Climate Change Goes Underground: Effects of Elevated CO_2 on Rhizosphere Priming of Soil Organic Matter

Submitted by

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List of Abbreviations

aCO_2	Ambient atmospheric CO_2 concentration
ANOVA	Analysis of variance
$\delta^{13}C$	the isotopic abundance of ^{13}C
eCO_2	Elevated atmospheric CO_2 concentration
EIN	Rhizosphere K_2SO_4-extractable inorganic nitrogen
EOC	Rhizosphere K_2SO_4-extractable organic carbon
FACE	Free-air CO_2 enrichment
FIA	A flow-injection analysis system
ICP-OES	An inductively coupled plasma optical emission spectrometer
IRMS	Isotope ratio mass spectrometer
K_2SO_4	Potassium sulphate
LN	Low N rate
LSD	the least significant difference test
MBC	Microbial biomass C
MBC:N	Microbial biomass C to N ratio
MBN	Microbial biomass N
Milli-Q	A Milli-Q® ultrapure water system
PE	Priming effect
PVC	Polyvinyl chloride
RO-H_2O	Reverse osmosis water
RPE	Rhizosphere priming effect
SIR	Substrate-induced respiration
SOC	Soil organic carbon
SOM	Soil organic matter
$SrCl_2$	Strontium chloride
$SrCO_3$	Strontium carbonate
SRO	Short-range-order
TOC	Total organic C
μ	the maximal specific growth rate of microorganism

List of Publications

Refereed papers

Xu Q, Wang X, Tang C (2017) Wheat and white lupin differ in rhizosphere priming of soil organic carbon under elevated CO_2. *Plant and Soil* 421:43–55 (Chapter 2)

Xu Q, Wang X, Tang C (2018) The effects of elevated CO_2 and nitrogen availability on rhizosphere priming of soil organic matter under wheat and white lupin. *Plant and Soil* 425:375–387 (Chapter 3)

Xu Q, O'Sullivan JB, Wang X, Tang C (2019) Elevated CO_2 alters the rhizosphere effect on crop residue decomposition. *Plant and Soil* (in press) (Chapter 4)

Xu Q, Jin J, Wang X, Armstrong R, Tang C (2019) Susceptibility of soil organic carbon to priming after long-term CO_2 fumigation is mediated by soil texture. *Science of the Total Environment* 657:1112–1120 (Chapter 5)

Conference and other presentations

Xu Q, Wang X, Tang C, Sale PWG (2015) The effect of elevated CO_2 concentration on rhizosphere priming effect. In: November 2015 *AgriBio Science Conference*. AgriBio, Centre for AgriBioscience, Bundoora, Victoria, Australia

Xu Q, Wang X, Tang C (2016) Elevated CO_2-induced rhizosphere priming of soil organic carbon is dependent on plant species and growth stages. In: November 2016, *Australia-China Symposium on Soil-Plant-Microbe Interactions*. AgriBio, Centre for AgriBioscience, Bundoora, Victoria, Australia

Xu Q, Wang X, Tang C (2017) Climate change goes underground. In: July 2017, *Three Minute Thesis Competition*. School of Life Sciences, La Trobe University, Bundoora, Victoria, Australia

Xu Q, Wang X, Tang C (2018) Climate change goes underground: effects of elevated CO_2 on rhizosphere priming of soil organic matter. In: November 2018 *AgriBio Science Conference*. AgriBio, Centre for AgriBioscience, Bundoora, Victoria, Australia

Xu Q, Jin J, Wang X, Armstrong R, Tang C (2018) Vulnerability of soil organic carbon to substrate-induced priming in three cropland soils after eight years of CO_2 enrichment. In November 2018, *National Soils Conference*. Canberra, ACT, Australia

Abstract

Increasing atmospheric CO_2 concentration associated with climate change stimulates plant photosynthesis and below-ground release of root exudates. As microbial energy and C sources, this enhanced root exudation could potentially increase microbial growth and activity and, in turn, increase rhizosphere priming effect (RPE) on the decomposition of soil organic matter. Using ^{13}C isotopic tracing techniques in both short- and long-term CO_2 enrichment (eCO_2) experiments, this thesis systematically examined the eCO_2 effect on rhizosphere priming decomposition of both soil organic carbon (SOC) and crop residues, and on SOC stability after 8 years of CO_2 enrichment. The thesis showed that the eCO_2 effect on RPE depended on plant species and growth stage. Elevated CO_2 generally increased RPE but it decreased the RPE under wheat at a later growth stage due to severe N deficiency which might limit both root exudation and microbial growth and activity. This thesis also found that eCO_2 increased residue decomposition via microbial activation and/or co-metabolism but this effect depended on growth stage and residue type. The thesis further discovered that eight years of eCO_2 did not increase SOC stock (0–5 cm) of three major cropland soils with contrasting basic properties at the SoilFACE (Free-air CO_2 Enrichment). The eCO_2 history escalated the susceptibility of SOC to ^{13}C-glucose-induced priming in the low-C and coarse-structured Calcarosol soil due to lack of physiochemical protection. A new and abiotic mechanism revealed in the final experiment explained that root exudates (mainly organic acid anions) could also increase RPE by liberating mineral-associated C and subsequently its decomposition by microorganisms. The thesis concluded that CO_2 enrichment could diminish Australian cropland SOC stock in the topsoil by enhancing RPE. Cultivating crop species and/or genotypes with less root exudation are promising to slow down RPE and SOC decomposition.

Statement of Authorship

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The extent and nature of collaborative efforts are specified below. The statement of collaborative input has been approved by all co-authors and their approval verified by Prof Caixian Tang in the Authority to Submit Form.

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Chapter 4: Xu Q[1], O'Sullivan JB[1], Wang X[1], Tang C[1]. Plant and Soil (in press) https://doi.org/10.1007/s11104–019–03940–2
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Xu was responsible for the design and execution of the experiments, acquisition and analysis of data, and drafting and revising the manuscripts. His supervisor Tang and co-authors Armstrong, Jin, O'Sullivan and Wang involved in the interpretation of data and revising the manuscripts.

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Chapter 1 Literature review and general introduction

1.1 Atmospheric CO_2 enrichment-an ongoing process

Carbon dioxide – one of the main greenhouse gases, is naturally present in the environment and could absorb and remit solar radiation to necessarily warm the earth's temperature (Chesney et al. 2013). Its concentration was quite stable before the Industrial Revolution, but anthropogenic activities, such as the burning of fossil fuels, agricultural productions and deforestation, have increased atmospheric CO_2 concentration dramatically afterwards (Stocker et al. 2013). The latest observation at Mauna Loa Observatory shows that the atmospheric CO_2 concentration has reached 411 ppm (parts per million) in June 2018 (Fig. 1.1A), which is about 1.5 times of the level (280 ppm) before the Industrial Revolution (Schmitt et al. 2012; Joos et al. 2015). Moreover, the annual mean growth rate of atmospheric CO_2 is also escalating and has passed 2 ppm per year in the past decade (Fig. 1.1B). By this trend, the atmospheric CO_2 concentration could be doubled at the end of this century (the IPCC A2 scenario) according to model projections.

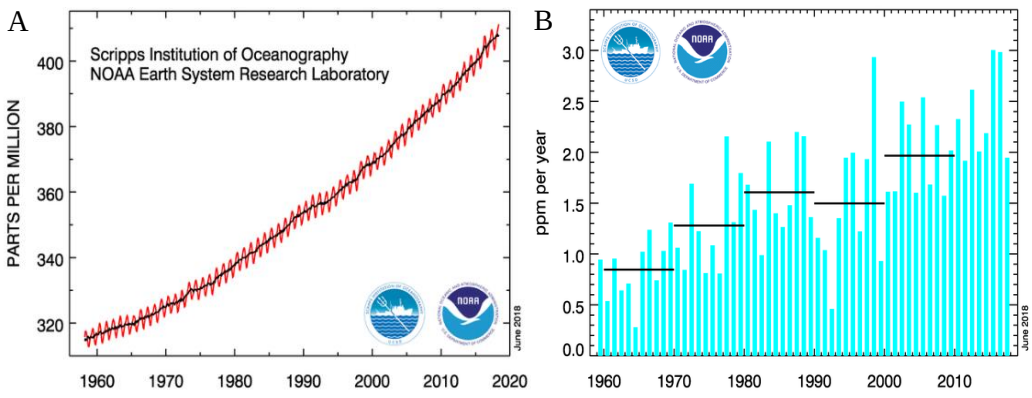

Figure 1.1 Changes in atmospheric CO_2 concentration (A) and annual mean growth rate (B) from March 1958 to June 2018 at Mauna Loa Observatory. The decadal averages of the growth rate were denoted by horizontal lines on Fig. 1.1B. https://esrl.noaa.gov/gmd/ccgg/trends/full.html (accessed in June 2018)

1.2 Effects of elevated CO_2 on C assimilation

Carbon dioxide, as the substrate of plant photosynthesis, manipulates terrestrial C assimilation. As a result, the effects of elevated CO_2 (eCO_2) on plant photosynthesis have been a core

research topic in plant physiology and ecology in the past several decades. Short-term CO_2 enrichment has been found to stimulate net photosynthetic rate in C3 plants based on the results from experiments using growth chambers, open-top chambers, greenhouses and Free-air Carbon Dioxide Enrichment (FACE) facilities (Cheng et al. 1998; Ainsworth and Long 2004; Pal et al. 2005; Ainsworth and Rogers 2007). The initial reaction of photosynthesis is catalysed by ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) in C3 plant mesophyll cells using CO_2 as substrate (Fig. 1.2). This enzyme can also initiate photorespiration with O_2 as substrate (Fig. 1.2). Elevated CO_2 stimulates C3 photosynthesis because the high CO_2/O_2 ratio activates the low catalytic activity of Rubisco which is often inhibited by O_2 (Bowes 1991) and therefore enhances CO_2 assimilation rate (A_s) (Xu et al. 2015). The fixed C is transformed into sucrose, starch and other metabolites, and accumulated in plant organs (Dong et al. 2018; Li et al. 2018). The increased CO_2 fixation accounts for the increases in biomass and/or yield under eCO_2 (Norby et al. 1986; Pal et al. 2005; Nie et al. 2015). According to a review by Ainsworth and Long (2004), eCO_2 increases aboveground biomass by 20% when averaged over 29 C3 species, however, there are little responses in the five C4 plants. Elevated CO_2 increased the productivity of C3 crops by 15–41% and that of C4 crops by only 5–10% (Madhu and Hatfeld 2013). The C4 plants have evolved an additional pathway to concentrate CO_2 (> 400 μmol mol^{-1}; Bowes 1991) in their bundle-sheath cells where the photosynthesis performs (Fig. 1.3). Low O_2 concentration inside the bundle-sheath cells also inhibits the oxygenase activity of Rubisco (photosynthesis). These mechanisms emphasize that C4 plants benefit much less than C3 plants from the eCO_2 environment, which has been evidenced in a variety of short-term experiments (see meta-analyses by Wand et al. (2001), Ainsworth and Long (2004) and Wang et al. (2012)).

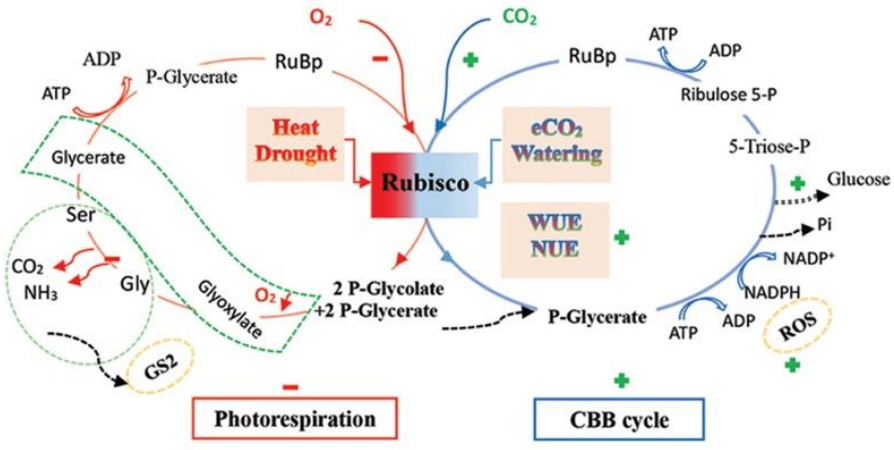

Figure 1.2 Diagrammatic outline of the effects of CO_2 enrichment with abiotic factors on plant Calvin-Benson-Bassham cycle and photorespiration pathway (Xu et al. 2015)

However, in long-term CO_2 experiments, the effects of eCO_2 on photosynthesis and biomass production are variable and could even be reversed (Makino and Mae 1999; Reich et al. 2018). For example, a recent study reported that eCO_2 increased biomass production for C3 grasses but not for C4 grasses over the first 12 years in a 20–year FACE experiment in Minnesota, USA (Reich et al. 2018). Over the last 8 years, biomass for C4 species was enhanced drastically by eCO_2 when compared to ambient CO_2 (aCO_2) but not for C3 species (Fig. 1.4). The reversal biomass responses of the grass species with different photosynthetic pathways to eCO_2 was explained by a similar shift in net nitrogen mineralization of the soil in that study. The down-regulation of biomass stimulation by eCO_2 in C3 species is termed negative photosynthetic acclimation (Li et al. 1999; Adam et al. 2000; Ghildiyal and Sharma-Natu 2000) and normally observed in nutrient-poor soils (Curtis 1996) where soil N could not support the enhanced biomass growth under eCO_2 and causes the so-called progressive N limitation (Luo et al. 2004; Finzi et al. 2006; Hu et al. 2006). The negative photosynthetic acclimation could be induced directly by repression of photosynthetic gene expression and difficulty of CO_2 diffusion due to excess carbohydrate accumulation (Makino and Mae 1999). Notably, plant photosynthetic acclimation to eCO_2 is species-specific. For example, Li et al. (1999) pointed out that even species shared with the same habit would exhibit markedly different photosynthetic acclimation to eCO_2. These results suggest that eCO_2 could enhance terrestrial C assimilation by stimulating plant photosynthesis but this effect is controlled by species and temporal scales.

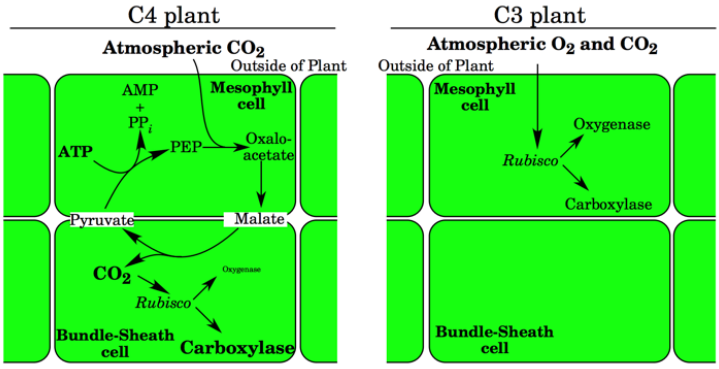

Figure 1.3 Diagram of the differences between C3 and C4 photosynthetic pathways. https://rose–hulman.edu/~brandt/Chem331/Photosynthesis_Carbon_Assimilation_Processes.pdf

1.3 Effects of elevated CO_2 on C allocation

1.3.1 Shoot and root allocation

Elevated CO_2 results in greater allocation of biomass below-ground to maintain a balance of resources given that carbon fixation has increased, frequently resulting in a larger root to canopy ratio (van Ginkel et al. 1996; Cotrufo and Gorissen 1997; Suter et al. 2002), especially in water and/or nutrient-limited systems (Madhu and Hatfeld 2013). Rogers et al. (1996) reviewed the responses of crop root-to-shoot ratio (R:S) to eCO_2 from 264 observations in a series of experimental systems and found the responses range from –37.5 to 59.5%. Elevated CO_2 generally increases plant growth, indicating more water and nutrient requirements to support the enhanced growth. The investment of more photosynthetic C to root growth under eCO_2 might represent a strategy of plants to explore greater soil volume to acquire more water and nutrients (Norby et al. 1987; Lutze and Gifford 1998; Daepp et al. 2001; Uddin et al. 2018). This is further proved by the findings that root length, branching and fine root biomass are commonly enlarged by eCO_2 (Pregitzer et al. 2000; Norby et al. 2004; Madhu and Hatfeld 2013).

Apart from soil nutrient and water status, other factors may also contribute to the inconsistent results regarding root-to-shoot ratio under eCO_2. First of all, full recovery of roots from soil samples is technically difficult in soil-cultured systems, in particular for fine roots (Norby 1994). Secondly, root mortality and decomposition can cause underestimation of root biomass. These factors are further different among species and changed by growth stages, which may cause huge variation for cross-comparison. Moreover, C allocation patterns cannot be thoroughly evaluated from above- and below-ground biomass only and carbon fluxes (e.g. in forms of root exudation and respiration) should also be quantified within the system (Medlyn et al. 2001), particularly root exudation as regard to its functions in driving microbial growth and activity and hence ecological C and N cyclings.

1.3.2 Root exudation

Root exudates are produced and secreted by plant roots as either waste or functioning molecules which can be classified as two main groups: small-molecular-weight metabolites (such as sugars, organic acids and amino acids) and secondary compounds like mucilage and proteins which contribute to a larger proportion of root exudates by mass (Bais et al. 2006). The magnitude of rhizodeposition and its main part – root exudation has been largely studied

(Table 1.1). According to Jones et al. (2009), rhizodeposits account for roughly 11% of photosynthetic C and 27% of total below-ground C allocation. In a recent synthesis of literature, Pausch and Kuzyakov (2018) found that net rhizodeposition represent 3% of fixed C for crops and 5% for grasses. Specifically, the proportion of net photosynthetic C lost from root exudation is estimated between 1% and 40% (Dilkes et al. 2004). The wide variation is derived from plant species and their physiological stage, soil type, nutrient availability and collecting and analytical methods adopted (Bais et al. 2006).

Studies on root exudation are of great difficulty because root exudates are easily degradable and re-uptake by plant roots. Two classes of distinctive methods have been largely applied to study root exudation. The first one is based on root exudation collection by growing plants in axenic hydroponic culture or sand matrix which differ significantly from soil conditions (Tarnawski and Aragno 2006). Combined with instrumental analysis (e.g. Total Organic Carbon Analyser, High-Performance Liquid Chromatography), this type of methods could quantify the total amount and component of root exudates. However, plants growing in solution culture may reduce the release of root exudation due to the absence of microorganisms and/or lack of mechanical impedance. In contrast, large amounts of root exudates in soil culture may be decomposed by microorganisms in the processes of collecting and storing, leading to an underestimation of root exudation while analysis. Moreover, root exudates could also bind with soil particles and thus reduce its extractability and detectability. ^{13}C or ^{14}C isotopic labelling is another type of methods, which detects photosynthetic C allocation *in situ* and quantifies C loss via rhizosphere and rhizo-microbial respiration. However, this indirect method gives little information on the composition of root exudates. As a result, caution should be given when interpreting and comparing measurements of root exudation under eCO_2 (Tarnawski and Aragno 2006).

Root exudation is regulated by photosynthesis and is, in turn, affected by eCO_2 (Kuzyakov and Cheng 2001; Kuzyakov 2002). In general, eCO_2 increases root exudation as indicated by enhanced rhizosphere respiration and C concentration in soil solutions (Hungate et al. 1997a; Cheng and Johnson 1998; van Hees et al. 2005; Phillips et al. 2011; Drake et al. 2015) (Table 1.1). The increase in root exudation associates mostly with larger root systems under eCO_2 and also with stimulated root activity in a number of studies (Lekkerkerk 1990; Cheng and Johnson 1998; Allard et al. 2006). However, the effect of eCO_2 on root exudation is not always positive (Hodge et al. 1998; Paterson et al. 1999). For example, using the ^{14}C-tracing technology,

Paterson et al. (1999) showed that eCO_2 induces higher root exudation of *Lolium perenne* L. in non-sterile soils but lower root exudation in sterile sands. Fewer results have been reported regarding the chemical composition of root exudates with higher C/N to eCO_2 was often noticed (Paterson et al. 1997; Hu et al. 1999) in non-leguminous species as eCO_2 increases plant C fixation and decreases tissue N concentration (Pritchard et al. 1999; Rasche et al. 2017). For leguminous plants, eCO_2 has been found to decrease the tissue C/N of legumes (Rodriguez et al. 2015; Rasche et al. 2017), probably due to enhanced biological N_2 fixation under eCO_2 (Hartwig and Sadowsky 2006; Lam et al. 2012; Butterly et al. 2016a; Tobita et al. 2016). This indicates eCO_2 may also decrease the C/N of root-secreted compounds by legumes. By foliar-feeding of ^{15}N-KNO_3, De Graaff et al. (2007) further reported that eCO_2 might increase N rhizodeposition in C3 but not C4 species.

Root exudation is not merely an important process for soil C sequestration but a primary process that could drive microbial responses in soils (Hu et al. 1999; Kuzyakov and Cheng 2001; Tarnawski and Aragno 2006; Nannipieri et al. 2008; Shi 2009;). As substrates to soil microorganism, the quantitative and qualitative changes of root exudation under eCO_2 could potentially alter microbial growth, activity and community structure as well as their decomposition of plant-derived C compounds and original soil organic carbon (SOC) (Carney et al. 2007; Langley et al. 2009; Bengtson et al. 2012).

1.4 Effects of elevated CO_2 on soil microbiota

Stimulated growth of plants and the subsequent higher quantity of root exudates under eCO_2 create a relatively C-rich rhizosphere which alleviates the general C-limitation of microorganisms in soils. The decline in rhizosphere N availability is a common phenomenon at eCO_2 due to extra plant N demand and uptake to favour enhanced growth under eCO_2 (Hovenden et al. 2008; Nie and Pendall 2016). As a result, microbial processes in the rhizosphere under eCO_2 is more limited by N relative to C when compared to aCO_2 (West et al. 2006). However, soil microorganisms may not subject to N limitation because of their stronger capacities in N uptake than plant roots. This is consistent with the findings that eCO_2 increases microbial N immobilisation (Billings and Ziegler 2005; De Graaff et al. 2007; Dijkstra et al. 2008). Microbial responses to these changes in rhizosphere under eCO_2 are discussed below.

Table 1.1 Summary of methods to quantify root exudation and its responses to eCO_2

Species	Growth matrix	CO_2 (ppm)	Method	Indicator	CO_2 effect	Specific effect	Reference
Lolium perenne L.	soil	350/700[a]	^{14}C-labelling	rhizosphere resp.	100%	50%	van Ginkel et al. 1997
Lolium perenne L.	sand/soil	450/720[a]	syringe suction	TOC	–		Hodge and Millard 1998
Lolium perenne L.	soil	450/720[b]	^{14}C-labelling	total root release	82%		Paterson et al. 1999
Lolium perenne L.	sterile sand	450/720[b]	^{14}C-labelling	total root release	–58%		Paterson et al. 1999
Lolium perenne L. *Agrostis capillarism* L. *Festuca ovina* L.	soil	350/700[a]	^{14}C-labelling	^{14}C in soil solution	69%		Cotrufo and Gorissen 1997
Plantago lanceolata L.	sand	400/800[b]	^{14}C-labelling	TOC		–	Hodge and Millard 1998
Avena, Bromus, Lolium spp.	soil	360/720[c]	^{13}C-pulse labelling	root C fluxes	56%		Hungate et al. 1997a
Triticum aestivum L.	soil	350/700[a]	^{14}C-labelling	rhizosphere resp.	74%	60%	Lekkerkerk 1990
Triticum aestivum L.	soil	350/700[a]	^{14}C-labelling	root-derived C	18%	+	Billes et al. 1993
Triticum aestivum L.	C4 soil	360/700[a]	^{13}C-labelling	rhizosphere resp.	60%	31%	Cheng and Johnson 1998
Triticum aestivum L.	C4 soil	360/700[a]	^{14}C-labelling	water-soluble-C	60%		
Lipinus albus L.	h.c.	350/700[a]	HPLC	organic acids			Watt and Evans 1999
Lipinus albus L.	h.c. –P	410/740[a]	HPLC	citrate	+		Campbell and Sage 2002
Lipinus albus L.	h.c. +P			malate	+	+	
Oryza satova L.	h.c.	355/650[c]	HPLC	TOC, formic acid, acetic acid	+		Wang and Lin 1999
Oryza sativa L.	soil	370/550[c]	collection	TOC	55%		Bhattacharyya et al. 2013
Pinus echinata L.	soil	368/695[a]	^{14}C-labelling	water soluble-C	38%		Norby et al. 1987
Populus tremuloides L.	soil	367/715[c]	^{14}C-pluse labelling	rhizosphere resp.			Mikan et al. 2000
Robinia pseudocacia L.	sand	350/700[a]	water-flushing	DOC			Uselman et al. 2000
Pinus taeda L.	soil	385/585[d]	in situ collection	DOC	50%	55%	Phillips et al. 2011

h.c., hydroponic culture; '–P' and '+P' means no or with phosphorus fertiliser; superscript lower-case letter 'a', 'b', 'c', 'd' represents growth chamber, controlled environment room, open-top chamber, and FACE, respectively; resp., respiration; '+' and '–' means positive and negative effect, respectively; 'Specific effect' represents the CO_2 effect per mass unit of root biomass.

1.4.1 Microbial biomass

Microbial biomass is considered a sensitive indicator of changes in the quality and quantity of rhizodeposits. It has been largely included in climatic change experiments, however, the results have been controversial. Some studies reported positive responses of microbial biomass to CO_2 enrichment (Cotrufo and Gorissen 1997; Williams et al. 2000; Anderson et al. 2011; Das et al. 2011). For example, Das et al. (2011) reported positive effects of eCO_2 on microbial biomass over the control with microbial biomass C being increased by 6.2%, 38.0% and 49.2% at 400, 500 and 600 μmol mol^{-1} CO_2 concentration, respectively. In a meta-analysis of 43 reports, Zak et al. (2000) found that for grasses, herbaceous dicots, and woody plants, eCO_2 increased microbial biomass by 17%, 29%, and 19%, respectively. Others reported no CO_2 effects on microbial biomass (Larson et al. 2002; Mitchell et al. 2003; Zheng et al. 2010). Albeit no increase in total microbial biomass, Mitchell et al. (2003) found that eCO_2 significantly increased bacterial biomass (by 48%), implying shifting in soil microbial community structure. The inconsistent results may derive from greater microbial activity and C turnover under eCO_2 (Williams et al. 2000), system nutrient limitation (e.g. N or P) (Hungate et al. 1999) and different plant responses (Zheng et al. 2010).

1.4.2 Microbial activity

The dynamics of soil organic matter is controlled by microbial metabolic activity regulated by microbial enzymes. Enzyme synthesis is governed by biotic and abiotic factors like soil microbial community, soil moisture, C and nutrient availabilities (Niklaus et al. 2003; Carney et al. 2007; Dijkstra and Cheng 2007a; Langley et al. 2009; Bengtson et al. 2012; Guenet et al. 2012). Elevated CO_2 could change the responses of these processes to mediate microbial enzymatic activity in responses of ecological C, N and P cycles. For example, Das et al. (2011) discovered that eCO_2 increased the activities of fluorescein diacetate hydrolase, dehydrogenase, β-glucosidase, urease, alkaline and acid phosphatases with a range of 1.3% (urease) to 53.2% (alkaline phosphatase) in four tropical paddy soils. Elevated CO_2 also increased invertase and dehydrogenase activities at all sampling times with an occasional stimulating effect on the activities of β-N-acetylglucosaminidase and acid phosphatase and an inhibiting effect on nitrifying enzyme activity (Zheng et al. 2010). Carney et al. (2007) reported eCO_2 stimulated the activity of phenol oxidase that degrades recalcitrant organic materials (e.g. lignin) but not that of β-glucosidase which is important in the degradation of cellulose. However, Guenet et al. (2012) found no significant effect of eCO_2 on the overall activity profile of enzymes (α- and

β-glucosidase, β-xylosidase and alkaline phosphatase), although eCO_2 increased acid phosphatase activity. When compared to aCO_2, microbial metabolic activity and/or microbial respiration are generally higher at eCO_2 (Chen et al. 2014a; Jin et al. 2014; Lam et al. 2014). The surplus of C in form of rhizodeposition under eCO_2 may stimulate microbial activity to mobilise organic P in P-limited soils (Jin et al. 2014), to mineralise either soil organic matter or plant residues to acquire N when N is insufficient (Carney et al. 2007; Chen et al. 2014b; Lam et al. 2014) or just to increase microbial respiration without organic matter decomposition (Lagomarsino et al. 2006).

