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Wheat and white lupin differ in rhizosphere priming of soil organic carbon under elevated CO_2

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Abstract

Elevated CO_2 (eCO_2) alters plant rhizosphere processes and soil microbial properties which in turn change the decomposition of native soil organic carbon (SOC), termed as rhizosphere priming effect (RPE). A column experiment was conducted to examine the effect of eCO_2 (800 ppm) on the RPEs of wheat (*Triticum aestivum* L.) and white lupin (*Lupinus albus* L.) at two growth stages. Plants were grown for 34 and 62 days in C4 soil, allowing the separation of SOC-derived CO_2 and root-derived CO_2. Elevated CO_2 increased the RPEs of white lupin at both growth stages by 78% and 47% respectively. It also increased the rhizosphere soil respiration (63%) and microbial biomass carbon (MBC, 43%) of white lupin at Day 62. . In contrast, no positive eCO_2 effect on wheat RPEs was found. Elevated CO_2 decreased wheat RPE by 22% at Day 62. It increased wheat root C: N ratio (18%) and decreased soil available N (23%), indicating poorer substrate quality and less microbial energy and nutrient. It is concluded that white lupin responds greater than wheat to eCO_2 in stimulating RPE due to its higher quantity and quality of root exudates.

Key words

Elevated CO_2, *Lupinus albus*, N limitation, rhizosphere priming effect, root exudates, stable isotopes, *Triticum aestivum*

Introduction

The CO_2 concentration in the atmosphere will reach 600-800 μmol mol^{-1} by the end of this century (Meinshausen *et al.*, 2011). High atmospheric CO_2 concentration stimulates plant photosynthesis and enhances photosynthetic inputs into below-ground via rhizodeposition (Pendall *et al.*, 2004; Bazot *et al.*, 2006; Paterson *et al.*, 2008; de Graaff *et al.*, 2009; Phillips *et al.*, 2011), which provide labile substrates to relative C-limited soil microorganisms to degrade indigenous soil organic carbon (SOC). This rhizodeposition-induced change in the decomposition of SOC is termed as rhizosphere priming effect (RPE) (Cheng *et al.*, 2014).

Root exudates accounts for 58-95% of the observed RPEs (Shahzad *et al.*, 2015). Therefore any changes in root exudation by eCO_2 would yield differences in RPEs. Greater RPE is anticipated under eCO_2 by comparison to aCO_2 due to an increased input of easily decomposable C under eCO_2. From another perspective, eCO_2 changes the chemical composition in plants to more production of non-structural carbohydrates, and the synthesis of more phenolics (Peñuelas *et al.*, 1996, 1997). The increases of such substances may facilitate soil C sequestration because they are biologically resistant to microbial decomposition. If eCO_2 would also alter the chemical composition of root exudates, less RPEs are expected to occur under eCO_2. Furthermore, eCO_2 enhances biological N_2 fixation in legumes, which in turn provides extra N for these plants (Hartwig & Sadowsky, 2006; Lam *et al.*, 2012; Butterly *et al.*, 2016; Tobita *et al.*, 2016). As a result, the responses of legumes and cereals to eCO_2 may differ in their N status, and hence chemical compositions of their root exudates, ultimately affecting RPEs.

Exceptthe changes in the amount and quality of root exudates, eCO_2 could also alter RPEs via its influences on soil variables such as soil N availability and pH. For example, eCO_2 has been reported to decrease soil N availability, mainly in the form of nitrate (NO_3^-) (Hovenden *et al.*, 2008) possibly by increasing microbial immobilization and reducing the rate of gross N mineralization (Berntson & Bazzaz, 1997; Hungate *et al.*, 1999). Stimulated labile C inputs and lower soil N availability under eCO_2 can increase SOC mineralisation because soil microorganisms utilize labile substrates as energy to acquire N from more stable soil organic matter (the so-called 'microbial N mining' theory) (Craine *et al.*, 2007). Moreover, pH is a dominant factor affecting soil microbial activities, community structures and functions, such as SOC decomposition (Blagodatskaya & Kuzyakov, 2008). Elevated CO_2 could change rhizosphere soil pH by affecting cation-anion uptake of plant roots (Guo *et al.*, 2012) through this way the RPEs would also be changed by eCO_2.

Plant species differ naturally in their releasing pattern of root exudates. For example, some legume plants (e.g. white lupin) exude large amounts of low molecular-weight anions (carboxylates) into their rhizospheres, especially when soil P is limited (Veneklaas *et al.*, 2003), in contrast, cereal plants (e.g. wheat) have extremely low amounts of root exudates (Weisskopf *et al.*, 2008). The release of root exudates is also controlled by plant developmental stages. For instance, white lupin secretes small amounts of organic acids (mainly malate) at early stages, however, it exudes larger amounts of citrate at the mature stage (Sugiyama & Yazaki, 2012). The changes in the composition and amounts of root exudates could drive distinct microbial growth and function as well as their decomposition of SOC. Besides, root architecture could be another plant trait that affects RPE. Cereal species such as wheat have longer and finer roots which can explore larger volume of soil than legumes such as white lupin. This possibly means more SOC in wheat rhizosphere are subjected to microbial decomposition than in white lupin rhizosphere (Weisskopf *et al.*, 2008).

