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The effects of elevated CO_2 and nitrogen availability on rhizosphere priming of soil organic matter under wheat and white lupin

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Abstract

Background and Aims: Plants grown under elevated CO_2 (eCO_2) demand more nitrogen from soil and invest more labile carbon (C) compounds into below-ground. This would potentially affect microbial decomposition of soil organic C (SOC) in the rhizosphere- namely rhizosphere priming effect (RPE). This study aims to reveal how eCO_2 and nitrogen supply affect the RPEs under wheat and white lupin.

Methods: Wheat (*Triticum aestivum* L. cv. Yitpi) and white lupin (*Lupinus albus* L. cv. Kiev) were grown under ambient CO_2 (400 μmol mol^{-1}) and eCO_2 (800 μmol mol^{-1}) for 32 and 52 days in a C4 soil. Rhizosphere priming of SOC was quantified using the stable ^{13}C isotopic tracing technique.

Results: Relative to high nitrogen supply, low nitrogen increased the RPEs under both species at Day 32, but decreased the RPEs under wheat while had no effect on RPE under white lupin at Day 52. Elevated CO_2 increased the RPE except that under wheat at Day 52.

Conclusions: The results imply that quantity and quality of root exudates have more effect than nitrogen availability on mediating RPE under eCO_2.

Key words
High CO_2 concentration; *Lupines albus* L.; N limitation; rhizosphere priming effect; stable isotope; *Triticum aestivum* L.

Introduction

Much of the climate change is related to increased CO_2 concentration in the atmosphere which has been increased by 40% since the industrial revolution (Ciais et al. 2013). The increased CO_2 concentration in the atmosphere has been shown to increase plant biomass production and the turnover of root-derived C via increased microbial activity (Cheng and Johnson, 1998; Health et al. 2005). Root-induced increase in the mineralization of indigenous soil organic carbon (SOC) in the rhizosphere, i.e. rhizosphere priming effect (RPE) may play a vital role in the decrease in terrestrial carbon sink (Bengtson et al. 2012) as elevated CO_2 (eCO_2) may potentially change root rhizosphere and environment.

Rhizosphere priming effect is a microbial process regulated by both the quantity and quality of labile C substrates in the form of root exudates and other rhizodeposits (Wang et al. 2015; Liu et al. 2017). Plants grown under elevated CO_2 (eCO_2) invest proportionally larger amounts of photosynthetic C below-ground via rhizodeposition (Cheng and Johnson 1998; Jin et al. 2014; Vestergård et al. 2016). For example, Cheng and Johnson (1998) found that eCO_2 increases the input of root-derived C substrates in the rhizosphere of a wheat plant via increased root growth and mass-specific exudation. In another respect, the composition of rhizodeposits under eCO_2 could differ from ambient CO_2 (aCO_2) given changes in root chemical properties like C:N ratio (Nie et al. 2015) and secondary metabolites e.g. phenolics and terpenoids (Peñuelas et al. 1996; Peñuelas et al. 1997; Poorter et al. 1997). The CO_2-induced changes in the quantity and quality of rhizodeposits are expected to affect the growth and/or activity of soil microorganisms and subsequently the direction and magnitude of RPE (Hoosbeek et al. 2004; Health et al. 2005).

Nitrogen availability in the rhizosphere is generally lower under eCO_2 than under aCO_2 due to enhanced N uptake by plants and changes in N transformation [e.g. stimulated microbial immobilisation (Dijkstra et al. 2010) and inhibited nitrification (Lagomarsino et al. 2008)]. Low N availability would drive soil microorganisms to mine N from soil organic matter, leading to a higher RPE (Kuzyakov 2002), especially in soils with low N availability (Cardon 1996; Dijkstra et al. 2009). It follows that increased soil N availability (e.g. increased N

fertiliser application or biological N_2 fixation) would potentially decrease the RPE due to reduced microbial N requirements from soil organic matter (Li et al. 2017) and thus the synthesis of extracellular enzymes which degrade SOC (Cheng and Kuzyakov 2005). However, in the POP/EuroFACE, Hoosbeek et al. (2004) found that higher soil N availability actually increased SOC decomposition while N limitation leads to a net accumulation of SOC. Similarly, eCO_2 increases SOC decomposition when N fertiliser had been added in a microcosm experiment (Cheng and Johnson 1998). This inconsistency may stem from different soil N status in distinctive soil systems (Bengtson et al. 2012). Our previous study found that eCO_2 decreased the RPE at a later growth stage (Day 62) of a wheat plant probably due to extreme N limitation (Xu et al. 2017), however it increased the RPE of white lupin because enhanced N_2 fixation provided extra N to plants and eased the N limitation. It is apparent that soil N status is of great importance in mediating RPE especially under eCO_2, but this has been poorly studied.

The objective of this study was to elucidate how soil N availability would affect RPE on decomposition of SOC under eCO_2 to better understand the effect of agricultural management to the environment in future higher CO_2 scenario and to better assess the capacity of soil C sequestration in mitigating climate change. We hypothesize that (i) eCO_2 would decrease the RPE when the soil system is extremely N-limited and this effect might be species-specific, (ii) as a result, reduced N supply would further decrease the RPE under eCO_2.

Materials and Methods

Soil

Surface soil (0-10 cm) was collected in September 2015 from a C4 kangaroo grassland (*Themeda triandra*) (32º 10′ S, 149º 34′ E), located at about 22 km north of Gulgong, New South Wales, Australia. The soil was air-dried and sieved (< 2 mm) with plant roots, rocks and granules being carefully removed. Soil physicochemical properties were: total organic C (SOC) 21 mg g^{-1}, total N 1.0 mg g^{-1}, mineral N 18.2 mg kg^{-1}, pH 4.8 (1:5 in 0.01 *M* $CaCl_2$), clay content 13% and $\delta^{13}C$ of SOC -20.9‰. Before packing into each soil column, 2.8 kg of soil was mixed with basal nutrients at following rates (mg kg^{-1} soil): $CO(NH_2)_2$, 32.1; K_2SO_4, 120; KH_2PO_4, 180; $MgSO_4 \cdot 7H_2O$, 50; $CaCl_2 \cdot 2H_2O$, 180; $MnSO_4 \cdot H_2O$, 15; $ZnSO_4 \cdot 7H_2O$, 9; $CuSO_4 \cdot 5H_2O$, 6; $Na_2MoO_4 \cdot 2H_2O$, 0.4; Fe-EDTA, 5.5.