1.4.3 Microbial community

The effects of eCO_2 on soil microbiota depend on C inputs through rhizodeposition, thereby rhizosphere is the main region where direct effects of eCO_2 on the mycorrhizal, bacterial, and fungal communities occur. There is little or no direct effect of eCO_2 on the microbial community of bulk soil (Drigo et al. 2008).

Elevated CO_2 alters the fungi to bacteria ratio with more fungal biomass being reported under eCO_2 (Lagomarsino et al. 2006; Carney et al. 2007; Drigo et al. 2008; Cheng et al. 2012; Guenet et al. 2012). Elevated CO_2 frequently stimulates mycorrhizal colonization as plants invest more C to mycorrhizal fungi and rely on mycorrhizal fungi to obtain nutrients (e.g. N and P). Low nitrogen availability at eCO_2 may help to explain the enhancement of fungi because the higher C:N ratios of fungi than bacteria allow them to grow continuously even in low-N conditions (Hu et al. 2001). In another study by Jones (1998), the changes in fungal abundance under eCO_2 is ascribed to changes in rhizosphere soluble organic C and N. This shift toward fungal pathway under eCO_2 may be beneficial to soil C sequestration, because fungi are thought beneficial to aggregate formation, and subsequently the physical protection of SOM. Contrarily, the stimulated fungi may also increase microbial decomposition of soil original C (Carney et al. 2007; Cheng et al. 2012) – i.e. increased rhizosphere priming effect (RPE, which will be discussed later in detail). In contrast to above findings, other studies did not find such changes under eCO_2 (Niklaus et al. 2003; van Groenigen et al. 2007), probably due to high N availability in those systems, which inhibits saprophytic fungal communities (Lagomarsino et al. 2006; van Groenigen et al. 2007). The responses of bacterial community to eCO_2 are different across the literature (Freeman et al. 2004; Tarnawski and Aragno 2006; Grover et al. 2015; Lee and Kang

2016; Liu et al. 2017a). There are also studies showing no significant effect of eCO_2 on the structure of microbial communities (Zak et al. 1996; Griffiths et al. 1998).

1.4.4 Ecological strategy and microbial grazing

According to their growth strategies, soil microbial community could be classified as fast-growing r-strategists (or r-selected microorganisms) which specialise for utilising easily-decomposable substrates and slow-growing K-strategists which favour recalcitrant C compounds. Microorganisms with different ecological strategies may respond differently to eCO_2. Blagodatskaya et al. (2010) stated that eCO_2 enhances the growth of r-selected microorganisms especially in microaggregates (Dorodnikov et al. 2009), even though the total soil organic matter and microbial biomass C are not influenced. The shift is caused by the increased input of labile substrates through rhizodeposition at eCO_2. The increase in r-selected microorganisms indicated an acceleration of available C mineralization in soil, which may counterbalance the additional C input by roots in a future eCO_2 environment (Dorodnikov et al. 2009; Blagodatskaya et al. 2010). Elevated CO_2 is favoured by r-strategists when compared to the K-strategists due to more labile C substrates, which also confirms the notion that r-selected microorganisms are more evolved to utilize easily decomposable substrates.

The increase in the growth of r-strategists may increase the population of bacterial grazers (or predators) (Drigo et al. 2008). Elevated CO_2 could affect the grazing activities of protozoa and nematodes. Protozoa prey on bacteria, their number in soils under eCO_2 affect soil bacterial composition directly. According to Rønn et al. (2003), CO_2 enrichment has no effect on the number of bacteria but increases the total number of bacterivorous protozoa. The increase in numbers of protozoa in wheat rhizosphere is due to a general increase in rhizodeposition, other than an increased root exudation per root mass given the unchanged density of protozoa in the rhizosphere at eCO_2. Neher et al. (2004) observed that eCO_2 can lead to shifts in nematode community structure, increase bacterivores and decrease fungivores. There are also studies showing that eCO_2 reduced the amounts of larger-diameter soil nematodes mainly caused by the reduced aggregate sizes and pore neck diameters at eCO_2 (Niklaus et al. 2003). The increased grazing speeds up the turnover of microbial biomass, resulting in either increasing release of N from the microbial biomass and SOM or C accumulation and N immobilization.

1.5 Effects of elevated CO_2 on soil processes

1.5.1 Carbon cycling

Elevated CO_2 generally increases plant C fixation by up to 300% (Ainsworth and Long 2004; Hill et al. 2006) with 50% more C being allocated below-ground (Suter et al. 2002; Hoosbeek et al. 2004). From this perspective, eCO_2 may be a solution to climate change by sequestrating more atmospheric C below-ground and mitigating the on-going increment of atmospheric CO_2 (Paustian et al. 1997; Lal 2004a, 2009). On the other hand, the increased input of recently fixed C below-ground (e.g. root exudates) is of great importance to microbial processes in soils, which may potentially stimulate microbial growth and/or activity and shift microbial community structure (Langley et al. 2009; Cheng et al. 2012; Vestergård et al. 2016) and further increase or decrease original soil C decomposition (i.e. RPE). Soil C sequestration and decomposition together determine whether eCO_2 would affect soil C stock and the capacity of soils to mitigate climate change (De Graaff et al. 2006a, 2009; Lal 2008).

Greater inputs of complex organic compounds to soils under eCO_2 than aCO_2 are supposed to increase soil organic C (SOC) stocks. However, this increase is quite small (van Kessel et al. 2000; van Groenigen et al. 2002, 2006; Xie et al. 2005). According to De Graaff et al. (2006a), although the increases in aboveground and below-ground biomass were 21.5% and 28.3%, respectively, the response of SOC content to eCO_2 is minimal, which was estimated at only 1.2% per year. The marginal change may derive from short-term durations (normally, 2–3 years) of most experiments. However, even though exposed to higher levels of CO_2 for 8 to 9 years, soil organic C in the Swiss FACE showed also no significant increases (van Groenigen et al. 2002; Xie et al. 2005). Only a few studies have reported significant stimulation of SOC by eCO_2 (Lutze and Gifford 1998; Williams et al. 2000; Luo et al. 2006) while other studies show no effect of CO_2 enrichment on SOC (van Groenigen et al. 2006; Keiluweit et al. 2015). Soil type and nutrient availability may be the main factors that regulate the responses of SOC to CO_2 enrichment (Hagedorn et al. 2001; Finzi et al. 2006; Hu et al. 2006).

The zero and even negative impacts of eCO_2 on SOC stock could be induced by enhanced microbial decomposition due to higher microbial growth and activity, leading to large amounts of C lost to the atmosphere. Elevated CO_2 has been found to enlarge below-ground soil respiration (root +microbial respiration) (Norby and Luo 2004; Hoosbeek et al. 2007; Jackson et al. 2009; Drake et al. 2011). A meta-analysis of 47 studies conducted by Zak et al. (2000)

showed that eCO_2 generally increases the total below-ground CO_2 efflux, with only one study, found a decline of 10%. On average, eCO_2 increased CO_2 efflux from soil by 51%, 49%, and 42%, respectively, in the grass-, herbaceous dicot-, and woody plant-soil systems. In the same study, results of microbial respiration from incubation of root-free soil are also synthesized with microbial respiration being increased by an average of 29% under eCO_2. The increased soil respiration at eCO_2 may derive from higher turnover rates of roots and/or root mass-specific microbial activities (Cheng and Johnson 1998; Cheng and Gershenson 2007). Few studies could have successfully separated root- and soil-derived respiration, which should be given a priority in future ecological studies.

1.5.2 Nitrogen cycling

Elevated CO_2 has been projected to decrease gross N mineralisation (Berntson and Bazzaz 1997; Hungate et al. 1999), increase N immobilization (Hagedorn et al. 2005; de Graaff et al. 2007) and potentially feedback and lead to progressive nitrogen limitation in field (Finzi et al. 2006; McKinley et al. 2009; Norby et al. 2010), particularly in systems that have already constrained by available N. However, in a review of 47 studies, eCO_2 had no obvious effect on net N mineralization (the difference between gross N mineralization and microbial immobilisation) (Zak et al. 2000).

Recent evidence has proven that eCO_2 could increase soil N cycling (Kelly et al. 2012; Phillips et al. 2012; Brenzinger et al. 2017; Sun et al. 2018) but in a more closed way which means closed cycling between N mineralisation and microbial immobilisation with small or no N loss to environment via leaching or volatilisation (Dijkstra et al. 2010). Soil N cycling could be reflected in C cycling (Williams et al. 2000; Phillips et al. 2012). Rhizosphere priming effect can increase soil N availability by microbial mining of N from mineralisation of soil organic matter (Phillips and Bernhardt 2008; de Graaff et al. 2009; Nie and Pendall 2016). This is caused by both enhanced root-derived substrates and reduced N availability under eCO_2, which drives microbial synthesis of extracellular enzymes to depolymerize organic N from soil organic matter (Cheng and Kuzyakov 2005; Phillips et al. 2011; Meier et al. 2014). Nitrogen immobilized in microbial biomass could also be released if eCO_2 stimulates microbial turnover via faunal grazing of microflora (Phillips 2007). Elevated CO_2 could also affect soil N transformation. For example, eCO_2 doubled the release of long-term N_2O emissions at the Giessen Free-Air Carbon Dioxide Enrichment (GiFACE) sites. Elevated CO_2 increased soil N-

fixers, although soil denitrifiers, archaeal and bacterial ammonia oxidizers, and dissimilatory nitrate reducers were not changed by eCO_2 (Brenzinger et al. 2017). Rakshit et al. (2012) found eCO_2 decreased soil nitrate by 14–20% and the population of denitrifiers by 4–14% with no effect on the population of ammonia (AOB) and nitrite oxidizers (NOB). Adopting the quantitative polymerase chain reaction and clone library sequencing, Kelly et al. (2012) stated that eCO_2 induced significant N loss which is associated with a dramatic increase in nirK-containing (nitrite reductase gene) bacteria and reflected by a decrease in abundance of bacterial amoA copy numbers.

1.6 Rhizosphere priming effect: factors and mechanisms

Rhizosphere priming effect is termed changes in soil organic matter decomposition in soils grown with or without plants. Rhizosphere priming effect is a key microbial process that regulates soil C and N dynamics, which might account for the declining and minimal responses of soil C stock under eCO_2. Soil harbours 1500 Gt (10^{15} g) of organic carbon to 1–m depth worldwide, making it the largest terrestrial ecosystem carbon pool (Batjes 1996; Scharlemann et al. 2014), which is about three times the amount of C in vegetation and twice the amount in the atmospheric pool (Lal 2004b). Any small change in terrestrial C stock could induce tremendous changes in global C cycling which would, in turn, speed up or slow down the climate change. As a result, many studies have been conducted aiming to examine the magnitude, factors and mechanisms of rhizosphere priming effect.

1.6.1 Methods to determine rhizosphere priming effect

The determination of RPE includes three main steps: 1) collection of total below-ground CO_2 efflux; 2) separation of soil-derived CO_2-C from total below-ground CO_2 efflux; 3) taking off basal soil respiration (control) from the soil-derived CO_2-C determined in step 2. There are two potential ways to separate below-ground respiration compartments. Both are based on isotopic labelling, the first one is growing plants in ^{14}C- or ^{13}C- labelled CO_2 environment which is either continuously or pulsed supplied, with the subsequent measuring of ^{14}C activity or ^{13}C enrichment in CO_2 evolved from soil. The second way is much cost-friendly and safe which is growing C3 plants in C4 soils or vice versa and then determining the ^{13}C natural abundance from the respired CO_2. Caution should be taken when adopting such methods because possible microbial fractionation when microorganisms utilize root-derived substrates may occur and induce more ^{13}C-depleted rhizosphere than root biomass (Werth and Kuzyakov 2010; Zhu and

Cheng 2011a). The development of these isotopic techniques has sprung up the research of RPE during the past two decades.

1.6.2 Factors that affect rhizosphere priming effect

Measured RPEs range from –70% to 380% as compared to root-free soil respiration (Cheng et al. 2014; Wang et al. 2016; Zhu and Cheng 2011b). In a review by Cheng et al. (2014), all the negative RPEs are present in short-term experiments (16–38 d) and seem to be caused by initial soil disturbance and may not persist in the long run. Generally, RPEs seem to be lower and even negative at early plant growing stages, increase to the highest at the flowering stage, and decline to lower levels thereafter (Fu et al. 2002; Cheng and Kuzyakov 2005). It is in accordance with the release of substrates and root exploration at different growing stages. Except for plant phenology, plant species is another plant trait that controls RPE. Species variation in the direction and magnitude of RPEs is associated with species' capacities to release root exudates (quantity and quality), to acidify rhizosphere soil and to explore volumes of soil body (Wang et al. 2016). The contribution of root exudates to observed RPEs is estimated at 58–95% (Shahzad et al. 2015). Rhizosphere priming effect is further controlled by soil properties, like C and N availabilities (Hamer and Marschner 2005a, 2005b; Kuzyakov et al. 2007), pH (Rousk et al. 2010; Aye et al. 2016; Wang et al. 2016), moisture (Dijkstra and Cheng 2007b), aeration (Davidson and Janssens 2006), and soil chemical and/or physical protection of SOC (Six et al. 2002; Reis et al. 2014) due to their effects on microbial activity.

1.6.3 Mechanisms of rhizosphere priming effect

In the past two decades, many works have been done and several mechanisms have been proposed to understand substrate-induced microbial priming of SOC. Well-developed mechanisms of RPE include microbial activation, N mining, co-metabolism, preferential substrate utilization and concurrence demand for mineral N between plant roots and microorganisms (Fig. 1.4, Table 1.2) (Horvath 1972; Cheng and Kuzyakov 2005; Mondini et al. 2006; Carney et al. 2007; Blagodatskaya et al. 2011). The first four mechanisms are associated with positive RPEs and the last two are normally used to explain negative RPEs. Other mechanisms like stoichiometric decomposition and C protection mechanisms are also discussed (Kuzyakov 2002; Chen et al. 2014b; Razanamalala et al. 2018) (Table 1.2).

The microbial activation mechanism suggests that certain low-molecular-weight organic substances secreted by roots can act as signalling molecules to trigger a shift of microbial community from a dormant to an active state (De Nobili et al. 2001). Microbial activation or triggering is, therefore, the most unique biotic mechanism as it does not necessarily require soil microorganisms to utilise root exudates as substrate or energy (Mason-Jones and Kuzyakov 2017) and it could also be the first step of all biotic mechanisms. All other biotic mechanisms are somehow linked to soil N availability except the co-metabolism (Table 1.2). When soil is deficient in available N, both microbial N mining and N competition could occur, with the first mechanism would result in an increase in RPE but the latter would lead to a reduction in RPE. It depends on whether soil N is lower enough to limit microbial growth or activity (Berntson and Bazzaz 1997; Mason-Jones and Kuzyakov 2017). In soils with sufficient N, microorganisms would prefer utilising easily-degradable root exudates to SOC and result in a negative RPE. However, this is not exclusive because high N with greater C substrate input could also increase microbial stoichiometric decomposition of soil organic matter. Interestingly, the microbial N mining and stoichiometric decomposition mechanisms seem to be mutually exclusive. This indicates that soil N is not the sole factor that drives the balance between these two mechanisms (Razanamalala et al. 2018). The two mechanisms may be driven by different microbial communities, for example, r-strategists are found to be responsible for stoichiometric decomposition, while K-strategists are more engaged in N mining (Chen et al. 2014b). Besides, the two strategists may also target different soil C pools, with the r-strategists mainly breaking down labile soil C and the K-strategists decomposing old and N-rich soil organic matter (Razanamalala et al. 2018).

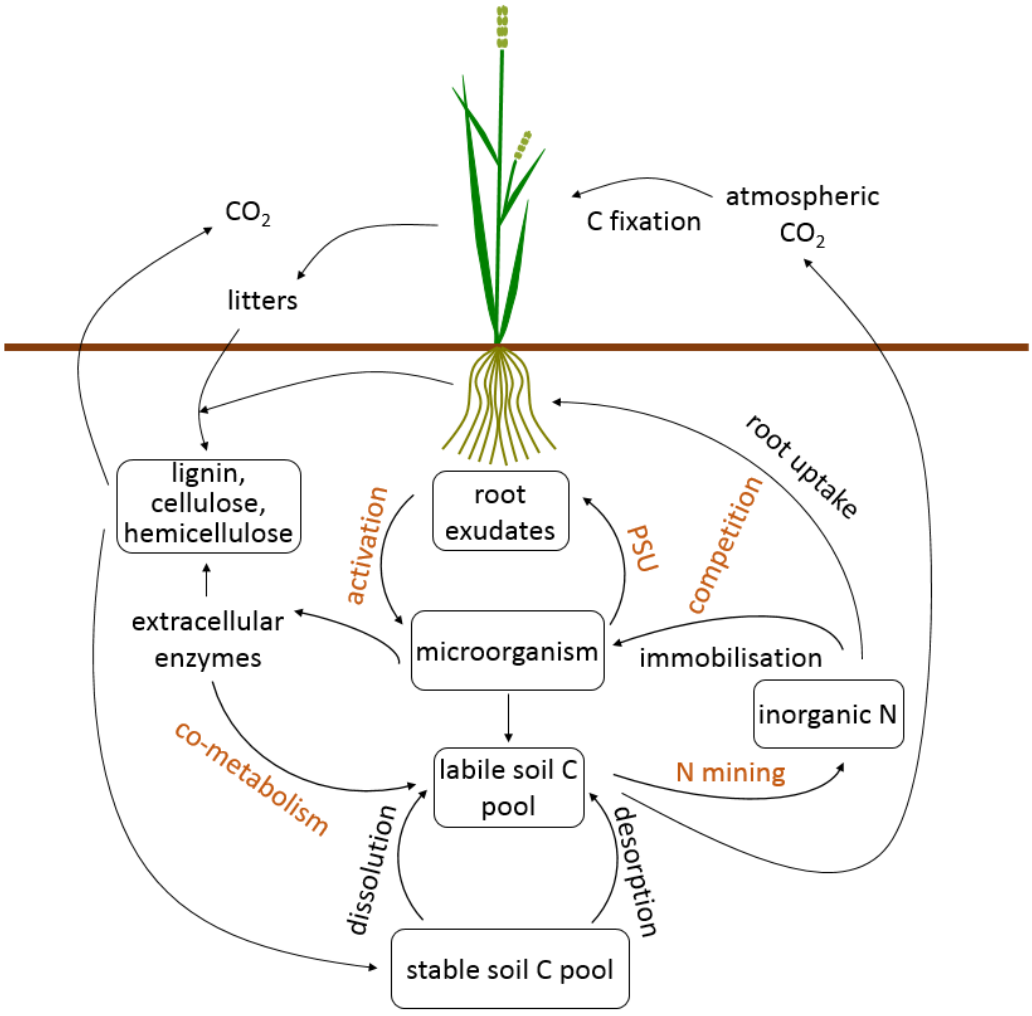

Figure 1.4 Diagrammatic denotation of below-ground C turnover and mechanisms (in brown) of rhizosphere priming effect. PSU, preferential substrate utilisation (see Table 1.2 for details of each mechanism of priming effect)

Table 1.2 Summary of biotic mechanisms of rhizosphere priming effect

Mechanism	Description
Microbial activation De Nobili et al. 2001	Easily available substrates from root exudates trigger microbial metabolism and the subsequent indigenous C mineralisation
N mining Fontaine et al. 2011	Activated microbiota are relatively limited by N other than C, leading to more decomposition of N-rich soil organic matter for N
Co-metabolism Kuzyakov et al. 2000	Stimulated synthesis and up-regulation of extracellular enzymes which are secreted to catalyse substrate decomposition unintentionally catalyse the degradation of soil organic matter
Preferential substrate utilisation Cheng 1999	With sufficient available N, soil microorganisms prefer root-derived labile C compounds rather than relatively recalcitrant soil organic matter for substrates and energy, leading to reduced decomposition of soil organic matter
N competition Cheng and Kuzyakov 2005 Kuzyakov and Xu 2013	In N-deficient soils, plants will outcompete microorganisms in N uptake in the long run, leading to microbial N limitation, reduction in microbial metabolism and growth and the subsequent decomposition of soil organic matter
Stoichiometric decomposition Chen et al. 2014b	This mechanism suggests that microbial activity is regulated by microbial stoichiometric C and N demands and the decomposing rate is the highest if N input matches with substrate C input. It indicates high N availability is more likely to enhance decomposition of soil organic matter

1.7 Possible eCO_2 effect on rhizosphere priming effect

Elevated CO_2 could affect rhizosphere priming effect through its impacts on soil microbial properties via changes in quantity and quality of root exudates, soil properties particularly N availability and pH, and root growth and exploration. First of all, greater RPEs are anticipated under eCO_2 by comparison to aCO_2 due to increased input of easily decomposable C to rhizosphere microbiota. Elevated CO_2 is also supposed to increase the complexity of plant tissues, leading to more aromatic structures and large-molecular-weight materials (Peñuelas et al. 1996, 1997; Poorter et al. 1997; Davidson and Janssens 2006). 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 to a similar pattern, less RPEs are expected to occur under eCO_2. For leguminous plants, however, eCO_2 usually boosts biological N_2 fixation, providing extra N for these plants (Hartwig and Sadowsky 2006; Lam et al. 2012; Butterly et al. 2016a; Tobita et al. 2016). As a result, the responses of legumes and non-legumes to eCO_2 may differ in their N status, and hence in the chemical composition of their root exudates, ultimately affecting RPEs. Secondly, rhizosphere N availability 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 immobilization in Dijkstra et al. (2010) and inhibited nitrification in Lagomarsino et al. (2008)). According to the N-mining mechanism, low soil available N would drive soil microorganisms to mine N from the N-rich soil organic matter, leading to a higher RPE (Kuzyakov 2002). Increasing soil N availability by applying N fertilizer and/or increased biological N_2 fixation would alleviate microbial N limitation and their synthesis of extracellular enzymes to degrade soil organic matter for N, resulting in a decrease in RPE. However, soil N availability may also be lowered by eCO_2 to the extent that the level would limit microbial decomposition and lead to a net accumulation of SOC when compared to aCO_2 (Hoosbeek et al. 2004). It is apparent that soil N status is of great importance in mediating RPE under eCO_2 and more works should be done to improve our understanding. Except for properties of substrates and nutrients, pH is another factor that affects soil microbial activities, community structures and functions (including SOC decomposition) (Blagodatskaya and Kuzyakov 2008). Elevated CO_2 could change rhizosphere soil pH by affecting cation-anion uptake of plant roots (Guo et al. 2012) and RPEs would also be changed by eCO_2 through this way. Lastly, plants grown under eCO_2 often exhibit larger root systems and hence larger rhizosphere volumes (Cheng and Johnson 1998; Dijkstra et al.

2009; Nie et al. 2013a; Butterly et al. 2016b), which implies larger volume of soil body is subjected to microbial decomposition under eCO_2 relative to aCO_2.

1.8 Thesis structure

Root exudates are supposed to differ naturally between plant species and developmental stages. The differences would be further changed by CO_2 enrichment from the discussion above. Whether these changes would lead to alternations in RPEs is studied in Chapter 2. The results showed that eCO_2 had opposite effects on RPE under white lupin and wheat plants at the early reproductive stage. The decrease in RPE under wheat by eCO_2 might result from N limitation of both plants and microbiomes. The conclusion is confirmed by the second experiment (Chapter 3) which showed no CO_2 × N interaction on RPEs and N limitation had an adverse effect on RPE in comparison to N mining. Rhizodeposits or root exudates can also fuel soil microbes to decompose other C sources in soil such as plant litters – a critical pathway to form stable SOC. A third experiment (Chapter 4) was conducted to examine the eCO_2–induced changes in rhizosphere effect on the decomposition of crop residues using a stable ^{13}C isotopic tracing technique. The results suggested that eCO_2 could also increase residue decomposition and N mineralization via microbial activation and/or co-metabolism but this effect depended on plant growth stage and residue type. In these short-term experiments, eCO_2 changed the decomposition of both residue and SOC. A further experiment (Chapter 5) aimed to study whether the RPE is a short-term microbial response to eCO_2 and how long-term CO_2 enrichment affects soil C distribution and stability based on the long-term (8 years) Free-air CO_2 Enrichment (FACE) facility at Horsham, Victoria, Australia. The 8–year eCO_2 history did not build up surface (0–5 cm) SOC stock in all three major Australian cropping soils, implicating enhanced decomposition. Elevated CO_2 history escalated the vulnerability of SOC to ^{13}C-glucose-induced priming but this effect was only significant in the sandy Calcarosol soil which had low and unprotected SOC. An additional experiment (Chapter 6) was included to explore any new and abiotic mechanism that might also contribute to the observed RPE, mainly focused on chemical liberation of C by root exudates from protected mineral-organic associations through dissolution and/or complexation reactions. The results showed that root exudates (organic acid anions) could increase microbial accessibility to previously-protected SOC and thus lead to a general increase in RPE. The thesis ends up with a general discussion (Chapter 7) of key findings from the PhD project.

Chapter 2 Wheat and white lupin differ in rhizosphere priming of soil organic carbon under elevated CO_2

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2.1 Summary

Elevated CO_2 (eCO_2) alters plant rhizosphere processes and soil microbial properties which, in turn, changes the decomposition of native soil organic carbon (SOC) – a process termed rhizosphere priming effect (RPE). This study examined the effect of eCO_2 on RPEs of plant species contrasting in root system and exudation. Specifically, two C3 species, wheat (*Triticum aestivum* L. cv. Yitpi) and white lupin (*Lupinus albus* L. cv. Kiev), were grown under ambient CO_2 (aCO_2, 400 ± 15 μmol mol^{-1}) and eCO_2 (800 ± 30 μmol mol^{-1}) for 34 and 62 days in a C4 soil. The amounts of CO_2 derived from SOC and plant roots were quantified. Elevated CO_2 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, eCO_2 decreased wheat RPE by 22% and did not affect rhizosphere soil respiration and microbial biomass carbon at Day 62. Moreover, eCO_2 increased the concentration of soluble organic carbon in the rhizosphere of white lupin but not wheat. The enhanced RPE of white lupin but not wheat under eCO_2 had resulted from an increase in root exudation of white lupin.

2.2 Introduction

The CO_2 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 CO_2 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 CO_2 (eCO_2) would yield differences in RPEs. Greater RPEs are anticipated under eCO_2 by comparison to aCO_2 due to

increased input of easily decomposable C. From another perspective, eCO_2 changes the chemical composition of plants to more production of carbon-based secondary compounds (e.g. phenolics) (Peñuelas et al. 1996; Peñuelas et al. 1997). The increases of such substances may facilitate soil C sequestration because they are biologically resistant to microbial decomposition. If eCO_2 alters the chemical composition of root exudates, RPEs are expected to change under eCO_2. Furthermore, eCO_2 enhances biological N_2 fixation in legumes, which in turn provides extra N for these plants (Hartwig and Sadowsky 2006; Lam et al. 2012; Butterly et al. 2016a; Tobita et al. 2016). As a result, eCO_2 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, 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 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 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 and 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 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 (jes, Mommer and Vries 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 eCO_2 alters RPE via its effect on root growth traits or exudation.

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 morphology. We hypothesized that 1) eCO_2 would stimulate the RPEs due to an increase in root exudation; 2) white lupin would have greater RPE stimulation under eCO_2 than wheat due to its higher quantity of root exudates.

2.3 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* $CaCl_2$) 5.0, clay 130 mg g^{-1}, and $\delta^{13}C$ –19.3‰.

2.3.1 Experimental design

A column experiment was carried out in four growth cabinets (Fitotron SGC 120, Loughborough, Leicestershire, UK). It consisted of two CO_2 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 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}). Pure CO_2 (Coregas, Yennora, New South Wales, Australia) was pumped into the growth cabinets and mixed with the air inside to get the desired CO_2 concentrations. The concentrations of CO_2 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 CO_2 on CO_2 partitioning, the growth cabinets were fluxed with fresh air and re-supplied with pure CO_2 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).