The objectives of this study were 1) to examine the effect of eCO_2 on RPE and 2) to compare the effects of cereal and legume species on RPEs under eCO_2. Wheat and white lupin were chosen as test plants. They represent common cereal and leguminous crop species, respectively, and differ substantially in root exudation and root architecture. We hypothesized that 1) eCO_2 would stimulate the RPEs due to an increase in root exudation; 2) white lupin responds greater than wheat to eCO_2 in stimulating RPE due to its higher quanity and quality of root exudates.

Materials and methods

Surface soil (0-10 cm) was collected from a native C4 kangaroo grassland (*Themeda triandra*) Merotherie, New South Wales, Australia (32˚11'S, 149˚33'E). The site had been dominated with C4 kangaroo grasses for more than 150 years. After collection, the soil was air-dried and sieved to pass a 2-mm mesh. The soil had total organic C 28 mg g^{-1}, total N 1.6 mg g^{-1}, pH ($CaCl_2$) 5.0, clay content 13%, and a $\delta^{13}C$ values of -14.9‰.

A column experiment was carried out in growth cabinets (Fitotron® SGC 120, Loughborough, Leicestershire, UK). It consisted of two CO_2 concentration levels, two plant species and seven replicates. Two growth cabinets (two replicates in each cabinets) were supplied with ambient CO_2 concentration (aCO_2, 400±15 μmol mol^{-1}) and another two with elevated CO_2 concentration (eCO_2, 800±30 μmol mol^{-1}). The concentrations of CO_2 inside the growth cabinets were monitored throughout the experiment using a portable carbon-dioxide analyzer. Temperatures were controlled at 22 °C day and 18 °C night with a day length of 14 h. Relative humidity was maintained at 70%. The photosynthetic active photon flux density at the top of the canopy was approximately 400 μmol

m^{-2} s^{-1}. The plant species were wheat (*Triticum aestivum* L. cv. Yitpi) and white lupin (*Lupines albus* L. cv. Kiev).

Growing system

Plants were grown in polyvinyl chloride (PVC) columns (diameter 7.5 cm, height 40 cm). Each column was bottom-capped and has an air-inlet and an air-outlet. To prevent anaerobic conditions and to facilitate CO_2 trapping, a pouch of 300 g plastic beads was packed at the bottom of each column before packing into 1.24 kg of air-dried soil. The soil was mixed with basal nutrients at the following rates (mg kg^{-1}): $CO(NH_2)_2$, 64.3; KH_2PO_4, 180; $CaCl_2 \cdot 2H_2O$, 180; K_2SO_4, 120; $MgSO_4 \cdot 7H_2O$, 50; $MnSO_4 \cdot H_2O$, 15; $ZnSO_4 \cdot 7H_2O$, 9; $CuSO_4 \cdot 5H_2O$, 6; $Na_2MoO_4 \cdot 2H_2O$, 0.4; FeEDTA, 5.5. Urea was further applied at the same rate (30 mg N kg^{-1} soil) at one-week interval from the fourth week. Soil water content was maintained at 80% field capacity by adding reverse-osmosis water daily based on weight loss.

Prior to planting, the seeds were pre-soaked in water and germinated at 25 °C for 48 hours. Eight pre-germinated seeds of wheat and four of white lupin were planted in a row into each column and thinned to four wheat and two white lupin plants after one week. The planted and control columns were transferred into growth cabinets. The soil columns were randomly reallocated between two replicate growth cabinets on a weekly basis to ensure homogenous growing conditions. Additional set of unplanted columns were used as controls. Plants were were harvested at 34 (3 replicates) and 62 (4 replicates) days after planting, representing the early vegetative and booting stages for wheat and the early vegetative and flowering stages for white lupin, respectively.

CO_2 trapping

CO_2 trapping was conducted to quantify below-ground CO_2 released from soil columns before each harvest. To form an air-tight entirety, the top of each column was sealed with two PVC plates around plant stems, using Blu-Tack (Bostik®, Thomastown, Australia). The integrity of the seal was tested by pumping CO_2-free air through the column and observing the bubbles produced in NaOH solution. Before each trapping, CO_2-free air was pumped through the columns for 30 min to remove the initial CO_2. Total below-ground CO_2 was trapped for two days in 150 ml of 0.3 M NaOH solution for 30 min between 9:00 and 23:00 and three times per day (6-h intervals). To accelerate the movement of gases, a vacuum was attached at the end of the trapping apparatus. More details about the CO_2-trapping system can be find in Wang *et al.* (2016). Total CO_2 trapped was determined by titrating the excessive NaOH solution with 0.25 M HCl using the phenolphthalein indicator after precipitation of the carbonate with 0.6 M $BaCl_2$. Another subsample of the trapping solution was mixed with

excessive $SrCl_2$ (0.6 M) solution to get $SrCO_3$ precipitates (Cheng *et al.*, 1998). To prevent the form of $Sr(OH)_2$ and to minimize the contamination of atmospheric CO_2, 0.25 M HCl was added drop by drop to neutralize the pH of the suspension. The precipitates were washed three times, dried in an oven at 60 °C and analyzed for $\delta^{13}C$ using an isotope mass spectrometer (Sercon® 20-22, Gateway, Crewe, UK).

The following equations were used to separate total below-ground CO_2 efflux (C_{total}) into SOC-derived CO_2 (C_{soil}) and root-derived CO_2 (C_{root}) (Cheng, 1996):

$$f = (\delta^{13}C_{total} - \delta^{13}C_{root}) / (\delta^{13}C_{soil} - \delta^{13}C_{root})$$

$$C_{soil} = C_{total} \times f$$

$$RPE = C_{soil} - C_{control}$$

where $\delta^{13}C_{root}$ is the $\delta^{13}C$ signature of roots. $\delta^{13}C_{total}$ is the $\delta^{13}C$ value of total below-ground CO_2 efflux (C_{total}) from planted columns. $\delta^{13}C_{soil}$ is the mean $\delta^{13}C$ value of CO_2 derived from SOC in unplanted treatments ($C_{control}$). f is the contribution of SOC decomposition to total below-ground CO_2 efflux.