Experimental design

A column experiment was conducted in four environmentally controlled growth cabinets (SGC 120, Fitotron, Loughborough, Leicestershire, UK) with two receiving ambient CO_2 (aCO_2, 400 ± 15 μmol mol^{-1}) and the other two receiving elevated CO_2 (eCO_2, 800 ± 30 μmol mol^{-1}). CO_2 concentrations inside the growth cabinets were achieved by mixing air with pure CO_2 (Coregas, Yennora, New South Wales, Australia) by a CO_2 controller. The growth cabinets were flushed with fresh air and re-supplied with pure CO_2 daily to reduce possible contamination of soil-derived CO_2 on plant growth. Wheat (*Triticum aestivum* L. cv. Yitpi) and white lupin (*Lupines albus* L. cv. Kiev) were chosen for their differences in the quantity and quality of root exudation (Weisskopf et al. 2008). Growing C3 plants in C4 soil provides a way to separate total below-ground CO_2 efflux into SOC-derived CO_2-C and root-derived CO_2-C (Cheng and Johnson 1998).

Within each growth cabinet, two N rates (low and adequate amounts of N, referred to LN and NN thereafter) with two replicates were performed by applying ^{15}N-labelled urea (2 atom% ^{15}N). The application rates and times were described in Table S1. Specifically, before the first below-ground CO_2 trapping, white lupin and wheat received the same amounts of N which were 25 and 60 mg N kg^{-1} soil in LN and NN, respectively. Wheat plants received another 60 mg N kg^{-1} soil, while white lupin received only 30 mg N kg^{-1} soil before the second CO_2 trapping in the LN treatment. Wheat and white lupin received the same amount of N in the NN treatment which was 90 mg N kg^{-1} soil before the second CO_2 trapping.

There were a total of 32 planted columns (2 CO_2 levels × 2 species × 2 N rates × 4 replicates). Eight unplanted soil columns (receiving LN or NN) were included as controls to determine basal soil respiration. At the same time, plants were also grown in carbon-free sands and inoculated with soil microorganisms to determine the possible isotopic fractionation between root tissue and root-derived CO_2.

Growing system

Bottom-capped polyvinyl chloride (PVC) column (diameter 10 cm, height 40 cm) was used as growing pot and drilled at the top and bottom to form an air inlet and outlet. To prevent anaerobic condition and facilitate gas-flowing, a pack of plastic beads (300 g) was placed at the bottom of each column before packing into soil. Soil was then rewetted to 80% field capacity with reverse osmosis water (RO-H_2O). Pre-germinated wheat (16) and white lupin

(8) seeds were sown in a row alongside the diameter of the column. White lupin seeds were inoculated with a commercial lupine inoculant (EasyRhiz, New-Edge Microbials, Albury, New South Wales, Australia). Wheat and white lupin were thinned to eight and four seedlings per column, respectively, one week after sowing.

All environmental parameters except CO_2 concentration in the growth cabinets were controlled at same levels, with relative humidity being set at 70% and temperature at 22 °C (day) and 18 °C (night) with a day-length of 14 h. The photosynthetic active photon flux density at the top of the canopy was approximately 350 μmol m^{-2} s^{-1} throughout the experiment. Soil inside each column was maintained at 80% field capacity by weighing and watering with RO-H_2O every two days before the first CO_2 trapping, and daily thereafter. Soil columns were randomly reallocated between two replicate growth cabinets on a weekly basis to ensure homogenous growing conditions.

Below-ground CO_2 trapping

Below-ground CO_2 were trapped at Day 32 when both species were at the early vegetative stage and Day 52 when wheat came to the late jointing stage while white lupin was at the late vegetative stage. Before CO_2 trapping, the top of each column was sealed with two PVC plates around plant stems and the interspaces were adhered with Blu-tack (Bostik, Thomastown, Victoria, Australia) (Wang et al. 2016). The integrity of the sealing was tested by pumping CO_2-free air through the column and observing the production of bubbles in NaOH solution until the bubbles were stable when pressing the sealing areas. Then initial CO_2 in soil cores was removed by pumping CO_2-free air through each soil column for 30 min. Total below-ground CO_2 released in 48 h was pumped and trapped in a 150 ml 0.5 *M* NaOH solution three times per day and 30 min each time. To speed up gas flowing, a vacuum was attached at the end of the trapping apparatus. The amount of below-ground CO_2 was determined by titrating the excessive NaOH solution with 0.25 *M* HCl using the phenolphthalein indicator after precipitation the carbonate with 0.25 *M* $BaCl_2$ standard solution. Another subsample of NaOH trap was mixed with excessive $SrCl_2$ (0.25 *M*) to get $SrCO_3$ precipitates. To prevent the formation of $Sr(OH)_2$ and the dissolution of $SrCO_3$, 0.1 *M* HCl was added drop by drop to get a neutral pH. The precipitates were washed three times with Milli-Q water, dried at 60 °C for 72 h and analysed for ^{13}C abundances using an isotope ratio mass spectrometer (IRMS) (Sercon Hydra 20-22, Crewe, Cheshire, UK).

Calculation of RPE

Rhizosphere priming effect was calculated as follows:

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

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

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

where f is the contribution of SOC decomposition (C_{soil}) to total below-ground CO_2 efflux (C_{total}). $\delta^{13}C_{root}$ is the $\delta^{13}C$ value of the CO_2 trapped from the sand column (Table 1) other than the $\delta^{13}C$ value of root material to eliminate the effect of possible isotopic fractionation. $\delta^{13}C_{total}$ is the $\delta^{13}C$ value of C_{total} in planted columns (Table 1). $\delta^{13}C_{soil}$ is the mean $\delta^{13}C$ value of CO_2 trapped from unplanted control columns ($C_{control}$) and the value was quite consistent (-19.5‰).

Plant and soil analysis

Immediately after the first CO_2 trapping (Day 32), 16 g of soil from each column was collected by a small corer for the determination of soil extractable N. No plants were harvested at this time. Plants were destructively harvested immediately after the second CO_2 trapping (Day 52) with shoots being cut at the soil surface and roots being collected by hand-picking using a 2-mm sieve. Plant roots were then washed and scanned with an image scanner (Seiko Epson EU-35, Suwa, Nagano, Japan) and analysed with WinRHIZO STD 1600+ (Regent Instruments, Quebec city, Quebec, Canada) to determine root length. Shoot and root samples were oven-dried at 60 °C for 48 h and ball-milled for the measurements of total organic C and N using CHNS/O Element Analyser (PerkinElmer EA2400, Branford, Connecticut, USA) and ^{13}C and ^{15}N abundances of plant tissues with IRMS (Sercon Hydra 20-22, Crewe, Cheshire, UK).

A thin layer of soil attached to the roots was sampled by shaking and defined as rhizosphere soil. Soil samples were separated into two parts with one being air-dried for chemical analysis and another being stored at -4 ºC for biological and biochemical analysis.