2.3.2 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 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.

Prior to planting, plant seeds were pre-soaked in Milli-Q water and germinated at 25 °C for 48 h. Eight pre-germinated seeds of wheat and four of white lupin were sown in a row into each column and seedlings were thinned to four for wheat and two for white lupin plants one week after emergence. Nitrogen was supplied as urea at a rate of 30 mg N kg^{-1} soil $week^{-1}$ from the fourth week. An additional set of unplanted columns was used as controls without urea application. The planted and control columns were transferred into growth cabinets. Soil water content was maintained at 80% field capacity by adding reverse-osmosis water daily based on weight loss. The soil columns were randomly reallocated between two replicated growth cabinets weekly to ensure homogenous growing conditions. Plants 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.

2.3.3 Below-ground CO_2 trapping

CO_2 trapping was conducted to quantify below-ground CO_2 released from soil columns before each harvest but 3–4 days after urea application. 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, Victoria, 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 gas movement, a vacuum was attached at the end of the trapping apparatus. More details can be found 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 1996). To prevent the formation 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 with Milli-Q water, dried in an oven at 60 °C and analysed 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 $\delta^{13}C$ value 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 ($C_{control}$) which was –14.91‰. f is the contribution of SOC decomposition to total below-ground CO_2 efflux. In this study, the ^{13}C abundance of roots other than the $\delta^{13}C$ 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.

2.3.4 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 analysed 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 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 ℃

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 gram of rhizosphere soil was incubated in a 1–L Mason jar at 25 ºC 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_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 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). Soil extracts from non-fumigated samples were also analysed 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, Colorado, USA).

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

2.3.5 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).

2.4 Results

2.4.1 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 2.1). 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. 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.

2.4.2 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 2.1). 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 CO_2 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 by18% at the second harvest. At both harvests, white lupin had lower C:N ratios than wheat (Table 2.1).

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 only. However, eCO_2 did not affect the root ^{13}C composition of either species at the second harvest (Table 2.1).

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

The magnitude of eCO_2 effect on below-ground respiration depended on plant species and growth stage (Fig. 2.1, Table 2.2). Elevated CO_2 had no significant effect on below-ground CO_2 evolved from wheat columns at both growth stages (Fig. 2.1). However, it increased total below-ground respirations under white lupin by 39% and 19% for the first and second harvest, respectively (Fig. 2.1). 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

Table 2.1 Shoot and root dry weights, N concentrations and C-to-N ratios, root length, and the $\delta^{13}C$ abundance of root and below-ground CO_2 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-to-N ratio		Root length	$\delta^{13}C$ abundance (‰)	
		Shoot	Root	Shoot	Root	Shoot	Root	(m $column^{-1}$)	Root	CO_2
					Day 34					
Wheat	aCO_2	2.12ab	1.46b	44.2b	27.0b	9.53a	14.0c	28.5b	–27.6c	–25.2c
	eCO_2	2.55 b	1.87c	31.9a	21.3a	13.4b	18.5d	30.4b	–28.9b	–26.2b
White lupin	aCO_2	1.71 a	0.58a	46.2b	28.8c	9.18a	12.3b	10.1a	–29.4a	–25.9b
	eCO_2	2.06ab	0.74a	42.2b	29.3c	10.0a	11.8a	10.2a	–29.5a	–26.5a
Significant level										
CO_2		*	*	***	***	**	***	_	**	**
Species		*	***	**	***	**	***	***	***	*
CO2 × species		–	–	*	***	*	***	–	**	–
					Day 62					
Wheat	aCO_2	10.5b	3.71c	17.2a	18.8b	25.3b	21.6b	589b	–26.8a	–22.2b
	eCO_2	11.1b	3.93c	16.0a	16.2a	26.7b	25.6c	615b	–27.3a	–22.8ab
White lupin	aCO_2	7.13a	1.58a	37.3c	29.0c	11.5a	14.7a	57 a	–26.5a	–24.7ab
	eCO_2	9.70b	2.83b	31.4b	29.7c	13.6a	14.4a	73 a	–27.2a	–25.2a
Significant level										
CO_2		**	***	**	*	_	*	_	_	_
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, $P < 0.05$

(Fig. 2.1, Table 2.2). Moreover, the below-ground respiration was 2.4 times greater at the second than the first harvest for white lupin (Fig. 2.1).

Elevated CO_2 increased the root-derived CO_2 from white lupin-soil system only, leading to a significant CO_2 × species interaction (Fig. 2.1, Table 2.2). The increases were 44% and 15% at the first and second harvest, respectively (Fig. 2.1). At the second harvest, the root-derived CO_2 from white lupin was 2.9 times greater than that from wheat.

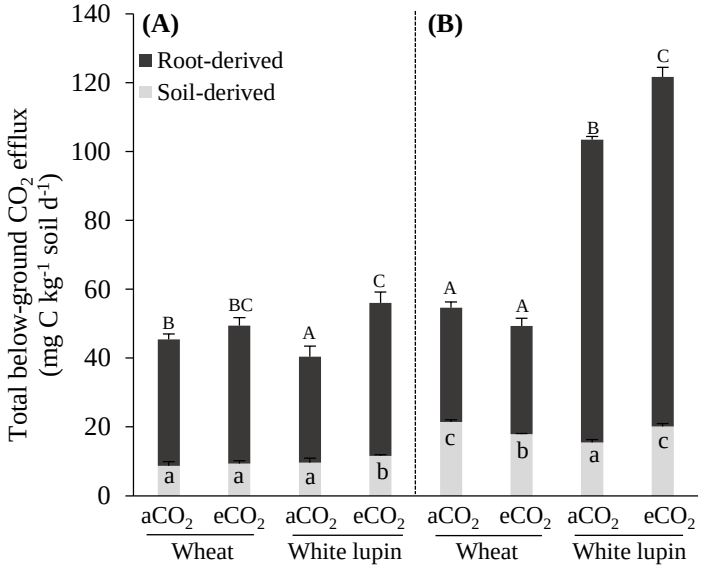

Figure 2.1 Total below-ground CO_2 efflux (soil-derived CO_2-C and root-derived CO_2-C) from soil columns with wheat and white lupin grown for 34 (A) and 62 (B) days under aCO_2 (400 μmol mol^{-1}) and eCO_2 (800 μmol mol^{-1}). Error bars represent standard errors of means of four replicates. Means with the same upper-case letter (root-derived CO_2-C) or lower-case letter (soil-derived CO_2-C) within the same harvest are not significantly different at $P = 0.05$ using Duncan's new multiple range test

The ^{13}C abundance of the CO_2 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 2.1). Elevated CO_2 decreased the ^{13}C abundance at Day 34, and tended to decrease it though not significant at Day 62. The CO_2 evolved from white lupin columns showed invariably lower $\delta^{13}C$ abundances when compared to those from wheat columns (Table 2.1).

2.4.4 Rhizosphere priming effect

Elevated CO_2 tended to increase the soil-derived CO_2 under wheat at the first harvest, but decreased it by 16% at the second harvest (Fig. 2.1). Elevated CO_2 increased the soil-derived CO_2 under white lupin and the increases were 21% and 30% at the first and second harvest, respectively (Fig. 2.1). On average, the soil-derived CO_2 was 18% higher under white lupin than under wheat at the first harvest but was 9% lower at the second harvest (Fig. 2.1, Table 2.2).

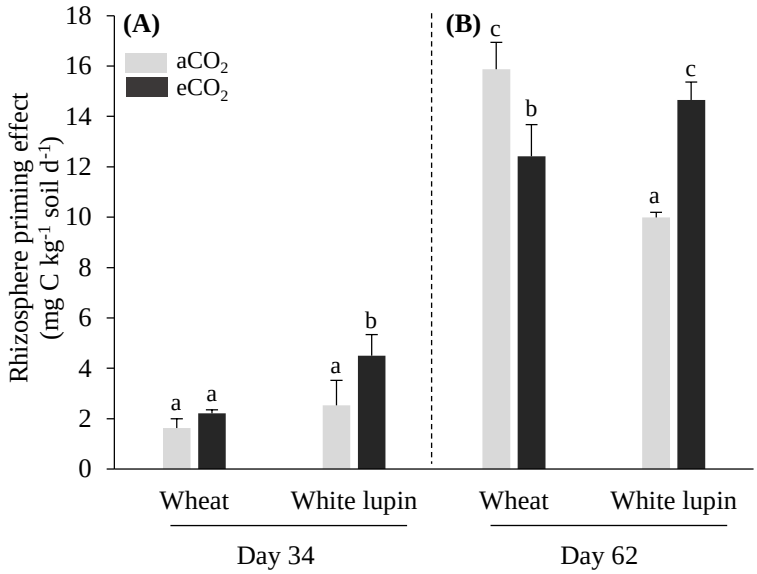

Figure 2.2 Rhizosphere priming effects (Primed soil C) of wheat and white lupin grown for 34 (A) and 62 (B) days under either aCO_2 (400 µmol mol^{-1}) or eCO_2 (800 µmol mol^{-1}). Error bars represent standard errors of means of four replicates. Means with the same letter within the same harvest are not significantly different at $P = 0.05$ using Duncan's new multiple range test

Rhizosphere priming effects were determined by taking the basal soil respiration rates out of the soil-derived CO_2. The basal soil respiration rates in the control amounted at 7.11 and 5.51 mg CO_2-C kg^{-1} soil d^{-1} at the first and second harvest, respectively. At the first harvest, eCO_2 showed no impact on the RPE of wheat but it increased white lupin RPE by 78% (Fig. 2.2A). On average, the RPE was 1.8 folds higher under white lupin than under wheat (Fig. 2.2, Table 2.2). At the second harvest, eCO_2 decreased the RPE under wheat by 22% but increased that under white lupin by 47% (Fig. 2.2B, Table 2.2). When averaged two CO_2 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. 2.2).

2.4.5 Rhizosphere soil respiration and 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. 2.3A, B, and Table 2.2). However, it increased the rhizosphere soil respiration of white lupin by 63% at the second harvest (Fig. 2.3B). 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. 2.3A, B, Table 2.2).

The significant CO_2 effect on soil MBC was only observed in the rhizosphere of white lupin at the second harvest (Fig. 2.3C, 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 by comparison with wheat (Fig. 2.3C, D, and Table 2.2). Additionally, MBC in the rhizosphere of white lupin was 115% higher at the second than the first harvest (Fig. 2.3C, D).

2.4.6 Rhizosphere K_2SO_4-extractable C

Elevated CO_2 had no significant effect on K_2SO_4-extractable C (EOC) in the rhizosphere of wheat at the first harvest (Fig. 2.4A**).** However, it increased the EOC in white lupin rhizosphere by 53% and 22% at the first and second harvest, respectively (Fig. 2.4). 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. 2.4, Table 2.2). Furthermore, rhizosphere EOC of white lupin increased 3 folds at the second harvest compared to the first harvest (Fig. 2.4).

2.4.7 Rhizosphere K_2SO_4-extractable N

The concentrations of K_2SO_4-extractable 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 extractable inorganic N were 59% and 45% lower under eCO_2 than aCO_2 for wheat and white lupin, respectively (Fig. 2.5A). At the second harvest, the concentration of extractable inorganic N in white lupin rhizosphere was 23% lower under eCO_2 (Fig. 2.5B). The concentrations of extractable inorganic N in the rhizosphere were higher under white lupin than under wheat (Fig. 2.5, Table 2.2). The decreases in the concentrations of extractable inorganic N under eCO_2 was mainly attributed to the decline in NO_x^-–N concentrations (Fig. 2.5).

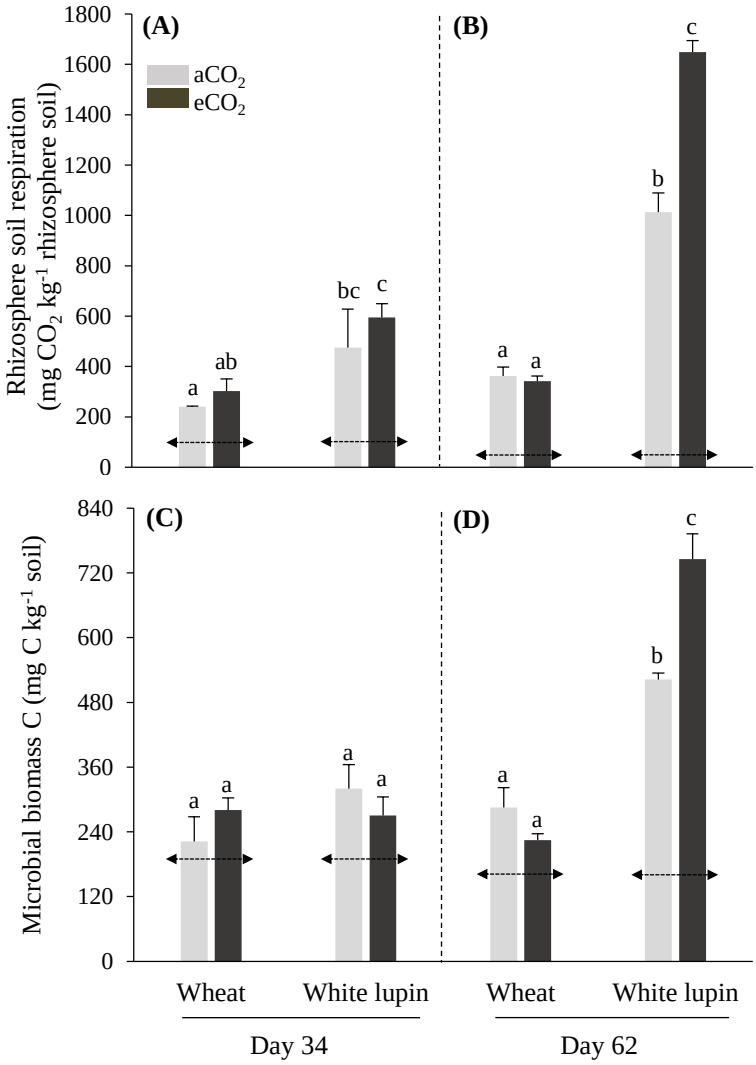

Figure 2.3 Rhizosphere soil respiration (A, B) and microbial biomass C (C, D) in soil with wheat and white lupin grown for 34 (A, C) and 62 (B, D) days under either aCO_2 (400 μmol mol^{-1}) or eCO_2 (800 μmol mol^{-1}). The arrow-ended short lines represent the values obtained from unplanted control soils. Error bars represent standard errors of means of three replicates for Day 34 and four replicates for Day 62. Means with the same letter within the same harvest are not significantly different at $P = 0.05$ using Duncan's new multiple range test

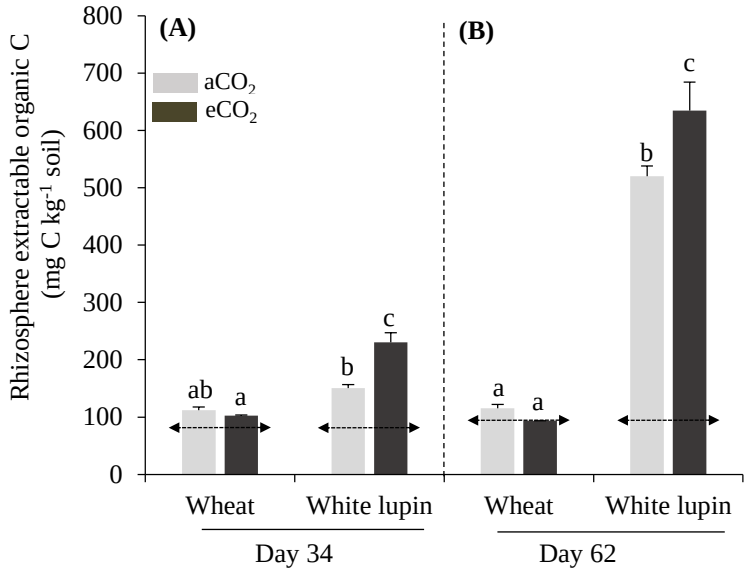

Figure 2.4 Concentrations of K_2SO_4-extractable organic C in the rhizospheres of wheat and white lupin grown for 34 (A) and 62 (B) days under either aCO_2 (400 μmol mol^{-1}) or eCO_2 (800 μmol mol^{-1}). The arrow-ended short lines represent the values obtained from unplanted control soils. Error bars represent standard errors of means of three replicates for Day 34 and four replicates for Day 62. Means with the same letter within the same harvest are not significantly different at $P = 0.05$ using Duncan's new multiple range test

Table 2.2 Two-way ANOVA analysis of total below-ground CO_2 efflux, root- and soil-derived CO_2, rhizosphere priming effect (RPE), rhizosphere soil respiration ($Rh_{resp.}$), rhizosphere K_2SO_4-extractable C (EOC), rhizosphere K_2SO_4-extractable N (NH_4^+–N and NO_x^-–N), and microbial biomass C (MBC) at Day 34 and 62

	CO_2 efflux	Root-derived C	Soil-derived C	RPE	$Rh_{resp.}$	EOC	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

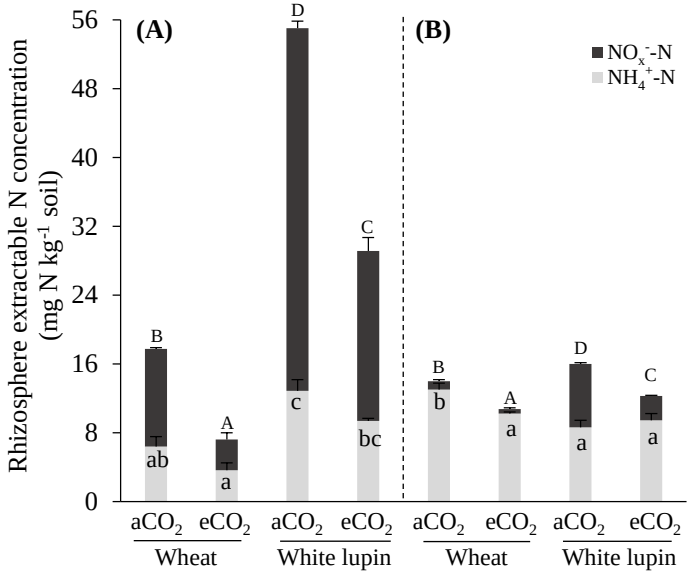

Figure 2.5 Concentrations of K_2SO_4-extractable inorganic N (NH_4^+–N and NO_x^-–N) in the rhizosphere of wheat and white lupin grown for 34 (A) and 62 (B) days under either aCO_2 (400 μmol mol^{-1}) or eCO_2 (800 μmol mol^{-1}). Error bars represent standard errors of means of three replicates for Day 34 and four replicates for Day 62. Means with the same upper-case letter (NO_x^-–N) or lower-case letter (NH_4^+–N) within the same harvest are not significantly different at $P = 0.05$ using Duncan's new multiple range test

2.5 Discussion

This study demonstrated that the CO_2 effect on the rhizosphere priming of SOC decomposition differed between plant species and between developmental stages. Elevated CO_2 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 possibly be attributed to variations in the quantity and quality of root exudates between the two species.

In contrast to our hypothesis, eCO_2 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, eCO_2–induced labile C input from wheat roots might be too small to induce a measurable change in microbial growth (Fig. 2.3A, C) and hence rhizosphere primed C. This is evidenced by no increases in root-derived CO_2 or extractable organic C in rhizosphere soil under eCO_2 relative to aCO_2. The decreased RPE under eCO_2 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. (1991) also reported that eCO_2 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 eCO_2. Soil microbial community shifted their substrate preference to readily decomposable root-derived C at eCO_2, decreasing their dependence on SOC and the decomposition of existing SOC. However, this explanation is not applicable to this study because eCO_2 did not change the quantities of root exudates as indicated by root-derived CO_2 and extractable organic C in the rhizosphere (Figs. 2.1B, 2.4B). 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 eCO_2 (Kuzyakov 2010; Cheng et al. 2014; Nie et al. 2015). For example, Cheng and Johnson (1998) found that eCO_2 (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 eCO_2 was considered as an important source of substrates for soil organisms to decompose SOC (Cheng and Gershenson 2007). This variation in CO_2 effect on plant growth between this and other studies is likely caused by difference in N availability (Billings and Ziegler 2008).

In contrast 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. This observation was consistent with the increases in rhizosphere soil respiration and microbial biomass C under eCO_2 (Fig. 2.3). In this study, eCO_2 stimulated the input of labile C, as indicated by the increases in root-derived CO_2 and extractable organic C (Figs. 2.1, 2.4), and hence the ability of soil microorganisms to decompose SOC (Health et al. 2005; Nie et al. 2013b; 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 eCO_2 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, 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. 2006b; Jin et al. 2012; Lam et al. 2012). The

changes in the quality alongside the quantity of root-derived substrates under eCO_2 possibly 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 (Berntson and Bazzaz 1997; Craine et al. 2007; Zang et al. 2016). Elevated CO_2 decreased the concentrations of K_2SO_4-extractable N in the rhizosphere of both species, mainly in the form of nitrate (Fig. 2.5) 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 eCO_2. The reduction of soil N availability under eCO_2 could also be caused by increased microbial N immobilisation (Billings and Ziegler 2005). The decrease in soil N availability under eCO_2 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. 2014b). However, in this study, eCO_2 decreased soil N availability, which might not have constrained soil microbes with white lupin because more N-rich root exudates under eCO_2 due to enhanced biological N_2 fixation (de Graaff et al. 2007) might have met the microbial N requirement. In the wheat-soil system, the negative effect of eCO_2 on N availability was stronger. This might have limited the activity of microorganisms to decompose SOC, which is in line with many other studies (Berntson and Bassaz, 1997; Bengtson et al. 2012). Moreover, lower N availability under eCO_2 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 eCO_2 was pertinent to the increase in fungal abundance which is possibly caused by the lower N availability under eCO_2 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 CO_2 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 CO_2 and extractable organic C in the rhizosphere soil (Figs. 2.1A, 2.4A) 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 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 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 explorer 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 CO_2, rhizosphere soil respiration and microbial biomass C were all greater at Day 62. However, there was less root-derived CO_2 ($P < 0.05$), 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 2.1). More studies are needed to cover the entire growth stages to better understand the effect and mechanisms of plant phenology on RPE.

2.6 Conclusions

Elevated CO_2 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 eCO_2 might

account for the species-specific responses in RPEs to eCO_2. This study suggests that future high atmospheric CO_2 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 eCO_2, and how microbes respond to these changes under various plant species at different growth stages.

Chapter 3 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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3.1 Summary

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 (N) supply affect the RPEs under wheat and white lupin. Specifically, C3 plants wheat (*Triticum aestivum* L. cv. Yitpi) and white lupin (*Lupinus albus* L. cv. Kiev) were grown at two N addition rates under ambient CO_2 (aCO_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. This study found that relative to adequate N supply, low N 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. It concluded that low N availability in soil increased the RPE probably via stimulated microbial N mining while eCO_2 and severe N limitation synergistically decreased the RPE under wheat but not under N_2–fixing white lupin.

3.2 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 enhance plant biomass production and the turnover of root-derived carbon (C) via stimulated microbial activity (Cheng and Johnson 1998; Health et al. 2005). Root-induced increase in the mineralization of indigenous soil organic C (SOC), i.e. rhizosphere priming effect (RPE), may play a vital role in the decrease in terrestrial carbon sink (Bengtson et al. 2012). Elevated CO_2 (eCO_2) could potentially change RPE via altering the rhizosphere properties which in turn affect microbial growth and activity.

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. 2017b). By comparison to ambient CO_2 (aCO_2), plants grown under 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. As a result, higher RPE is anticipated under eCO_2 due to enhanced C substrates for microorganisms. In another respect, the composition of rhizodeposits under eCO_2 could differ from that under 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, stimulated microbial immobilisation (Dijkstra et al. 2010) and/or 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 to 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 showed 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 the 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) low N availability in soil would enhance microbial N mining and hence increase the RPE, (ii) eCO_2 would increase the RPE due to enhanced root exudation, and (iii) eCO_2 would lead to extreme N limitation, which in turn decreased the RPE under wheat but not white lupin.

3.3 Materials and methods

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 the 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.

3.3.1 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 CO_2 controllers. 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 soil-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 3.1. 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 at 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.

Table 3.1 Rates of N (mg urea-N kg^{-1} soil) 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

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.

3.3.2 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 the 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 diameters of the columns. 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 in the 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.

3.3.3 Below-ground CO_2 trapping

Below-ground CO_2 were trapped at 32 days after sowing (Day 32) when both species were at the early vegetative stage and 52 days (Day 52) when wheat came to the late jointing stage while white lupin was at the late vegetative stage. Before each CO_2 trapping, the top of each column was sealed with two PVC plates around plant stems and the interspaces were closed 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 of the carbonate with 0.25 *M* $BaCl_2$ standard solution. The total below-ground CO_2 efflux was then partitioned as root-derived CO_2-C and soil-derived CO_2-C based on the differences in their ^{13}C isotopic abundances. Another subsample of NaOH trap was mixed with excessive $SrCl_2$ (0.25 *M*) to form $SrCO_3$ 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).

3.3.4 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 3.2) 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 3.2). $\delta^{13}C_{soil}$ 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‰). The small difference (6.4‰) between the $\delta^{13}C$ values of soil-derived CO_2 and root-derived CO_2 could have lowered the detection limit. However, the standard errors of $\delta^{13}C$ values ranged from 0.04 to 1.15‰, with a mean of 0.59‰, which gives a coefficient of variance (CV) of 9.2%. It means any treatment difference that is higher than 18% of the mean would be detected as statistically significant. The treatment effect in this study (23%–84%) is higher than this value, which ensures that the method is feasible in separation of plant-derived and soil-derived CO_2-C.

Table 3.2 $\delta^{13}C$ 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	–22.4 ± 0.2	–22.1 ± 1.1	–24.5 ± 1.2	–24.9 ± 0.5
eCO_2	LN	–22.3 ± 0.7	–21.8 ± 0.4	–24.2 ± 0.4	–24.5 ± 0.5
aCO_2	NN	–23.7 ± 0.5	–22.7 ± 0.4	–24.5 ± 1.2	–24.9 ± 0.4
eCO_2	NN	–23.4 ± 1.0	–22.3 ± 0.0	–24.2 ± 0.4	–24.3 ± 0.4
		White lupin			
aCO_2	LN	–22.6 ± 0.5	–22.8 ± 0.8	–24.6 ± 0.9	–25.5 ± 0.4
eCO_2	LN	–22.3 ± 0.3	–23.1 ± 1.0	–25.1 ± 0.6	–26.0 ± 0.5
aCO_2	NN	–23.5 ± 0.7	–23.6 ± 1.0	–24.6 ± 0.9	–25.3 ± 0.4
eCO_2	NN	–23.2 ± 0.6	–24.0 ± 0.7	–25.1 ± 0.6	–25.9 ± 0.5

3.3.5 Plant and soil analyses

Immediately after the first CO_2 trapping (Day 32), a composite sample of 16 g soil was collected by a small corer (diameter 1 cm, length 10 cm) from each soil column for the determination of extractable C and N. No plant was 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 2–mm sieves. 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 tissue ^{15}N abundances with an 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. Air-dried soil samples were ball-milled and analysed for 13C abundances using the IRMS.

Rhizosphere soil respiration ($Rh_{resp.}$) 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 short-time 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).

Fresh rhizosphere soil was extracted in 0.5 M K_2SO_4 (soil: solution, 1: 5) by end-to-end shaking for 1 h. Soil extracts were then filtered through Whatman No. 42 filter paper and analysed for extractable organic C (EOC) using a TOC analyser (GE Sievers InnovOx, Boulder, Colorado, USA). The extractable inorganic N (EIN, NH_4^+–N + NO_3^-–N) was determined by a flow-injection analysis system (Lachat's QuickChem 8500, Loveland, Colorado, USA). At the second harvest, fresh soils with or without chloroform fumigation in dark for 24 h (Vance et al. 1987) were extracted and determined for EOC and EIN using the above procedures.

Microbial biomass C (MBC) was calculated as the differences in EOC between the fumigated and non-fumigated samples with a conversion factor 0.45 (Brookes et al. 1985).

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 CaCl2 (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).