Plant and soil analyses

Plants were destructively harvested after each CO_2 trapping. Shoots were cut at the soil surface and roots were collected by hand-picking using a 2-mm sieve. Roots were washed and scanned with an EPSON EU-35 scanner (Seiko Epson Corp.®, Suwa, Japan). The root images were then analyzed using WinRHIZO Pro 2003b (Regent Instruments®, Quebec City, Canada). Shoot and root materials were oven-dried at 70 ºC for 48 h and weighed.

The soil attached to root mass was collected and defined as rhizosphere soil. The soil samples were divided into two parts, with one part being stored at -4 ℃ for the analysis of microbial biomass C (MBC) and N (MBN), and rhizosphere soil respiration, and the other being air-dried for total organic C and N measurements. Immediately after harvesting, a subsample of fresh rhizosphere soil was incubated for 14 h and the microbial respiration was measured using a Servomex 4210 Industrial Gas Analyser (Servomex®, Cowborough, UK).

The chloroform fumigation-extraction method was adopted to measure MBC and MBN. Briefly, a subsample of fresh rhizosphere soil was extracted with 0.5 M K_2SO_4 solution. Another subsample of soil was fumigated with chloroform for 24 h in dark, and subsequently extracted with 0.5 M K_2SO_4 solution. Fumigated and non-fumigated extracts were digested and analyzed for total organic C using a Varian Cary 50 Bio UV-Visible Spectrophotometer (Agilent Technologies®, Santa Clara, CA,

USA). All N forms in each extract were oxidized to mineral N in a autoclave using a persulfate digestion method (Cabrera & Beare, 1993) and then analyzed for NH_4^+ and NO_x^- (NO_2^- and NO_3^-) using a Lachat's QuikChem 8500 Series 2 Flow Injection Analysis System (Lachat Instruments®, Loveland, CO, USA). MBC and MBN were calculated as the differences in total organic C or N concentrations between the fumigated and non-fumigated extracts with a conversion factor of 0.45 for MBC and 0.54 for MBN (Brookes *et al.*, 1985; Vance *et al.*, 1987).

Oven-dried shoot and root samples were ball-milled and analyzed by a Sercon 20-22 Isotope Ratio Mass Spectrometer (Sercon®, Gateway, Crewe, UK) for $\delta^{13}C$ and ^{15}N abundances. A CHNS/O analyser (PerkinElmer® EA2400, Shelton, CT, USA) was used to determine total C and N contents in all soil and plant samples.

Statistical analysis

The effects of CO_2 levels, species and their interaction were assessed separately at two harvests using a two-way ANOVA with a block design. Differences between means were tested using Duncan's multiple rang test at P=0.05. The statistics was conducted using GENESTAT (v11; VSN International, Hemel Hempstead, UK).

Results

Plant growth

Elevated CO_2 tended to increase the shoot and root biomass of both species at the first harvest though not significant at P=0.05 (Table 1). At the second harvest, the two plant species differed in their responses to CO_2 treatment. Elevated CO_2 increased the shoot and root biomass of white lupin by 36% and 80%, respectively, but it had no effect on the biomass of wheat, leading to a significant CO_2×species interaction on root biomass. Elevated CO_2 did not change the root length of either species throughout the experiment. The total root length of wheat was 3 and 10 folds greater than those of white lupin at the first and second harvest, respectively.

Plant C and N

Elevated CO_2 deceased the N concentrations in both shoot and root of wheat at the first harvest, and decreased the root N concentration (by 14%) at the second harvest (Table 1). It decreased the shoot N concentration in white lupin by 16% at the second harvest. On average, white lupin had higher N concentrations than wheat. At the second harvest, wheat was N-limited as shown by an apparent N deficiency symptom (pale leaves).

Elevated CO_2 had no effect on C: N ratio in either shoot or root of white lupin throughout the experiment. However, it increased the shoot and root C: N ratios of wheat by 41% and 32%, respectively, at the first harvest, and the root C: N ratio by18% at the second harvest (Table 1).

Elevated CO_2 did not affect shoot ^{15}N abundance of wheat at either harvest, but it tended to decrease white lupin shoot ^{15}N abundance at the second harvest (Table 1). Wheat incorporated more ^{15}N in their shoots than white lupin at both harvests (Table 1).

A significant CO_2 × species interaction on root $\delta^{13}C$ occurred at the first harvest with eCO_2 decreasing the $\delta^{13}C$ values of wheat but not white lupin. However, eCO_2 did not affect root ^{13}C composition of either species at the second harvest (Table 1).

Total below-ground respiration and its ^{13}C signature

The effect of eCO_2 on below-ground respiration depended on both plant species (Fig. 1, Table S1). Elevated CO_2 had no significant effect on below-ground CO_2 evovled from wheat columns at both growth stages (Fig. 1a,b). However, it increased white lupin total below-ground respirations by 39% and 19% for the first and seond harvest respectively (Fig. 1a,b). The total below-ground CO_2 efflux did not differ between two species at the first harvest, but was 2.2 times higher for white lupin than for wheat at the second harvest (Fig. 1a,b, Table S1). Moreover, the below-ground respiration were 2.4 times greater at the second than the first harvest for white lupin.

