Wheat and white lupin differ in rhizosphere priming of soil organic carbon under elevated CO₂

Qiao Xu · Xiaojuan Wang · Caixian Tang

Abstract

**Background and aims** Elevated CO₂ (eCO₂) alters plant rhizosphere processes and soil microbial properties which in turn change the decomposition of native soil organic carbon (SOC), the process termed rhizosphere priming effect (RPE). This study examined the effect of eCO₂ on RPEs of plant species contrasting in root system and exudation.

**Methods** Two C3 species, wheat (*Triticum aestivum* L. cv. Yitpi) and white lupin (*Lupinus albus* L. cv. Kiev), were grown under ambient CO₂ (400 ± 15 μmol mol⁻¹) and eCO₂ (800 ± 30 μmol mol⁻¹) for 34 and 62 days in a C4 soil. The amounts of CO₂ derived from SOC and plant roots were quantified.

**Results** Elevated CO₂ increased the RPEs of white lupin by 78% and 47% at days 34 and 62, respectively. It increased microbial respiration (63%) and biomass carbon (43%) in the rhizosphere soil of white lupin at Day 62. In contrast, eCO₂ decreased wheat RPE by 22% and did not affect rhizosphere soil respiration and microbial biomass carbon at Day 62. Moreover, eCO₂ increased the concentration of soluble organic carbon in the rhizosphere of white lupin but not wheat.

**Conclusions** The enhanced RPE of white lupin but not wheat under eCO₂ had resulted from an increase in root exudation of white lupin.

**Keywords** High CO₂ concentration · *Lupinus albus* L. · N limitation · Rhizosphere priming effect · Root exudation · Stable isotope · *Triticum aestivum* L

**Abbreviations**

- aCO₂ ambient atmospheric CO₂ concentration
- eCO₂ elevated atmospheric CO₂ concentration
- EOC rhizosphere K₂SO₄-extractable C
- MBC microbial biomass C
- RPE rhizosphere priming effect
- SOC soil organic carbon

**Introduction**

The CO₂ concentration in the atmosphere is expected to reach 600–800 μmol mol⁻¹ by the end of this century (Meinshausen et al. 2011). High atmospheric CO₂ concentration stimulates plant photosynthesis and enhances photosynthetic inputs into below-ground via rhizodeposition (Pendall et al. 2004; Paterson et al. 2008; De Graaff et al. 2009; Phillips et al. 2011), which provides labile substrates to relative C-limited soil microorganisms to degrade indigenous soil organic carbon (SOC) (Van Groenigen et al. 2014). This rhizodeposition-induced change in the decomposition...
of SOC is termed rhizosphere priming effect (RPE) (Cheng et al. 2014).

The contribution of root exudates accounts for 58–95% of the observed RPEs (Shahzad et al. 2015). Therefore, any changes in root exudation by elevated CO2 (eCO2) would yield differences in RPEs. Greater RPEs are anticipated under eCO2 by comparison to aCO2 due to an increased input of easily decomposable C. From another perspective, eCO2 changes the chemical composition of plants to more production of carbon-based secondary compounds (e.g. 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 eCO2 alters the chemical composition of root exudates, RPEs are expected to change under eCO2. Furthermore, eCO2 enhances biological N2 fixation in legumes, which in turn provides extra N for these plants (Hartwig and Sadowsky 2006; Lam et al. 2012; Butterly et al. 2016; Tobita et al. 2016). As a result, eCO2 may affect the N status of legumes and cereals differently, which in turn affects the chemical composition of their root exudates and hence RPEs.

Except for the changes in the amount and quality of root exudates, eCO2 could also alter RPEs via its influences on soil variables such as soil N availability and pH. For example, eCO2 has been reported to decrease soil N availability, mainly in the form of nitrate (NO3−) (Hovenden et al. 2008) possibly by increasing microbial immobilisation and reducing the rate of gross N mineralisation (Berntson and Bazzaz 1997; Hungate et al. 1999). Stimulated labile C inputs and lower soil N availability under eCO2 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 and Kuzyakov 2008). Elevated CO2 could change rhizosphere soil pH by affecting cation-anion uptake of plant roots (Guo et al. 2012) and through this way the RPEs would also be changed by eCO2.