Ball-milled soil samples were tested for total organic C and N using CHNS/O Element Analyser (PerkinElmer EA2400, Branford, Connecticut, USA). The ^{13}C abundances of soil samples were determined by IRMS (Sercon Hydra 20-22, Crewe, Cheshire, UK).

Rhizosphere soil respiration (Rhresp.) was used as an indicator of the amount of root-derived substrates and rhizosphere microbial activity (Wang et al. 2016). It was determined as cumulative microbial respiration from a laboratory incubation. Briefly, 8 g of fresh rhizosphere soil was incubated in dark at 25 ºC for 14 h. The CO_2 released was measured after the incubation using an industrial gas analyser (Servomex 4210, Crowborough, East Sussex, UK).

The chloroform-fumigation-extraction method was used to measure microbial biomass C (MBC) (Vance et al. 1987). Briefly, 8 g soil was extracted with 0.5 *M* K_2SO_4 by end-to-end shaking for 1 h. Another 8 g soil was firstly fumigated with chloroform in dark for 24 h and then extracted with the same procedure. Soil extracts were filtered and analysed for total organic carbon using a TOC analyser (GE Sievers InnovOx, Boulder, Colorado, USA). Total organic C (TOC) in non-fumigated extracts was determined as rhizosphere extractable organic C (EOC) to give an indication of the quantity of root-derived substrates. Microbial biomass C was calculated as the differences in TOC between the fumigated and non-fumigated samples with a conversion factor 0.45 (Brookes et al. 1985). The extractable inorganic N was determined by a flow-injection analysis system (Lachat's QuickChem 8500, Loveland, Colorado, USA).

The activity of β-glucosidase enzyme was measured colourimetrically based on the protocol of Tabatabai (1994). Briefly, 0.5 g of fresh rhizosphere soil was mixed with 2 ml modified universal buffer and 0.5 ml Nitrophenyl-β-D-glucoside (0.05 *M*) and incubated at 37 ºC in dark. After 1 h, 0.5 ml $CaCl_2$ (0.5 *M*) and 2 ml THAM buffer were added separately to develop the colour which was measured at 420 nm with a UV-Vis spectrophotometer (Agilent Technologies Varian Cary 50, Santa Clara, California, USA).

Substrate-induced respiration (SIR)

According to Blagodatskaya et al. (2010), 10 g of fresh soil was amended with glucose (10 mg g^{-1}), talcum (20 mg g^{-1}), and mineral nutrients (mg g^{-1}) as follows: $(NH_4)_2SO_4$, 1.9; K_2HPO_4, 2.25; and $MgSO_4 \cdot 7H_2O$, 3.8. Soil samples were incubated in 250 ml Mason jars at 22 °C in dark and the CO_2 produced was measured hourly for a period of 16 h using an industrial gas analyser (Servomex 4210, Crowborough, East Sussex, UK).

Microbial specific growth rate (μ) was estimated by fitting the parameters of the following equation to the measured CO_2 release rate (Panikov and Sizova 1996) by minimizing the least-square sum in Excel 2013 (Microsoft Office, Redmond, Washington, USA):

$$CO_2\ (t) = A + B \exp\ (\mu \times t)$$

where 't' is time, 'CO_2 (t)' is the CO_2 release rate at time 't', 'A' is the initial rate of non-growth respiration, 'B' is the initial rate of the growth respiration, and 'μ' is the maximal specific growth rate of soil microbial community. Higher μ indicates relative domination of r-strategists, whilst lower μ reflects relative domination of K-strategists (Pianka 1970; Andrews and Harris 1986).

Statistical analysis

A two-way ANOVA was conducted to assess the effects of CO_2 level, N rate and their interaction on all measurements using Duncan's multiple range test at $P = 0.05$. The analysis was performed separately for wheat and white lupin and for Day 32 and Day 52 using Genstat (v.17; VSN International, Hemel Hempstead, UK). Significant differences between means were further distinguished at $P = 0.05$ with Duncan's multiple range test. Pearson correlation coefficients (r) among RPE, MBC, Rhresp. and EOC were determined separately for wheat and white lupin using Microsoft Excel (Microsoft 2013, Redmond, USA).

Results

Plant growth

Elevated CO_2 increased the shoot and root biomass of wheat by 28% and 56%, respectively, at Day 52 (Table 2). Low N rate (LN) did not affect the root growth but decreased the shoot biomass of wheat by 10% under eCO_2 (Table 2). Elevated CO_2 enhanced the growth of white lupin only when NN was supplied, with 21% and 79% increases in shoot and root biomass, respectively (Table 2). Similar to wheat, LN had no significant effect on root biomass but reduced the shoot growth of white lupin by 16% when averaged on two CO_2 levels (Table 2).

Elevated CO_2 increased the root length of wheat and white lupin by 80% and 27%, respectively, irrespective of N rates. (Table 2). The eCO_2-induced increase in the root length of wheat was greater under NN, resulting in a significant $CO_2 \times N$ interaction.

Shoot and root N concentrations and ^{15}N abundances

The N concentrations in shoot and root tissues of both species were generally higher under NN than LN (Table 2). Elevated CO_2 had no effect on the root N concentration of wheat but decreased the shoot N concentration by 24% (Table 2). In general, eCO_2 decreased the shoot and root N concentrations of white lupin by 13% and 11%, respectively (Table 2).

Elevated CO_2 had no effect on the ^{15}N atom% in plant shoot (Table 2). The ^{15}N abundances of both shoot and root increased consistently in response to adequate N application.

Total below-ground CO_2 efflux

Elevated CO_2 increased the total below-ground CO_2 efflux by 30% and 65% in the LN and NN treatments, respectively, under wheat at Day 32. (Fig. 1A, Table S2). Compared with NN, LN decreased the total below-ground CO_2 efflux from the wheat-soil system by 26% (Fig. 1A, Table S2). However, total below-ground CO_2 efflux under white lupin was not affected by either CO_2 or N application rate.

At Day 52, eCO_2 decreased the below-ground CO_2 efflux from the wheat-soil system by 23% when averaged on two N levels (Fig. 1B, Table S2). However, it increased the below-ground CO_2 efflux in the white lupin-soil system by 26% (Fig. 1B, Table S2). Low N rate decreased the total below-ground CO_2 efflux from the wheat-soil system by 44% (Fig. 1B, Table S2).

Rhizosphere priming effect

The basal soil respiration rates were measured from control columns and amounted at 1.86-2.26 mg C kg^{-1} soil d^{-1} soil and 3.24-3.95 mg C kg^{-1} soil d^{-1}, respectively, for Days 32 and 52 (arrow-ended dash lines on Fig. 1, Table S3).

At Day 32, eCO_2 increased the RPEs of wheat and white lupin by 92% and 50%, respectively, when averaged on two N rates (Fig. 2A, Table S2). Low N rate increased the RPEs of wheat by 194% and that of white lupin by 102% under aCO_2 (Fig. 2A, Table S2).

