). At Day 62, the $\delta^{13}C$ value of total below-ground CO_2 was -7.9‰PDB and -9.9‰PDB, respectively, for the treatments of wheat straw and field pea residue. Total CO_2 effluxed from the soil amended with canola residue showed the lowest $\delta^{13}C$ value, which was -5.8‰PDB (Fig. S2). On average, eCO_2 decreased the $\delta^{13}C$ value of CO_2 evolved from residue-amended columns at both stages and the decrease was 37% and 21% at Days 34 and 62, respectively (Fig. S2).

Rhizosphere soil respiration

Rhizosphere soil respiration (12 h) was 163-284 μg CO_2 g^{-1} soil at Day 34 (Fig. 3A). Elevated CO_2 had no significant effect on rhizosphere soil respiration. A strong effect of residue type was found with field pea residue inducing, on average, 1.3-1.5-fold higher rhizosphere soil respiration than other residues (Fig. 3A). At Day 62, the soil respiration from unplanted soils was only 0-38.4 μg CO_2 g^{-1} soil (the arrow-ended dash lines on Fig. 3B). The amounts of CO_2 released from rhizosphere soils ranged 318-434 μg CO_2 g^{-1} soil (Fig. 3B). Elevated CO_2 increased the rhizosphere soil respiration by 18% and 25% in the wheat and field pea residue-

amended soils, respectively, but not in the canola residue and no residue-amended soils, leading to a CO_2 × residue interaction (Fig. 3B).

Soil pH in the rhizosphere

White lupin acidified its rhizosphere by decreasing the original soil pH from 6.20 to 5.29-6.03 at Day 34 (Table 3). The rhizosphere soil pH was further decreased to 4.33-4.76 by Day 62 (Table 3). At Day 34, eCO_2 decreased the rhizosphere soil pH by around 0.3 units except for the wheat straw-amended soil (Table 3). The amendment of residues yielded higher pH when compared to no-residue control (Table 3) probably due to the alkalinity effect of crop residues (Wang et al. 2017). Elevated CO_2 also decreased the rhizosphere soil pH (by an average of 0.18 units) at Day 62 with the greatest reduction being 0.4 units when wheat straw was amended (Table 3). On average, the soil amended with field pea residue showed the highest rhizosphere soil pH which was 0.33 units higher than the no-residue control (Table 3).

Rhizosphere K_2SO_4-extractable organic C (EOC) and inorganic N (EIN)

At Day 34, eCO_2 increased the concentrations of EOC by 42% and 49% in the canola residue-amended and no-residue soils, respectively (Table 3). On average, soils amended with field pea residues showed the highest EOC, followed by canola residue, no-residue controls and wheat straw (Table 3). The concentration of EOC was 2.7 to 6.9-fold greater at Day 62 than at Day 34. Elevated CO_2 increased the EOC concentration in all residue-amended treatments, with the increases being 80%, 63%, 44% and 21% in the wheat straw, no-residue control, field pea residue and canola residue-amended soils, respectively (Table 3). Moreover, residue decomposition rate was positively correlated with rhizosphere extractable C at Day 62 ($P < 0.01$, Fig. 4).

The original soil EIN was 10.5 μg g^{-1}. Growing white lupin dropped the value to 0.05-1.93 μg g^{-1} and 0.67-1.09 μg g^{-1} at Day 34 and 62, respectively. The treatment effect was signifcant at Day 34 with a CO_2 × residue interaction being detected as eCO_2 increased the EIN in the wheat straw and canola residue-amended soils (Table 3). In general, the EIN was higher at Day 62 when compared to Day 34. There tended to be higher EIN under eCO_2 when compared to aCO_2 at Day 62, but the difference was not statistically significant.

Microbial biomass C (MBC), N (MBN) and C to N ratio (MBC:N)

Soil MBC in the rhizosphere ranged from 73-176 μg C g^{-1} soil at Day 34 (Table 3). Neither CO_2 level nor residue type had a significant effect on MBC (Table 3). At Day 62, the MBC was increased to 284-506 μg C g^{-1} soil (Table 3). On average, eCO_2 increased the MBC by 20% at this stage. Soil MBN was higher in residue-amended soils at both harvests, leading to lower MBC:N when compared to no-residue control columns. On average, eCO_2 increased the MBN by 23% at Day 62. The MBC:N was higher at Day 62 than Day 34 (Table 3). It correlated negatively with residue decomposition rate ($R^2 = 0.64$, $P < 0.01$) across the two growth stages.