Plant species differ naturally in their releasing patterns of root exudates. For example, some legume plants (e.g. white lupin) exude large amounts of low-molecular-weight organic anions (e.g. carboxylates) into their rhizospheres, especially when soil P is limited (Veneklaas et al. 2003). In contrast, cereal plants (e.g. wheat) release 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 but exudes larger amounts of citrate at the mature stage (Sugiyama and 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 (Bardgett et al. 2014). Cereal species such as wheat have longer and finer roots which can explore larger volumes of soil than legumes (e.g. white lupin). This possibly means more SOC in wheat rhizosphere are subjected to microbial decomposition than in white lupin rhizosphere (Weisskopf et al. 2008). However, it is unknown whether eCO2 alters RPE via its effect on root growth traits or exudation.

The objectives of this study were 1) to examine the effect of eCO2 on RPE and 2) to compare the effects of cereal and legume species on RPEs under eCO2. 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 morphology. We hypothesized that 1) eCO2 would stimulate the RPEs due to an increase in root exudation; 2) white lupin would have greater RPE stimulation than wheat under eCO2 due to its higher quantity and quality of root exudates.

Materials and methods

Surface soil (0–10 cm) was collected from a native C4 kangaroo grassland at Merotherie, New South Wales, Australia (32°11’ S, 149°33’ E). The site had been dominated by C4 kangaroo grasses (Themeda triandra) for more than 150 years. After collection, the soil was air-dried and sieved to pass a 2-mm mesh. The basic properties of the soil were: SOC 28 mg g−1, total N 1.6 mg g−1, pH (0.01 M CaCl2) 5.0, clay 130 mg g−1, and δ13C −19.3‰.

A column experiment was carried out in four growth cabinets (Fitotron SGC 120, Loughborough, Leicestershire, UK). It consisted of two CO2 concentration levels, two plant species and three replicates for the first harvest and four replicates for the second harvest. Two growth cabinets were supplied with ambient CO2 concentration (aCO2, 400 ± 15 μmol mol−1) and another two with elevated CO2 concentration (eCO2, 550 ± 15 μmol mol−1). The CO2 concentration of eCO2 and aCO2 cabinets were automatically controlled by infrared analyzers (Ecosense, AirSciences, USA).
800 ± 30 μmol mol⁻¹). Pure CO₂ (Coregas, Yennora, New South Wales, Australia) was pumped into the growth cabinets and mixed with the air inside to get the desired CO₂ concentrations. The concentrations of CO₂ inside the growth cabinets were also monitored throughout the experiment using portable carbon-dioxide analysers (Extech SD800, Nashua, New Hampshire, USA). To eliminate possible effects of below-ground CO₂ on CO₂ partitioning, the growth cabinets were flushed with fresh air and re-supplied with pure CO₂ daily. 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 (Lupinus 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 had an air-inlet and an air-outlet. To prevent anaerobic conditions and to facilitate CO₂ bottom-capped and had an air-inlet and an air-outlet. (diameter 7.5 cm, height 40 cm). Each column was growing system

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In this study, the $^{13}$C abundance of roots other than the δ$^{13}$C value of root-derived CO$_2$ was used to calculate RPE. This might lead to bias in absolute rhizosphere priming effect as isotopic fractionation might occur during microbial utilisation of root-derived labile C (Werth and Kuzyakov 2010), but this should not affect the treatment difference.

Plant and soil analysis

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 analysed using WinRHIZO Pro 2003b (Regent Instruments, Quebec City, Canada). Shoot and root materials were oven-dried at 70 °C for 48 h and weighed.