At Day 52, eCO_2 decreased the RPE of wheat by 37% regardless of N levels (Fig. 2B, Table S2). However, eCO_2-induced increase in primed C (by 56%) was still detected for white lupin at Day 52 (Fig. 2B, Table S2). Low N decreased the RPE by 65% for wheat but did not affect the RPE of white lupin (Fig. 2B, Table S2). Rhizosphere priming effect was positively correlated with microbial biomass C under both wheat and white lupin ($P < 0.01$, Table 3).

Rhizosphere soil respiration -

No significant treatment effect was found for rhizosphere soil respiration under wheat (Fig. 3, Table S2). Elevated CO_2 increased the rhizosphere soil respiration under white lupin by 40% when received NN, resulting in a significant CO_2 × N interaction. Low N rate decreased the rhizosphere soil respiration under white lupin by 35% when the plants were exposed to eCO_2 (Fig. 3, Table S2).

Microbial biomass C

Elevated CO_2 tended to decrease the MBC in the rhizosphere soil of wheat but increased the MBC by 67% ($P < 0.05$) in the rhizosphere soil of white lupin under NN (Fig. 3B, Table S2). Low N rate decreased the MBC in the rhizosphere of wheat by 48% but had no significant effect on the MBC in the rhizosphere of white lupin.

Rhizosphere K_2SO_4-extractable C (EOC) and N

Elevated CO_2 increased the EOC (39-102%) in the rhizosphere of both species except for wheat supplied with LN (Fig. 4, Table S2). The effect of LN on EOC was only apparent under eCO_2, with 38% and 29% increases for wheat and white lupin, respectively (Fig. 4). K_2SO_4-extractable C was positively correlated with rhizosphere soil respiration ($P < 0.05$, Table 3).

Elevated CO_2 yielded lower concentrations of inorganic N in the rhizospheres of both species especially under NN, leading to a significant CO_2 × N interaction (Fig. 5, Table S2). At Day 32, the inorganic N concentrations (under NN) were 82% and 48% lower under eCO_2 than aCO_2 in the wheat- and white lupin-soil systems, respectively (Fig. 5, Table S2). At Day 52, the concentrations of inorganic N (under NN) were 24% and 39% lower under eCO_2 than under aCO_2 in the wheat and white lupin-soil systems, respectively (Fig. 5). Higher inorganic N was invariably detected in the rhizosphere of white lupin than wheat.

Kinetics of substrate-induced respiration

The amendment of substrates exponentially increased soil respiration within a few hours. Elevated CO_2 had minimal effects on the specific microbial growth rate (Table 2). Low N rate decreased the specific microbial growth rate (μ) under white lupin by 17% when averaged the data from two CO_2 levels (Table 2).

β-glucosidase activity in rhizosphere

Elevated CO_2 had no effect on the activity of *β*-glucosidase in both soil systems (Table 2). Low N rate decreased the activity of *β*-glucosidase by 24% in the white lupin-soil system (Table 2).

Discussion

Effect of N availability on RPE

Low N availability increased the RPEs under both species at Day 32 (i.e. positive LN effect), but this effect did not last and differed between two species by Day 52. This inconsistency may be attributed to differences in soil N availabilities under two plant species at different growth stages.

Nitrogen competition between roots and soil microorganisms in the rhizosphere should be low at Day 32 when plants were small and soil available N was not depleted (Fig. 5A). As a result, when supplied with LN, the soil microorganisms might be under moderate N limitation which increased RPE (Fig. 2A) probably by microbial N mining (Billings and Ziegler 2005; Billings and Ziegler 2008; Dijkstra et al. 2008). By contrast, soil microorganisms could have down-regulated the synthesis of extracellular enzymes in response to higher N supply, leading to a less priming (Fig. 2A) as previously shown by Craine et al. (2007) and Phillips et al. (2011). Similar results have been generated from temperate ecosystems where lower heterotrophic decomposition was detected under N fertilisation when compared to no-N controls (Loiseau and Soussana 1999; Burton et al. 2004; Olsson et al. 2005). The NN-induced decrease in SOC decomposition is in line with the decrease in the activity of soil oxidative enzymes (e.g. phenol oxidase) (Saiya-Cork et al. 2002; Waldrop and Zak 2006) which are produced mainly by fungi to acquire nutrients from complex compounds (Cusack et al. 2010). Additionally, under high N supply, soil microorganisms might prefer to utilise easily degradable C compounds (e.g. root exudates and dissolved organic C) other than SOC (Kuzyakov 2002), which might also lead to the reduction in RPE.

Greater RPEs at Day 52 than at Day 32 are consistent with the finding that highest RPEs occurred at the flowering stage when root growth and exudation peaked (Cheng and Kuzyakov 2005, Cheng 2009). Due to vigorous growth of plants at Day 52, the competition

for N between plants and soil microorganisms could be severe, particularly in the LN treatment, as evidenced by the extremely low amount of extractable N in both plant-soil systems (Fig. 5B). Besides, LN could have limited the input of root-derived labile C, especially under eCO_2 as indicated by the lower rhizosphere extractable-C when compared to NN (Fig. 4). The limitation of both substrates and N under LN might have constrained the growth of soil microorganisms (Fig. 3B), resulting in a lower RPE when compared to NN in the wheat-soil system. Low N supply had no effect on total microbial biomass in the white lupin-soil system (Fig. 3B), probably due to biological N_2 fixation which potentially alleviated the N limitation. However, LN might have constrained the growth of fast-growing r-strategists (Table 2 and Blagodatskaya et al. 2010) and the activity of microbial community as evidenced by the reduction in the activity of β-glucosidase enzyme (Table 2). As a result, the positive LN effect on SOC decomposition (Fig. 2A) was diminished by Day 52 (Fig. 2B). These results indicate that soil N availability regulates RPE by directly affecting microbial growth and activity and indirectly influencing the amount of root-derived substrates.

It can be concluded that RPE is regulated by both external C availability and soil N status. Under moderate N limitation, rhizodeposition enhances microbial growth and/or activity to decompose soil organic matter for N whereas sufficient N alleviates the microbial demand for N and decreases the synthesis of extracellular enzymes to degrade soil organic matter, resulting in a decreased RPE. However, when soil is extremely deficient in N, the priming decreases accordingly due to microbial limitation of both C and N (the case of wheat under LN in this study).