Total soil C in rhizosphere

At Day 34, eCO_2 had no significant effect on the concentration of SOC in the rhizosphere. Not surprisingly, the residue amendments raised the rhizosphere SOC when compared to the no-residue controls, with the largest and smallest increases being 94% and 55% in field pea and canola residue-amended soils, respectively (Table 3). Higher SOC was observed in the rhizosphere at Day 62 than at Day 34, indicating a net C deposition. On average, eCO_2 enhanced SOC, with the increases being 6%, 9%, 6% and 19% for wheat straw, field pea residue, canola residue-amended and non-amended soils, respectively. Residue amendments increased the rhizosphere SOC by an average of 46% (Table 3).

Discussion

Rhizosphere effects on residue decomposition

This present study showed that the direction and magnitude of rhizosphere effects on residue decomposition were different at two growth stages. Specifically, the presence of white lupin increased the decomposition of all three crop residues (positive rhizosphere effects) at Day 34. However, the rhizosphere effects declined and even became negative in soils amended with wheat and field pea residues at Day 62. The decreased decomposition is consistent with some previous studies (Cotrufo and Ineson 1996; Lam et al. 2014; Butterly et al. 2016). For example, Butterly et al. (2016) reported a decreased decomposition of both wheat and field pea residues in the rhizosphere of either wheat or field pea at about 7-8 weeks after planting when compared with controls without plants. The negative rhizosphere effect was explained by preferential

substrate utilisation (Blagodatskaya et al. 2011) in their studies. In this study, the roots of white lupin might have released larger amounts of low-molecular-weight substrates at Day 62 as indicated by the greater rhizosphere extractable organic C and rhizosphere soil respiration (Table 3; Fig. 3B). However, it did not seem to have changed the pattern of microbial substrate utilisation as a positive relationship between residue decomposition and EOC was observed (Fig. 4). Other mechanisms must exist accounting for the change in the direction of decomposition.

The labile portion of residue C degrades faster in plant rhizospheres than in bulk soil probably due to stimulated microbial activity by root exudation (Cheng and Kuzyakov 2005). Therefore, less variable dissolved organic C would have been left in the planted columns at the later decomposing stage by comparison to the unplanted controls. This could partly explain the reduced positive rhizosphere effects on all residue decomposition at Day 62. Similarly, in a meta-analysis, Luo et al. (2016) discovered that the decomposition of SOC at a specific time correlated positively with the instantaneous quantity of remaining fresh C. Besides, the decreased rhizosphere effect on residue decomposition could be associated with rhizosphere N status. Compared to Day 34, soil microorganisms were extremely N-limited at Day 62 as shown by enhanced microbial C-to-N ratio (Table 3) as plants and/or microbes depleted soil of available N. This might have inhibited microbial decomposing capacity as residue decomposition rate was negatively correlated with microbial C to N ratio ($R^2 = 0.64$, $P < 0.01$, data not shown) across the two growth stages.

Moreover, the significant decrease in rhizosphere soil pH may have also contributed to the decreased decomposition at Day 62. The Tenosol used in this study has a low soil pH buffer capacity (6 $mmol_c\ kg^{-1}\ pH^{-1}$). Growing white lupin acidified the rhizosphere soil possibly by excess uptake of cations over anions (Tang et al. 1999). Rhizosphere soil pH dropped greatly in this study especially at the later growth stage (Table 3) when white lupin showed vigorous root growth and activity (Table 2, Fig. 3B). Residue decomposition was greater in high rhizosphere soil pH conditions ($R^2 = 0.82$, $P < 0.01$, data not shown). Previous studies have shown that low soil pH could strongly affect the composition and/or activity of the soil microbial community, thereby decreasing microbial degrading ability (Andersson et al. 2000; Kemmitt et al. 2006; Rousk et al. 2009). For example, Rousk et al. (2009) showed that low pH (4.5) favoured fungal

over bacterial growth and induced a fungal functional redundancy, leading to a decreased in C mineralization. A recent study further confirmed that residue decomposition dropped linearly with the decrease of soil pH (Aye et al. 2016). Moreover, low soil pH could also affect the growth of plants and their rhizodeposition/root exudation, but this may not be valid in the case of acid-tolerant species (e.g. white lupin in this study) (Huyghe 1997). This study, for the first time, showed that rhizosphere acidification might also be a factor that regulates residue decomposition in the rhizosphere.