Elevated CO_2 decreased the ^{13}C abundance of the total below-ground respiration at the first harvest (Table 1). It tended to decrease the ^{13}C abundance though not significant at the second harvest. The CO_2 evolved from white lupin columns showed invariably lower $\delta^{13}C$ values when compared to those from wheat columns (Table 1).

Rhizosphere primed soil C

At the first harvest, eCO_2 showed no impact on the RPE of wheat but it increased white lupin RPE by 78% (Fig. 2a). On average, the RPE was 1.8 folds higher under white lupin than under wheat (Fig. 2, Table S1). At the second harvest, eCO_2 decreased the wheat RPE by 22%, but it increased the white lupin RPE by 47% (Fig. 2b, Table S1). Under aCO_2, the RPE of white lupin was 37% less than that of wheat, while the opposite was true at eCO_2 with a 18% higher RPE under white lupin than wheat (Fig. 2b). Furthermore, the RPEs were 7.4 and 3.5 folds higher at the second harvest than at the first harvest for wheat and white lupin, respectively (Fig. 2).

Rhizosphere soil respiration & microbial biomass C

Elevated CO_2 had no significant effect on the rhizosphere soil respirations of either species at the first harvest and that of wheat at the second harvest (Fig. 3a,b, Table S1). However, it increased the rhizosphere soil respiration of white lupin by 63% at the second harvest (Fig. 3b). On average, the rhizosphere soil respiration of white lupin was 2.0 and 3.8 times greater than that of wheat at the first and second harvest, respectively (Fig. 3a,b, Table S1).

The significant CO_2 effect on soil MBC was only observed in the rhizosphere of white lupin at the second harvest (Fig. 3c,d). Elevated CO_2 induced a 43% increase in MBC in white lupin rhizosphere when compared to aCO_2. Although there was no difference in MBC between wheat and white lupin at the first harvest, growing white lupin resulted in a 149% increase in MBC at the second harvest compare to wheat (Fig. 3c,d, Table S1). Additionally, MBC in the rhizosphere of white lupin was 115% higher at the second than the first harvest (Fig. 3c,d).

Rhizosphere K_2SO_4-extractable C

Elevated CO_2 had no significant effect on K_2SO_4-extractable C (EOC) in wheat rhizosphere at the first harvest (Fig. 4a). However, it increased the EOC in white lupin rhizosphere by 53% and 22% at the first and second harvest, respecitvely (Fig. 4). On average, the concentrations of EOC in the rhizosphere of white lupin were 1.8 and 5.5 times greater than those of wheat at the first and second harvest, respectively (Fig. 4, Table S1). Furthermore, rhizosphere EOC of white lupin increased 3 folds at the second harvest compared to the first harvest (Fig. 4).

Inorganic N in rhizosphere

The concentrations of inorganic N (NH_4^+-N + NO_x^--N) in the rhizosphere of wheat and white lupin were lower under eCO_2 than aCO_2. For example, at the first harvest, the inorganic N concentrations were 59% and 45% lower under eCO_2 than aCO_2 for wheat and white lupin, respectively (Fig. 5a). At the second harvest, the inorganic N concentration in white lupin rhizosphere was 23% lower under eCO_2 (Fig. 5b). The concentrations of inorganic N in the rhizosphere were higher under white lupin than under wheat (Fig. 5, Table S1). The decreases in the concentrations of total inorganic N under eCO_2 was mainly attributed to the reduction in NO_x^--N concentration (Fig. 5).

Discussion

Greater rhizosphere priming effect was anticipated under eCO_2, but the impact of eCO_2 on the rhizosphere priming of SOC differed between plant species and between developemental stages. Elevated CO_2 increased the RPEs of white lupin at both growth stages but decresed the RPE of wheat at the later growth stage. This could be mainly attributed to possible variations in the quantity and

quality of root exudates between two species.In contrast to our hypothesis, eCO_2 had no effect on RPE of wheat plant at the vegetative stage (Day 34), besides it decreased the primed C under wheat by 22% at the booting stage (Day 62). (Kuikman *et al.*, 1991) also reported that eCO_2 deceased SOC decomposition at the late stage (Day 49) but not at the early stage (Day 22). They suggested that soil microbial community shifted their substrate preference to readily decomposable root-derived C at eCO_2 decreasing the dependence on SOC and the decomposition of existing SOC. In this present study, although eCO_2 stimulated wheat root growth by 28% at Day 34, the input of labile C (Fig. 4a) might not be proportional to root growth and not be enough to enhance the overall growth and activity of microorganisms (Fig. 3c,a) and thus their decomposition of SOC.