3.3.6 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 the following mineral nutrients (mg g^{-1}): $(NH_4)_2SO_4$, 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).

3.3.7 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 new multiple range test. Pearson

correlation coefficients (r) among RPE, MBC, rhizosphere soil respiration and EOC were determined separately for wheat and white lupin using Microsoft Excel (Microsoft 2013, Redmond, Washington, USA).

3.4 Results

3.4.1 Plant growth

Elevated CO_2 increased the shoot and root biomass of wheat by 28% and 56%, respectively, at Day 52 (Table 3.3). Low N rate (LN) did not affect the root growth but decreased the shoot biomass of wheat by 10% under eCO_2 (Table 3.3). 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 3.3). 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 3.3).

Elevated CO_2 increased the root length of wheat and white lupin by 80% and 27%, respectively, irrespective of N rates (Table 3.3). The eCO_2–induced increase in root length of wheat was greater under NN, resulting in a significant CO_2 × N interaction.

3.4.2 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 3.3). Elevated CO_2 had no effect on the root N concentration of wheat but decreased the shoot N concentration by 24% (Table 3.3). In general, eCO_2 decreased the shoot and root N concentrations of white lupin by 13% and 11%, respectively (Table 3.3).

Elevated CO_2 had no effect on ^{15}N atom% in plant shoot (Table 3.3). The ^{15}N abundances of shoot and root increased consistently in response to adequate N application.

3.4.3 Root-derived CO_2-C

At Day 32, eCO_2 increased the root-derived CO_2-C of wheat by 33% and 68% under LN and NN, respectively, but decreased that of white lupin by an average of 24% (Fig. 3.1A, Table 3.4). When compared to NN, LN decreased the root-derived CO_2-C by 48% and 25% for wheat and white lupin, respectively (Fig. 3.1A, Table 3.4).

By Day 52, eCO_2 decreased the root-derived CO_2-C of wheat by 30% (Fig. 3.1B, Table 3.4). However, it increased that of white lupin by 38% under LN but not NN, causing a significant CO_2 × N interaction (Fig. 3.1B, Table 3.4). As compared to NN, LN decreased the root-derived CO_2-C of wheat by 56% irrespective of CO_2 level and that of white lupin by 32% under aCO_2 (Fig. 3.1B, Table 3.4).

3.4.4 Soil-derived CO_2-C

At Day 32, eCO_2 increased the soil-derived CO_2-C by an average of 39% and 26% for wheat and white lupin, respectively (Fig. 3.1A, Table 3.4). When compared to NN, LN increased the soil-derived CO_2-C by 84% and 58% under wheat and white lupin, respectively (Fig. 3.1A, Table 3.4).

At Day 52, eCO_2 decreased the soil-derived CO_2-C under wheat by 23%, but increased that under white lupin by 41% (Fig. 3.1B, Table 3.4). The low rate of N had no effect on the soil-derived C under white lupin but decreased that under wheat by 30% when compared to NN (Fig. 3.1B, Table 3.4).

3.4.5 Rhizosphere priming effect

The basal soil respiration rates were 2.1 and 2.0 mg C kg^{-1} soil d^{-1} soil at Day 32 under LN and NN, respectively. The corresponding values were 3.41 and 3.95 mg C kg^{-1} soil d^{-1} at Day 52 (arrow-ended dash lines in Fig. 3.1).

At Day 32, eCO_2 increased the RPEs of wheat and white lupin by 57% and 34%, respectively, when averaged across the two N rates (Fig. 3.2A, Table 3.4). The low rate of N increased the RPEs of wheat by 167% and that of white lupin by 97% (Fig. 3.2A, Table 3.4).

At Day 52, eCO_2 decreased the RPE of wheat by 34% regardless of N level (Fig. 3.2B, Table 3.4). However, the eCO_2–induced increase in primed C (by 59%) was still detected for white lupin (Fig. 3.2B, Table 3.4). The low rate of N decreased the RPE by 37% for wheat but did not affect the RPE of white lupin (Fig. 3.2B, Table 3.4). Rhizosphere priming effect was positively correlated with microbial biomass C under both wheat and white lupin ($P < 0.01$, Table 3.4).

3.4.6 Rhizosphere soil respiration

No significant treatment effect was found for rhizosphere soil respiration under wheat (Fig. 3.3A, Table 3.4). 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 29% when the plants were exposed to eCO_2 (Fig. 3.3A, Table 3.4).

3.4.7 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. 3.3B, Table 3.4). 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 (Fig. 3.3B, Table 3.4).

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

At Day 32, eCO_2 only increased the EOC under wheat by 49% when received NN (Fig. 3.4A, Table 3.4). By Day 52, eCO_2 increased the EOC (by 39–102%) in the rhizosphere of both species except for wheat supplied with LN (Fig. 3.4B, Table 3.4). Increasing N rate increased the EOC concentration only under eCO_2 for both plant species, leading to significant CO_2 × N interactions (Fig. 3.4B). The EOC concentration in the rhizosphere was positively correlated with rhizosphere soil respiration ($P < 0.05$, Table 3.5).

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. 3.4C, D, Table 3.4). 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. 3.4C, Table 3.4). At Day 52, the concentrations of inorganic N (under NN) were 24% and 39% lower under eCO_2 than under aCO_2 in the rhizospheres of wheat and white lupin, respectively (Fig. 3.4D). Higher inorganic N was invariably detected under white lupin than under wheat.

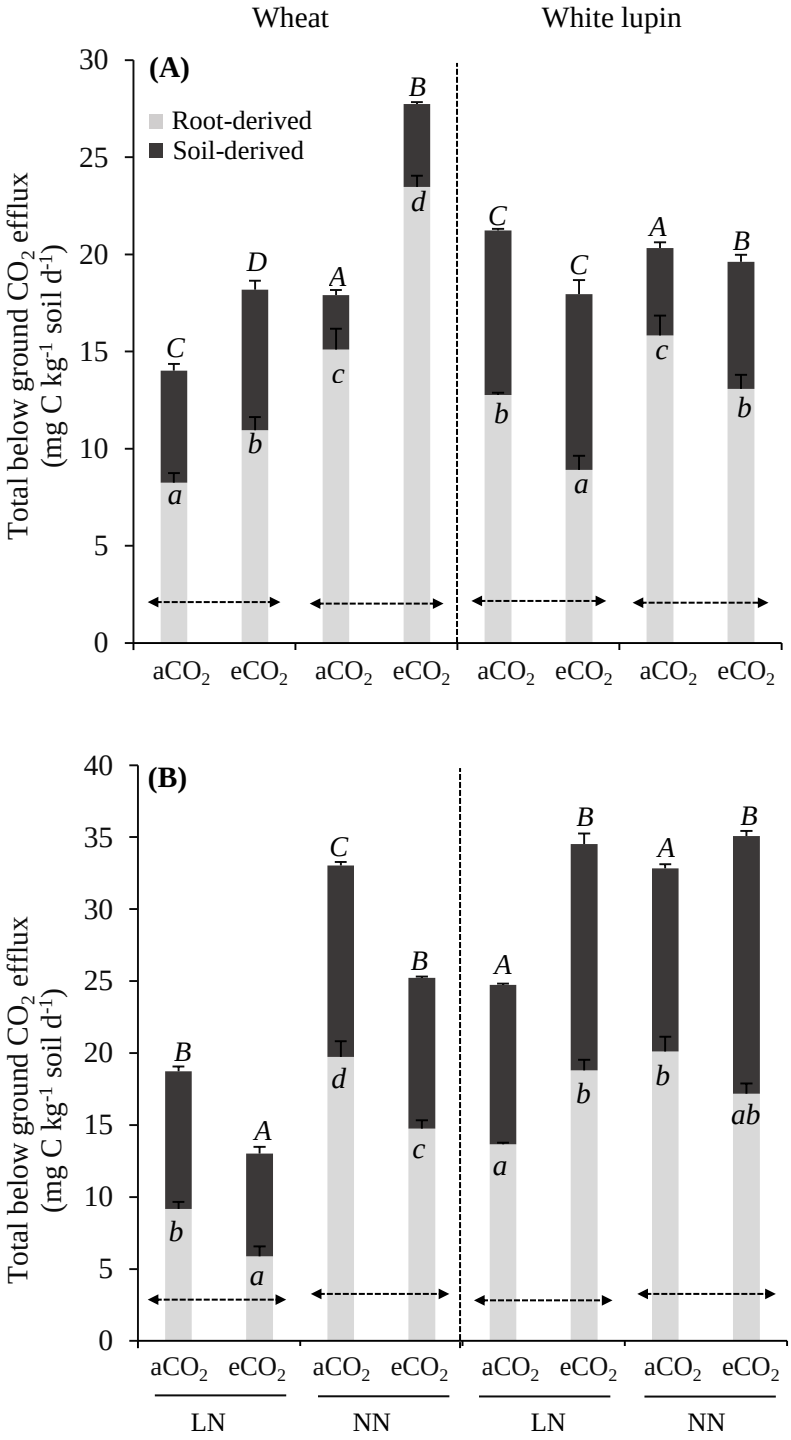

Figure 3.1 Root-derived and soil-derived CO_2 efflux from soil columns with wheat and white lupin grown for 32 (A) and 52 (B) days, supplied with low (LN) or adequate (NN) rate of N fertiliser under either aCO_2 (400 µmol mol^{-1}) or eCO_2 (800 µmol mol^{-1}). The arrow-ended dash lines represent the values of non-planted soil. Error bars indicate standard errors of means of four replicates. Means with a common italic 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

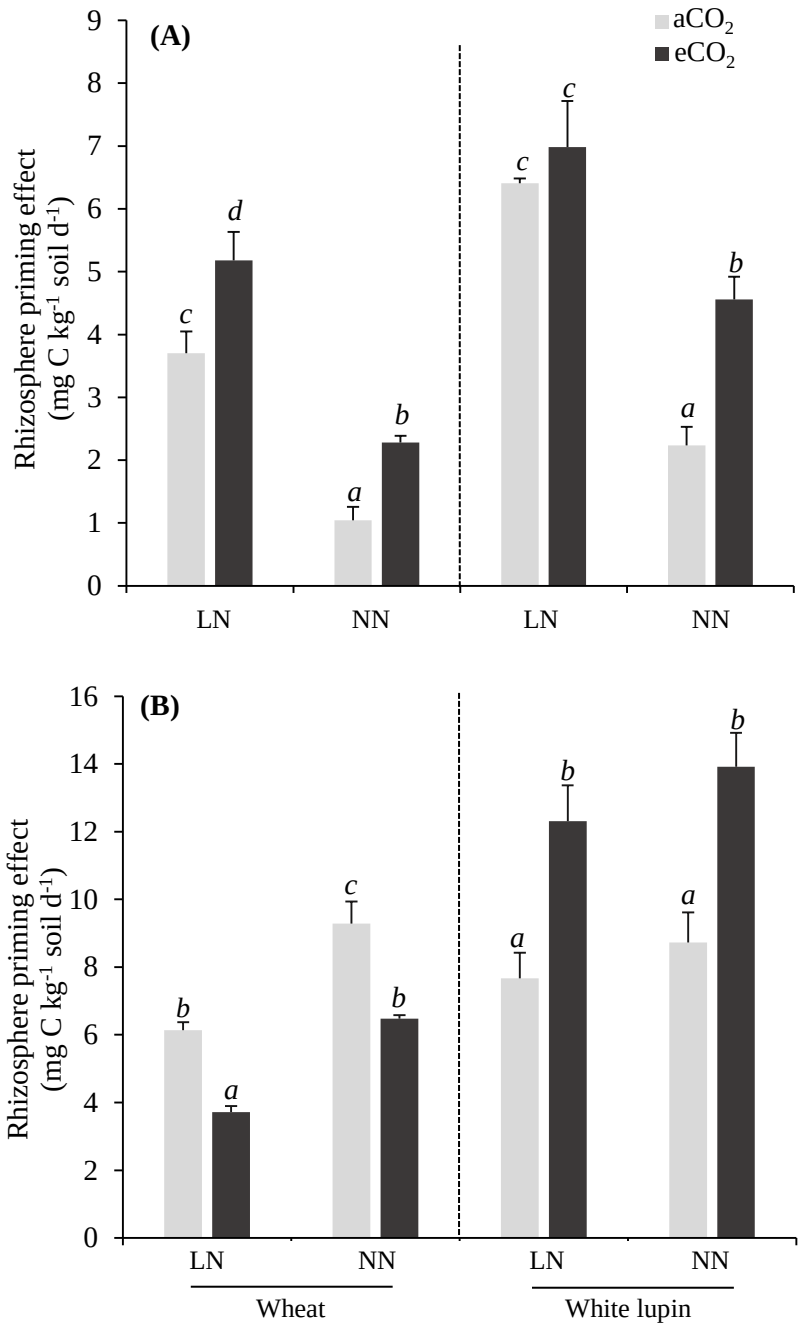

Figure 3.2 Rhizosphere priming effect (mg C kg^{-1} soil d^{-1}) in soil columns with wheat and white lupin grown for 32 (A) and 52 (B) days with low (LN) or adequate (NN) rate of N fertiliser under either aCO_2 (400 µmol mol^{-1}) or eCO_2 (800 µmol mol^{-1}). Error bars indicate standard errors of means of four replicates. Means with a common lower-case letter are not significantly different at $P = 0.05$

Table 3.3 Shoot and root dry mass (DM, g column^{-1}), N concentration (N conc., mg g^{-1}) and ^{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.		^{15}N		Root length	μ	β-glucosidase
		Shoot	Root	Shoot	Root	Shoot	Root			
					Wheat					
aCO_2	LN	8.78a	3.20a	17.5ab	8.2b	1.45a	1.21a	321a	0.15	180
eCO_2	LN	10.32b	4.55b	13.7a	8.1b	1.44a	1.24a	442b	0.16	176
aCO_2	NN	9.07a	3.18a	28.2c	14.2a	1.64b	1.58b	263a	0.16	182
eCO_2	NN	12.48c	5.41b	20.6b	11.8ab	1.65b	1.50b	585c	0.16	189
Significant level										
CO_2		***	***	**	n.s.	n.s.	n.s.	***	n.s.	n.s.
N		**	n.s.	**	**	***	**	n.s.	n.s.	n.s.
$CO_2 \times N$		*	n.s.	n.s.	n.s.	n.s.	n.s.	**	n.s.	n.s.
					White lupin					
aCO_2	LN	6.92a	1.46ab	26.7a	22.1a	0.86a	0.73a	24.5a	0.13a	192a
eCO_2	LN	7.63a	1.82bc	23.3a	19.4b	0.94a	0.82a	30.4b	0.15ab	198a
aCO_2	NN	7.86a	1.22a	46.3c	24.8a	1.38b	1.34b	22.8a	0.18bc	236b
eCO_2	NN	9.54b	2.19c	40.1b	22.1a	1.39b	1.27b	29.5b	0.17c	250b
Significant level										
CO_2		**	***	*	*	n.s.	n.s.	**	n.s.	n.s.
N		**	n.s.	***	*	***	***	n.s.	**	***
$CO_2 \times N$		n.s.	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

n.s., $P > 0.05$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. For each column, different letters indicate significant differences between means ($P <$ 0.05

Table 3.4 Significant levels of main effects and interaction terms from the analysis of variance, for CO_2 level and N rate on root-derived CO_2-C (Root-C), soil-derived CO_2-C (Soil-C), rhizosphere K_2SO_4-extractable C (EOC) and inorganic N (EIN) and rhizosphere priming effect (RPE) at Day 32 (D32) and Day 52 (D52) and rhizosphere soil respiration ($Rh_{resp.}$) and microbial biomass C (MBC) at Day 52 (D52)

Factors	Root-C		Soil-C		EOC		EIN		RPE		$Rh_{resp.}$	MBC
	D32	D52	D32	D52	D32	D52	D32	D52	D32	D52	D52	D52
						Wheat						
CO_2	***	***	**	***	n.s.	n.s.	**	**	**	***	n.s.	n.s.
N	***	***	***	***	n.s.	*	**	***	***	***	n.s.	**
$CO_2 \times N$	**	n.s.	n.s.	n.s.	*	*	*	***	n.s.	n.s.	n.s.	n.s.
						White lupin						
CO_2	**	n.s.	*	***	n.s.	***	**	**	*	***	*	*
N	**	n.s.	***	n.s.	n.s.	*	***	***	***	n.s.	*	n.s.
$CO_2 \times N$	n.s.	**	n.s.	n.s.	*	*	**	*	n.s.	n.s.	*	n.s.

n.s., $P > 0.05$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$

3.4.9 β-glucosidase activity in rhizosphere

Elevated CO_2 had no effect on the activity of β-glucosidase in both soil systems (Table 3.3). Low N rate decreased the activity of β-glucosidase by 20% in the white lupin-soil system (Table 3.3).

Table 3.5 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	$Rh_{resp.}$	EOC
Wheat	RPE	0.81^{**}	0.30	0.25
	MBC		0.49	0.58
	$Rh_{resp.}$			0.79^{*}
White lupin	RPE	0.89^{**}	0.76^{**}	0.89^{**}
	MBC		0.68^{*}	0.96^{**}
	$Rh_{resp.}$			0.73^{*}

*, $P < 0.05$; **, $P < 0.01$

3.4.10 Kinetics of substrate-induced respiration

The amendment of substrates exponentially increased soil respiration rate within a few hours. Elevated CO_2 had minimal effects on the specific microbial growth rate (Table 3.3). Low N

rate decreased the specific microbial growth rate (μ) under white lupin by 20% when averaged the data from two CO_2 levels (Table 3.3).

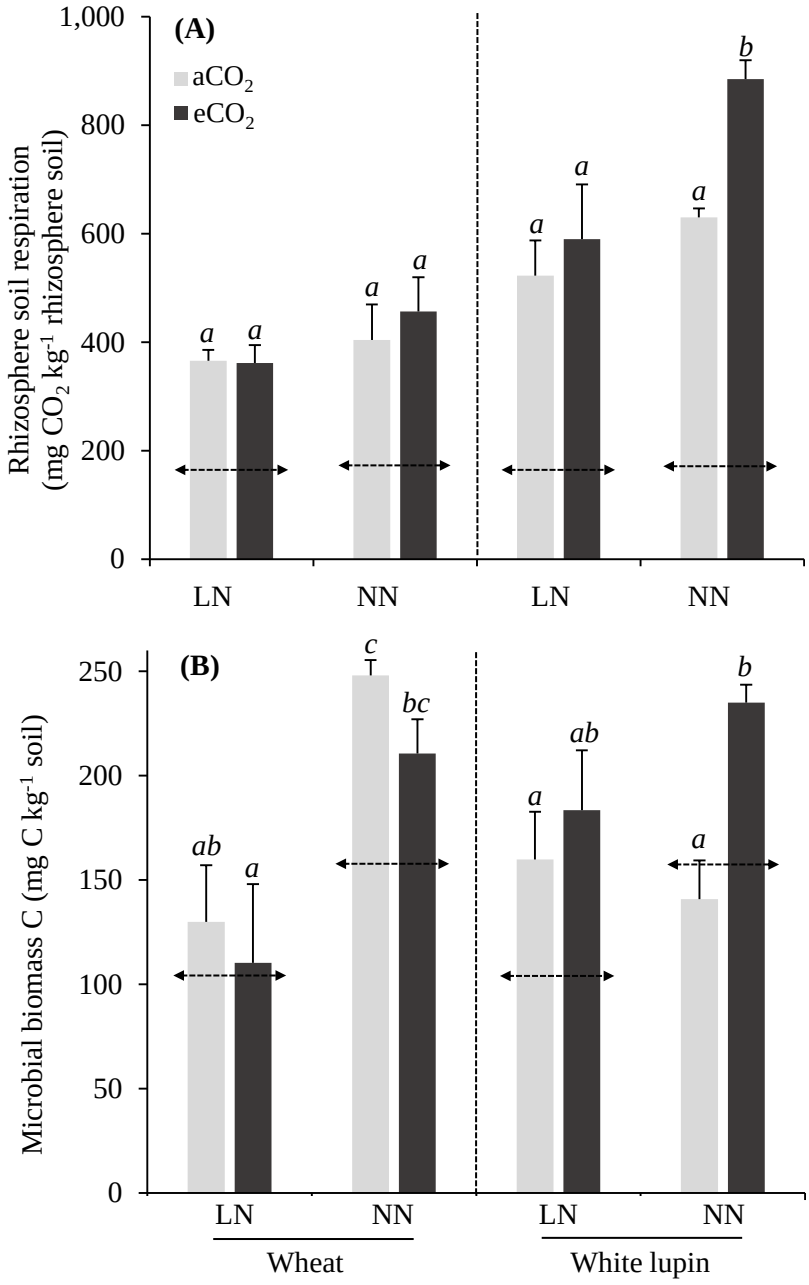

Figure 3.3 Rhizosphere soil respiration (14 h) (A) and microbial biomass C (B) in soils with wheat and white lupin grown for 52 days with low (LN) or adequate (NN) rate of N fertiliser under either aCO_2 (400 µmol mol^{-1}) or eCO_2 (800 µmol mol^{-1}). The arrow-ended dash lines represent the values of non-planted soil. Error bars indicate standard errors of means of four replicates. Means with a common lower-case letter are not significantly different at $P = 0.05$

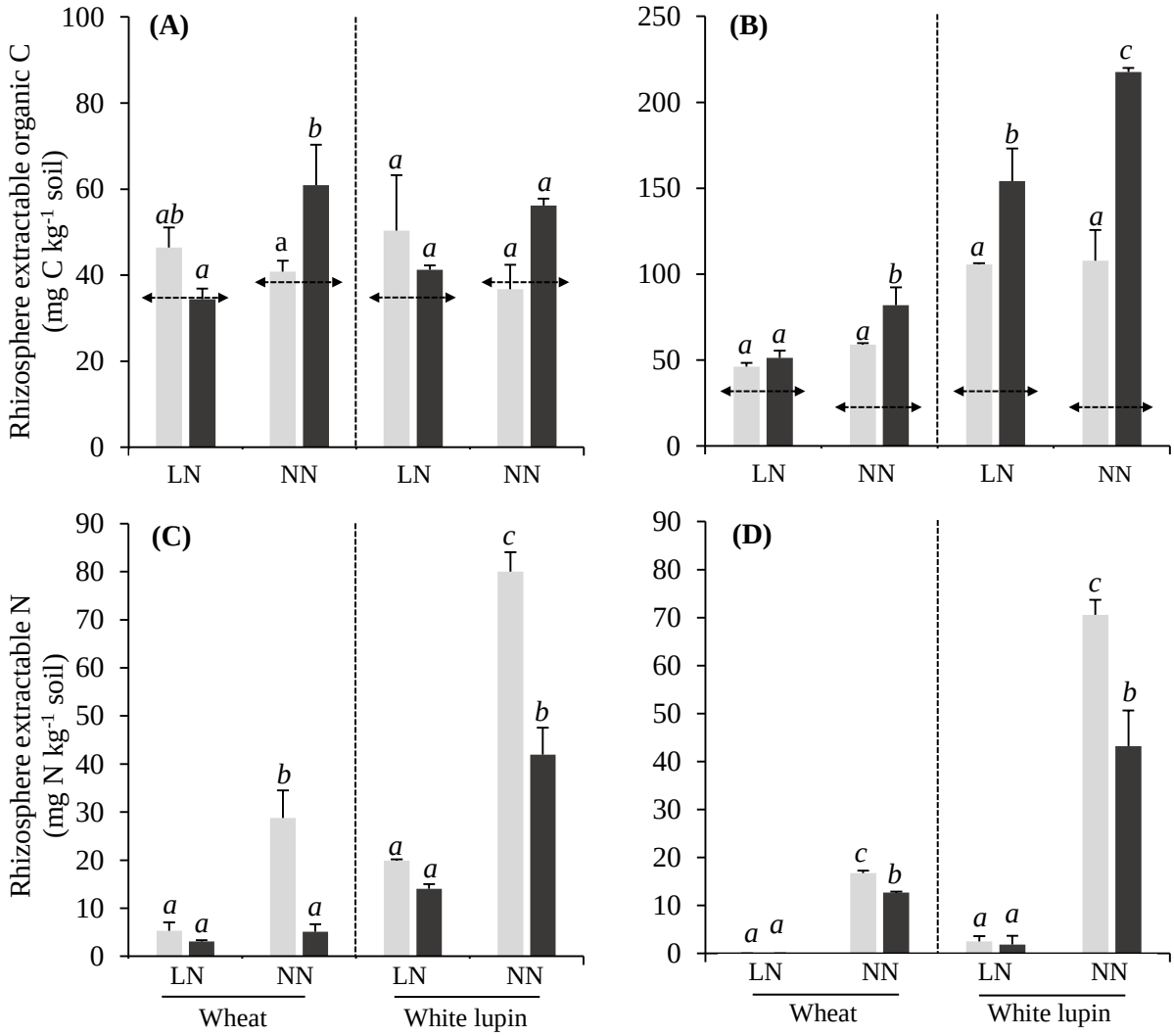

Figure 3.4 K_2SO_4-extractable organic C (EOC) (A, B) and inorganic N (EIN) (C, D) in the rhizospheres of wheat and white lupin grown for 32 (A, C) and 52 (B, D) days with low (LN) or adequate (NN) rate of N fertiliser under either aCO_2 (400 µmol mol^{-1}) or eCO_2 (800 µmol mol^{-1}). The arrow-ended dash lines represent the values of non-planted soil. Error bars indicate standard errors of means of four replicates. Means with the same lower-case letter are not significantly different at $P = 0.05$

3.5 Discussion

3.5.1 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 to Day 52 and differed between two species. Thus our first hypothesis is partly supported. Previous studies have shown that low N supply could either increase, decrease or have no effect on the RPE (Cheng and Johnson 1998; Cheng et al. 2003; Liljeroth

et al. 1994), and contradictory mechanisms have been proposed (Kuzyakov et al. 2000; Fontaine et al. 2003). In this study, the greater RPE under low N relative to adequate N supply at day 32 was likely a consequence of microbial N mining of the relatively N-rich soil organic matter (Craine et al. 2007; Chen et al. 2014b). However, the extent of N limitation in the rhizosphere could vary between the species and at different growth stages, which possibly accounts for the different effects of N addition on RPE between Day 32 and 52.

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. 3.4C). As a result, when supplied with LN, the soil microorganisms might be under moderate N limitation which increased RPE (Fig. 3.2A) 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 less priming (Fig. 3.2A) as previously shown by Craine et al. (2007) and Phillips et al. (2011). Similar results have been generated from temperate ecosystems where heterotrophic decomposition was lower under N fertilisation compared to no-N controls (Loiseau and Soussana 1999; Burton et al. 2004; Olsson et al. 2005). The NN-induced decrease in SOC decomposition in this study is in line with the decreases in the activities of soil oxidative enzymes by N deposition in other studies (Saiya-Cork et al. 2002; Waldrop and Zak 2006). Additionally, under relatively 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 is 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 extractable N in both plant-soil systems (Fig. 3.4C, D). Besides, LN could have limited the input of root-derived labile C as indicated by the lower rhizosphere extractable-C and root-derived CO_2-C when compared to NN (Figs. 3.1B, 3.4A, B). The limitation of both C substrates and N under LN might have constrained the growth of soil microorganisms (Fig. 3B), resulting in a lower RPE as compared to NN in the wheat-soil system. In comparison, 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 (Blagodatskaya et al. 2010) and the activity of microbial community as evidenced by the reduction in specific microbial growth rate and the activity of β-glucosidase enzyme (Table 3.3). As a result, the positive LN effect on SOC decomposition (Fig. 3.2A) was diminished by Day 52 (Fig. 3.2B). 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 (Day 32), the input of labile C substrates through 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 the soil is extremely N-deficient (Day 52, soil extractable N < 5 mg kg^{-1}), the priming decreases accordingly due to microbial limitation of both C and N (the case of wheat under LN).