The soil attached to the root was collected and defined as rhizosphere soil. The soil samples were sieved to less than 2 mm and divided into two parts, with one part being stored at -4 °C when necessary for the analysis of microbial biomass C (MBC) and rhizosphere extractable inorganic N, and the other being air-dried for chemical measurements.

Rhizosphere soil respiration was measured as cumulative microbial respiration from a laboratory incubation (Wang et al. 2016). Briefly, 8 g of rhizosphere soil was incubated in a 1-L Mason jar at 25 °C for 14 h. The amount of CO$_2$ released during this period was measured using an infrared gas analyser (Servomex 4210, Crowborough, East Sussex, UK).

The chloroform-fumigation extraction method was adopted to measure MBC. Briefly, a subsample of 8 g fresh rhizosphere soil was extracted with 40 ml of 0.5 $M$ K$_2$SO$_4$ solution. Another subsample of soil was fumigated with chloroform for 24 h in dark, and subsequently extracted with 0.5 $M$ K$_2$SO$_4$ solution. Fumigated and non-fumigated extracts were digested and analysed for total organic C using a Varian Cary 50 Bio UV-Visible Spectrophotometer (Agilent Technologies, Santa Clara, California, USA). The MBC was calculated as the differences in total organic C between the fumigated and non-fumigated extracts with a conversion factor of 0.45 (Vance et al. 1987).

Statistical analysis

The effects of CO$_2$ levels, species and their interaction were assessed at each harvest using a two-way ANOVA with a block design. Differences between means were tested using Duncan’s multiple range test at $P = 0.05$. The ANOVA was conducted using Genstat (v17; VSN International, Hemel Hempstead, UK).

Results

Plant growth

While wheat had greater biomass than white lupin, eCO$_2$ tended to increase the shoot and root biomass of both species at the first harvest (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$_2 \times$ species interaction. Elevated CO$_2$ did not change the root length of either species throughout the experiment although the total root length of wheat was 3 and 9 folds greater than those of white lupin at the first and second harvest, respectively.

Plant C and N

Elevated CO$_2$ decreased 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 N concentration in the shoot by 16% but not in the root of white lupin at the second harvest. On average, white lupin had 16–107% higher N concentrations than wheat. At the second harvest, wheat was N-limited as shown by an apparent N-deficiency symptom (pale leaves).
Elevated CO₂ had no effect on C:N ratio in either shoot or root of white lupin throughout the experiment except root C:N at the first harvest. However, it increased the shoot and root C:N ratios of wheat by 41% and 32% at the first harvest, and the root C:N ratio by 18% at the second harvest. At both harvests, white lupin had lower C:N ratios than wheat (Table 1). A significant CO₂ × species interaction on root δ¹³C occurred at the first harvest with eCO₂ decreasing the δ¹³C values of wheat only. However, eCO₂ did not affect the root 13C composition of either species at the second harvest (Table 1).

Total below-ground respiration and its ¹³C signature

The magnitude of eCO₂ effect on below-ground respiration depended on plant species and growth stage (Fig. 1, Table 2). Elevated CO₂ had no significant effect on below-ground CO₂ evolved from wheat columns at both growth stages (Fig. 1). However, it increased total below-ground respirations under white lupin by 39% and 19% for the first and second harvest, respectively (Fig. 1). The total below-ground CO₂ 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. 1, Table 2). Moreover, the below-ground respiration was 2.4 times greater at the second than the first harvest for white lupin (Fig. 1). Elevated CO₂ increased the root-derived CO₂ from white lupin-soil system only, leading to a significant CO₂ × species interaction (Fig. 1, Table 2). The increases were 44% and 15% at the first and second harvest, respectively (Fig. 1). At the second harvest, the root-derived CO₂ from white lupin was 2.9 times greater than that from wheat.