At the booting stage, eCO_2 had no stimulating effect on wheat growth (Table 1). Similarly, no eCO_2 effect was found on total below-ground CO_2 efflux, labile C input and rhizosphere soil respiration (Fig. 1b, Fig. 4b, Fig. 3b). Our results were inconsistent with other findings that plants often exhibit stimulated growth and higher root to shoot ratio, greater rhizodeposition and higher rhizosphere respiration under eCO_2 (Kuzyakov, 2010; Cheng *et al.*, 2014; Nie *et al.*, 2015). For example, Cheng & Johnson (1998) found that eCO_2 (700 μmol mol^{-1}) stimulated the total mass of wheat by 49% and the rhizosphere soluble C concentration by 60%. The larger input of rhizodeposits under eCO_2 was considered as an important sources of substrates for soil organisms to decompose SOC (Cheng & Gershenson, 2007). The inconsistency in our and previous studies might stem from the differences in experimental conditions such as N status (Billings & Ziegler, 2008). Elevated CO_2 might have decreased the quality of root-dervied substrates, as suggested by the increased root C: N ratio in this and other studies (Jin *et al.*, 2015) and by the increased phenolics and non-structural carboydrates (Goufo *et al.*, 2014). In our present study, the amount of N applied was probably not sufficient to meet the requirements of both wheat plant and microorganisms under eCO_2, as indicated by the N-deficiency sympotoms in plants and the lower available N in soil under eCO_2. This might have led to lower quantity and/or quality of rhizodeposits at eCO_2, which constrained the responses of soil microorganisms and their decomposition of SOC. Although some studies proved that low-N status in soil would favor microbes to decompose SOC for N (Craine *et al.*, 2007; Zang *et al.*, 2016), extreme N limitation would yield less positive priming (Berntson & Bazzaz, 1997). It appears that the lower RPE of wheat plant under eCO_2 than aCO_2 is likely to be caused by microbial limitation of substrates and/or nutrients.

Constrast to wheat, eCO_2 increased the RPE of white lupin by 78% and 47% at the vegetative stage (Day 34) and the flowering stage (Day 62) respectively (Fig. 2).This observation was consistent with the increases in both rhizosphere soil respiration and microbial biomass C under eCO_2 at Day 62 (Fig. 3b,d). In this study, eCO_2 possibly stimulated the input of labile C, as indicated by the increases in

K_2SO_4-extractable C and rhizosphere soil respiration (Fig. 4, Fig. 3b,d), and hence microbial growth and decomposition of SOC. The prominent capacity of white lupin in releasing organic acids/anions such as citrate has been documented (Watt & Evans, 1999; Wasaki *et al.*, 2005). Previous hydroponic culture experiments showed that eCO_2 increased the release of malate or citrate via enhanced specific release or biomass production of this species (Watt & Evans, 1999; Campbell & Sage, 2002). Furthermore, eCO_2 is also expected to alter the composition of the root exudates of legumes with relatively higher N-rich substances due to the increased N_2 fixation at eCO_2 (de Graaff *et al.*, 2006; Jin *et al.*, 2012; Lam *et al.*, 2012). These changes in the quality alongside the quantity of root-derived substrates under eCO_2 stimulated microbial growth (Fig. 3d) which increased the decomposition of SOC by co-metabolism and/or enhanced extracellular enzyme production (Kuzyakov *et al.*, 2000).

Elevated CO_2 decreased the concentrations of soil mineral N in both plant rhizospheres, mainly in the form of nitrate (Fig. 5) which concurredwith those reported previously (Hovenden *et al.*, 2008; Nie & Pendall, 2016). This was mainly attributed to the extra N demand and uptake by plants to favor the enhanced photosynthesis under eCO_2. The reduction of soil N availability under eCO_2 could also be caused by increased microbial N immobilization (Billings & Ziegler, 2005). The decrease in soil N availability under eCO_2 was thought to increase the intensity and duration of RPE (Cheng & Kuzyakov, 2005). The relatively lower N availability in white lupin rhizosphere at eCO_2 relative to aCO_2 in this study might have driven the soil microorganisms to decompose the more N-rich organic matter for N (Chen *et al.*, 2014) and increased the RPE at eCO_2. Moreover, lower N availability under eCO_2 could aslo shift soil microbial community, as a result, changing the decomposition of SOC. For example, Carney *et al.* (2007) found that the increased PE at eCO_2 was pertinent to the incrase in fungal abundance which is possibly caused by the lower N availability under eCO_2 as fungi demand less N than bacteria due to their higher C: N ratio (Billings & Ziegler, 2005). However, under extremely N-limited conditions, less or no RPE would occur (Bengtson *et al.*, 2012), as is the case of wheat plant at eCO_2 in this study.

In general, the RPE of white lupin was higher than that of wheat except that wheat elicited higher RPE than white lupin under aCO_2 at the second harvest. Although white lupin has less root biomass and shorter root length, the specific release of root exudates is higher than wheat (Nuruzzaman *et al.*, 2006; Pearse *et al.*, 2006; Weisskopf *et al.*, 2008), as indicated by higher rhizosphere soil respiration and K_2SO_4-extractable C (Fig. 3a,b, Fig. 4). The more input of easily decomposable substrates into white lupin rhizosphere accounted for the higher RPE of white lupin than wheat, which is similar to the case at eCO_2 (Groenigen *et al.*, 2014). Besides, white lupin relying on N_2 fixation had higher root N concentrations than wheat, indicating the rhizodeposits of white lupin were also more N-enriched

than wheat. Nitrogen-rich labile compounds were thought to produce higher degree of priming than C substrates alone (Dalenberg & Jager, 1989; Cheng, 2009). For instance, Knorr *et al.* (2005) found that the addition of N-rich substrates increased the litter decomposition. The higher RPE under wheat than white lupin under aCO_2 at the second harvest could be explained by the 10-fold higher root length of wheat than white lupin in the small soil columns, that is wheat had more rhizosphere soil than white lupin (Weisskopf *et al.*, 2008). Rhizosphere soil microbial communities can be distinct among plant species due to species' selective influences (yielded by the differences in quantity and quality of root exudates) on different microbial communities (Marschner *et al.*, 2001; Hartmann *et al.*, 2009; Hinsinger *et al.*, 2011). Future work is needed to explore plant traits and root exudation, and their impacts on soil microbial function and community structure to better understand the species' variation in eliciting RPE.