Interestingly, negative rhizosphere effects on residue decomposition were found for wheat straw and field pea residue but not for canola residue at Day 62. Canola residue has the highest C:N ratio (54) and the least labile C (36.4 mg g^{-1}) when compared to wheat and field pea residues. In addition, it contains relatively more lignin (Table 1) and structural carbohydrates (Lupwayi et al. 2004), which could make it more recalcitrant to microbial degradation (Ruiz-Dueñas and Martínez 2009) because these materials are more energetically costly to decompose (Saar et al. 2016). This assumption is supported by the lower decomposition rates of canola residue in the no-plant controls when compared to wheat and field pea residues (arrow-ended dash lines on Fig. 1). However, this substrate quality-suppressed microbial decomposition of canola residue vanished at the presence of white lupin (canola residue, Fig. 1B) probably because root-derived C compounds highly stimulated the growth of soil microbes and/or their degrading ability as stated by de Graaff et al. (2009) and Bengtson et al. (2012), leading to the positive rhizosphere effect on canola residue decomposition at Day 62. Moreover, residue amendment tended to decrease root and/or rhizosphere respiration with enhanced residue decomposition as it did not affect the total below-ground CO_2 efflux when compared to the no-residue control (Fig. 2). This could be caused by possible allelopathic effects of the residues and by microbial N competition with growing plants (Lam et al. 2013).

The effects of elevated CO_2 on rhizosphere residue decomposition

Residue decomposition was higher under eCO_2 than aCO_2 at Day 62, but the results were distinct for different residues at Day 34, indicating both residue type and CO_2 concentration could affect residue decomposition in the rhizosphere. The discrepancy might derive from the different CO_2-

effect on the amount and quality of root exudates at the two growth stages and from the different properties of residues, such as C availability, C to N ratio and biochemical recalcitrance.

Greater root exudation is expected under eCO_2 especially at the later growth stage when plant roots secrete more labile C substrates (Sugiyama and Yazaki 2012), expand and explore larger volumes of soil (Paterson et al. 2008). This is evidenced by the greater EOC under eCO_2 when compared to aCO_2, particularly at Day 62 (Table 3) and the greater rhizosphere respiration at Day 62 than Day 34 (Fig. 3B). In comparison with aCO_2, residue decomposition was higher under eCO_2 at Day 62 and the decomposition rates were positively correlated with rhizosphere EOC ($P < 0.01$) (Fig. 4), which concurs with the results of Bengtson et al. (2012) when linking the decomposition of SOC with the rate of root exudation. The increased decomposition is due to microbial responses to enhanced root exudates. For example, eCO_2 might have activated soil microbial activity and/or growth as shown by the increased rhizosphere soil respiration and the increasing trend of MBC in residue-amended soils, complying with the co-metabolism theory (Kuzyakov et al. 2000; Cheng and Kuzyakov et al. 2005; Zhu et al. 2014). Moreover, the presence of more root exudates by the development of white lupin under eCO_2 may also chemically liberate soil mineral-protected nutrients via complexation and dissolution (Keiluweit et al. 2015; Yuan et al. 2018) and therefore could alleviate microbial nutrient limitation to some extent, leading to greater decomposition of crop residues. Furthermore, eCO_2 could potentially change the composition of root exudates with more N rhizodeposition (de Graaff et al. 2007). The greater inputs of these N-rich root exudates could activate the growth and/or activity of the soil microbial community to degrade SOC and/or residue C (Butterly et al. 2016; Xu et al., 2018) as N in exudates can alleviate microbial constraints (Drake et al. 2013).

Except the quantity and quality of root exudates, residue properties (e.g. C:N ratio and molecular degradability) could also affect the decomposition under eCO_2 and this effect is more pronounced at Day 34 when the eCO_2 effect on plant growth was relatively minor. Although plant growth was not significantly enhanced by eCO_2, root exudation could still be stimulated due to disproportional distribution of photosynthetic C below-ground (Cheng and Johnson 1998; Butterly et al. 2016). The increased root exudation under eCO_2 only stimulated field pea residue decomposition probably due to the presence of more labile materials and N (C:N ratio of 29).

The higher C:N ratio (46) wheat straw, however, could have shifted the microorganisms to utilise the root-derived C compounds under eCO_2, leading to a decrease in decomposition–the so-called 'preferential substrate utilisation' theory (Blagodatskaya et al. 2011). However, the canola residue has a similar C:N ratio (54) to wheat straw but its decomposition was not affected by eCO_2. It may relate to the biochemical recalcitrance of the canola residue (e.g. less labile C, more lignin) as low-quality residue could delay its responses to microbial decomposition (Partey et al. 2013). The eCO_2 effect could also be missed from the two small windows of measurements in this present study. As a result, a system that enables better temporal resolution of gas measurements is required in future studies.