3.5.2 Effect of elevated CO_2 on RPE

Elevated CO_2 generally increased the RPEs (Fig. 3.2), supporting our second hypothesis. 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. 3.4A, B). The increase in root-derived labile substrates under eCO_2 increased total microbial biomass (white lupin, Fig. 3B) and activity (Fig. 3.3A). Although eCO_2 did not increase the activity of β-glucosidase, the activities of other soil enzymes might have been stimulated by eCO_2 (Saiya-Cork et al. 2002; Waldrop and Zak 2006), leading to the higher RPE (Cheng and Johnson 1998; Dijkstra et al. 2008; Nie and Pendall 2016). In addition, previous studies have shown that eCO_2 could potentially change the composition of root-derived substrates (Tarnawski and Aragno 2006; Jin et al. 2015). This effect would inherently differ among plant species, leading to the species variation in RPEs under eCO_2.

In this study, eCO_2 decreased wheat tissue N concentration (Table 3.2), indicating low N or high C:N ratio rhizodeposits under eCO_2 (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 decreasing trend of microbial biomass C under eCO_2

(wheat, Fig. 3.3B). 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 (Table 3.3) and eCO_2 could increase the amount of these substrates to a greater extent (Fig. 3.4B) 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. 3.3). The changes in RPE by eCO_2, however, did not affect the contribution of soil-derived N to total plant N uptake, possibly due to microbial N immobilization as shown in Hungate et al. (1997b) and Hagedorn et al. (2005).

Elevated CO_2 could also alter RPE via additional impacts on N availability (Table 3.5). It led to a low N concentration in the rhizosphere (Fig. 3.4C, D). This might drive the soil microbial community to mine the relatively N-rich soil organic matter for N (Kuzyakov 2002), resulting in increases in RPE. On the contrary, reduction in RPE had occurred under eCO_2 when soil N availability was extremely low as the case of wheat at Day 52 (Fig. 3.4D), which could limit microbial decomposition and hence decreased RPE under wheat. Due to The RPE under white lupin was still stimulated by eCO_2 due to additional N source (N_2 fixation). The results support our third hypothesis. Nevertheless, the rate of N supply did not alter the CO_2 effect on SOC decomposition (Fig. 3.2A). Low 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 the different N application rates and plant species which affect the quantity and quality of rhizodeposits and thus microbial responses. These results imply that in future higher atmospheric CO_2 scenario, the quantity and quality of substrates are more important than soil N availability in regulating RPE.

3.6 Conclusions

The effect of N availability on RPE differed between plant species and growth stages. Relative to NN, LN increased the RPEs at an early plant growth stage, probably by microbial N mining. This positive effect diminished at the later growth stage possibly by limited microbial growth or activity due to severe N scarcity. This study shows that extreme N limitation would slow down the microbial decomposition of SOC, and suggests that 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 due to poor-quality substrates and limited N availability. The quantity and quality of substrates could be more important than N availability in mediating RPE under eCO_2.

Chapter 4 Elevated CO_2 alters the rhizosphere effect on crop residue decomposition

(Accept for Publication in Plant and Soil, in press)

4.1 Summary

Elevated atmospheric CO_2 (eCO_2) can affect microbial decomposition of native soil organic carbon (SOC) via enhanced root exudation and rhizosphere activity. Few studies have examined the effect of eCO_2 on the decomposition of newly-added crop residues which are important to understanding below-ground C changes. A soil microcosm experiment was conducted to examine whether eCO_2 would enhance the rhizosphere effects on the decomposition of crop residues. White lupin (*Lupinus albus* L. cv. Kiev) was grown for 34 or 62 days under ambient CO_2 (aCO_2, 400 μmol mol^{-1}) or eCO_2 (800 μmol mol^{-1}) in a low-C (2.0 mg g^{-1}) soil which was amended with or without dual ^{13}C and ^{15}N labelled wheat, field pea or canola crop residues. An isotopic tracing technique was adopted to partition residue-derived CO_2 from total below-ground CO_2 efflux. Two independent groups of data were analysed statistically at either Day 34 or 62. The presence of white lupin increased the decomposition of all residues at Day 34. This positive rhizosphere effect on residue decomposition decreased and was even reversed at Day 62, probably due to depletion of labile C, or microbial N limitation, or rhizosphere acidification. The eCO_2-induced decomposition depended on residue type at Day 34. Specifically, when compared to aCO_2, eCO_2 did not affect the decomposition of canola residue, increased that of field pea residue by 13.5% but decreased wheat straw decomposition by 7.4%. However, residue decomposition was, on average, 13% higher under eCO_2 at Day 62, which was correlated positively with the increase in rhizosphere extractable C ($P < 0.01$). Elevated CO_2 generally increased residue decomposition in the rhizosphere, but this effect was mediated by residue type at Day 34. Enhanced residue decomposition under legumes at eCO_2 may favour C turnover and the release of residue N.

4.2 Introduction

Soil organic carbon (SOC) sequestration has been proposed as an appropriate way to improve soil quality and productivity (Lal 2004; Smith et al. 2012) and to potentially mitigate the increasing CO_2 emissions (Meinshausen et al. 2009). The return of crop residues to soils is a technically simple and effective agricultural management practice to achieve this purpose. A

fraction of the incorporated residue is stabilised as soil organic matter by physical, chemical, and biochemical mechanisms (Six et al. 2002), with the remaining fraction being lost to the environment via microbial decomposition. The effectiveness of SOC stabilisation depends on the quality and quantity of residues returned to the soil and soil microbial responses. Elevated CO_2 (eCO_2) could potentially change the rhizosphere environment and therefore microbial parameters, which may further alter the decomposition of soil organic matter including crop residues.

Elevated CO_2 is anticipated to alter rhizosphere processes and affect organic matter decomposition in three ways. Firstly, eCO_2 could alter both the quantity and quality of root-derived C substances (Phillips et al. 2011; Jia et al. 2014; Butterly et al. 2016a; Calvo et al. 2017) which can regulate the microbial processing of other C sources such as crop residues and native SOC. The difference in SOC decomposition induced by plant roots is termed the rhizosphere priming effect (RPE) (Zhu et al. 2014; Nie and Pendall 2016; Wang et al. 2016). Higher priming effects are linked to increased root exudation under eCO_2 (Bengtson et al. 2012; Nie and Pendall 2016). Secondly, plants growing under eCO_2 often exhibit larger root systems and hence large rhizosphere volumes (Dijkstra et al. 2009; Nie et al. 2013a), which indicates more organic matter would be susceptible to microbial decomposition. Lastly, eCO_2 could change the soil environment. For example, eCO_2 could induce rhizosphere acidification, especially in soils with low pH buffer capacity, possibly via enhanced efflux of carboxylates and/or unbalanced cation-anion uptake by plant roots **(**Haynes 1990; Guo et al. 2012**)**. Low pH can directly inhibit microbial activity, microbial community size and structure and/or substrate availability (Andersson et al. 2000; Kemmitt et al. 2006; Rousk et al. 2009), leading to decreased decomposition of SOC (Wang et al. 2016; Wang and Tang 2018). Soil moisture could also be altered by eCO_2 due to improvement in plant water-use efficiency (Cruz et al. 2016). Although several studies have examined the effect of eCO_2 on SOC decomposition (Cheng and Johnson 1998, Nie and Pendall 2016; Xu et al. 2017, 2018), the eCO_2–induced changes in the rhizosphere on residue decomposition received less attention. Apart from rhizosphere responses, distinctive types of crop residues differ intrinsically in properties like N concentration and C complexity which may affect the decomposition under eCO_2. High N could potentially ease microbial N constraints (Drake et al. 2013) and labile C is easier to decompose than chemically-complex compounds (Ruiz-Dueñas and Martínez 2009).

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

Plants actively secrete root exudates into rhizosphere soil, which could affect soil C dynamics. Approximately 11–17% of the photo-assimilates are distributed below-ground as root exudates (Nguyen 2003; Jones et al. 2009). Soil microbes utilise such C substrates for respiration and biomass production and the subsequent turnover of microbial biomass helps to build up SOC (Lloyd et al. 2016). Moreover, root exudates can also fuel soil microbes to decompose other C sources (e.g. crop residues and native SOC). Previous studies have demonstrated that the RPE on SOC decomposition can be stimulated by up to 380% and repressed by 50% (Zhu et al. 2014), depending on plant species, soil properties and environmental parameters (Huo et al. 2017; Xu et al. 2017, 2018). The positive RPEs are mostly explained by 'co-metabolism' (Kuzyakov et al. 2000) and 'microbial N mining' (Kuzyakov and Xu 2013), while 'preferential substrate utilization' and 'nutrient competition' (Cheng and Kuzyakov 2005) account for the negative RPEs. However, it is still largely unknown whether the presence of rhizosphere of growing plants affects the decomposition of crop residues and how it interacts with eCO_2.

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

4.3 Materials and methods

4.3.1 Soil description

Subsurface soil (10–30 cm) of a Tenosol (Isbell and NCST 2016) was collected from a grass pasture. The soil was air-dried, sieved to pass a 2–mm sieve with plant roots and gravels removed, and then thoroughly mixed. The soil was selected for its low SOC and N content, and a similar ^{13}C abundance to the roots of white lupin. A preliminary experiment showed that a low amount of CO_2 (< 2.7 μg C g^{-1} soil d^{-1}, which was only 6% of total below-ground CO_2 efflux) had been released from this soil when amended with crop residues. The plant- and soil-derived CO_2 was integrated as one pool with the same ^{13}C abundance, which could further be discriminated from CO_2 derived from ^{13}C-enriched residues. The soil is a sandy loam (sand 81%, silt 6%, clay 13%, Butterly et al. 2013). Other basic properties were: pH 6.2 (1:5 w/v in 0.01 *M* $CaCl_2$), pH buffer capacity 6.0 $mmol_c$ kg^{-1} pH^{-1}, SOC 1.8 mg g^{-1}, total N 0.28 mg g^{-1}, K_2SO_4-extractable inorganic N 10.5 μg g^{-1} and $\delta^{13}C$ –25.6‰ PDB. Soil was supplied with the following basal nutrients before the experiment (μg g^{-1}): KH_2PO_4, 180; K_2SO_4, 120; $CaCl_2.2H_2O$, 180; $MgSO_4.7H_2O$, 50; $MnSO_4.H_2O$, 15; $ZnSO_4.7H_2O$, 8; $CuSO_4.5H_2O$, 6; FeEDTA, 1.3; $CoCl_2.6H_2O$, 0.4; $Na_2MoO_4.2H_2O$, 0.4.

4.3.2 Crop residues

Dual ^{13}C and ^{15}N-labelled plant materials (wheat, field pea and canola shoot residues) were generated as described in Butterly et al. (2015). Briefly, wheat (*Triticum aestivum* L.), field pea (*Pisum sativum* L.) and canola (*Brassica napus* L.) plants were fertilized with ^{15}N-labelled $Ca(^{15}NO_3)_2$ (20% atom excess) and pulse-labelled 7 times with $^{13}CO_2$ by injecting 12 ml of 9.2 *M* H_2SO_4 into 90 ml of 1.23 *M* $Na_2{}^{13}CO_3$ (98% atom excess) throughout the growing season. At maturity, plant shoots were collected, oven-dried at 70 ºC and finely ground (< 2 mm). Basic chemical properties of the residues were listed in Table 4.1.

Table 4.1 Basic chemical properties of ^{13}C and ^{15}N dual-labelled shoot residues used in this experiment. Water-soluble C (1:25, w/v)

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

4.3.3 Experimental design

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

Four pre-germinated seeds of white lupin were inoculated with a lupin rhizobial inoculant (EasyRhiz, New-Edge Microbials, Albury, Australia) and sown to a 2–cm depth in a line into each column. The planted columns were then transferred into four growth cabinets (SGC 120, Fitotron, Loughborough, UK) with two receiving elevated CO_2 (eCO_2, 800 ± 30 µmol mol^{-1}, within the range of published studies) and the other two receiving ambient CO_2 (aCO_2, 400 ± 15 µmol mol^{-1}). All growth cabinets were set at temperature regimes of 18 °C night (10 h) and 22 °C day (14 h) and relative humidity of 60%. The photosynthetic active photon flux density at the plant canopy was approximately 350 µmol m^{-2} s^{-1}. The columns were weighed and kept at 80% field capacity by adding reverse osmosis water daily. Plants were thinned to two seedlings per column two weeks from sowing. The columns were randomly reallocated within the two replicated growth cabinets weekly to ensure homogenous growing conditions. No additional fertiliser was applied throughout the experiment.

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

Because the residues might not be uniformly labelled and microbial discrimination might occur (Zhu and Cheng 2011), the ^{13}C abundances of the residues might thus change over the decomposing processes, leading to variations in the ^{13}C abundances of the residues and the residue-derived CO_2. To minimise such an effect when partitioning residue-derived CO_2 from total below-ground CO_2, we used the ^{13}C abundances of residue-derived CO_2 from a concurrent incubation experiment other than the original ^{13}C abundances of the residues. The incubation experiment was conducted under the same conditions except that there is no headspace CO_2 treatment. Briefly, 40 g of sands were firstly mixed with pre-incubated Tenosol soil at a rate of 50 mg g^{-1} soil for microbial inoculation and then amended with one of the three residues. After adjusting the water content to 80% field capacity, the sand and residue mixtures were placed into 1–L Mason jars. To maintain the moisture, a vial with 8 ml of Milli-Q water was included. The CO_2 released was trapped in 8 ml of 1 *M* NaOH solution. The NaOH traps were replaced with new ones every week for 9 weeks. Two ml of the trapped solution at the end of the fourth and ninth week was added with 0.5 *M* $SrCl_2$ to form precipitates for the quantification of the ^{13}C abundance of the respired CO_2, which was used to represent the ^{13}C abundance of crop residues at each time point (Table 4.2).

4.3.4 Below-ground CO_2 trapping

At Days 34 and 62 (representing the early vegetative and early flowering stage respectively), the tops of the columns were enclosed with two clear PVC plates around plant stems, and the open spaces were sealed with Blu-tack (Bostik, Thomastown, Australia) (Wang et al. 2016, Fig. 4.1). The seal was checked by vacuuming CO_2–free air through each column into a 150–ml NaOH solution (1 *M*). No air leak was indicated if the bubbles formed in the solution were stable and consistent before and after pressing the adhered area.

Before trapping, the initial CO_2 inside soil pores was removed by pumping CO_2–free air through all soil columns for 30 min. The CO_2 generated inside the column during a 48–h period was then trapped into 150 ml of 0.5 *M* NaOH solution by pumping and vacuuming CO_2–free air through each column. The total below-ground CO_2 was trapped 30 min at each time and three times per day with a 6–h interval between 9:00 am and 11:00 pm. To determine the total CO_2 trapped, a subsample of the NaOH trap was firstly added with 0.5 *M* $BaCl_2$ standard solution to precipitate the carbonate and the excessive NaOH was back-titrated with 0.25 *M* HCl using the phenolphthalein indicator. Another subsample of the trapping solution was

mixed with excessive 0.25 *M* $SrCl_2$ to form $SrCO_3$ precipitates (Cheng and Johnson 1998) at a pH of 7.0 to prevent the formation of $Sr(OH)_2$. The precipitates were rinsed and centrifuged three times with Milli-Q water before being oven-dried at 70 °C. The ^{13}C abundance of the $SrCO_3$ precipitate was analysed by an Isotope Ratio Mass Spectrometer ('IRMS', SerCon Hydra 20–22, Crewe, UK).

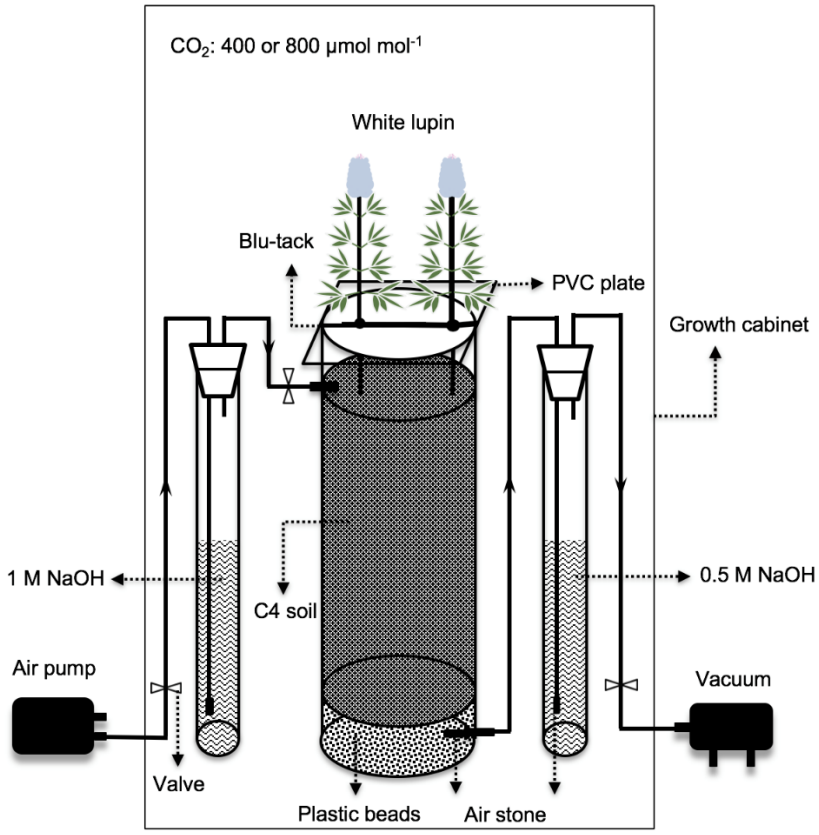

Figure 4.1 Diagram of the below-ground CO_2 trapping system

4.3.5 Plant and soil analyses

After each CO_2 trapping, plants were harvested and rhizosphere soils were sampled. The rhizosphere soil is of particular interest as it has high microbial abundance, diversity and growth rate due to root exudation (Blagodatskaya et al. 2014). Plant shoots were cut at the soil surface and roots were collected on a 2–mm soil sieve after collecting the rhizosphere soil. Roots were then washed and scanned with an EPSON EU-35 Scanner (Seiko Epson Corp, Suwa, Japan) with root length being generated from a WinRHIZO STD 1600+ Image Analysis System (Regent Instruments, Quebec City, Canada). Shoots and root materials were oven-dried at 70 °C for 48 h to determine dry mass.

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

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

4.3.6 Calculation

Residue-derived CO_2-C

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

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

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

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

where '$\delta^{13}C_{residue\text{-}amended\ soil}$' and '$\delta^{13}C_{no\text{-}residue\ control}$' are the $\delta^{13}C$ values of CO_2 derived from planted columns with and without residue amendment, respectively (Fig. 4.4); '$\delta^{13}C_{residue}$' is the $\delta^{13}C$ value of CO_2 released from residues when incubated with sands (Table 4.2).

4.3.7 Statistical analysis

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

4.4 Results

4.4.1 Plant growth

The effect of eCO_2 on plant growth depended on growth stages. There was no CO_2 treatment or residue type effect on plant biomass, root length, shoot C and N concentrations as well as shoot ^{13}C abundance at Day 34 (Table 4.2). However, eCO_2 decreased the ^{15}N atom% of plant shoot when field pea residue was amended, leading to a significant CO_2 × residue interaction (Table 4.3). Residue amendments increased shoot ^{15}N abundances when compared to non-amended controls with the highest increase being 5.3% in soil amended with field pea residue (Table 4.3).

At Day 62, eCO_2 increased shoot and root biomass by 23–36% and 40–48%, respectively (Table 4.2), indicating white lupin distributed more photosynthesized C below-ground under eCO_2. Elevated CO_2 increased the root length by 16%, 19% and 33% in the wheat, field pea and no residue-amended soils, respectively (Table 4.2). Residue amendments abated the eCO_2 effect on root length, particularly when canola residue was added. Elevated CO_2 did not affect shoot N concentration or C:N ratio but decreased the $\delta^{13}C$ value of shoots generally, with the largest reduction being 2.0‰ when the field pea residue was incorporated and the smallest

Table 4.2 Shoot and root dry weights, root length, shoot C, N concentration and ^{13}C abundance of white lupin growing in Tenosol soil with or without the amendment of wheat, field pea and canola residues in aCO_2 (400 µmol mol^{-1}) or eCO_2 (800 µmol mol^{-1}) environment for 34 or 62 days. $\delta^{13}C_{residue}$ represented the $\delta^{13}C$ values of CO_2 evolved from incubated sands ($\delta^{13}C_{residue}$) amended with either wheat, field pea or canola residue

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

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

being 0.8‰ in the wheat straw-amended soil (Table 4.2). Elevated CO_2 reduced the shoot ^{15}N (atom%) by 0.5% in the wheat straw-amended soil, but increased it by 1.6% when field pea residue was amended (Table 4.3).

4.4.2 Residue decomposition

The rhizosphere effect accelerated residue decomposition when compared to the no-plant control. A positive rhizosphere effect on residue decomposition was found at Day 34. Specifically, the residue-derived CO_2 was 7.0–8.8 μg C g^{-1} soil d^{-1} from planted columns which was higher than the amount of CO_2 (2.7–4.8 μg C g^{-1} soil d^{-1}) evolved from their corresponding unplanted columns (Fig. 4.2A). When compared to aCO_2, eCO_2 did not affect the decomposition of canola residue, decreased that of wheat straw by 7.4% and increased that of field pea residue by 13.5%, leading to a CO_2 × residue interaction (Fig. 4.2A).

The positive rhizosphere effect on residue decomposition decreased and was even reversed at Day 62 (Fig. 4.2B). On average, the decomposition rates of wheat and field pea residues were both 3.9 μg C g^{-1} soil d^{-1} in unplanted columns (the arrow-ended dash lines on Fig. 4.2B), while their corresponding decomposition rates were only 2.6 and 2.4 μg C g^{-1} soil d^{-1} in planted soils. The decomposition rate of canola residue was 2.0 μg C g^{-1} soil d^{-1} in the unplanted column which was still lower than that (2.9 μg C g^{-1} soil d^{-1}) in the planted column (Fig. 4.2B). The decomposition of residue was 13%, 15% and 11% higher under eCO_2, compared to aCO_2 for the wheat, field pea and canola residue, respectively (Fig. 4.2B). Besides, canola residue exhibited the highest decomposition rate at this stage under both CO_2 concentrations.

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

The presence of white lupin increased the total below-ground CO_2 efflux (Fig. 4.3). For example, at Day 34, soil respiration from the unplanted columns (the arrow-ended dash lines on Fig. 4.3A) was only 5.3–5.7 μg C g^{-1} soil d^{-1} when residues were amended, however, the respiration rate amounted up to 33.2–45.3 μg C g^{-1} soil d^{-1} in the corresponding planted columns. This positive rhizosphere effect on below-ground CO_2 efflux was further increased by 19–36% by eCO_2 when compared to aCO_2 (Fig. 4.3A). At Day 62, the total below-ground CO_2 efflux increased to 48.4–64.1 μg C g^{-1} soil d^{-1} in the planted columns (Fig. 4.3B). Elevated CO_2 increased the total below-ground CO_2 efflux by 33% and 13% in field pea and canola residue-amended soils, respectively (Fig. 4.3B).

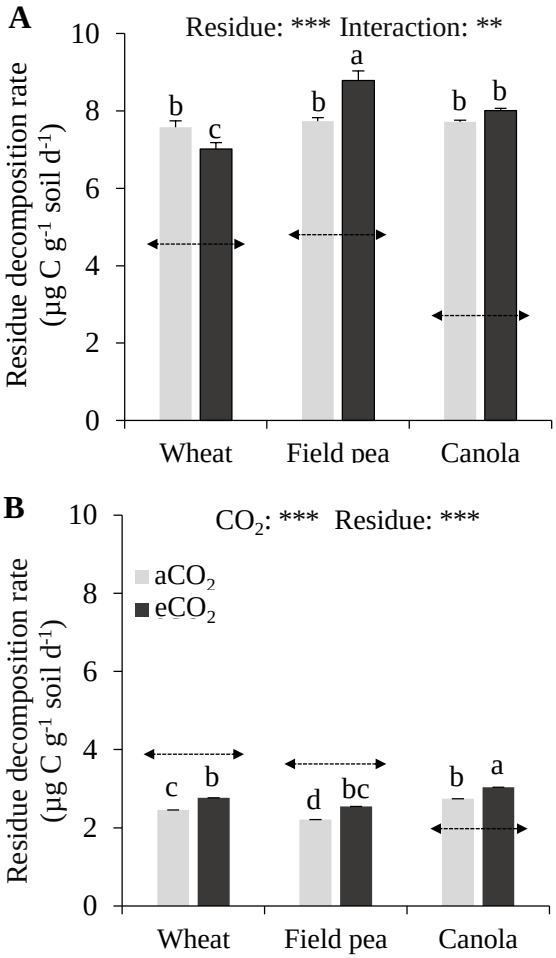

Figure 4.2 The rhizosphere effect of white lupin under aCO_2 (400 µmol mol^{-1}) or eCO_2 (800 µmol mol^{-1}) on the decomposition of wheat, field pea and canola residues at Day 34 (**A**) and 62 (**B**). The arrow-ended dash lines represent residue decomposition rates in unplanted controls. Error bars represented standard errors of means. Means with a common lower-case letter represent no significant difference within a panel. The main effects of CO_2, residue and the interaction are shown as: ** ($P < 0.01$) and *** ($P < 0.001$)

The $\delta^{13}C$ value of CO_2 from no-residue control was similar between the two CO_2 levels and was constant throughout the experiment (–23.3 to –25.6‰) (Fig. 4.4). The amendment of ^{13}C-labelled residue yielded a much higher $\delta^{13}C$ value of below-ground CO_2 and the value decreased with time. At Day 34, the field pea residue treatment showed the highest ^{13}C abundance of total below-ground CO_2 (55.2‰), followed by the wheat straw (43.2‰) and the canola residue treatment (14.5‰). At Day 62, the $\delta^{13}C$ value of total below-ground CO_2 was –7.9‰ and –9.9‰, respectively, for the treatments of wheat straw and field pea residue. Total CO_2 effluxed from the soil amended with canola residue showed the lowest $\delta^{13}C$ value, which was –5.8‰ (Fig. 4.4). On average, eCO_2 decreased the $\delta^{13}C$ value of CO_2 evolved from residue-amended columns at both stages and the decrease was 37% and 21% at Days 34 and 62, respectively (Fig. 4.4).