The 13C abundance of the CO₂ released via below-ground respiration of planted columns ranged from −26.5 to −25.2‰ at Day 34 and from −25.2 to −22.2‰ at Day 62 (Table 1). Elevated CO₂ decreased the ¹³C abundance at Day 34, and tended to decrease it though not significant at Day 62. The CO₂ evolved from white lupin columns showed invariably lower δ¹³C

### Table 1

| Species        | CO₂ level | Weight (g column⁻¹) | N conc. (g kg⁻¹) | C-to-N ratio | Root length (m column⁻¹) | δ¹³C abundance (‰) |
|----------------|-----------|---------------------|-----------------|--------------|--------------------------|-------------------|
|                |           | Shoot   | Root   | Shoot | Root | Shoot | Root | Shoot | Root |
| Day 34         |           |         |        |       |      |       |      |       |      |
| Wheat          | aCO₂      | 2.12ab  | 1.46b  | 44.2b | 27.0b | 9.53a | 14.0c | 28.5b | −27.6c | −25.2c |
|                | eCO₂      | 2.55b   | 1.87c  | 31.9a | 21.3a | 13.4b | 18.5d | 30.4b | −28.9b | −26.2b |
| White lupin    | aCO₂      | 1.71a   | 0.58a  | 46.2b | 28.8c | 9.18a | 12.3b | 10.1a | −29.4a | −25.9b |
|                | eCO₂      | 2.06ab  | 0.74a  | 42.2b | 29.3c | 10.0a | 11.8a | 10.2a | −29.5a | −26.5a |
| Significant level | CO₂         | *    | *     | ***  | ***  | ***  | ***  | _    | **   | **   |
|                | Species    | *    | ***   | **   | ***  | ***  | ***  | ***  | *    | *    |
|                | CO₂ × species | _ | _     | *    | ***  | *    | ***  | _    | **   | _    |
| Day 62         |           |         |        |       |      |       |      |       |      |
| Wheat          | aCO₂      | 10.5b  | 3.71c  | 17.2a | 18.8b | 25.3b | 21.6b | 589b  | −26.8a | −22.2b |
|                | eCO₂      | 11.1b  | 3.93c  | 16.0a | 16.2a | 26.7b | 25.6c | 615b  | −27.3a | −22.8ab |
| White lupin    | aCO₂      | 7.13a  | 1.58a  | 37.3c | 29.0c | 11.5a | 14.7a | 57 a   | −26.5a | −24.7ab |
|                | eCO₂      | 9.70b  | 2.83b  | 31.4b | 29.7c | 13.6a | 14.4a | 73 a   | −27.2a | −25.2a |
| Significant level | CO₂         | **   | ***   | **   | *    | _    | _    | _    | _    | _    |
|                | Species    | ***  | ***   | **   | _    | *    | _    | _    | _    | _    |
|                | CO₂ × species | * | _     | **   | _    | _    | _    | _    | _    | _    |

- , 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.
abundances when compared to those from wheat columns (Table 1).

Rhizosphere priming effect

Elevated CO₂ tended to increase the soil-derived CO₂ under wheat at the first harvest, but decreased it by 16% at the second harvest (Fig. 1). Elevated CO₂ increased the soil-derived CO₂ under white lupin and the increases were 21% and 30% at the first and second harvest, respectively (Fig. 1). On average, the soil-derived CO₂ was 18% higher under white lupin than under wheat at the first harvest, but was 9% lower at the second harvest (Fig. 1, Table 2).

Rhizosphere priming effects were determined by taking the basal soil respiration rates out of the soil-derived CO₂. The basal soil respiration rates in the control amounted at 7.11 and 5.51 mg CO₂-C kg⁻¹ soil d⁻¹ at the first and second harvest, respectively (Fig. 2A). On average, the RPE was 1.8 folds higher under white lupin than under wheat (Fig. 2B, Table 2). When averaged two CO₂ treatments, the RPEs were 6.6 and 3.5 folds higher at the second harvest than at the first harvest for wheat and white lupin, respectively (Fig. 2).