Consistently, the RPEs were lower at the early growth stage which is confirmed by Cheng & Kuzyakov (2005) and Cheng (2009) who reported that in general, the priming effects were lower and even negative at early growing stages, increased to the highest at the flowering stage, and decline thereafter. According to them, the responses of priming effects are associated with the the release of substrates in the rhizosphere which is also regulated by the plant phenological stages. This could account for the higher RPE of white lupin at Day 62 than Day 34 in present study as greater rhizosphere extractable C, rhizosphere soil respiration and MBC were detected at Day 62. However, there were no such changes in wheat rhizosphere at the two growth stages. The increase of wheat RPE at later growth stage was caused by the increase of ^{13}C abundance of the below-ground CO_2 efflux which increased from -25.7‰ at Day 34 to -22.5‰ at Day 62 averagely (Table 1). Although the mechanisms behind the responses of ^{13}C abundance of the below-ground CO_2 to wheat growth stages are still not clear, several speculations could be make. One is the decline of below-ground input in assimilates which have more negative ^{13}C signature than the C4 soil or the decrease in root activity at later growth stage. Another possibility is the microbial utilization of SOC was increased at Day 62 leading to more release of SOC-derived higher ^{13}C-CO_2. More studies need to be done to cover the whole growth stage to understand the effect and inner mechanisms of plant phenology on RPE.

Conclusion

Elevated CO_2 increased the RPEs of white lupin at both growth stages but decreased the RPE of wheat at later growth stage. Changes in root exudate quality and quantity at eCO_2 might account for the species-specific reponses in RPEs to eCO_2. This study suggestes that future high atmospheric CO_2 may favor the decomposition of native SOC under legumes. The selection of proper crop species which release small amounts of root exudates could conducive to SOCsequestration, particulary in

N-limited conditions. Future studies on mechanistic understanding of the RPE should focus on the effect of eCO_2 on root exudate quantity and composition, and how they interacts with soil N status and plant phenology.

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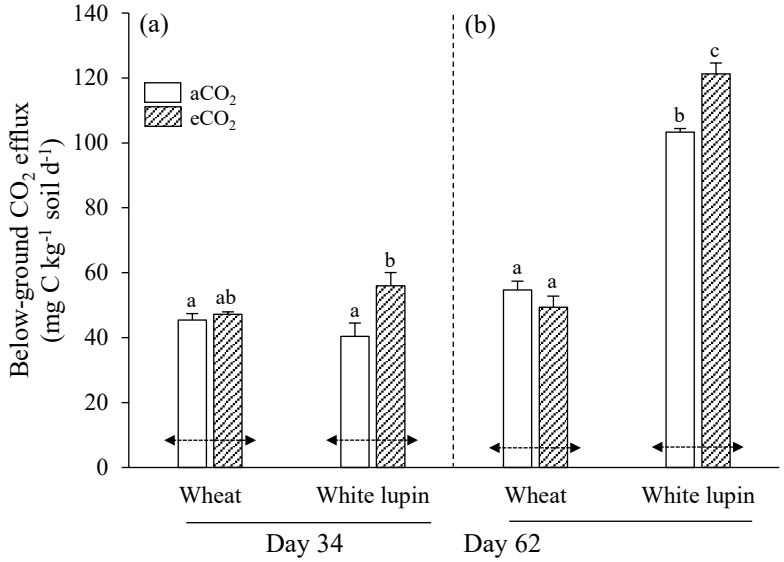

Figure 1. Total below-ground CO_2 efflux from soil columns with wheat and white lupin grown for 34 **(a)** and 62 **(b)** days under aCO_2 (400 µmol mol^{-1}) and eCO_2 (800 µmol mol^{-1}). The arrow-ended short lines represent the values obtained from unplanted control soils. Error bars represent standard errors of means of four replicates. Means with a same letter within the same harvest are not significantly different at P=0.05 using the Duncan's new multiple range test.

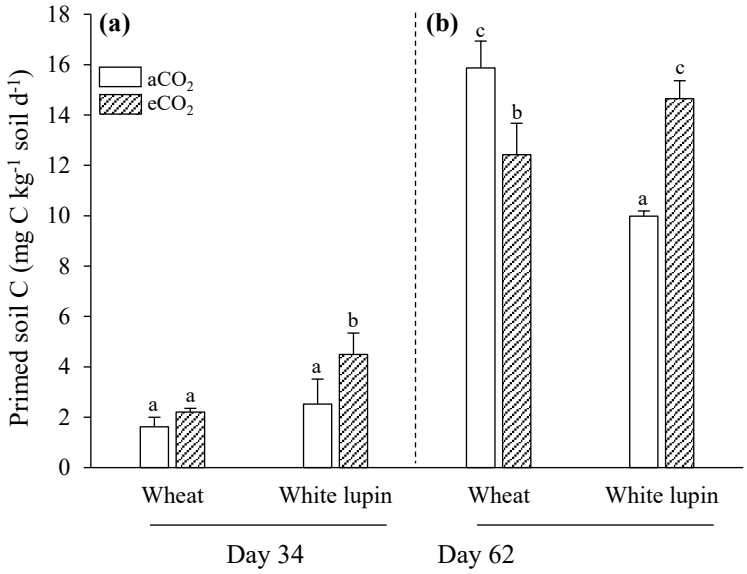

Figure 2. Primed soil C from soils with wheat and white lupin grown for 34 **(a)** and 62 **(b)** days under either aCO_2 (400 µmol mol^{-1}) or eCO_2 (800 µmol mol^{-1}). Error bars represent standard errors of means of three replicates for Day 34 and four replicates for Day 62. Means with a same letter within the same harvest are not significantly different at P=0.05 using the Duncan's new multiple range test.