Conclusions

Using the stable isotope tracing technique, this study examined the rhizosphere effects on crop residue decomposition under eCO_2. Residue decomposition was different at two growth stages and further regulated by residue type. The decomposition rate was enhanced at Day 34 but inhibited at Day 62 by the presence of white lupin, because of changes in labile residue-C content, microbial N limitation and rhizosphere soil pH over time. In general, eCO_2 induced higher decomposition of crop residues at Day 62 via microbial responses to enhanced root exudation. Moreover, residue C:N ratio and degradability also affected the decomposition under eCO_2 at Day 34. Our results imply that the greater residue decomposition induced by eCO_2 in the rhizosphere of leguminous plants may stimulate C turnover and release of residue-N for later plant uptake. Further research is required to improve temporal resolution of gas measurements and to simultaneously examine the effects of eCO_2 on the decomposition of both residue and native soil C to better understand below-ground C cycling.

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Table 1 Basic chemical properties of ^{13}C and ^{15}N dual-labelled shoot residues used in this experiment

Residue type	Water-soluble C (mg g^{-1})	Total C (mg g^{-1})	Total N (mg g^{-1})	C:N	Klason lignin (mg g^{-1})	$\delta^{13}C$ (‰PDB)	^{15}N (atom%)
Wheat	43.7	421	9.2	46	203	497	15.7
Field pea	46.4	415	14.4	29	183	500	11.6
Canola	36.4	431	8.0	54	238	222	15.8

Water-soluble C (1:25, w/v)

Table 2 Shoot and root dry weights, root length, shoot C, N concentration and ^{13}C abundance of white lupin growing in Tenosol soil with or without the amendment of wheat, field pea and canola residues in aCO_2 (400 μmol mol^{-1}) or eCO_2 (800 μmol mol^{-1}) environment for 34 or 62 days. $\delta^{13}C_{residue}$ represented the $\delta^{13}C$ values of CO_2 evolved from incubated sands ($\delta^{13}C_{residue}$) amended with either wheat, field pea or canola residue

Residue	CO_2	Shoot (g $column^{-1}$)	Root (g $column^{-1}$)	Root length (m $column^{-1}$)	Shoot C (mg g^{-1})	Shoot N (mg g^{-1})	Shoot $\delta^{13}C$ (‰PDB)	$\delta^{13}C_{residue}$ (‰PDB)
				Day 34				
Wheat	aCO_2	1.93	0.87	11.9	441	40.5	-23.9	341
	eCO_2	1.69	0.80	11.7	433	43.2	-24.8	
Field pea	aCO_2	1.68	0.75	11.5	430	41.3	-23.0	340
	eCO_2	1.85	0.95	13.9	435	42.1	-24.6	
Canola	aCO_2	1.85	0.82	11.8	437	46.7	-24.6	158
	eCO_2	1.92	0.91	10.6	436	44.7	-25.8	
No-residue	aCO_2	1.84	0.74	10.2	435	45.0	-25.9	
	eCO_2	2.30	1.00	11.6	435	42.7	-26.6	
Significance level								
CO_2		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	
Residue		n.s.	n.s.	n.s.	n.s.	ns	n.s.	
CO_2 × residue		n.s.	n.s.	n.s.	n.s.	ns	n.s.	
				Day 62				
Wheat	aCO_2	7.96d	3.06b	35.8cde	442b	36.0	-25.2ab	307
	eCO_2	9.90ab	4.29a	41.4ab	443b	37.1	-26.0bc	
Field pea	aCO_2	7.49d	2.70b	33.4de	448a	38.6	-24.5a	303
	eCO_2	9.25bc	3.85a	39.7abc	442b	37.2	-26.5bc	
Canola	aCO_2	7.73d	2.79b	37.7bcd	450a	40.0	-24.0a	134
	eCO_2	10.50a	4.14a	39.4abc	443b	36.9	-25.9bc	
No-residue	aCO_2	8.37cd	2.93b	32.6e	449a	39.3	-25.1ab	
	eCO_2	10.50a	4.19a	43.4a	447ab	36.7	-26.8c	
Significance level								
CO_2		***	***	***	**	n.s.	***	
Residue		*	n.s.	n.s.	*	n.s.	n.s.	
CO_2 × residue		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	

The values followed by a common lower-case letter represent no significant difference within one column at each growth stage. n.s., *, ** and *** represent P >0.05, <0.05, <0.01 and <0.001, respectively.