Effect of elevated CO_2 on RPE

Elevated CO_2 generally increased the RPEs (Fig. 2). The eCO_2-induced higher RPE was possibly caused by greater root-derived C input into rhizosphere as indicated by the greater EOC under eCO_2 (Fig. 4). The increase in root-derived labile substrates under eCO_2 stimulated the decomposing activity of microorganisms (Billings and Ziegler 2005; Billings and Ziegler 2008; Dijkstra et al. 2008), inducing greater decomposition of SOC (Cheng and Johnson 1998; Dijkstra et al. 2008; Nie and Pendall 2016). In addition, eCO_2 could potentially change the composition of root-derived substrates (Tarnawski and Aragno 2006; Jin et al. 2015), and the effect would differ among plant species.

In this study, eCO_2 decreased wheat tissue N concentration, especially at NN (Table 2), which might indicate a low N or high C:N ratio in rhizodeposits (Paterson et al. 1997; Hungate et al. 1999). The poorer quality of root-derived compounds would constrain microbial decomposition of SOC (Drake et al. 2013) because the energy acquired from these substrates could not sustain long-term microbial growth as evidenced by the decrease trend of microbial biomass C under eCO_2 (Fig. 3B). This may be the reason why eCO_2 decreased the RPE under wheat at the later growth stage, which is consistent with Xu et al. (2017). Unlike wheat, legumes (e.g. white lupin) might contain more N-rich rhizodeposits due to their higher tissue N than wheat (Table 2) and the amount of these substrates could be increased more greatly by CO_2 enrichment (Fig. 4) as eCO_2 normally enhances biological N_2 fixation, providing extra N to plants (Jin et al. 2015). As a result, soil microorganisms and their decomposing capacity in the rhizosphere of white lupin would be less limited by both C and N under eCO_2, which is evidenced by the higher microbial biomass and rhizosphere respiration (Fig. 3). These results supported our first hypothesis.

Elevated CO_2 could also alter RPE via additional impacts on N availability because eCO_2 normally leads to a lower N rhizosphere (Fig. 5) due to enhanced plant N uptake. This may drive the soil microbial community to mine the relatively N-rich soil organic matter for N (Kuzyakov 2002), which increases the RPE. On the contrary, reduction in RPE would also occur under eCO_2 when soil N is extremely low which limits microbial decomposition. In this present study, N availability did not alter the CO_2 effect on SOC decomposition, except that LN decreased the CO_2 effect on RPE under white lupin at the early vegetative stage (Fig. 2A, Table S2). As a result, the second hypothesis was rejected. However, N availability was previously found to increase or decrease SOC decomposition under eCO_2. For example, in a microcosm study, Cheng and Johnson (1998) found higher N (51.7 mg kg^{-1} soil) increased the CO_2 effect on SOC decomposition. By contrast, Cardon (1996) reported that the application of N fertiliser (83 mg N kg^{-1} soil) decreased the CO_2 effect on the decomposition of SOC when compared to zero N control. More recently, N fertilisation was found to decrease the heterotrophic decomposition under elevated CO_2 and temperature (Choi et al. 2017). The inconsistency in this and other studies might result from different N application rates and plant species which affect the quantity and quality of rhizodeposits and thus microbial responses. These results imply that the quantity and quality of substrates is more important than soil N availability in regulating RPE under eCO_2.

Conclusions

The effect of N availability on RPE differed between plant species and growth stages. Relative to NN, LN increased the RPEs when plants are small possibly by N mining. This positive effect decreased and even reversed (under wheat) at the later growth stage possibly by limited growth or activity of microorganisms due to severer N scarcity. This study shows that extremely N limitation would slow down microbial decomposition of SOC, suggesting stoichiometric analysis of microbial nutrient demand is critical to predicting SOC decomposition. Elevated CO_2 decreased the RPE under wheat at the later growth stage probably by secreting of poorer microbial substrates and LN did not worsen this reduction, indicating the quantity and quality of substrates are more important than N availability in mediating RPE under eCO_2.

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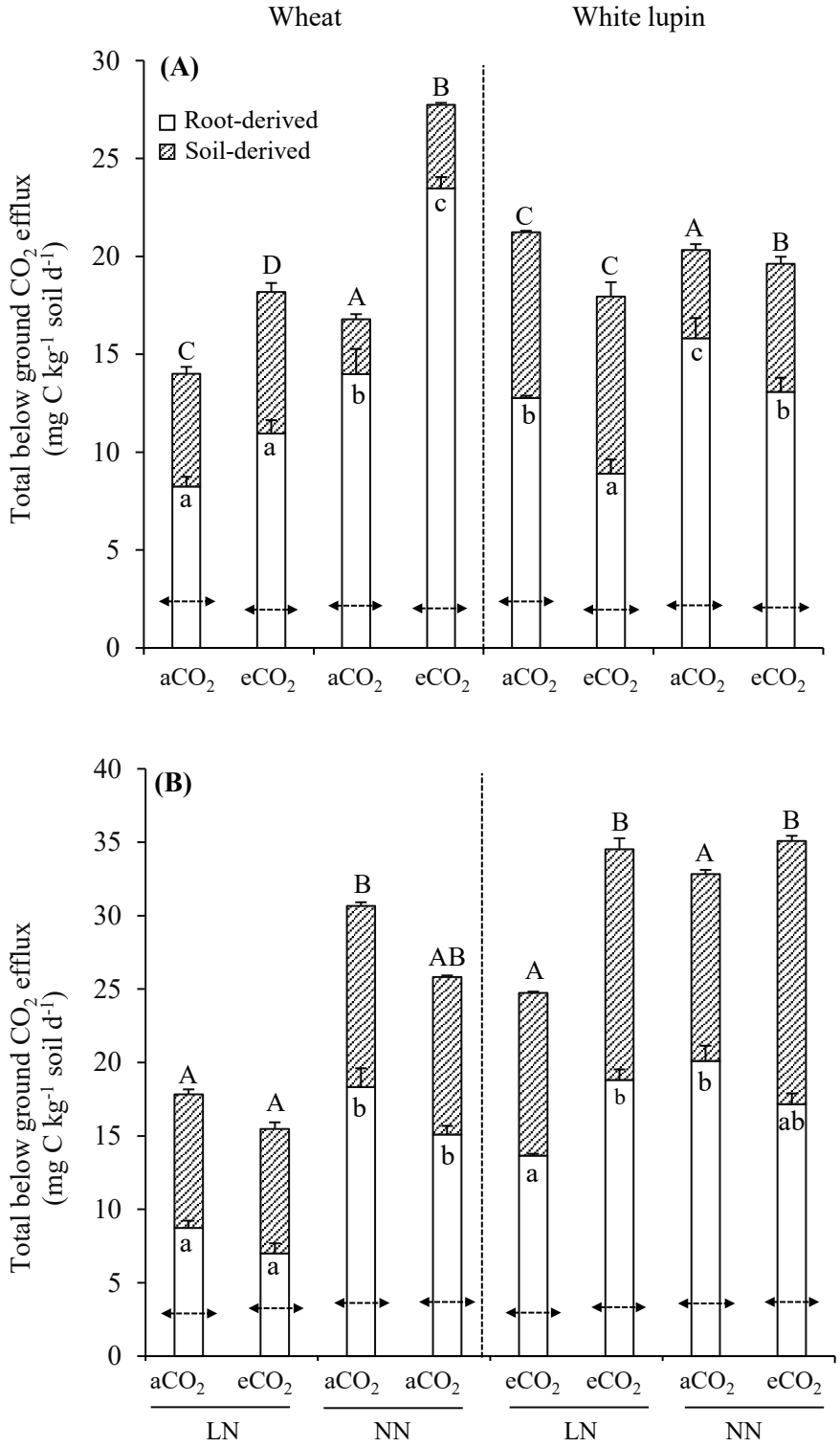