Table 2 Two-way ANOVA analysis of total below-ground CO₂ efflux, root- and soil-derived CO₂, rhizosphere priming effect (RPE), rhizosphere soil respiration (Rｈ.resp.), rhizosphere K₂SO₄-extractable C (K₂SO₄-C), rhizosphere K₂SO₄-extractable N (NH₄⁺-N and NO₃⁻-N), and microbial biomass C (MBC) at Day 34 and 62.

| CO₂ efflux | Root-derived C | Soil-derived C | RPE | Rℎ.resp. | K₂SO₄-C | NH₄⁺-N | NO₃⁻-N | MBC |
|------------|----------------|----------------|-----|----------|--------|--------|--------|-----|
| Day 34     |                |                |     |          |        |        |        |     |
| CO₂        | *              | ***            | *   | ***      |       |        |        |     |
| Species    |                |                |     |          |        |        |        |     |
| CO₂ × species |        |                | *** | ***      | ***    |        |        |     |
| Day 62     |                |                |     |          |        |        |        |     |
| CO₂        | *              | ***            | *   | ***      | *      |        |        | *** |
| Species    | ***            | ***            | *   | ***      | ***    |        |        | *** |
| CO₂ × species | ***        | ***            | *** | ***      | ***    |        |        | *** |

-, P > 0.05; *, P < 0.05; **, P < 0.01; ***, P < 0.001. Two-way ANOVA, Duncan’s new multiple range test.
Rhizosphere soil respiration and microbial biomass C

Elevated CO₂ 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, and Table 2). 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 those of wheat at the first and second harvest, respectively (Fig. 3A, B, Table 2).

The significant CO₂ effect on soil MBC was only observed in the rhizosphere of white lupin at the second harvest (Fig. 3C, D). Elevated CO₂ induced a 43% increase in MBC in white lupin rhizosphere when compared to aCO₂. 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 by comparison with wheat (Fig. 3C, D, and Table 2). Additionally, MBC in the rhizosphere of white lupin was 115% higher at the second than the first harvest (Fig. 3C, D).

Rhizosphere K₂SO₄-extractable C

Elevated CO₂ had no significant effect on K₂SO₄-extractable C (EOC) in the rhizosphere of wheat 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, respectively (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 2). Furthermore, rhizosphere EOC of white lupin increased 3 folds at the second harvest compared to the first harvest (Fig. 4).

Rhizosphere K₂SO₄-extractable N

The concentrations of K₂SO₄-extractable inorganic N (NH₄⁺-N + NOₓ⁻-N) in the rhizosphere of wheat and white lupin were lower under eCO₂ than aCO₂. For example, at the first harvest, the extractable inorganic N were 59% and 45% lower under eCO₂ than aCO₂ for wheat and white lupin, respectively (Fig. 5A). At the second harvest, the concentration of extractable inorganic N in white lupin rhizosphere was 23% lower under eCO₂ (Fig. 5B). The concentrations of extractable inorganic N in the rhizosphere were higher under white lupin than under wheat (Fig. 5, Table 2). The decreases in the concentrations of extractable inorganic N under eCO₂ was mainly attributed to the decline in NOₓ⁻-N concentrations (Fig. 5).

Discussion

This study demonstrated that the CO₂ effect on the rhizosphere priming of SOC decomposition differed between plant species and between developmental
stages. Elevated CO₂ increased the RPEs of white lupin at both growth stages but decreased the RPE of wheat at the later growth stage, partly supporting our hypothesis. This species variation could be mainly attributed to possible variations in the quantity and quality of root exudates between the two species.