Table 3 Rhizosphere pH, K_2SO_4-extractable organic C (EOC) and inorganic N (EIN), microbial biomass C (MBC), N (MBN) and C to N ratio (MBC:N), and soil organic C (SOC) in white lupin-planted Tenosol with or without wheat, field pea and canola residue amendments under aCO_2 (400 μmol mol^{-1}) or eCO_2 (800 μmol mol^{-1}) at Day 34 or 62

Residue type	CO_2	pH	EOC (μg g^{-1})	EIN (μg g^{-1})	MBC (μg g^{-1})	MBN (μg g^{-1})	MBC:N	SOC (mg g^{-1})
				Day 34				
Wheat	aCO_2	5.36de	75bc	0.09c	133	11.8ab	11.2c	3.00cd
	eCO_2	5.48bcd	71bc	1.93a	129	10.3ab	12.7bc	3.26abc
Field pea	aCO_2	6.03a	116a	0.48bc	146	15.7ab	9.2c	3.56a
	eCO_2	5.71b	124a	0.05c	138	13.1ab	10.6c	3.54ab
Canola	aCO_2	5.66bc	88bc	0.19c	159	17.3a	9.0c	2.61d
	eCO_2	5.35de	125a	0.84b	176	19.6a	11.5c	3.06bc
No-residue	aCO_2	5.41cde	65c	0.30bc	73	2.3b	31.7a	1.74e
	eCO_2	5.18e	98ab	0.84b	117	6.0ab	21.6b	1.93e
Significance level								
CO_2		*	*	**	n.s.	n.s.	n.s.	n.s.
Residue		***	***	*	n.s.	*	***	***
CO_2 × residue		n.s.	n.s.	**	n.s.	n.s.	n.s.	n.s.
				Day 62				
Wheat	aCO_2	4.76a	231e	0.84	393ab	11.6bcd	33.6ab	3.39a
	eCO_2	4.36cd	417ab	0.67	463a	20.8a	22.3b	3.59a
Field pea	aCO_2	4.72ab	311cd	0.75	284b	14.5bcd	19.6b	3.51a
	eCO_2	4.66ab	449a	1.09	365ab	17.3ab	21.0b	3.81a
Canola	aCO_2	4.54bc	295cde	0.86	418ab	14.8abcd	28.5b	3.55a
	eCO_2	4.35cd	358bc	1.01	447a	15.9abc	28.2b	3.76a
No-residue	aCO_2	4.40cd	254de	0.78	391ab	10.8cd	36.2ab	2.26b
	eCO_2	4.33d	416ab	1.06	505a	9.6d	52.6a	2.68b
Significance level								
CO_2		***	***	n.s.	*	*	n.s.	*
Residue		***	*	n.s.	n.s.	*	*	***
CO_2 × residue		n.s.	*	n.s.	n.s.	n.s.	n.s.	n.s.

The values followed by a common lower-case letter represent no significant difference within one column at each stage. The main effects of CO_2 and residue type, and their interactions were shown as n.s. ($P > 0.05$), * ($P < 0.05$), ** ($P < 0.01$), or *** ($P < 0.001$)