Fig. 1 Total below-ground CO_2 efflux (root-derived and soil-derived CO_2-C, mg C kg^{-1} soil d^{-1}) from soil columns with wheat and white lupin grown for 32 **(A)** and 52 **(B)** days with low (LN) or adequate (NN) rate of N fertiliser under either aCO_2 (400 µmol mol^{-1}) or eCO_2 (800 µmol mol^{-1}). The arrow-ended dash lines represent soil respiration of non-planted soil. Error bars indicate standard errors of means of four replicates. Means with a same lower-case or upper-case letter are not significantly different at $P = 0.05$ for root-derived CO_2-C and soil-derived CO_2-C, respectively, using the Duncan's new multiple range test.

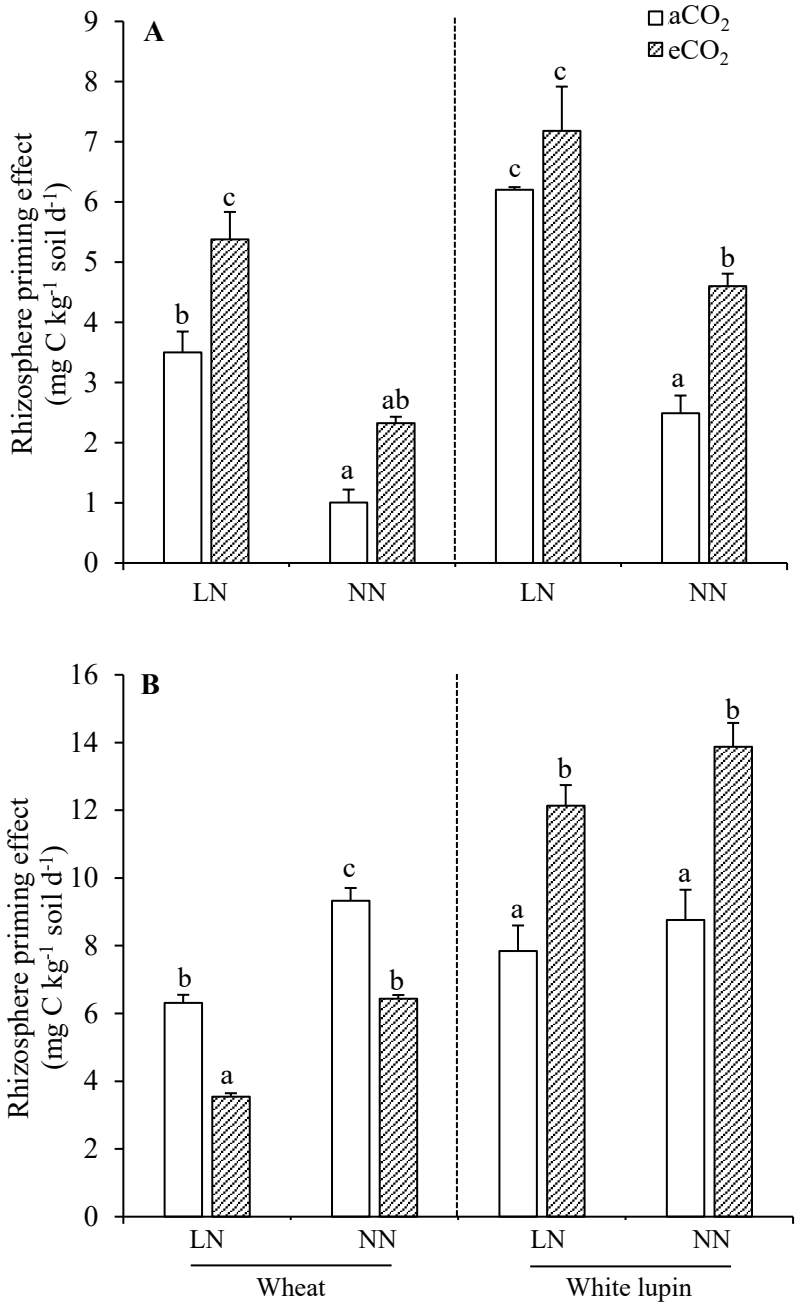

Fig. 2 Rhizosphere priming effect (mg C kg^{-1} soil d^{-1}) from soil columns with wheat and white lupin grown for 32 (**A**) and 52 (**B**) days with low (LN) or adequate (NN) rate of N fertiliser under either aCO_2 (400 µmol mol^{-1}) or eCO_2 (800 µmol mol^{-1}). Error bars indicate standard errors of means of four replicates. Means with a same lower-case letter are not significantly different at $P = 0.05$ using the Duncan's new multiple range test.