Table 4.3 Shoot ^{15}N abundance (atom%) of white lupin growing in Tenosol soil with or without the amendment of wheat, field pea and canola residues in aCO_2 (400 µmol mol^{-1}) or eCO_2 (800 µmol mol^{-1}) environment for 34 or 62 days

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

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

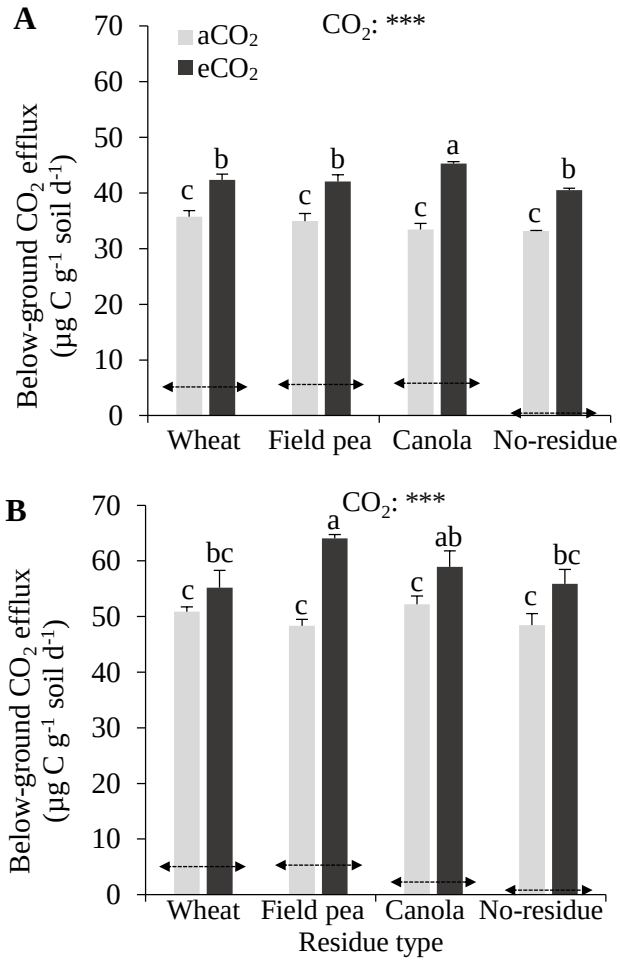

Figure 4.3 Below-ground CO_2 efflux from Tenosol soil with or without amendment of wheat, field pea and canola residues under white lupin grown in aCO_2 (400 µmol mol^{-1}) or eCO_2 (800 µmol mol^{-1}) environment for 34 (**A**) or 62 days (**B**). The arrow-ended dash lines represent CO_2 released from unplanted soils with or without residue amendment. Error bars represented standard errors of means. Means with a common lower-case letter represent no significant difference within a panel. The main effect of CO_2 was highly significant (***, $P < 0.001$) but the main effect of residue or the interaction was not significant ($P > 0.05$)

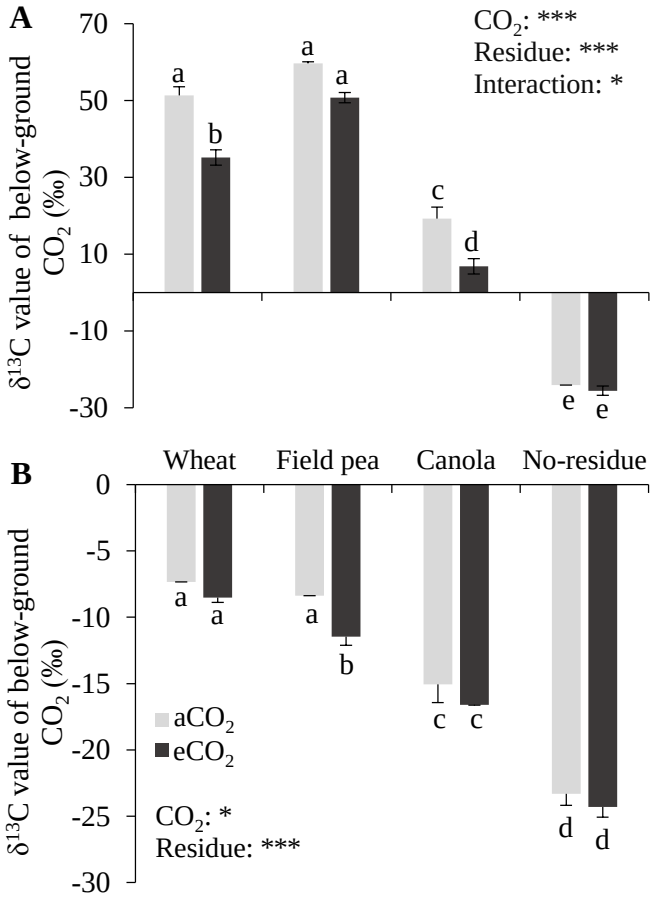

Figure 4.4 $\delta^{13}C$ value of total below-ground CO_2 trapped from Tenosol soil with or without amendment of wheat, field pea and canola residues under white lupin grown in aCO_2 (400 µmol mol^{-1}) or eCO_2 (800 µmol mol^{-1}) environment for 34 (**A**) or 62 days (**B**). Error bars represented standard errors of means. Means with a common lower-case letter represent no significant difference within a panel. The main effect of CO_2, residue and their interaction were shown as: * ($P < 0.05$) and *** ($P < 0.001$)

4.4.4 Rhizosphere soil respiration

Rhizosphere soil respiration (12 h) was 163–284 µg CO_2 g^{-1} soil at Day 34 (Fig. 4.5A). Elevated CO_2 had no significant effect on rhizosphere soil respiration. A strong effect of residue type was found with field pea residue inducing, on average, 1.3–1.5–fold higher rhizosphere soil respiration than other residues (Fig. 4.5A). At Day 62, the soil respiration from unplanted soils was only 0–38.4 µg CO_2 g^{-1} soil (the arrow-ended dash lines on Fig. 4.5B). The amounts of CO_2 released from rhizosphere soils ranged from 318 to 434 µg CO_2 g^{-1} soil (Fig. 4.5B). Elevated CO_2 increased the rhizosphere soil respiration by 18% and 25% in the

wheat and field pea residue-amended soils, respectively, but not in the canola residue and no residue-amended soils, leading to a CO_2 × residue interaction (Fig. 4.5B).

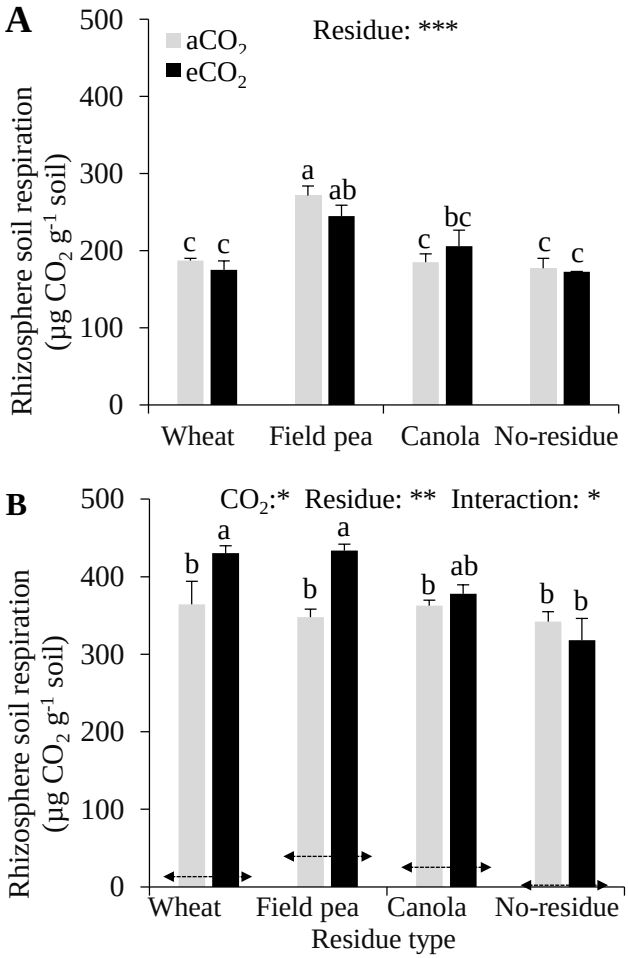

Figure 4.5 Respiration (12 h) of rhizosphere soil under white lupin grown in Tenosol soil with or without amendment of wheat, field pea and canola residues in aCO_2 (400 µmol mol^{-1}) or eCO_2 (800 µmol mol^{-1}) environment for 34 (**A**) or 62 (**B**) days. The arrow-ended dash lines represent microbial respiration (12 h) of unplanted soils with or without residue amendment at Day 62. Error bars represented standard errors. Means with a common lower-case letter represent no significant difference within a panel. The main effects of CO_2, residue or the interaction were shown as * ($P < 0.05$), ** ($P < 0.01$) and *** ($P < 0.001$)

4.4.5 Soil pH in the rhizosphere

White lupin acidified its rhizosphere by decreasing the original soil pH from 6.20 to 5.29–6.03 at Day 34 (Table 4.4). The rhizosphere soil pH was further decreased to 4.33–4.76 by Day 62 (Table 4.4). At Day 34, eCO_2 decreased the rhizosphere soil pH by around 0.3 units except for the wheat straw-amended soil (Table 4.4). The amendment of residues yielded higher pH when compared to no-residue control (Table 4.4) probably due to the alkalinity effect of crop residues

(Wang et al. 2017). Elevated CO_2 also decreased the rhizosphere soil pH (by an average of 0.18 units) at Day 62 with the greatest reduction being 0.4 units when wheat straw was amended (Table 4.4). On average, the soil amended with field pea residue showed the highest rhizosphere soil pH which was 0.33 units higher than the no-residue control (Table 4.4).

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

At Day 34, eCO_2 increased the concentrations of EOC by 42% and 49% in the canola residue-amended and no-residue soils, respectively (Table 4.4). On average, soils amended with field pea residues showed the highest EOC, followed by canola residue, no-residue controls and wheat straw (Table 4.4). The concentration of EOC was 2.7 to 6.9–fold greater at Day 62 than at Day 34. Elevated CO_2 increased the EOC concentration in all residue-amended treatments, with the increases being 80%, 63%, 44% and 21% in the wheat straw, no-residue control, field pea residue and canola residue-amended soils, respectively (Table 4.4). Moreover, residue decomposition rate was positively correlated with rhizosphere extractable C at Day 62 (P < 0.01, Fig. 4.6).

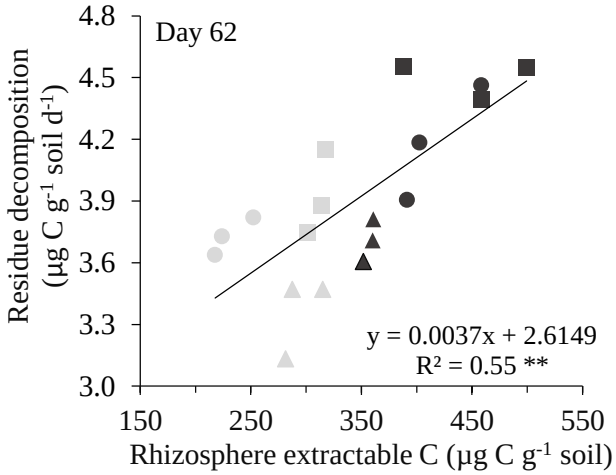

Figure 4.6 The relationship between K_2SO_4-extractable C in rhizosphere soil and residue decomposition at Day 62 (**, P < 0.01). Wheat, field pea and canola residue treatments were indicated by circles, triangles and squares, respectively. Grey and black symbols represent aCO_2 and eCO_2, respectively

The original soil EIN was 10.5 μg g^{-1}. Growing white lupin dropped the value to 0.05–1.93 μg g^{-1} and 0.67–1.09 μg g^{-1} at Day 34 and 62, respectively. The treatment effect was significant at Day 34 with a CO_2 × residue interaction being detected as eCO_2 increased the EIN in the wheat straw and canola residue-amended soils (Table 4.4). In general, the EIN was higher at

Day 62 when compared to Day 34. There tended to be higher EIN under eCO_2 when compared to aCO_2 at Day 62, but the difference was not statistically significant.

Table 4.4 Rhizosphere pH, K_2SO_4-extractable organic C (EOC) and inorganic N (EIN), microbial biomass C (MBC), N (MBN) and C to N ratio (MBC-to-N), and soil organic C (SOC) in white lupin-planted Tenosol with or without wheat, field pea and canola residue amendments under aCO_2 (400 µmol mol^{-1}) or eCO_2 (800 µmol mol^{-1}) at Day 34 or 62

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

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

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

Soil MBC in the rhizosphere ranged from 73–176 µg C g^{-1} soil at Day 34 (Table 4.4). Neither CO_2 level nor residue type had a significant effect on MBC (Table 4.4). At Day 62, the MBC was increased to 284–506 µg C g^{-1} soil (Table 4.4). On average, eCO_2 increased the MBC by

20% at this stage. Soil MBN was higher in residue-amended soils at both harvests, leading to lower MBC:N when compared to no-residue control columns. On average, eCO_2 increased the MBN by 23% at Day 62. The MBC:N was higher at Day 62 than Day 34 (Table 4.4). It correlated negatively with residue decomposition rate ($R^2 = 0.64$, $P < 0.01$, data not shown) across the two growth stages.

4.4.8 Total soil C in the rhizosphere

At Day 34, eCO_2 had no significant effect on the concentration of SOC in the rhizosphere. Not surprisingly, the residue amendments raised the rhizosphere SOC when compared to the no-residue controls, with the largest and smallest increases being 94% and 55% in field pea and canola residue-amended soils, respectively (Table 4.4). Higher SOC was observed in the rhizosphere at Day 62 than at Day 34, indicating a net C deposition. On average, eCO_2 enhanced SOC, with the increases being 6%, 9%, 6% and 19% for wheat straw, field pea residue, canola residue-amended and non-amended soils, respectively. Residue amendments increased the rhizosphere SOC by an average of 46% (Table 4.4).

4.5 Discussion

4.5.1 Rhizosphere effects on residue decomposition

This present study showed the direction of rhizosphere effects on residue decomposition differed at two plant growth stages. Specifically, the presence of white lupin increased the decomposition of all three crop residues (positive rhizosphere effects) at Day 34. However, the rhizosphere effects declined and even became negative in soils amended with wheat and field pea residues at Day 62. The decreased decomposition is consistent with some previous studies (Cotrufo and Ineson 1996; Lam et al. 2014; Butterly et al. 2016b). For example, Butterly et al. (2016b) reported a decreased decomposition of both wheat and field pea residues in the rhizosphere of either wheat or field pea at about 7–8 weeks after planting when compared with controls without plants. The negative rhizosphere effect was explained by preferential substrate utilisation (Blagodatskaya et al. 2011) in their studies. In this study, the roots of white lupin might have released larger amounts of low-molecular-weight substrates at Day 62 as indicated by the greater rhizosphere extractable organic C and rhizosphere soil respiration (Table 4.4; Fig. 4.5B). However, it did not seem to have changed the pattern of microbial substrate utilisation as a positive relationship between residue decomposition and EOC was observed

(Fig. 4.6). Other mechanisms must exist accounting for the change in the direction of decomposition.

The labile portion of residue C degrades faster in plant rhizospheres than in bulk soil probably due to stimulated microbial activity by root exudation (Cheng and Kuzyakov 2005). Therefore, less labile residue C would have been left in the planted columns at the later decomposing stage by comparison to the unplanted controls. This could partly explain the reduced positive rhizosphere effects on all residue decomposition at Day 62. Similarly, in a meta-analysis, Luo et al. (2016) pointed out that the decomposition of SOC at a specific time correlated positively with the instantaneous quantity of remaining fresh C. Besides, the decreased rhizosphere effect on residue decomposition could be associated with rhizosphere N status. Compared to Day 34, soil microorganisms were extremely N-limited at Day 62 as shown by enhanced microbial C-to-N ratio (Table 4.4) as plants depleted soil available N. This might have inhibited microbial decomposing capacity as residue decomposition rate was negatively correlated with microbial C to N ratio ($R^2 = 0.64$, $P < 0.01$, data not shown) across the two growth stages.

Moreover, the significant decrease in rhizosphere soil pH may have also contributed to the decreased decomposition at Day 62. The Tenosol used in this study has a low soil pH buffer capacity (6 $mmol_c\ kg^{-1}\ pH^{-1}$). Growing white lupin acidified the rhizosphere soil possibly by excess uptake of cations over anions (Tang et al. 1999). Rhizosphere soil pH dropped significantly in this study especially at the later growth stage (Table 4.4) when white lupin showed more vigorous root growth and activity (Table 4.2, Fig. 4.5B). Previous studies have shown that low soil pH could strongly affect the composition and/or activity of the soil microbial community, thereby decreasing microbial degrading ability (Andersson et al. 2000; Kemmitt et al. 2006; Rousk et al. 2009). For example, Rousk et al. (2009) showed that low pH (4.5) favoured fungal over bacterial growth and induced a fungal functional redundancy, leading to a decreased in C mineralization. A recent study further confirmed that residue decomposition decreased linearly with the drop of soil pH (Aye et al. 2016). Moreover, low soil pH could also affect the growth of plants and their rhizodeposition/root exudation, but this may not be valid in the case of acid-tolerant species (e.g. white lupin in this study) (Huyghe 1997). This study showed that rhizosphere acidification might also be a factor regulating residue decomposition in the rhizosphere.

Interestingly, negative rhizosphere effects on residue decomposition were found for wheat straw and field pea residue but not for canola residue at Day 62. Canola residue has the highest C-to-N ratio (54) and the least labile C (36.4 mg g^{-1}) when compared to wheat and field pea residues. In addition, it contains relatively more lignin (Table 4.1) and structural carbohydrates (Lupwayi et al. 2004), which could make it more energetically costly and more recalcitrant to microbial degradation (Ruiz-Dueñas and Martínez 2009) (Saar et al. 2016). This assumption is supported by the lower decomposition rates of canola residue in the no-plant controls when compared to wheat and field pea residues (arrow-ended dash lines on Fig. 4.2). However, this substrate quality-suppressed microbial decomposition of canola residue vanished at the presence of white lupin (canola residue, Fig. 4.2B) probably because root-derived C compounds highly stimulated the growth of soil microorganisms and/or their degrading ability (de Graaff et al. 2009; Bengtson et al. 2012), leading to the positive rhizosphere effect on canola residue decomposition at Day 62. Moreover, residue amendment tended to decrease root and/or rhizosphere respiration with enhanced residue decomposition as it did not affect the total below-ground CO_2 efflux when compared to the no-residue control (Fig. 4.3). This could be caused by possible allelopathic effects of the residues and by microbial N competition with growing plants (Lam et al. 2013).

4.5.2 The effects of elevated CO_2 on rhizosphere residue decomposition

Residue decomposition was higher under eCO_2 than aCO_2 at Day 62, but was residue-type-dependent at Day 34. The discrepancy might derive from the different CO_2-effect on the amount and quality of root exudates between the two growth stages, and/or from the different properties of residues, such as C availability, C to N ratio and biochemical recalcitrance.

In comparison with aCO_2, eCO_2 increased residue decomposition at Day 62. Greater root exudation was expected under eCO_2 especially at the later growth stage when plant roots secreted more labile C substrates (Sugiyama and Yazaki 2012), expanded and explored larger volumes of soil (Paterson et al. 2008). This is evidenced by the greater EOC under eCO_2 when compared to aCO_2, particularly at Day 62 (Table 4.4) and the greater rhizosphere respiration at Day 62 than Day 34 (Fig. 4.5B). The decomposition rates of residues were positively correlated with rhizosphere EOC ($P < 0.01$) (Fig. 4.6), which concurs with the results of Bengtson et al. (2012) that decomposition of SOC increased with the rate of root exudation. Possibly, eCO_2 activated soil microbial activity and/or growth, as shown by the increased

rhizosphere soil respiration and the increasing trend of MBC, and hence enhanced SOC decomposition, complying with the co-metabolism theory (Kuzyakov et al. 2000; Cheng and Kuzyakov et al. 2005; Zhu et al. 2014). Moreover, root exudates enhanced under eCO_2 may also chemically liberate soil mineral-protected nutrients via decomplexation and dissolution (Keiluweit et al. 2015; Yuan et al. 2018) and therefore could partly alleviate microbial nutrient limitation, leading to greater decomposition of crop residues. Furthermore, eCO_2 could potentially alter the composition of root exudates, given more N-enriched rhizodeposition was previously detected under eCO_2 by de Graaff et al. (2007). The greater inputs of N-rich root exudates could activate the growth and/or activity of the soil microbial community to degrade SOC and/or residue-C (Butterly et al. 2016b; Xu et al. 2018) as N in exudates can alleviate microbial N constraints in the rhizosphere (Drake et al. 2013).

Except for the quantity and quality of root exudates, the chemical properties of residue (e.g. C-to-N ratio and molecular degradability) could also affect its decomposition under eCO_2. Although plant growth was not significantly enhanced by eCO_2, root exudation could still be stimulated due to the disproportional distribution of photosynthetic C below-ground (Cheng and Johnson 1998; Butterly et al. 2016a). The increased root exudation under eCO_2 only stimulated the decomposition of field pea residue with lower C-to-N ratio, probably due to enhanced N availability. The higher C-to-N ratio (46) wheat straw, however, could have shifted the microorganisms to utilise the root-derived C compounds under eCO_2, leading to a decrease in decomposition according to the preferential substrate utilisation theory (Blagodatskaya et al. 2011). However, the canola residue has a similar C-to-N ratio (54) to wheat straw but its decomposition was not affected by eCO_2. It might relate to the biochemical recalcitrance of the canola residue as low-quality residue could delay its responses to microbial decomposition (Partey et al. 2013). The eCO_2 effect could also be missed from the two small windows of measurements in this present study. As a result, a system that enables a better temporal resolution of gas measurements is required in future studies.

4.6 Conclusions

Using the stable isotope tracing technique, this study examined the rhizosphere effects on crop residue decomposition under eCO_2. Residue decomposition in the presence of white lupin differed at two growth stages and between residue types. Specifically, the decomposition of crop residue was enhanced at Day 34 but inhibited at Day 62, possibly due to depletion of labile

residue-C, enhanced microbial N limitation or decreased rhizosphere soil pH over time. In general, eCO_2 increased the decomposition of crop residues at Day 62 possibly via microbial activation. The C-to-N ratio of residue and its degradability also affected the decomposition under eCO_2 at Day 34. Our results imply that eCO_2–induced increase in residue decomposition in the rhizosphere of leguminous plants may stimulate C turnover and release of residue-N for later plant uptake. Further research is required to improve the temporal resolution of gas measurements, and also to simultaneously examine the effects of eCO_2 on the decomposition of both residue and native soil C to better understand below-ground C cycling.

Chapter 5 Susceptibility of soil organic carbon to priming after long-term CO_2 fumigation is mediated by soil texture

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5.1 Summary

Elevated CO_2 (eCO_2) may enhance soil organic carbon (SOC) sequestration via greater input of photosynthetic carbon (C). However, greater rhizodeposits under eCO_2 may stimulate microbial decomposition of native SOC. This study aimed to examine the status and stability of SOC in three Australian cropping soils after long-term CO_2 enrichment. Samples (0–5 cm) of Chromosol, Vertosol and Calcarosol soils were collected from an 8–year Free-air CO_2 Enrichment (SoilFACE) experiment and were used to examine SOC dynamics by physical fractionation and incubation with ^{13}C-glucose. Compared to the ambient CO_2 (aCO_2) (390–400 μmol mol^{-1}), 8 years of elevated CO_2 (eCO_2) (550 μmol mol^{-1}) did not increase the SOC concentration of all soils but changed SOC distribution with 12% more C in coarse soil fractions and 5% less C in fine fractions. Elevated CO_2 also enhanced the susceptibility of SOC to ^{13}C-glucose-induced priming, but this effect was only significant in the coarse-textured Calcarosol topsoil. The eCO_2 history increased labile C (coarse C fraction, +13%), soil pH (+0.25 units) and decreased available N (–30%) in the Calcarosol, which stimulated microbial biomass C by 28%, leading to an enhanced specific priming effect. Despite with greater total primed C, the Chromosol that had the highest amount of native C, had lower primed C per unit of SOC when compared to the low-C Calcarosol. In conclusion, the effect of long-term eCO_2 enrichment on soil C and N availability in cropping soils depended on soil type with the coarse-textured Calcarosol soil being more susceptible to substrate-induced decomposition of its SOC.

5.2 Introduction

Anthropogenic activities have resulted in unceasing increases in atmospheric CO_2 concentration since the Industrial Revolution and the concentration will continue to rise in the foreseeable future (Chesney et al. 2013). Elevated atmospheric CO_2 (eCO_2) generally stimulates plant photosynthesis and enhances plant production (Ainsworth and Long 2004; Ellsworth et al. 2004; Kimball 2016). It also enhances the input of photosynthetic carbon (C) to belowground components in the form of root exudates (Phillips et al. 2006), root biomass and other rhizodeposits (Pendall et al. 2004). Increased input of this recently fixed C

belowground (e.g. root exudates) is important to a range of soil microbial processes, leading to potential stimulation of microbial growth and/or activity and shifts in microbial community structure (Langley et al. 2009; Cheng et al. 2012; Vestergård et al. 2016). These changes may affect the decomposition of indigenous soil C (Xu et al. 2017, 2018). Soil C sequestration and decomposition together determine whether eCO_2 would affect soil C stocks and the capacity of soils to mitigate climate change (de Graaff et al. 2006a, 2009; Lal 2008).

The net effect of CO_2 enrichment on SOC stock has been examined in several long-term experiments of Free-air CO_2 Enrichment (FACE). Soil organic C contents have been found to be either unaffected (van Groenigen et al. 2006; Keiluweit et al. 2015), increased (Luo et al. 2006) or decreased (Hoosbeek et al. 2004; Carney et al. 2007; Langley et al. 2009) by CO_2 enrichment. A recent meta-analysis suggested that eCO_2 increases the accumulation of new soil C in the short term (< 1 year) but not in the longer term (1–4 years) (van Groenigen et al. 2017), suggesting that microbial decomposition might be a long-term soil process which could offset short-term gains in SOC under eCO_2. The relative contribution of soil C input and decomposition under eCO_2 may also affect SOC turnover (Loiseau and Soussana 1999; Sulman et al. 2014). For example, Xie et al. (2005) found eCO_2 caused more C stored in > 53 μm fractions after 9 years of fumigation in a grassland system. The ^{13}C isotopic tracing technique further revealed that new C replaced 26.5% of the soil C pool after 10 years of CO_2 enrichment at the same site (van Kessel et al. 2006). These findings suggest that, in the long term, eCO_2 could renew SOC pools with more newly-added photosynthetic C in less-protected soil fractions while the native C (old C) is progressively decomposed until an equilibrium is achieved (van Kessel et al. 2006; Keiluweit et al. 2015). The newly-added C is readily utilised by microorganisms due to low chemical complexity (Ruiz-Dueñas and Martínez 2009) and less physiochemical protection (Six et al. 2002; Rasmussen et al. 2006). The renewed SOC pools resulting from long-term eCO_2 would, therefore, be more susceptible to microbial decomposition when compared to aCO_2. It remains unclear, however, whether changes in SOC content and turnover after long-term CO_2 enrichment would affect the susceptibility of SOC to further microbial decomposition.

Soil type and properties such as C and N status, pH and texture can affect microbial growth, activity and/or community structure directly and indirectly via influences on substrate and nutrient availability to soil microorganisms (Hamer and Marschner 2005a, 2005b; Kuzyakov

et al. 2007). For example, soil C:N ratio and N content could affect the direction and magnitude of priming effect (PE) – the difference in microbial decomposition of SOC with or without external substrate addition (Fontaine et al. 2003; Kuzyakov et al. 2000) and higher PEs are normally found in soils with high C and N (Kuzyakov et al. 2000; Qiao et al. 2015; Liu et al. 2017). Low soil pH normally decreases the relative abundance and diversity of bacteria but not fungi (Rousk et al. 2010) and/or reduces bacterial activity (Fernández-Calviño and Bååth 2010), thus limiting microbial decomposition (Aye et al. 2016; Wang et al. 2016). Soils with fine textures often exhibit high C retention capacity and stability because small pores harbour less O_2 for aerobic decomposers and SOC is largely bound with clays and/or protected within aggregates (Six et al. 2002; Reis et al. 2014; Procter et al. 2015). Most CO_2 enrichment experiments, however, examine only one soil type, especially in FACE experiments. It is still elusive how soil type and CO_2 concentration interactively influence SOC dynamics.

The objective of this study was to investigate the effect of long-term CO_2 enrichment on SOC stability in three cropping soils with contrasting physicochemical properties. Soil samples (0–5 cm) were collected from an 8–year FACE experiment (SoilFACE) established in a semi-arid temperate environment. Dynamics of SOC decomposition was examined by incubation with ^{13}C-labelled glucose. We hypothesised that (1) long-term eCO_2 would shift SOC pools with increases in newly-input C and decreases in protected C; (2) as a result, the SOC under long-term eCO_2 would be more susceptible to substrate-induced microbial decomposition and (3) this effect would be more pronounced in the low-C and coarse-textured Calcarosol soil than heavier-textured soils.

5.3 Materials and methods

5.3.1 Study site – the field experiment

The SoilFACE experiment is located in Horsham, Victoria, Australia (36º44′57″S, 142º06′50″E). The SoilFACE had been running continuously for 8 years before sampling in June 2017. The experiment comprised two CO_2 concentrations which were replicated four times and thus eight soil bunkers were established. Intact soil cores of a Chromosol, Vertosol, and Calcarosol (Isbell and NCST 2016) were collected from paddocks, packed into soil columns (diameter 30 cm, length 100 cm) and then sank into the soil bunkers to keep the surfaces of the soil at the ground level of the paddock. Four soil columns were included for each soil type within each soil bunker. These soils represented three major soil types in dryland

cropping systems in South-Eastern Australia. The starting soil pH, total C and N were characterised in Jin et al. (2017). Specifically, the Chromosol had pH 4.5, total C 48.9 mg g^{-1} and total N 4.0 mg g^{-1}, the Vertosol pH 7.3, total C 9.4 mg g^{-1} and total N 0.8 mg g^{-1} and the Calcarosol pH 5.7, total C 4.4 mg g^{-1} and total N 0.4 mg g^{-1}. Soil physical properties were reported previously by Butterly et al. (2016). The Chromosol had 16% sand, 66% silt and 18% clay, the Vertosol 12% sand, 37% silt and 51% clay and the Calcarosol 85% sand, 10% silt and 6% clay. Field capacity of the Chromosol, Vertosol and Calcarosol soil was 0.46, 0.44 and 0.12, respectively.