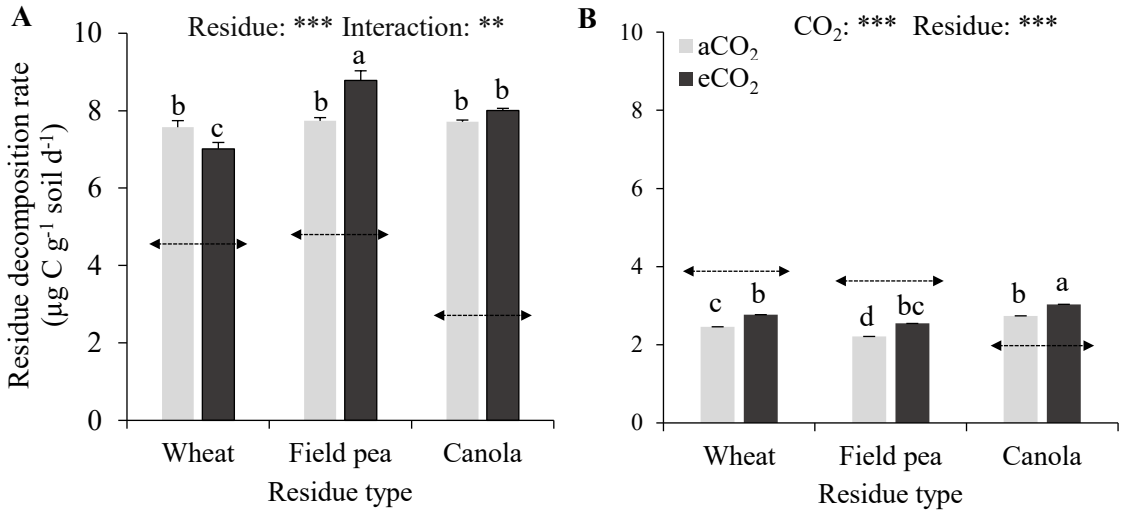

Fig. 1 The rhizosphere effect of white lupin under aCO_2 (400 μmol mol^{-1}) or eCO_2 (800 μmol mol^{-1}) on the decomposition of wheat, field pea and canola residues at Day 34 (**A**) and 62 (**B**). The arrow-ended dash lines represent residue decomposition rates in unplanted controls. Error bars represented standard errors of means. Means with a common lower-case letter represent no significant difference within a panel. The main effects of CO_2, residue and the interaction are shown as: ** ($P < 0.01$) and *** ($P < 0.001$)

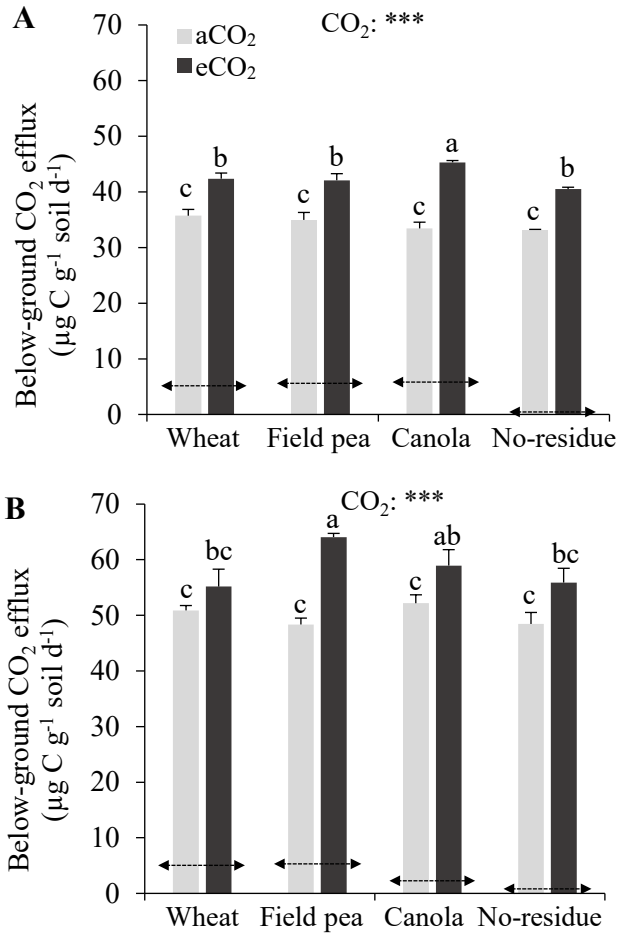

Fig. 2 Below-ground CO_2 efflux from Tenosol soil with or without amendment of wheat, field pea and canola residues under white lupin grown in aCO_2 (400 µmol mol^{-1}) or eCO_2 (800 µmol mol^{-1}) environment for 34 (**A**) or 62 days (**B**). The arrow-ended dash lines represent CO_2 released from unplanted soils with or without residue amendment. Error bars represented standard errors of means. Means with a common lower-case letter represent no significant difference within a panel. The main effect of CO_2 was highly significant (***, $P < 0.001$) but the main effect of residue or the interaction was not significant ($P > 0.05$)