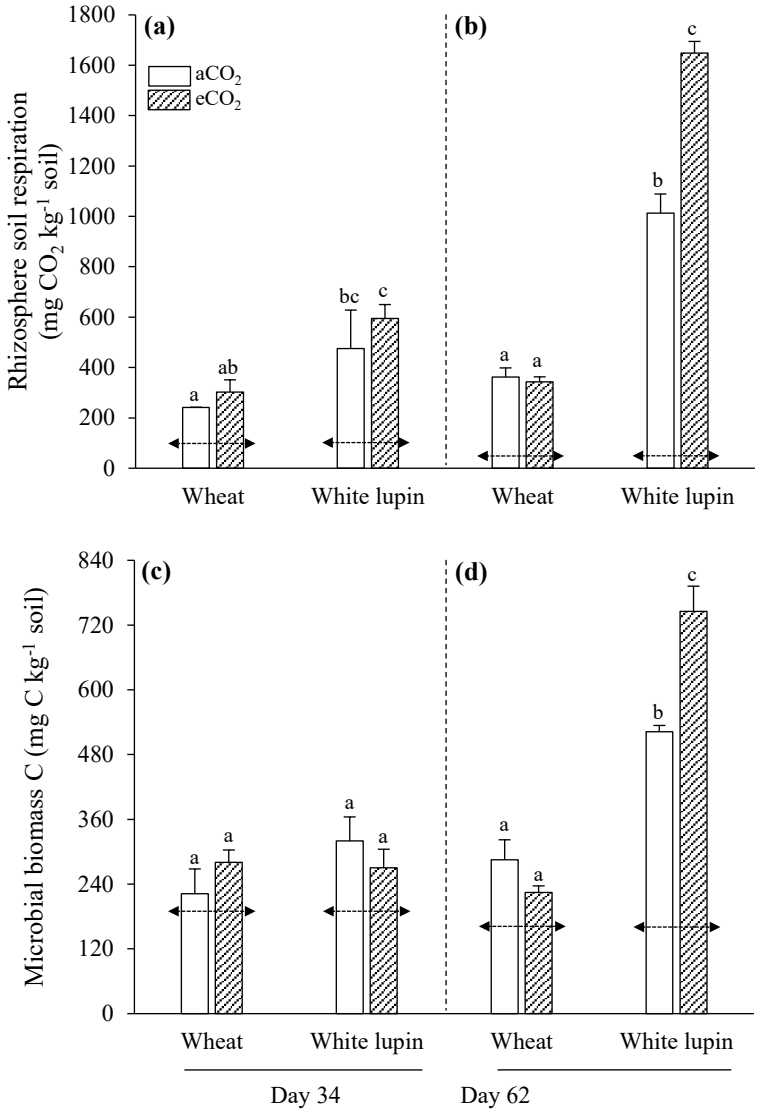

Figure 3. Rhizosphere soil respiration **(a, b)** and microbial biomass C **(c, d)** in soil with wheat and white lupin grown for 34 and 62 days under either aCO_2 (400 µmol mol^{-1}) or eCO_2 (800 µmol mol^{-1}). The arrow-ended short lines represent the values obtained from unplanted control soils. Error bars represent standard errors of means of three replicates for Day 34 and four replicates for Day 62. Means with a same letter within the same harvest are not significantly different at P=0.05 using the Duncan's new multiple range test.

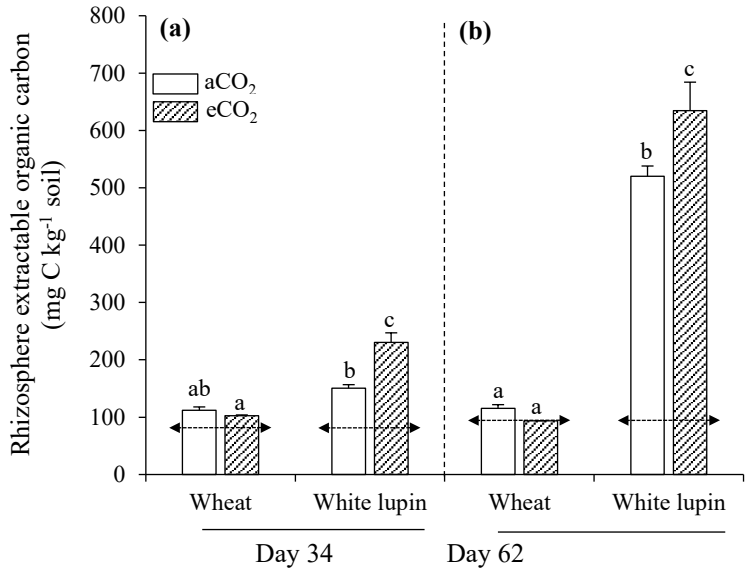

Figure 4. K_2SO_4-extractable C (mg C kg^{-1} soil) in the rhizospheres of wheat and white lupin grown for 34 **(a)** and 62 **(b)** days under either aCO_2 (400 µmol mol^{-1}) or eCO_2 (800 µmol mol^{-1}). The arrow-ended short lines represent the values obtained from unplanted control soils. Error bars represent standard errors of means of three replicates for Day 34 and four replicates for Day 62. Means with a same letter within the same harvest are not significantly different at P=0.05 using the Duncan's new multiple range test.