In contrast to our hypothesis, eCO₂ had no effect on RPE of wheat plants at the vegetative stage (Day 34), but decreased the amount of primed soil C under wheat by 22% at the booting stage (Day 62). At the early vegetative growth stage, eCO₂-induced labile C input from wheat roots might be too small to induce a measurable change in microbial growth (Fig. 3A, C) and hence rhizosphere primed C. This is evidenced by no increases in root-derived CO₂ or extractable organic C in rhizosphere soil under eCO₂ relative to aCO₂. The decreased RPE under eCO₂ at the booting stage might be attributed to the poor quality of root-derived substrates as indicated by the increased root C:N ratio in this and previous studies (e.g. Jin et al. 2015) or increased phenolic and non-structural carbohydrates (Goufo et al. 2014). Poor substrate quality might restrict microbial decomposition of SOC (Cotrufo and Ineson 1996; Viswanath et al. 2010).

Kuikman et al. (1990) also reported that eCO₂ decreased SOC decomposition under wheat (Triticum aestivum L. cv. Ralle) at a late growth stage (Day 49) but not at an early stage (Day 22). They suggested that
the decrease could be caused by microbial preferential utilisation of root-released labile C that was stimulated by eCO2. Soil microbial community shifted their substrate preference to readily decomposable root-derived C at eCO2, decreasing their dependence on SOC and the decomposition of existing SOC. However, this explanation is not applicable to this study because eCO2 did not increase the quantities of root exudates as indicated by root-derived CO2 and extractable organic C in the rhizosphere (Figs. 1B, 4B). Our results were inconsistent with previous findings that plants often exhibit stimulated growth and higher root-to-shoot ratio, greater rhizodeposition and higher rhizosphere respiration under eCO2 (Kuzyakov 2010; Cheng et al. 2014; Nie et al. 2015). For example, Cheng and Johnson (1998) found that eCO2 (700 μmol mol⁻¹) increased the total mass of wheat by 49% and the concentration of rhizosphere soluble C by 60%. The larger input of rhizodeposits
under eCO2 was considered as an important source of substrates for soil organisms to decompose SOC (Cheng and Gershenson 2007). This difference in CO2 effect on plant growth between this study and others is likely due to a difference in N availability (Billings and Ziegler 2008).

In contrast to wheat, eCO2 increased the RPE of white lupin by 78% and 47% at the vegetative stage (Day 34) and the flowering stage (Day 62), respectively. This observation was consistent with the increases in rhizosphere soil respiration and microbial biomass C under eCO2 (Fig. 3). In this study, eCO2 stimulated the input of labile C, as indicated by the increases in root-derived CO2 and extractable organic C (Figs. 1 and 4), and hence the ability of soil microorganisms to decompose SOC (Health et al. 2005; Nie et al. 2013; Van Groenigen et al. 2017). The prominent capacity of white lupin in releasing organic acids/anions such as citrate has been documented (Watt and Evans 1999; Wasaki et al. 2005). Previous hydroponic culture experiments showed that eCO2 increased the release of malate or citrate from white lupin roots via enhanced biomass production or specific release rate per unit of root biomass (Watt and Evans 1999; Campbell and Sage 2002). Furthermore, eCO2 is also expected to alter the composition of the root exudates of legumes with relatively higher N-rich substances due to the increased N2 fixation at eCO2 (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 eCO2 stimulated microbial growth and activity which increased the decomposition of SOC by co-metabolism and/or enhanced extracellular enzyme production (Kuzyakov et al. 2000; Carney et al. 2007).