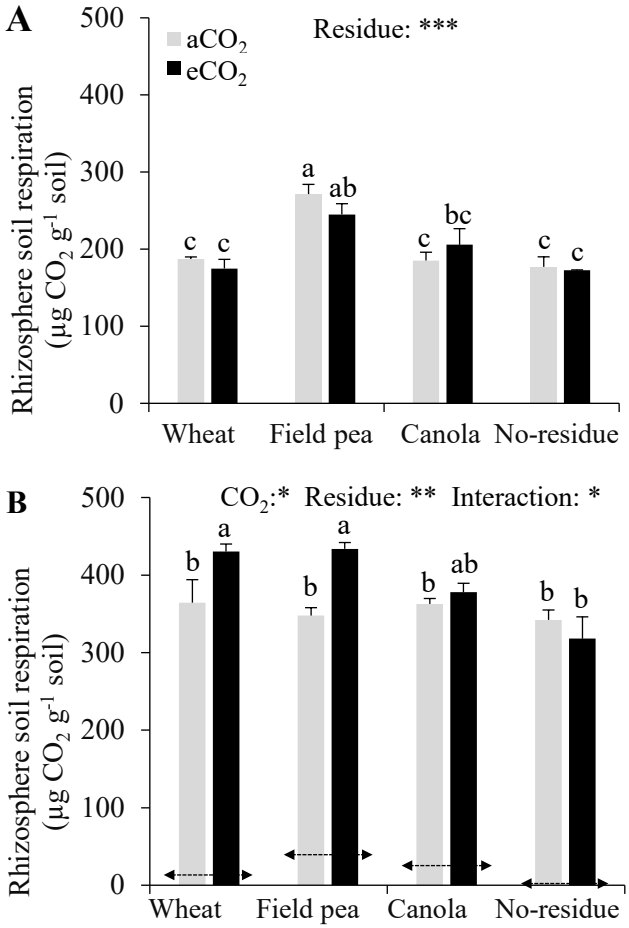

Fig. 3 Respiration (12 h) of rhizosphere soil collected from soil columns planted with white lupin with or without amendment of wheat, field pea and canola residues under either aCO_2 (400 µmol mol^{-1}) or eCO_2 (800 µmol mol^{-1}) environment for 34 (**A**) or 62 (**B**) days. The arrow-ended dash lines represent microbial respiration (12 h) of unplanted soils with or without residue amendment at Day 62. Error bars represented standard errors. Means with a common lower-case letter represent no significant difference within a panel. The main effects of CO_2, residue or the interaction were shown as * ($P < 0.05$), ** ($P < 0.01$) and *** ($P < 0.001$)

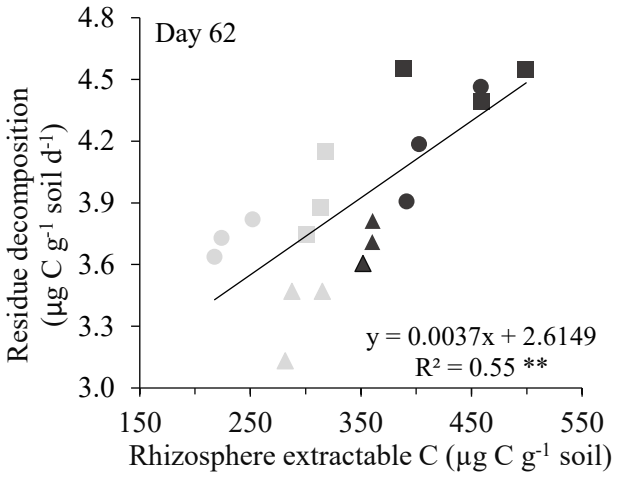

Fig. 4 The relationship between K_2SO_4-extractable C in rhizosphere soil and residue decomposition at Day 62 (**, $P < 0.01$). Wheat, field pea and canola residue treatments were indicated by circles, triangles and squares, respectively. Grey and black symbols represent aCO_2 and eCO_2, respectively.