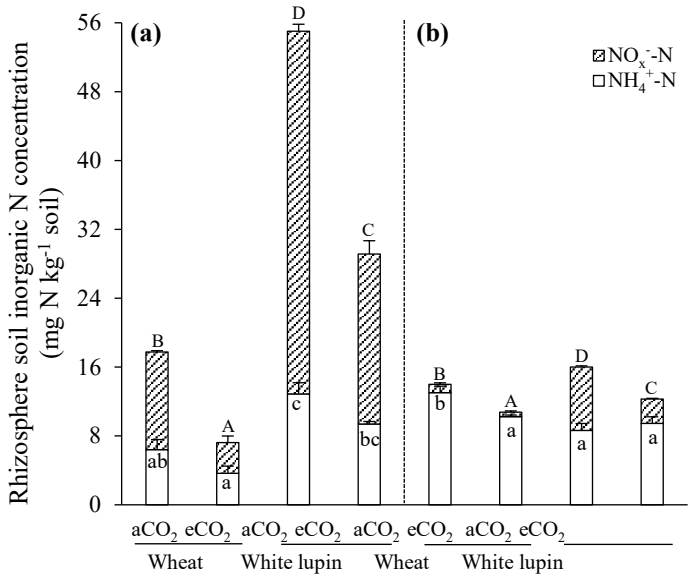

Figure 5. Concentrations of inorganic N (NH_4^+-N and NO_x^--N) in the rhizosphere of wheat and white lupin grown for 34 **(a)** and 62 **(b)** days under either aCO_2 (400 µmol mol^{-1}) or eCO_2 (800 µmol mol^{-1}). Error bars represent standard errors of means of three replicates for Day 34 and four replicates for Day 62. Means with a same upper-case letter (NO_3) or lower-case letter (NH_4) within the same harvest are not significantly different at P=0.05 using the Duncan's new multiple range test.

Table 1. Shoot and root dry weights, N concentrations (N conc.) and C-to-N ratios (C: N), root length, shoot ^{15}N signature, and root and below-ground CO_2 $\delta^{13}C$ abundances of wheat and white lupin grown for 34 and 62 days under either aCO_2 (400 µmol mol^{-1}) or eCO_2 (800 µmol mol^{-1}) levels.

Species	CO_2 level	Weight (g $column^{-1}$)		N conc. (g kg^{-1})		C: N		Root length	Shoot ^{15}N	$\delta^{13}C$ abundance (‰)	
		Shoot	Root	Shoot	Root	Shoot	Root	(m $column^{-1}$)	(atom%)	Root	CO_2
					Day 34						
Wheat	aCO_2	2.12ab	1.46b	44.2b	27.0b	9.53a	14.0c	28.5b	0.80b	-27.6c	-25.2c
	eCO_2	2.55 b	1.87c	31.9a	21.3a	13.4b	18.5d	30.4b	0.81b	-28.9b	-26.2b
White lupin	aCO_2	1.71 a	0.58a	46.2b	28.8c	9.18a	12.3b	10.1a	0.58a	-29.4a	-25.9b
	eCO_2	2.06ab	0.74a	42.2b	29.3c	10.0a	11.8a	10.2a	0.57a	-29.5a	-26.5a
Significant level											
CO_2		*	*	***	***	**	***	-	-	**	**
Species		*	***	**	***	**	***	***	***	***	*
CO_2×Species		-	-	*	***	*	***	-	-	**	-
					Day 62						
Wheat	aCO_2	10.5b	3.71c	17.2a	18.8b	25.3b	21.6b	589b	1.06c	-26.8a	-22.2b
	eCO_2	11.1b	3.93c	16.0a	16.2a	26.7b	25.6c	615b	1.07c	-27.3a	-22.8ab
White lupin	aCO_2	7.13a	1.58a	37.3c	29.0c	11.5a	14.7a	57 a	0.84b	-26.5a	-24.7ab
	eCO_2	9.70b	2.83b	31.4b	29.7c	13.6a	14.4a	73 a	0.77a	-27.2a	-25.2a
Significant level											
CO_2		**	***	**	*	-	*	-	-	-	-
Species		***	***	***	***	***	***	***	***	-	*
CO_2×Species		*	**	-0.055	**	-	*		-	-	-

-, $P>0.05$; *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$. For each column, different letters indicate significant differences between means (Two-way ANOVA, Duncan's new multiple range test, $P<0.05$).

Table S1. Two-way Anova analysis of total below-ground CO_2 efflux, rhizosphere priming effect (RPE), rhizosphere soil respiration ($Rh_{resp.}$), rhizosphere inorganic N (NH_4^+-N and NO_x^--N), and microbial biomass C at Day 34 and 62.

	CO_2 efflux	RPE	$Rh_{resp.}$	K_2SO_4-C	NH_4^+-N	NO_x^--N	MBC
			Day 34				
CO_2	*	**	-	**	-	***	-
Species	-	**	***	***	*	***	-
CO_2×Species	*	-	-	***	-	**	-
			Day 62				
CO_2	*	-	***	*	-	***	**
Species	***	*	***	***	*	***	***
CO_2×Species	***	***	***	**	*	***	***

-, $P>0.05$; *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$ (Two-way ANOVA, Duncan's new multiple range test, $P<0.05$)