Pure CO_2 was injected daily on the upwind side of soil bunker from sunrise to sunset during the growing seasons to achieve an elevated CO_2 concentration (eCO_2, 550 ± 30 μmol mol^{-1}) than the ambient CO_2 concentration (aCO_2, 390–400 μmol mol^{-1}). The research site has an annual rainfall of 462 mm, minimum mean temperature of 7.6 ºC and maximum mean temperature of 22.3 ºC (Jin et al. 2017), which is characterised as a Mediterranean type climate (Mollah et al. 2009). More details of the research site and facility can be found in Mollah et al. (2009).

Field pea-canola-wheat rotation was adopted in the SoilFACE. Briefly, field pea (*Pisum sativum* L. cv. PBA Twilight) was grown in the year of 2009, 2011, canola (*Brassica napus* L. cv. Hyola 50) in 2013 and 2015 and wheat (*Triticum aestivum* L. cv. Yitpi) in 2010, 2012, 2014 and 2016. Urea-N was supplied at an annual rate of 50 kg N ha^{-1} since 2011 for wheat and canola and P fertiliser was applied as triple superphosphate at 15 kg P ha^{-1} y^{-1} for all crops. Crop residues were chopped to < 2 mm and returned to their respective soil columns after removing grains at maturity. Possible residue loss during summer time was avoided by mesh-netting.

5.3.2 Soil sampling

Surface soils (0–5 cm) were taken from the four soil columns of each soil type within each bunker and composited as one soil sample in June 2017. Soil samples were kept cool, sieved through a 2–mm sieve and stored field-moist at 4 ºC after transporting back to laboratory. Subsamples were taken for measurements of basic properties, i.e. pH, available N, total C and N, and microbial biomass C and N.

5.3.3 Physical fractionation

Soil organic C was partitioned into coarse and fine fractions according to Baldock et al. (2013). Briefly, 5 g of each sample was sieved (≤ 2 mm) and mixed with 20 mL of sodium hexametaphosphate solution (5 g L^{-1}). The soil and solution mixture was shaken for 16 h on a reciprocating shaker (OM6, Ratek Instruments, Boronia, Australia) at 180 rpm with an amplitude of 2.5 cm to disperse soil aggregates before sieving with a 53 μm-size sieve. Soil particles on the surface of the sieve (coarse fraction > 53 μm) was back-washed into pre-weighed container and samples that passed through the sieve (fine fraction < 53 μm) were also recovered and transported into pre-weighed containers. Both fractions were dried at 50 °C to get the dry weights. The coarse fraction was homogenised and ball-milled and the fine fraction was ground by hand using a mortar and pestle. Both fractions were analysed for organic C concentration (mg C g^{-1} particle) using an Elemental Analyser (PerkinElmer EA2400, Branford, Connecticut, USA). The proportion (%) of total soil organic C allocated to coarse or fine fraction was calculated as the percentage of coarse organic C or fine organic C (mg C [g soil]$^{-1}$) to the sum of coarse and fine organic C.

5.3.4 Laboratory incubation

Soil samples were pre-incubated at 25 ºC for 16 days to acclimate microorganisms prior to application of treatments. During the pre-incubation, fresh air was flushed daily to maintain the aerobic condition and the soil moisture was kept at 50% field capacity by weighing and adding Milli-Q water.

After the pre-incubation, ^{13}C-enriched glucose (99 atom%, Sigma-Aldrich, USA) was mixed with equivalent ^{12}C-glucose to produce a 5 atom% ^{13}C-glucose solution. Two sets of 30 g (fresh mass equivalent) pre-incubated soils were weighed into mesh-ended soil cores. One set of the soils was mixed with the glucose solution at the rate of 500 μg C [g soil]$^{-1}$ soil weekly over 6 consecutive weeks. The glucose solution was added to bring the soil moisture to 80% of field capacity. Another set of the pre-incubated soils was brought to 80% of field capacity using Milli-Q water only and regarded as controls. During the first addition, the substrate solution was applied evenly onto the soil surface, allowed to settle for 15 min and then mixed with a spatula. The same mixing procedure was conducted for the control samples.

Soil cores were then transferred into 1–L air-tight Mason jars, each containing a vial of 8 mL NaOH (1 *M*) that acted as a CO_2 trap and another vial of 8 mL Milli-Q water to maintain humidity during the incubation. In the subsequent weeks, the same amount of glucose solution or water was added evenly on the surface of soils using syringes but without thorough mixing to avoid perturbation. Four jars containing no soil cores were also included as blanks. The sealed jars were incubated at 25 ºC for 6 weeks. The NaOH traps were renewed weekly, and NaOH solutions in the old traps were transferred into 10 mL air-tight vials and kept at 4 ºC to avoid CO_2 absorbance from the atmosphere. The incubation jars were opened and allowed to dry at 25 ºC overnight to aid water evaporation so that the addition of glucose and water did not over-saturate the soil cores (Morrissey et al. 2017). Soil samples were destructively harvested and then stored at 4 ºC at the end of the incubation for subsequent chemical analysis.

5.3.5 Soil respiration and its ^{13}C abundance

Two millilitres of NaOH solution was back-titrated with 0.25 *M* HCl to determine the CO_2 released from each soil core using the phenolphthalein indicator after precipitation of the carbonate with 8 mL of 0.25 *M* $BaCl_2$. Another 2 mL of NaOH was precipitated with 2 mL $SrCl_2$ (1 *M*) solution to form $SrCO_3$ precipitates. The suspension was then mixed with 15 mL Milli-Q water and adjusted to a neutral pH using 0.25 *M* HCl to prevent the absorbance of CO_2 and the formation of $Sr(OH)_2$. The precipitates were centrifuged, rinsed three times with Milli-Q water, oven-dried at 60 °C for 72 h, and analysed for $\delta^{13}C$ value in triplicate using an Isotope Ratio Mass Spectrometer (Sercon 20–22, Gateway, Crewe, UK) at the 1st, 2nd, 3rd, 4th and 6th week after incubation.

5.3.6 Determination of priming effect (PE)

A two-pool mixture model (Cheng 1996) was used to separate the total soil respiration (C_{total}) into glucose-derived CO_2-C (C_{glu}) and soil-derived CO_2-C (C_{soil}) based on the following equations:

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

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

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

where $\delta^{13}C_{glu}$ is the $\delta^{13}C$ value of the glucose solution which is 5 atom% throughout the incubation; $\delta^{13}C_{total}$ is the $\delta^{13}C$ value of the total soil respiration measured from the $SrCO_3$ precipitates; $\delta^{13}C_{soil}$ is the ^{13}C natural abundance of the soils (Table 5.1); $C_{control}$ is the basal soil

respiration. Total primed C (μg C [g soil]$^{-1}$ d^{-1}) due to glucose addition was calculated as the difference between soil-derived CO_2-C (C_{soil}) from the glucose-amended soil and soil basal respiration ($C_{control}$) and defined as potential PE as it was measured in laboratory incubation but not in the field. The total primed C was then normalised based on the total SOC content and defined as specific PE (mg C [g SOC]$^{-1}$ d^{-1}) to provide information of SOC stability due to glucose addition.

5.3.7 Soil analyses

Soil samples before and after the incubation were analysed for chemical and biological properties. Briefly, soil pH was determined after shaking the air-dried soil in 0.01 *M* $CaCl_2$ (1: 5 w/v) for 1 h. Finely-ground samples were analysed for C and N using an elemental analyser (PerkinElmer EA2400, Branford, Connecticut, USA), and $\delta^{13}C$ value by the Isotope Ratio Mass Spectrometer. Fresh soils after field sampling were extracted with 2 *M* KCl (1:5 w/v) to determine available N (NO_3^-–N + NH_4^+–N). Fresh soils were also analysed for moisture content and K_2SO_4-extractable organic C (EOC) and inorganic N (EIN). The chloroform-fumigation and extraction method was adopted to determined soil microbial biomass C (MBC) and N (MBN) (Brookes et al. 1985; Vance et al. 1987). Briefly, two sets of soil samples with or without chloroform fumigation were extracted with 0.5 *M* K_2SO_4 (1: 5, w/v) and then filtered through Whatman no. 42 filter paper before determination of soluble organic C in extracts using a TOC Analyser (GE Sievers InnovOx, Boulder, Colorado, USA). Microbial biomass C was calculated as the difference in soluble organic C between K_2SO_4-extracts with and without fumigation before extraction with a conversion factor of 0.45 (Brookes et al. 1985). The fumigated and non-fumigated soil extracts were also oxidised with $K_2S_2O_8$ solution according to Cabrera and Beare (1993) in an autoclaved at 120 ºC for 30 min and then analysed for total inorganic N (mainly NO_3^-–N) using a Flow-inject Analysis System (Lachat Instruments, Loveland, Colorado, USA). The MBN was calculated as the difference in total inorganic N between K_2SO_4-extracts with and without fumigation a conversion coefficient of 0.54 (Vance et al. 1987).

5.3.8 Statistical analysis

Data were presented as means of four replicates except for the results of $\delta^{13}C$ which were means of triplicates. Tests of normal distribution and homogeneous variances were conducted with skewed data being either log_{10}– or square-root-transformed. Two-way ANOVA was

adopted to test the effects of eCO_2 history, soil type and their interactions on soil properties before incubation and soil respiration-related measurements. Three-way ANOVA was adopted to test the effects of eCO_2 history, soil type, substrate and their interactions on soil properties after incubation. Significant differences of means were determined at $P < 0.05$ using the least significant difference test (LSD) and signified using different low-case letters. All statistical analyses were conducted in Genstat (v17, VSN International, Hemel Hempstead, UK).

5.4 Results

5.4.1 Soil properties after long-term field CO_2 enrichment

The concentrations of total soil C and N differed between the soils at the beginning of the CO_2 enrichment (Jin et al. 2017). After 8 years of CO_2 enrichment, the average SOC concentration of the Chromosol, Vertosol and Calcarosol was 39.2 mg g^{-1}, 10.9 mg g^{-1} and 4.0 mg g^{-1}, respectively, and the average total N concentration of the correspondent soil was 3.22 mg g^{-1}, 0.89 mg g^{-1} and 0.35 mg g^{-1}, respectively (Table 5.1). The effect of 8 years of eCO_2 treatment on soil C and N concentrations varied with soil type. Soil C was not increased by eCO_2 despite with greater biomass production in this treatment (data not shown). The eCO_2 history decreased soil total N concentration in the Chromosol by 9.5% but not in other soils. Elevated CO_2 had no effect on the ^{13}C abundances of the three soils. The Vertosol had the highest $\delta^{13}C$ value (–24.7‰), followed by the Calcarosol (–25.6‰) and the Chromosol (–26.7‰) (Table 5.1).

The Chromosol showed the highest soil available N (27.8 μg g^{-1}) which was 8.1 and 6.2 folds greater than that of the Vertosol and Calcarosol, respectively (Table 5.1). On average, soils under long-term eCO_2 had lower available N when compared to aCO_2, in particular, for the Chromosol (Table 5.1). The EOC of the Chromosol was 3.4 and 4.6 times higher than that of the Vertosol (46 μg g^{-1}) and Calcarosol (34 μg g^{-1}), respectively (Table 5.1). Elevated CO_2 decreased the EOC in the Chromosol by 20% (Table 5.1).

Elevated CO_2 increased the MBC in the Calcarosol soil by 28% but did not affect either the MBN or the microbial C:N ratio (Table 5.1). The highest MBC (400 μg g^{-1}) was found in the Vertosol, followed by the Chromosol (250 μg g^{-1}) and then the Calcarosol (54 μg g^{-1}) (Table 5.1). However, the Chromosol and the Vertosol had similar MBN (37.1 and 34.8 μg g^{-1}, respectively), leading to a higher MBC:N ratio in the Vertosol than the Chromosol. The MBN of the Calcarosol was 11.8 μg g^{-1}, resulting in an MBC:N of 4.5.

The three soils differed greatly in their soil pH. The Vertosol had a neutral pH of 7.2 compared to pH 5.0 in the Calcarosol and 4.4 in the Chromosol (Table 5.1). Long-term CO_2 enrichment increased the soil pH in the Calcarosol by 0.25 units (Table 5.1).

5.4.2 Soil organic carbon fractionation

The Calcarosol had the highest proportion of SOC in the coarse fraction and lowest in the fine fraction among all the soils. The Chromosol and Vertosol showed similar C distribution between the two fractions (Table 5.2). When averaged across soil types, eight years of CO_2 enrichment increased the proportion of SOC in the coarse fractions by 12% whereas it decreased that in the fine fraction by 5% (Table 5.2). Long-term eCO_2 treatment decreased the N concentration in both soil fractions of the Calcarosol and the C concentration in the fine fraction. It also decreased the N concentration in the fine fraction of the Chromosol.

5.4.3 Soil respiration, its $\delta^{13}C$ value, priming effect and SOC stability

The eCO_2 history had no effect on the total substrate-induced CO_2 efflux (Fig. 5.1A). The total CO_2 efflux in the glucose treatments was the largest for the Chromosol and the lowest for the Calcarosol; except during the last 2 weeks of the incubation when the Vertosol had similar CO_2 efflux rates to the Chromosol (Fig. 5.1B). The cumulative CO_2 efflux was 2.6, 2.2 and 1.6 mg CO_2-C [g soil]$^{-1}$ for the Chromosol, Vertosol and Calcarosol, respectively, by the end of 6–week incubation (Fig. 5.1B). Moreover, the weekly addition of glucose raised the CO_2 efflux rate after 3 weeks of incubation, with increases being greater for the Vertosol and Calcarosol (Fig. 5.1A). Total CO_2 efflux from all controls decreased gradually with incubation time and was in the order of Chromosol > Vertosol > Calcarosol (Fig. 5.1a). The cumulative CO_2 efflux from the controls totalled 706, 447 and 224 µg CO_2-C [g soil]$^{-1}$ for the Chromosol, Vertosol and Calcarosol, respectively, with no eCO_2 history effect (Fig. 5.1B).

Table 5.1 Physicochemical properties prior to incubation of the topsoil (0–5 cm) of Chromosol, Vertosol and Calcarosol soils under either aCO_2 (390 µmol mol^{-1}) or eCO_2 (550 µmol mol^{-1}) for 8 consecutive years

Soil	CO_2	pH	Total C (mg g^{-1})	Total N (mg g^{-1})	$\delta^{13}C$ (‰PDB)	Available N (µg g^{-1})	EOC (µg g^{-1})	MBC (µg g^{-1})	MBN (µg g^{-1})	MBC:MBN
Chromosol	aCO_2	4.37d	40.8a	3.38a	–26.6b	30.7a	175a	258b	34.3a	7.6b
	eCO_2	4.52d	37.5a	3.06b	–26.8b	24.9b	140b	242bc	39.9a	6.4bc
Vertosol	aCO_2	7.16a	10.6b	0.86c	–24.8a	3.3d	47c	391a	33.6a	12.3a
	eCO_2	7.23a	11.2b	0.92c	–24.6a	3.6d	46c	409a	36.0a	12.1a
Cthelcarosol	aCO_2	4.95c	4.3c	0.36d	–25.4ab	5.3c	37d	47d	11.6b	4.1c
	eCO_2	5.20b	3.8c	0.33d	–25.9ab	3.7cd	32d	60c	12.1b	4.8bc
Significance level										
CO_2		**	n.s.	n.s.	n.s.	*	*	n.s.	n.s.	n.s.
Soil		***	***	***	**	***	***	***	***	***
CO_2×Soil		n.s.	n.s.	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

EOC, K_2SO_4-extractable soil C; MBC and MBN, microbial biomass C and N, respectively.

Total C, total N, available N, EOC and MBC were log_{10}–transformed before statistical analysis.

Means (n = 4) with the same letters represent no significance at 0.05 level using the LSD test. n.s. $P > 0.05$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

Table 5.2 Concentrations of C and N (mg g^{-1} particle), C-to-N ratio (C:N) and the proportion (%) of soil organic C allocated to the coarse (> 53 µm) and fine fraction (< 53 µm) separated from samples under either aCO_2 (390 µmol mol^{-1}) or eCO_2 (550 µmol mol^{-1}) for 8 consecutive years

Soil	CO_2	Coarse fraction (> 53 µm)				Fine fraction (< 53 µm)			
		C	N	C:N	%	C	N	C:N	%
Chromosol	aCO_2	50.4a	3.75a	13.5a	25.5c	36.5a	3.26a	11.2a	74.5a
	eCO_2	47.9a	3.49a	13.7a	28.9bc	32.9a	2.91b	11.3a	71.1ab
Vertosol	aCO_2	8.7b	0.70b	12.5ab	27.2c	9.5d	0.93e	10.3ab	72.8a
	eCO_2	8.5b	0.72b	11.6b	29.6bc	10.2d	1.00e	10.3ab	70.4ab
Calcarosol	aCO_2	1.4c	0.14c	9.6c	32.9ab	24.3b	2.46c	9.9b	67.1bc
	eCO_2	1.3c	0.11d	11.7b	37.3a	19.5c	2.05d	9.5b	62.7c
Significance level									
CO_2		n.s.	n.s.	n.s.	*	*	*	n.s.	*
Soil		***	***	***	***	***	***	**	***
CO_2×Soil		n.s.	n.s.	*	n.s.	n.s.	n.s.	n.s.	n.s.

Carbon and N in the coarse fraction were log_{10}–transformed and C in the fine fraction was square-root-transformed before ANOVA.

Means (n = 4) with the same letters represent no significance at 0.05 level using the LSD test. n.s. $P > 0.05$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

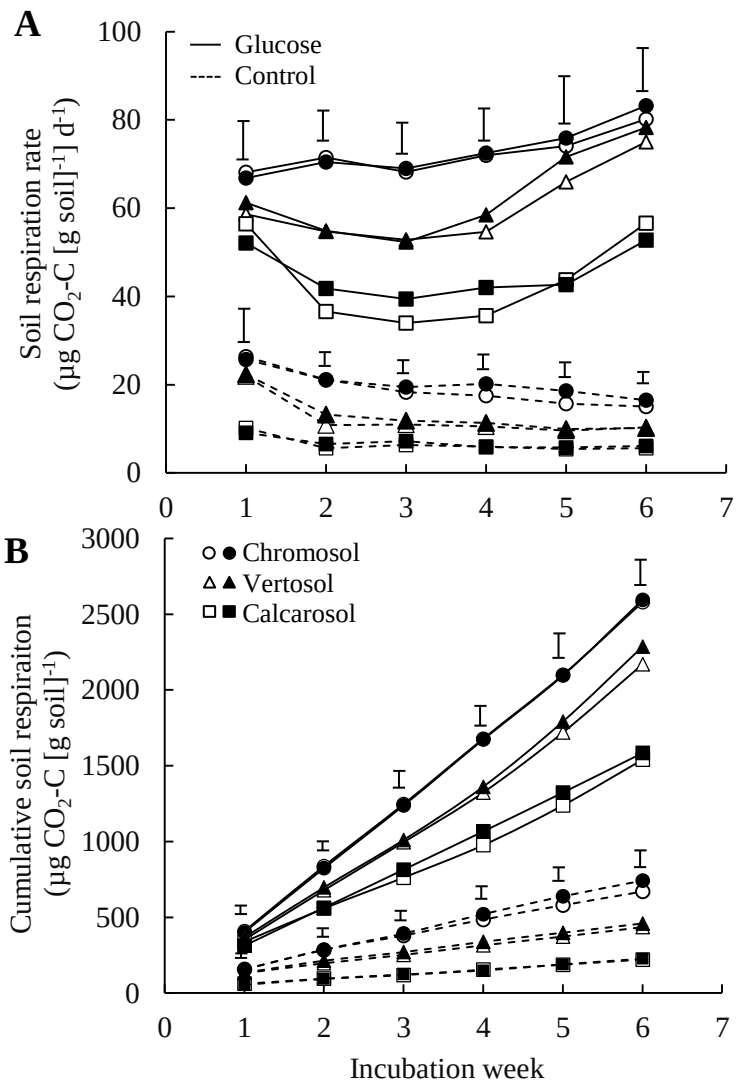

Figure 5.1 Changes of soil respiration rates (µg CO_2-C [g soil]$^{-1}$ d^{-1}) (a) and cumulative soil respiration (µg CO_2-C [g soil]$^{-1}$) (b) over 6 weeks for samples that received weekly ^{13}C-glucose (solid lines) and samples that did not (dash lines). Open symbols represent aCO_2 (390 µmol mol^{-1}), solid symbols represent eCO_2 (550 µmol mol^{-1}), solid lines represent glucose-added soils and dash lines represent control soils. Vertical bars represent the least significant difference (P = 0.05) between means (n = 4) for each week, which were analysed separately for the glucose-added soils and controls

The δ^{13}C value of the total CO_2 efflux increased gradually over time for the Calcarosol and Vertosol soils, indicating an increase of contribution of glucose-derived CO_2 to total CO_2 efflux with time. However, the δ^{13}C value of total CO_2 efflux from the Chromosol showed a slight decrease after the third week (Fig. 5.2). The Calcarosol had the highest δ^{13}C value of total CO_2 efflux throughout the incubation, followed by the Vertosol soil and then the Chromosol soil;

the exception was during the first week when the Vertosol soil and the Chromosol soil had similar ^{13}C abundances of total CO_2 (Fig. 5.2). The effect of CO_2 history on the $\delta^{13}C$ value of total CO_2 depended on both soil type and sampling time. Specifically, the long-term CO_2 enrichment decreased the $\delta^{13}C$ value of total CO_2 from all soils at week two, and that of the Chromosol at Weeks 3 and 4 (Fig. 5.2).

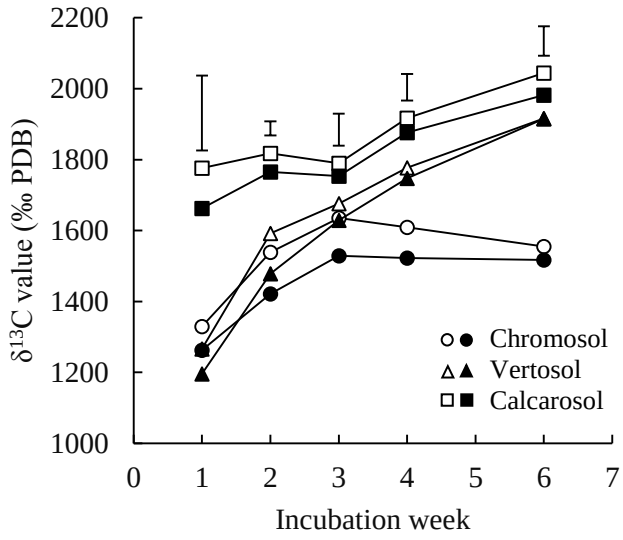

Figure 5.2 Changes of $\delta^{13}C$ values (‰PDB) of total CO_2 efflux for samples that received weekly ^{13}C-glucose over 6 weeks. Open symbols represent aCO_2 (390 µmol mol^{-1}); solid symbols represent eCO_2 (550 µmol mol^{-1}). Vertical bars represent the least significant difference (P = 0.05) between means (n = 3) for each sampling time

A positive priming effect (total primed C) was found for all treatments and the PEs increased with incubation time after the fourth week (Fig. 5.3A). The effect of soil type was significant across time with the Chromosol having the highest PE, followed by the Vertosol and the Calcarosol (Figs. 5.3A, 5.4). Although the Calcarosol tended to have higher PE under eCO_2, the difference was only statistically significant at week four (Figs. 5.3A, 5.4). By the end of the incubation, weekly addition of glucose yielded more soil-derived CO_2-C compared to its corresponding control, with an increase of 827, 739 and 554 µg C [g soil]$^{-1}$ for the Chromosol, Vertosol and Calcarosol soil, respectively (Fig. 5.4).

Primed C per gram of indigenous SOC (specific PE) (Fig. 5.3B) reflects the susceptibility of native SOC to microbial decomposition upon external substrate addition. The Calcarosol exhibited the highest primed C per SOC, followed by the Vertosol soil and the Chromosol soil

lowest (Fig. 5.3B). The CO_2 history had no effect on the primed C per SOC for the Chromosol and Vertosol, increased it for the Calcarosol, especially at Weeks 2–4 (Fig. 5.3B).

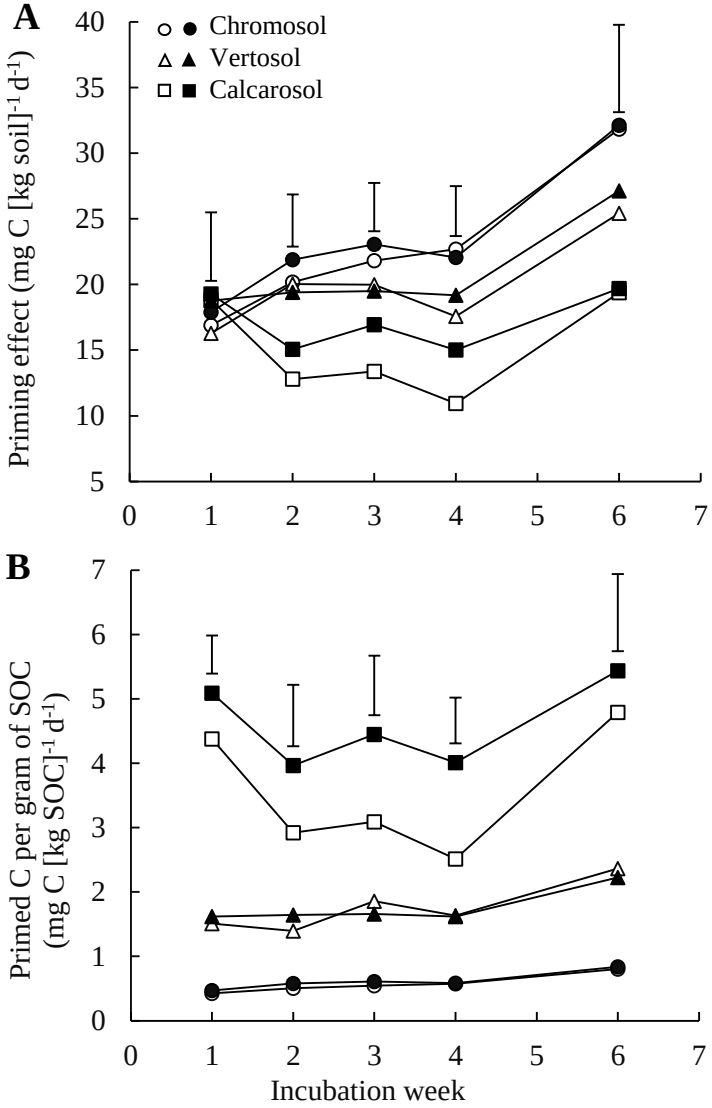

Figure 5.3 Changes of priming effect (µg C [g soil]$^{-1}$ d^{-1}) (a) and primed C per SOC (mg C [g SOC]$^{-1}$) (b) caused by weekly glucose amendment over 6 weeks. Open symbols represent aCO_2 (390 µmol mol^{-1}); solid symbols represent eCO_2 (550 µmol mol^{-1}). Vertical bars represent the least significant difference (P = 0.05) between means (n = 4) for each sampling time

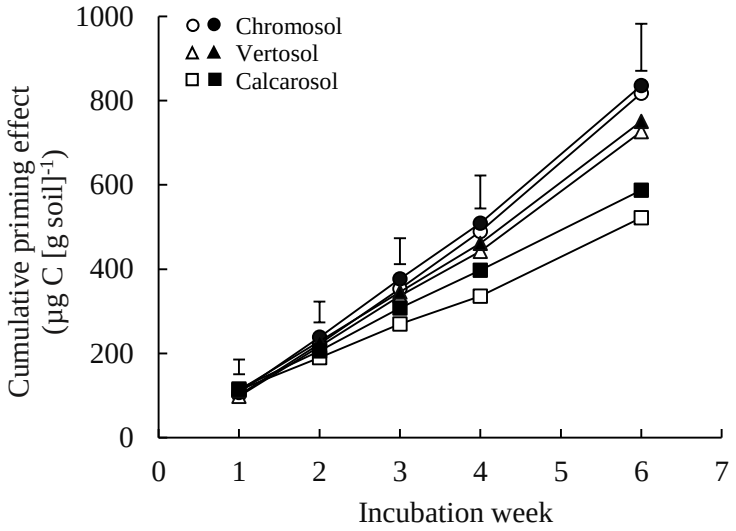

Figure 5.4 Changes of cumulative priming effect (µg C [g soil]$^{-1}$) caused by weekly glucose amendment over 6 weeks. Open symbols represent aCO_2 (390 µmol mol^{-1}); solid symbols represent eCO_2 (550 µmol mol^{-1}). Vertical bars represent the least significant difference (P = 0.05) between treatment means (n = 4) for Weeks 3, 4 and 6

5.4.4 Soil properties after incubation

After 6–weeks of incubation, the EOC decreased by 11%, 25% and 29% for the Chromosol, Vertosol and Calcarosol soils, respectively (Tables 5.1 and 5.3). Elevated CO_2 history had no significant effect on EOC. The addition of glucose substantially increased the EOC in the Calcarosol (Table 5.3), indicating that microorganisms did not deplete this large pool of substrate C and/or labile soil C.