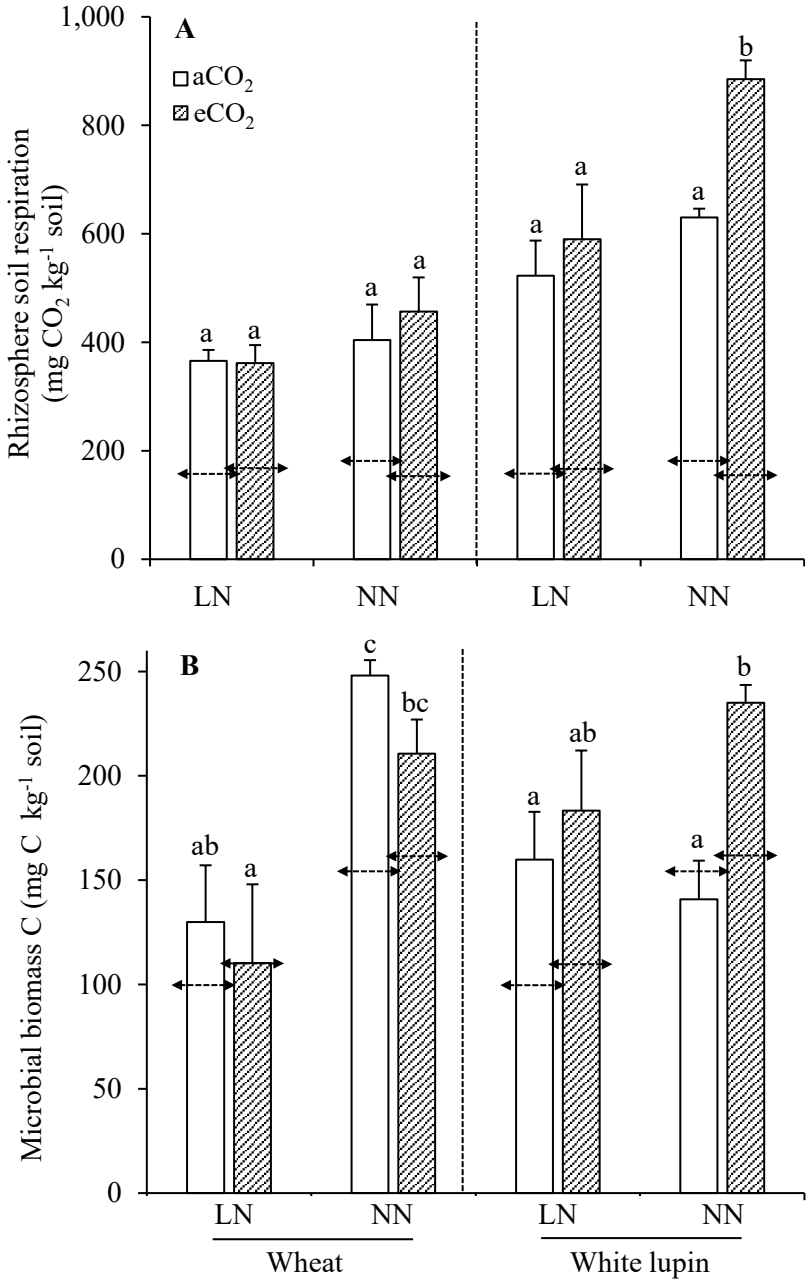

Fig. 3 Rhizosphere soil respiration (**A**) (14 h, mg CO_2 kg^{-1} soil) and microbial biomass C (**B**) (mg C kg^{-1} soil) in soils with wheat and white lupin grown for 52 days with low (LN) or adequate (NN) rate of N fertiliser under either aCO_2 (400 µmol mol^{-1}) or eCO_2 (800 µmol mol^{-1}). The arrow-ended dash lines represent soil respiration of non-planted soil. Error bars indicate standard errors of means of four replicates. Means with a same lower-case letter are not significantly different at $P = 0.05$ using the Duncan's new multiple range test.

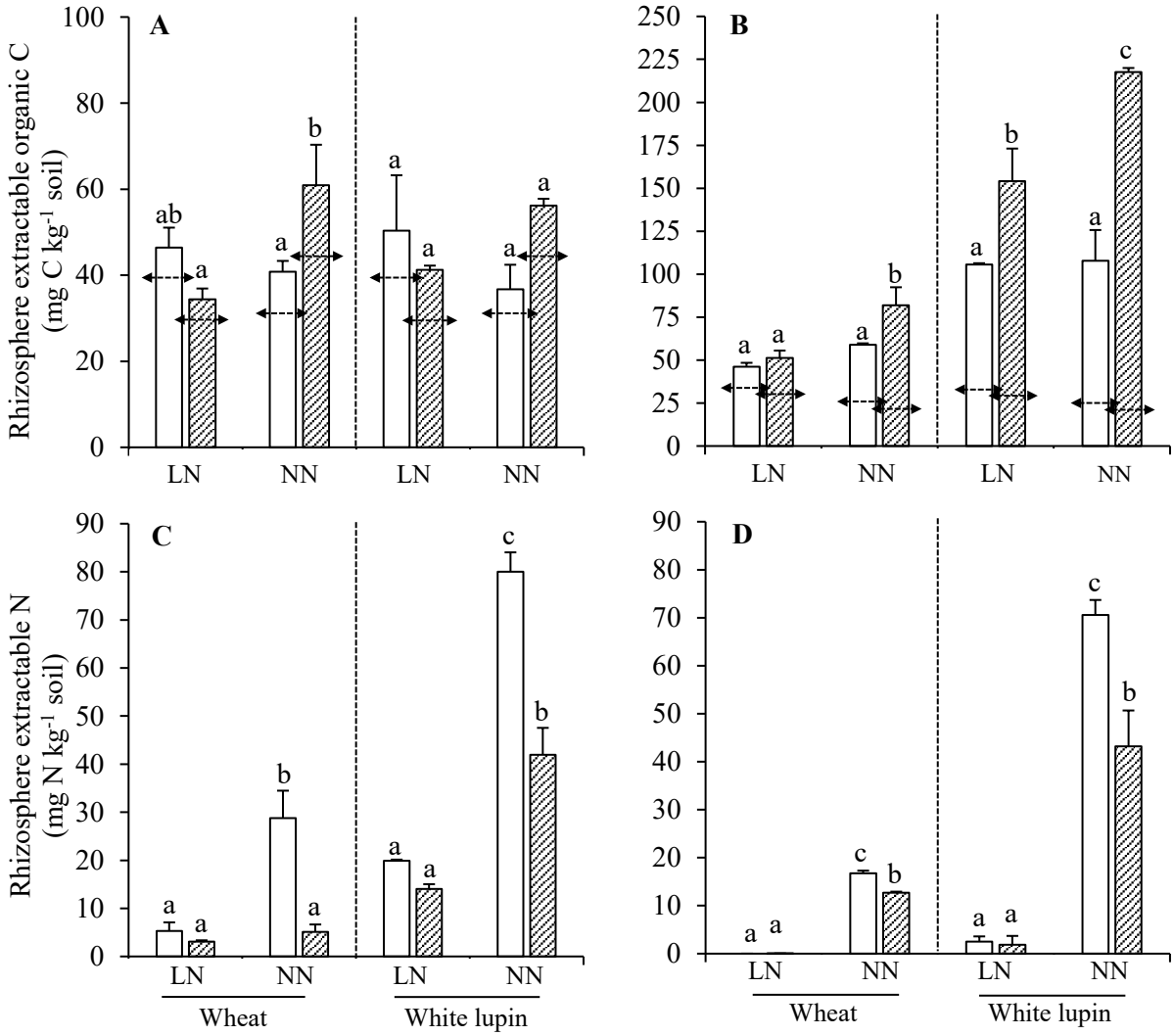

Fig. 4 K_2SO_4-extractable organic C (EOC) (**A**, **B**; mg C kg^{-1} soil) and inorganic N (**C**, **D**; mg N kg^{-1} soil) in the rhizospheres of wheat and white lupin grown for 32 (**A**, **C**) and 52 (**B**, **D**) days with low (LN) or adequate (NN) rate of N fertiliser under either aCO_2 (400 µmol mol^{-1}) or eCO_2 (800 µmol mol^{-1}). The arrow-ended dash lines represent soil respiration of non-planted soil. Error bars indicate standard errors of means of four replicates. Means with a same lower-case letter are not significantly different at $P = 0.05$ using the Duncan's new multiple range test.