Except for the quantity and quality of root exudates, soil N availability might also affect the rhizosphere priming effect, as reported by other studies (Bernston and Bazzaz 1997; Craine et al. 2007; Zang et al. 2016). Elevated CO2 decreased the concentrations of K2SO4 extractable N in the rhizosphere of both species, mainly in the form of nitrate (Fig. 5) which concurred with those reported previously (Hovenden et al. 2008; Nie and Pendall 2016). This was mainly attributed to the extra N demand and uptake by plants to favour the enhanced plant growth under eCO2. The reduction of soil N availability under eCO2 could also be caused by increased microbial N immobilisation (Billings and Ziegler 2005). The decrease in soil N availability under eCO2 was thought to increase the intensity and duration of RPE (Cheng and Kuzyakov 2005) due to microbial N mining from soil organic matter (Chen et al. 2014). However, in this study, eCO2 decreased soil N availability, which might not have constrained soil microbes with white lupin because more N-rich root exudates under eCO2 due to enhanced biological N2 fixation might have met the microbial N requirement. In the wheat-soil system, the negative effect of eCO2 on N availability was stronger. This might have limited the activity of microorganisms to decompose SOC, which is in line with many other studies (Bernston and Bazzaz 1997; Bengtson et al. 2012). Moreover, lower N availability under eCO2 could also shift the composition of soil microbial community, as a result, changing the decomposition of SOC. For example, Carney et al. (2007) found that the increased priming effect at eCO2 was pertinent to the increase in fungal abundance which is possibly caused by the lower N availability under eCO2 as fungi demand less N than bacteria (Billings and Ziegler 2005).

The RPE of white lupin was higher than that of wheat at the first harvest. Conversely, there was more RPE under wheat than under white lupin when averaged across two CO2 levels at the second harvest. When both species had small root systems at the first harvest, white lupin might have secreted more easily decomposable C compounds than wheat as indicated by the higher root-derived CO2 and extractable organic C in the rhizosphere soil (Figs. 1A, 4A) because its specific release rate of root exudates per unit of root biomass is higher than wheat (Nuruzzaman et al. 2006; Pearse et al. 2006; Weisskopf et al. 2008). Besides, white lupin relying on N2 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 are thought to yield higher priming effects than sole C substrates (Dalenberg and Jager 1989; Cheng 2009). For instance, Knorr et al. (2005) found that the addition of N-rich substrates increased the decomposition of plant litters. The increased input of labile C compounds with higher N content from white lupin roots could have activated soil microbial growth and activity (Carney et al. 2007; Drake et al. 2013), leading to higher RPE under white lupin than under wheat. The higher RPE of wheat than white lupin at the second harvest might be due to its longer roots than white lupin in the small soil columns, that is, wheat roots explore more volume of soil than white lupin (Weisskopf et al. 2008). Moreover, soil microbial communities can be
distinct between plant species due to species selective influences (due to the differences in quantity and quality of root exudates) on different microbial communities (Marschner et al. 2001; Hartmann et al. 2009). 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.

The RPEs were consistently lower at the early than the late growth stage. The results are consistent with the findings by Cheng and Kuzyakov (2005) and Cheng (2009) reporting that the priming effects are lower and even negative at early growing stages, increase to the highest at the flowering stage, and decline thereafter. According to them, plant growth stage regulates the release of substrates in the rhizosphere and hence the pattern of RPE. This could account for the higher RPE of white lupin at Day 62 than Day 34 in our present study as rhizosphere extractable C, root-derived CO2, rhizosphere soil respiration and microbial biomass C were all greater at Day 62. However, there was less root-derived CO2 (P < 0.05), similar MBC and EOC in wheat rhizosphere at Day 62 when compared to Day 34. As stated above, the increase of wheat RPE at the later growth stage was caused by more rhizosphere soil when wheat roots expanded and explored more soil volumes than the first harvest (Table 1). More studies are needed to cover the entire growth stages to better understand the effect and mechanisms of plant phenology on RPE.

Conclusions

Elevated CO2 increased the RPEs of white lupin at both growth stages but decreased the RPE of wheat at the later growth stage. Changes in root exudate quality and quantity at eCO2 might account for the species-specific responses in RPEs to eCO2. This study suggests that future high atmospheric CO2 concentration may favour the decomposition of native SOC under legumes. The selection of proper crop species which release small amounts of root exudates could be conducive to SOC sequestration. Future studies on mechanistic understandings of the RPE should focus on the changes in the quantity and quality of root exudates at eCO2, and how microbes respond to these changes under various plant species at different growth stages.

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