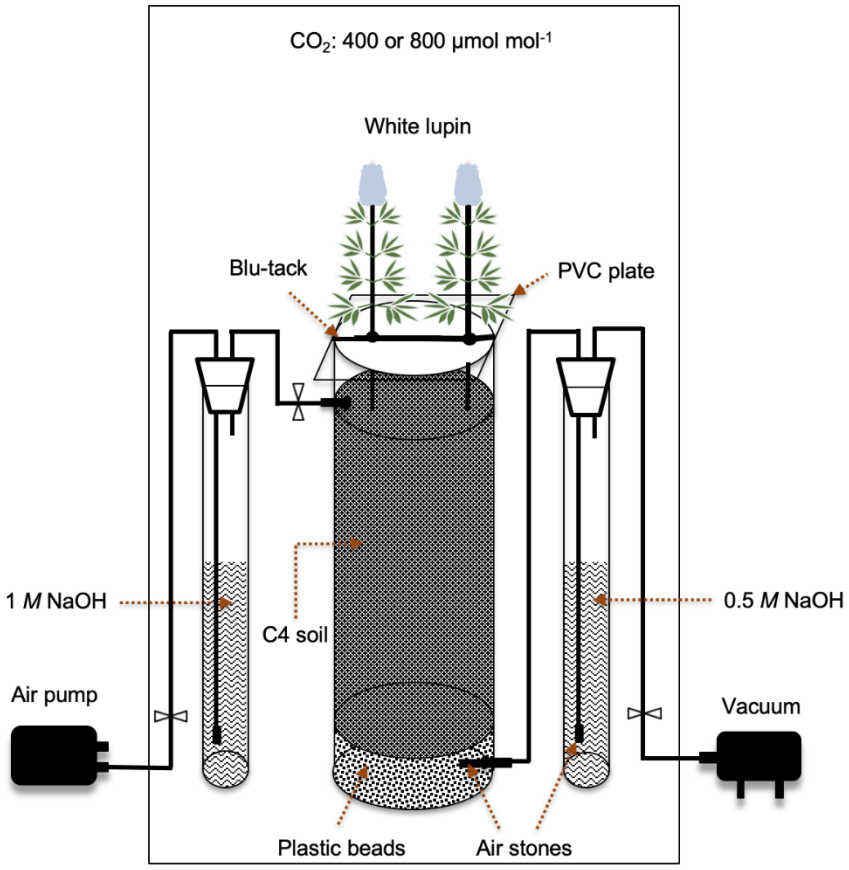

Fig. S1 Diagram of below-ground CO_2 trapping system (modified from Wang et al. 2016)

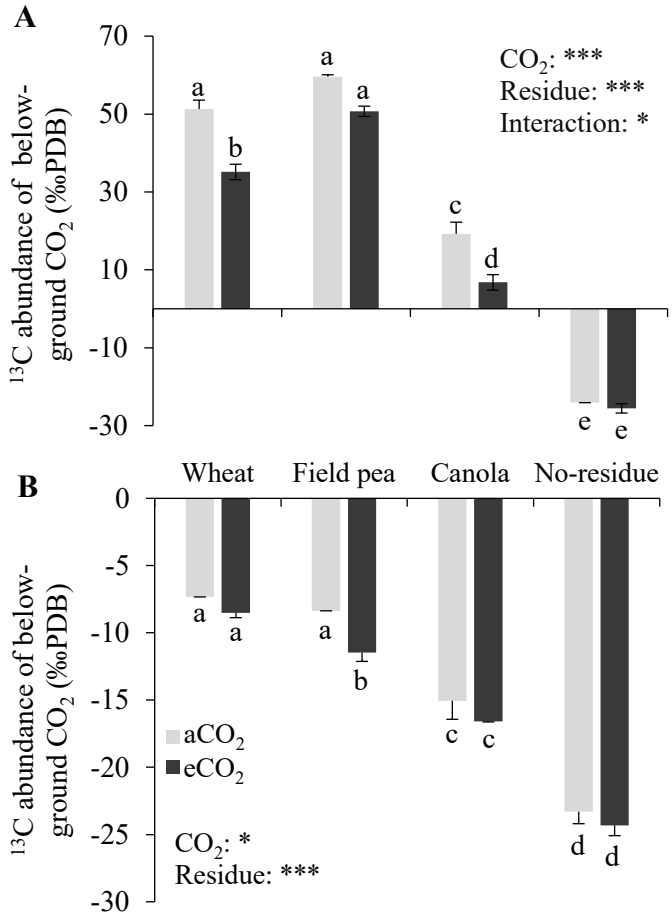

Fig. S2 ^{13}C abundance of total below-ground CO_2 trapped from Tenosol soil with or without amendment of wheat, field pea and canola residues under white lupin grown in aCO_2 (400 µmol mol^{-1}) or eCO_2 (800 µmol mol^{-1}) environment for 34 (**A**) or 62 days (**B**). Error bars represented standard errors of means. Means with a common lower-case letter represent no significant difference within a panel. The main effect of CO_2, residue and their interaction were shown as: * ($P < 0.05$) and *** ($P < 0.001$)

Table S1 Shoot ^{15}N abundance (atom%) of white lupin growing in Tenosol soil with or without the amendment of wheat, field pea and canola residues in aCO_2 (400 µmol mol^{-1}) or eCO_2 (800 µmol mol^{-1}) environment for 34 or 62 days

Residue	CO_2	Shoot ^{15}N (atom%)	
		Day 34	Day 62
Wheat	aCO_2	0.378c	0.375c
	eCO_2	0.378c	0.373d
Field pea	aCO_2	0.388a	0.384b
	eCO_2	0.384b	0.390a
Canola	aCO_2	0.379c	0.374cd
	eCO_2	0.379c	0.373d
No-residue	aCO_2	0.367d	0.366e
	eCO_2	0.366d	0.366e
Significance level			
CO_2		*	*
Residue		***	***
CO_2×Residue		**	***