Table 1. $\delta^{13}C$ values (‰) of the CO_2 trapped from soil columns ($\delta^{13}C_{total}$) and sand columns ($\delta^{13}C_{root}$) planted with wheat and white lupin for 32 and 52 days under either aCO_2 (400 µmol mol^{-1}) or eCO_2 (800 µmol mol^{-1}) receiving low N (LN) or adequate N (NN).

CO_2	N	$\delta^{13}C_{total}$		$\delta^{13}C_{root}$	
		Day 32	Day 52	Day 32	Day 52
		Wheat			
aCO_2	LN	-22.44	-22.14	-24.49	-24.89
eCO_2	LN	-22.31	-21.76	-24.17	-24.50
aCO_2	NN	-23.66	-22.72	-24.49	-24.88
eCO_2	NN	-23.45	-22.32	-24.17	-24.33
		White lupin			
aCO_2	LN	-22.56	-22.83	-24.59	-25.54
eCO_2	LN	-22.26	-23.06	-25.06	-26.03
aCO_2	NN	-23.46	-23.62	-24.59	-25.26
eCO_2	NN	-23.21	-23.99	-25.06	-25.86

Table 2. Shoot and root dry mass (DM, g column^{-1}), N concentration (N conc., mg g^{-1}), ^{15}N (atom%), root length (m column^{-1}), specific microbial growth rate (μ, h^{-1}) and β-glucosidase activity (μg *p*-nitrophenol g^{-1} h^{-1}) in the rhizospheres of wheat and white lupin grown for 52 days under either aCO_2 (400 μmol mol^{-1}) or eCO_2 (800 μmol mol^{-1}) receiving low N (LN) or adequate N (NN).

CO_2	N	DM		N conc.		Root length	μ	β-glucosidase
		Shoot	Root	Shoot	Root			
					Wheat			
aCO_2	LN	8.78a	3.20a	17.5ab	8.2b	321a	0.15	180
eCO_2	LN	10.32b	4.55b	13.7a	8.1b	442b	0.16	176
aCO_2	NN	9.07a	3.18a	28.2c	14.2a	263a	0.16	182
eCO_2	NN	12.48c	5.41b	20.6b	11.8ab	585c	0.16	189
Two-way ANOVA								
CO_2		***	***	**	ns	***	ns	ns
N		**	ns	**	**	ns	ns	ns
CO_2 × N		*	ns	ns	ns	**	ns	ns
					White lupin			
aCO_2	LN	6.92a	1.46ab	26.7a	22.1a	24.5a	0.13a	192a
eCO_2	LN	7.63a	1.82bc	23.3a	19.4b	30.4b	0.15ab	198a
aCO_2	NN	7.86a	1.22a	46.3c	24.8a	22.8a	0.18bc	236b
eCO_2	NN	9.54b	2.19c	40.1b	22.1a	29.5b	0.17c	250b
Two-way ANOVA								
CO_2		**	***	*	*	**	ns	ns
N		**	ns	***	*	ns	**	***
CO_2 × N		ns	*	ns	ns	ns	ns	ns

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

Table 3. Pearson correlation coefficient (*r*) and significance among rhizosphere priming effect (RPE), microbial biomass C (MBC), rhizosphere soil respiration (Rhresp.) and rhizosphere-extractable C (EOC) in wheat and white lupin-soil systems at Day 52.

		MBC	Rhresp.	EOC
Wheat	RPE	0.812**	0.300	0.251
	MBC		0.488	0.584
	Rhresp.			0.786*
White lupin	RPE	0.887**	0.764**	0.889**
	MBC		0.681*	0.955**
	Rhresp			0.729*

∗, $P < 0.05$; ∗∗, $P < 0.01$

Table S1. Rates of N amended at each application time.

N	Species	D0	D10	D27	D29	1st	D34-35	D44	D50	2nd	Total
LN	White lupin	15	0	10	0	**25**	30	0	0	**30**	55
	Wheat	15	0	10	0	**25**	30	30	0	**60**	85
NN	White lupin	15	15	0	30	**60**	30	30	30	**90**	150
	Wheat	15	15	0	30	**60**	30	30	30	**90**	150

'LN' represents the low N rate; "NN" represents the adequate rate of N. 'D0' represents the day before sowing; '1st' represents the total amount of N added before the first CO_2 trapping; '2nd' represents the total amount of N applied before the second CO_2 trapping and 'Total' represents the total amount of N added throughout the experiment.

Table S2. Significant levels of main effects and interaction terms from the analysis of variance, for CO_2 and N rate on root-derived CO_2-C (Root-C), soil-derived CO_2-C (Soil-C), rhizosphere inorganic N and rhizosphere priming effect (RPE) at Day 32 (D32) and Day 52 (D52) and rhizosphere soil respiration ($Rh_{resp.}$), microbial biomass C (MBC) and rhizosphere K_2SO_4-extractable C (EOC) at Day 52.

Factors	Root-C		Soil-C		Inorganic N		RPE		$Rh_{resp.}$	MBC	EOC
	D32	D52	D32	D52	D32	D52	D32	D52			
					Wheat						
CO_2	***	ns	**	ns	**	**	**	***	ns	ns	ns
N	***	***	***	**	**	***	***	***	ns	**	*
CO_2 × N	**	ns	ns	ns	*	***	ns	ns	ns	ns	*
					White lupin						
CO_2	**	ns	*	***	**	**	***	***	*	*	***
N	**	ns	***	ns	***	***	***	ns	*	ns	*
CO_2 × N	-	**	ns	ns	**	*	*	ns	*	ns	*

ns, $P > 0.05$; ∗, $P < 0.05$; ∗∗, $P < 0.01$; ∗∗∗, $P < 0.001$.

Table S3. Basal soil respiration rates ($C_{control}$, mg C kg^{-1} soil d^{-1}) measured from unplanted control columns under either aCO_2 (400 µmon mol^{-1}) or eCO_2 (800 µmon mol^{-1}) with low N (LN) or adequate N (NN) supply.

CO_2	N	$C_{control}$	
		Day 32	Day 52
aCO_2	LN	2.26	3.24
eCO_2	LN	1.86	3.59
aCO_2	NN	2.02	3.95
eCO_2	NN	1.94	4.03