For the controls, the Chromosol had a K_2SO_4-extractable inorganic N (EIN) of 86 µg g^{-1} which was 3.2– and 1.3– fold higher than that of the Vertosol and Calcarosol soil, respectively (Table 5.3). The addition of glucose led to depletion of the EIN (Table 5.3).

After the 6–week incubation with glucose, the MBC was 2.0, 1.6 and 3.7 times greater than that measured in the no-glucose added treatments in the Chromosol, Vertosol and Calcarosol soils, respectively (Table 5.3), suggesting microbes in the Calcarosol soil were more limited by substrate before the incubation. The MBN in glucose-added samples was 2.0, 1.2 and 1.2 times higher than the MBN measured when no glucose was added to the Chromosol, Vertosol and Calcarosol soils, respectively (Table 5.3). As a result, glucose addition increased the MBC:N of the Calcarosol by 95% (Table 5.3), meaning that the microorganisms were more

Table 5.3 Concentrations of K_2SO_4-extractable C (EOC) and inorganic N (EIN), and microbial biomass C (MBC) and N (MBN), and MBC-to-MBN ratio (MBC:N) in soil after 6–week incubation with or without glucose amendment to cropping Chromosol, Vertosol and Calcarosol un under ambient (aCO_2) (390 µmol mol^{-1}) or elevated CO_2 (eCO_2) (550 µmol mol^{-1}) for 8 years

Soil	CO_2	EOC (µg g^{-1})		EIN (µg g^{-1})		MBC (µg g^{-1})		MBN (µg g^{-1})		MBC:N	
		Control	Glucose	Control	Glucose	Control	Glucose	Control	Glucose	Control	Glucose
Chromosol	aCO_2	153b	172b	86.2a	8.8cd	221cd	505ab	30.8b	69.9a	7.7bc	6.8c
	eCO_2	128b	159b	85.6a	13.3c	278bc	501ab	41.6ab	72.7a	7.1c	6.9c
Vertosol	aCO_2	36d	53c	29.9b	7.3d	442ab	724a	49.9ab	61.2ab	10.0bc	11.8abc
	eCO_2	34d	57c	24.0b	7.8d	450ab	660a	48.7ab	59.6ab	9.8bc	11.0bc
Calcarosol	aCO_2	26de	602a	22.7b	4.8e	36e	154d	3.8d	5.4c	11.0bc	16.2a
	eCO_2	23e	472a	19.2b	4.4e	51e	168d	8.3c	8.7d	7.1c	12.2ab
Significance level											
CO_2		n.s.		n.s.		n.s.		*		n.s.	
Soil		***		***		***		***		***	
Substrate		***		***		***		*		*	
CO_2×Soil		n.s.		n.s.		n.s.		*		n.s	
Soil×Substrate		***		***		*		n.s.		*	

The CO_2× Substrate and CO_2×Soil×Substrate interactions are not significant at $P \leq 0.05$.

The data of EOC, EIN, MBC and MBN are log_{10}–transformed before ANOVA.

Means (n = 4) with the same letters represent no significance at 0.05 level using the LSD test. n.s. $P > 0.05$; * $P < 0.05$; *** $P < 0.001$

N-limited in the glucose-added Calcarosol soil. Moreover, in comparison to the other two soils, the Chromosol soil had the lowest MBC:N (6.9, Table 5.3) probably due to the highest available N and total N.

5.5 Discussion

5.5.1 Effects of eCO_2 history on SOC dynamics

Long-term (8 years) CO_2 enrichment did not affect SOC of all three cropland soils. Plants growing under the eCO_2 condition generally exhibit increased rates of photosynthesis and biomass production. At the SoilFACE, eCO_2 increased the above-ground biomass by 26.5%, 14.8% and 18.5%, respectively, for the Chromosol, Vertosol and Calcarosol over the growing seasons of 2009 to 2012 (Butterly et al. 2018). Elevated CO_2 could have also increased belowground C input via increased root growth and rhizodeposition as previously found (Pendall et al. 2004; Nie et al. 2013a; Phillips et al. 2011). It is assumed that the potentially greater input of C into belowground compartments under eCO_2 would result in a net overall increase in SOC stocks, but we found that concentrations of SOC in the top 5-cm soil remained unchanged. This neutral eCO_2 effect on SOC stock might result from eCO_2–induced changes in microbial decomposition. The priming effect has been regarded as a general process that could be stimulated by CO_2 enrichment due to a greater supply of easily-metabolised substrates, which escalates the decomposition of SOC under elevated CO_2 (Langley et al. 2009; van Groenigen et al. 2014; Black et al. 2016). Increased total primed C under elevated CO_2 is attributed to changes in quantity and quality of rhizodeposits (Phillips et al. 2011; Xu et al. 2017, 2018) and the subsequent effects on microbial growth, activity (Billings and Ziegler 2005; Janus et al. 2005; Jin et al. 2014) and/or community composition (Carney et al. 2007). No increment in SOC under eCO_2 even after eight years of cultivation possibly reflects a counter-balancing effect of PE on SOC gains from enhanced productivity.

The enhanced input of C and increased decomposition under eCO_2 could potentially accelerate SOC turnover (van Groenigen et al. 2014, 2017). This is confirmed by the finding that eCO_2 generally increased C distributed in particulate soil organic matter rather than the more-protected fine fractions after eight years of fumigation, which confirmed our first hypothesis. Similar results have been reported previously. For example, Hofmockel et al. (2011) reported that four years of CO_2 enrichment (200 μmol mol^{-1} above ambient concentration) speeded up SOC turnover in a forest soil with more C in the coarse particulate fraction when compared to

aCO_2. Xie et al. (2005) also found more retention of newly-added C in particulate soil organic matter and enhanced decomposition of indigenous soil C in a clay-loamy Cambisol after 9 years of exposure to eCO_2 (600 relatives to 350 μmol mol^{-1}) in a Swiss grassland. These results imply that the inevitable higher atmospheric CO_2 in future climates may actually accelerate soil C loss rather than sequestration from the atmosphere, resulting in a positive feedback effect. Whether the renewed SOC fractions by long-term CO_2 enrichment affects SOC stability to environmental changes in future is discussed below.

5.5.2 Effects of eCO_2 history on SOC susceptibility to microbial decomposition

Glucose addition was expected to induce more C primed from cropping soils treated with long-term eCO_2 (Hypothesis 2), but the increase was only significant for the Calcarosol soil (Fig. 5.3A). Soil organic C of the Calcarosol topsoil was more sensitive to the substrate-induced priming under eCO_2 than aCO_2 as shown by the greater primed C per unit mass of SOC (specific PE) (Fig. 5.3B) (Hypothesis 3). Elevated CO_2 increased the proportion of coarse organic C, a relatively labile C fraction primarily composed of plant residues, living organisms and debris of dead organisms. The increase might have contributed to the greater (28%) microbial biomass C under eCO_2 when compared to aCO_2 (Table 5.1), thereby leading to the higher PE under eCO_2. In addition, the Calcarosol soil had 30% less available N under eCO_2 than under aCO_2. The relative N-limitation (higher C:N ratio of soil and microbes) under eCO_2 might have increased the decomposition of residue and soil organic matter for N requirement through biosynthesis of more extracellular enzymes (Craine et al. 2007; Chen et al. 2014b). The higher microbial biomass N and lower microbial C-to-N ratio following the substrate incubation (Table 5.3) could thus result from the enhanced SOC decomposition and utilisation of soil-derived N. Lastly, long-term CO_2 enrichment increased soil pH by ~0.25 units in the Calcarosol soil, which might also contribute to the higher substrate-induced priming of SOC. In other studies, low pH has been found to affect microbial community composition and enzyme activity (Kemmitt et al. 2006; Rousk et al. 2010) and to decrease microbial degradation of soil organic matter (Aye et al. 2016; Wang et al. 2016). The increase in surface soil pH might result from more incorporation of plant residues under eCO_2 as residue alkalinity and mineralisation of residue N could increase soil pH especially in surface soil with more plant litter input(Xu et al. 2006; Butterly et al. 2013). Long-term CO_2 enrichment could have also changed the SOC composition of the Calcarosol soil, as indicated by the higher C:N ratio in the coarse C fraction. Elevated CO_2 has been found to increase plant C:N (Nie et al. 2015) and

perhaps the amounts of recalcitrant C compounds (Peñuelas et al. 1996, 1997; Poorter et al. 1997) of plant tissues. However, the changes in residue quality under eCO_2 is too small to induce any changes in microbial growth and/or their decomposing capacity (de Graaff et al. 2006b). The result corroborates with some previous findings that despite with lower quality, residues from eCO_2 do not exhibit lower decomposition than those from aCO_2 (Hirschel et al. 1997; Norby et al. 2001; Knnops et al. 2007).

However, both total primed C and specific PE did not differ between aCO_2 and eCO_2 in the Chromosol and Vertosol soils which had relatively higher C, N and clay contents than the Calcarosol soil. Soil texture affects the stability and microbial accessibility of SOC. The Vertosol soil (and Chromosol soil), with a relatively higher specific surface area and higher amounts of reactive sites, could retain more organic materials (Parfitt et al. 2003) inside of soil aggregates and thus reduce microbial accessibility and enzymatic decomposition of SOC (Six et al. 2002). This might account for the lower primed C per unit mass of SOC in the Vertosol and Chromosol soils than in the Calcarosol soil. The Chromosol and Vertosol soils, however, lost more C per unit of soil weight when compared to the Calcarosol during the due to their higher C concentration. The result is consistent with the notion that PEs are normally greater in soils that have more C and N (Kuzyakov et al. 2000; Qiao et al. 2015; Liu et al. 2017b). There was no net SOC gain in all cropped soils, albeit more C input under eCO_2, suggesting eCO_2 might have stimulated microbial decomposing capacities and the decomposition of crop residues and/or soil C in the field.

5.5.3 Temporal responses of priming effect

The weekly supply of glucose-induced higher potential PE at a later stage of incubation when labile SOC became less in amount. Multiple glucose pulses might have changed the composition and/or C-use plasticity of microbial community with activation of previously inactive SOC-utilising decomposers which could secrete larger quantities of extracellular enzymes to decompose recalcitrant C (Kuzyakov 2010; Mau et al. 2015; Morrissey et al. 2017). For example, using the quantitative stable isotope probing of DNA, Morrissey et al. (2017) discovered that the increase in PE by multiple glucose pulses was related to the shift of a wide phylogenetical group of taxa using SOC for growth. This could be attributed to microbial mining of soil organic matter for N (Fontaine and Barot 2005; Chen et al. 2014b) at the later stage of incubation as no synchronous N was supplied to the incubated soils in this and other

studies (Mau et al. 2015; Morrissey et al. 2017). In contrast, small and even negative PEs have been reported in response to a single pulse addition, which has been related to preferential utilisation of glucose (Blagodatskaya et al. 2011; Mau et al. 2015; Morrissey et al. 2017) by opportunistic organisms and their nutritional competition with SOC-decomposers (Mau et al. 2015).

5.6 Conclusions

Eight years of exposure to eCO_2 did not increase SOC concentration in the topsoil (0–5 cm) of three agriculturally important soil types in South-Eastern Australia, implying greater microbial decomposing capacities to mineralise plant residue and soil native C under eCO_2. Soil organic C would be more susceptible to microbial decomposition in future higher CO_2 atmosphere, probably due to a higher proportion of the coarse SOC fraction, less available N and higher soil pH which could stimulate microbial growth and depletion of N. The decrease in SOC stability induced by eCO_2 history, however, was only observed in the Calcarosol soil which had less clay and SOC concentration and a higher proportion of unprotected C than the Chromosol and Vertosol soils. Our results imply that the coarse-textured Calcarosol is more likely to be net CO_2 sources than sinks in future higher CO_2 scenarios. Meanwhile, the priming effect appears to be a long-term soil process which would potentially affect terrestrial ecosystem C sequestration. It should be included in global biochemical models to better understand global C cycling and its responses to climate change. This study focused on the effects of eCO_2 history on microbial priming of native SOC and only surface soil (0–5 cm) with the highest microbial abundance and activity was studied. Future studies are needed to examine the effect of long-term eCO_2 on SOC dynamics at various depths of soil profiles.

Chapter 6 Rhizosphere priming of two near-isogenic wheat lines varying in citrate efflux under different levels of phosphorus supply

(Submitted to Annals of Botany)

6.1 Summary

The traditional explanation of rhizosphere priming effect (RPE) is mainly from the perspective of microbial responses to root exudates and nutrient availability (e.g. microbial activation, co-metabolism and microbial N mining). This study introduced an abiotic process that could also contribute to RPE, which puts forward that root exudates (organic acids) could make previously mineral-protected C more available to microbial degradation through mineral dissolution. A pair of wheat (*Triticum aestivum* L.) near-isogenic lines with or without citrate efflux were grown for 44 days in a C4 soil supplied with either low P (10 μg g^{-1}) or high P (40 μg g^{-1}) in an environmental-controlled growth cabinet. Total below-ground CO_2 was trapped and partitioned for determination of SOC decomposition and RPE using a stable isotopic technique. Soil mineral dissolution was examined by incubating soil with either citric acid or Tri-potassium citrate at a series of concentrations. High P decreased rhizosphere inorganic N, increased RPE (81%), wheat biomass (shoot 32%, root 57%), root-derived CO_2-C (20%), microbial biomass C (28%) and N (100%), rhizosphere soil respiration (20%) and concentrations of water-extractable P (30%), Fe (43%) and Al (190%). Compared to Egret-Burke (no citrate efflux), wheat line Egret-Burke *TaMATE1B* with citrate efflux had lower rhizosphere inorganic N, microbial biomass C (16%) and N (30%) but greater RPE (18%), shoot biomass (12%), root-derived CO_2-C (low P 36%, high P 13%). It also tended to have higher concentrations of water-extractable P, Fe and Al in the rhizosphere. Citrate ligand facilitated Fe and Al releases from the soil with Fe and Al concentrations increasing with ligand concentration and incubation time. Citrate efflux may have facilitated the liberation of mineral-bounded C, which led to the higher RPE under Egret-Burke *TaMATE1B*. The higher RPE under high P is related to both mineral dissolution and microbial activation or co-metabolism by root exudates. Mineral dissolution is an important process that regulates RPE and should not be neglected in future RPE research.

6.2 Introduction

Plants can affect microbial decomposition of soil organic matter (SOM). The change in SOM decomposition by growing plants is termed rhizosphere priming effect (RPE) which is mainly caused by microbial responses to rhizodeposits derived from living roots. Root exudates (mainly sugars and molecular organic acids) are the most important primers in rhizosphere due to their solubility in water, mobility, as well as ability to direct incorporation into growing microbial cells (Kuzyakov 2002). Plants export proportionally 2–5% photosynthetic carbon (C) into its rhizosphere as root exudates (Jones et al. 2004; Pausch and Kuzyakov 2018). Although low in glucose and organic acids, root exudates could trigger microbial growth and activity (De Nobili et al. 2001) and their subsequent decomposition of SOM via microbial activation or co-metabolism (Kuzyakov et al. 2000).

Rhizosphere priming effect is additionally regulated by soil nutrient availability. When soil is with moderate N deficiency, root exudates could stimulate RPE by activating microorganisms to mine N from N-rich SOM (the so-called 'microbial N mining' mechanism, Fontaine et al. 2011). In severe N-limited systems, however, RPE could be reduced due to constraints for microbial growth and/or activity. For example, Xu et al. (2017) found that eCO_2 decreased the RPE under wheat plants where soil N is severely limited but not under white lupin where biological fixation provided additional N. Soil phosphorus (P) availability can also affect root exudation directly or indirectly via influences on plant photosynthesis and biomass production, and hence RPE but fewer studies have examined the effects of P availability on RPE. Low P availability could up-regulate the secretion of organic anions (e.g. citrate and malate) and the synthesis of phosphatase activity to facilitate P release via dissolution and desorption of inorganic P and hydrolysis of organic P (Kamh et al. 1998; Nuruzzaman et al. 2006; Weisskopf et al. 2006). Previous hydroponic studies with small plantlets have suggested that this up-regulation of organic anions under P deficiency is normally found in leguminous species, like chickpea (*Cicer arietinum* L.), field pea (*Pisum sativum* L.) and white lupin (*Lupinus albus* L.) but not in cereal plants such wheat (*Triticum aestivum* L.) (Neumann and Römheld, 1999; Nuruzzaman et al. 2005 Nuruzzaman et al. 2006). A recent study examined root secretion of organic anions by wheat (cv. Krabat) from the three leaves stage to the flowering stage at a long-term P experimental site with or without an annual P supply at 48 kg P ha^{-1} (Wang et al. 2017). The results show that the P deficiency increases citrate efflux but this effect depends on plant developmental stage. It is largely unknown whether soil P availability would affect RPE

via its effect on root exudation of wheat.

Labile soil organic C (SOC) has been regarded as the main soil C pool that undergoes microbial decomposition due to low chemical complexity (Ruiz-Dueñas and Martínez 2009) and lack of physical and chemical protection (Rasmussen et al. 2006). Soil organic C is chemically protected by adsorption on secondary mineral phases, the formation of metal-containing complexes (Kleber et al. 2015; Finley et al. 2018) and further occlusion inside of soil aggregates (Six et al. 2002). Soil short-range-order (SRO) materials such as aluminosilicates, and Fe- and Al-oxyhydroxides, are amorphous minerals that have a high reactive surface area and can form complexes with SOM through ligand change and render SOM unavailable for microbial degradation (Elliott and Sparks 1981; Chatterjee et al. 2013; Finley et al. 2018). The presence of SRO minerals has also been found conducible to soil aggregation (Duiker et al. 2003). Mineral protection of SOC by SRO minerals has been associated with weak priming effect (Rasmussen et al. 2006; Rasmussen et al. 2007). However, organic acid anions released from roots could increase mineral dissolution (Jones et al. 2004) by either breaking down organic-mineral associations and/or displacement of SOC on mineral surfaces (Kleber et al. 2015). The C released during these processes is further utilised by microorganisms and leads to an increased priming effect in a laboratory incubation experiment (Keiluweit et al. 2015). Likely, the function of root exudates to mobilise mineral-associated C could also occur in plant rhizospheres and thus affect SOM decomposition. Closely related plant genotypes with genetically tractable introgressed diversity (e.g. organic acid efflux) would be excellent materials to examine this abiotic mechanism.

In this study, a pair of near-isogenic wheat lines (Egret-Burke and Egret-Burke *TaMATE1B*) differing in citrate efflux were supplied with two P levels to study how soil P level and citrate exudation would impact RPE. This study aimed to further understand biotic and abiotic (mineral dissolution by root exudates) mechanisms of RPE. We hypothesised that i) P deficiency would increase root-specific RPE due to the up-regulation of organic anions secretion, and ii) wheat line with citrate efflux would induce greater RPE possibly via mineral dissolution of organic-mineral associations.

6.3 Materials and methods

6.3.1 Soil description

Surface soil (0–10 cm) was collected from a C4 *Themeda triandra* grassland (32°10'44" S, 149°33'45" E) in Gulgong, Australia. The soil was a sandy loam with total SOC 27 mg g^{-1}, total N 1.6 mg g^{-1}, Olsen P 9.2 mg g^{-1}, pH 4.7, $\delta^{13}C_{SOC}$ –19.8‰ and clay 13%. The soil was air-dried, homogenized and sieved (< 2 mm) with grass roots being removed. Basal nutrients were added to the soil at the following composition (µg g^{-1} soil): $CO(NH_2)_2$ 64.2, K_2SO_4 120, $MgSO4.7H_2O$ 50, $CaCl_2.2H_2O$ 180, $MnSO_4.H_2O$ 15, $ZnSO_4.7H_2O$ 9, $CuSO_4.5H_2O$ 6, $Na_2MoO_4.2H_2O$ 0.4 and Fe-EDTA 5.5. Two P levels were low P (10 µg P g^{-1} soil) and high P (40 µg P g^{-1} soil) added as KH_2PO_4. The nutrients were then thoroughly mixed with the soil before potting.

6.3.2 Wheat near-isogenic lines

A pair of wheat (*Triticum aestivum* L.) near-isogenic lines were Egret-Burke (Mat.) and Egret-Burke *TaMATE1B* (Mcit.), respectively. The expression of *TaMATE1B* in Egret-Burke *TaMATE1B* renders the line with constitutive citrate efflux at root apex (Ryan et al. 2009; Tovkach et al. 2012). The wheat line with *TaMATE1B* expression exudes 5–8 fold more citrate ligands than Egret-Burke (Ryan et al. 2014).

6.3.3 Growing system

A soil column experiment consisted of two wheat lines (Mat. and Mcit.) and two P levels (10 µg P g^{-1} and 40 µg P g^{-1}) in four replicates, and four unplanted columns were included as controls. It was conducted using bottom-capped polyvinyl chloride (PVC) tubes (diameter 7.5 cm, height 40 cm). One hundred grams of plastic beads were enclosed by micro mesh (0.45 µm) and then placed inside of each soil column before packing into 1.25 kg of the air-dried C4 soil. Soil moisture was adjusted to 80% field capacity with reverse osmosis water. Six pre-germinated wheat seeds were sown in a row alongside the diameter of the soil column. The columns were then transferred into growth chambers (Thermoline, Climatron-1100-SL-H, Wetherill Park, Australia). The temperature inside the growth chamber was set at 22 °C day (14 h) and 18 °C night (10 h) and the relative humidity maintained at 70%. The photosynthetic active photon flux density was around 350 µE m^{-2} s^{-1} at canopy level. After six days, the plants were thinned to three seedlings per column. Soil moisture was maintained at 80% field capacity by daily watering to the target weight. The columns were randomly reallocated weekly to avoid inhomogeneous growing conditions. Additional N was supplied at the rate of 30 µg urea-N g^{-1} soil weekly from the fourth week till harvest.

6.3.4 Below-ground CO_2 trapping

At 42 days after sowing, the top of each column was enclosed with two PVC plates around plant stems, and the open spaces were sealed with Blu-tack (Bostik, Thomastown, Australia). The air-tightness of the sealing was tested by vacuuming air through the column and observing the status of the bubbles formed in water. It was considered airtight if the bubbles were stable when pressing the jointing area, if not, more Blu-tack was added (Wang et al. 2016).

Below-ground CO_2 released was trapped for two days after the sealing. Firstly, CO_2-free air was generated by pumping air through 150 ml 1.0 *M* NaOH solution. Secondly, the CO_2–free air was pumped through the soil columns for 30 min to remove existing CO_2 in soil pores. The CO_2 exiting the column was then trapped in 150 ml of 0.5 *M* NaOH. A vacuum pump was attached at the other end of the NaOH trap to accelerate gas movement (Xu et al. 2018). The trapping was performed three times and 30 min each time between 0900 h and 2300 h with an interval of 6 h.

The total below-ground CO_2 released was determined by back-titration of the excessive alkali using 0.25 *M* HCl after precipitating the carbonate with $BaCl_2$ solution. The endpoint of the titration was indicated using phenolphthalein. The trap was also mixed with excessive $SrCl_2$ to form $SrCO_3$ precipitates (Cheng 1999) which were further washed and oven-dried for determination of the $\delta^{13}C$ value of the below-ground CO_2 using an isotope ratio mass spectrometer (SerCon Hydra 20–22, Gateway, UK).

6.3.5 Plant and soil analyses

Soil columns were destructively harvested after below-ground CO_2 trapping. Plant shoots were cut at ground level. Plant roots were picked up after collecting rhizosphere soil and washed. The roots were scanned using an EPSON EU-35 Scanner (Seiko Epson Corp., Suwa, Japan) before determination of root length using a WinRhizo STD 1600+ Image Analysis System (Regent Instruments, Quebec City, Canada). Dry weights of shoot and roots masses were recorded after oven-drying at 70 °C for 72 h. Dry plant samples were ball-milled and analysed for C and N concentrations using a CHNS/O Analyser (PerkinElmer EA2400, Branford, USA). Plant roots were also measured for $\delta^{13}C$ values by the IRMS.

Immediately after harvest, 10 g of rhizosphere soil was incubated in dark for 12 h to determinate rhizosphere soil respiration which was the amount of CO_2 produced during the incubation period and measured by a Sermex 4200 Industrial Gas Analyser (Servomex, Crowborough, UK). The fresh rhizosphere soil was also analysed for microbial biomass C (MBC) and N (MBN) using the extraction-fumigation method (Brookes et al. 1985). Briefly, one set of 8 g rhizosphere soil was fumigated with chloroform in dark for 24 h. The fumigated soil together with another set of soil without fumigation was then extracted with 0.5 *M* K_2SO_4 solution (1:5, w/v). The soil extracts were filtered through Whatman no. 42 filter paper and frozen at –20 °C before analysis of extractable organic C (EOC) and inorganic N. The EOC was measured using a TOC Analyser (GE Sievers InnovOx, Boulder, USA) and the MBC was calculated as the differences in EOC concentrations between the fumigated and non-fumigated soils with a conversion factor of 0.45 (Vance et al. 1987). The concentration of inorganic N in the extracts pf non-fumigated soils was determined as the sum of ammonium (NH_4^+), nitrite and nitrate (NO_x^-)using a Flow-Injection Analysis System (FIA) (Lachat's QuickChem 8500, Loveland, USA). The soil extracts of the fumigated and unfumigated soils were further autoclaved at 120 °C for 30 min and analysed for total NO_3^- using the FIA, and the MBN was calculated as the differences in total inorganic N concentrations between the fumigated and non-fumigated soil extracts with a conversion factor of 0.54 (Brookes et al. 1985; Vance et al. 1987).

The remaining rhizosphere soil was air-dried and analysed for pH after shaking the soil with 0.01 *M* $CaCl_2$ (1:5, w/v) for 1 h. Mineral dissolution was determined according to Yu et al. (2017). Briefly, air-dried soil was extracted with Milli-Q water (1:5, w/v) for 24 h at 25 °C on an orbital shaker (Ratek OM6, Boronia, Australia) at 170 rpm. The suspension was centrifuged at 3000 *g* for 10 min, filtered through 22–µm filter (Millex-GP SLGP033RS, Bayswater, Australia) and analysed for water-extractable P, Fe and Al using an inductively coupled plasma optical emission spectrometer (ICP-OES) (Optima 8000, PerkinElmer, Waltham, USA). The difference in water-extractable elements from planted and unplanted columns was used to estimate mineral dissolution due to root exudates (Yu et al. 2017).

6.3.6 Soil material dissolution experiment

The C4 soil with organic acid ligand was incubated to examine the effects of organic acid ligands on dissolution of soil minerals. Briefly, 1 g soil was weighed into opaque polyvinyl

chloride containers and then amended with 200 ml either Milli-Q water, tri-potassium citrate or citric acid in three replicates. The concentrations of the acids were 0.5, 1, 5 and 10 mM, representing the localised concentration range of root exudates by a variety of plant species (Ryan et al. 2014; Menezes-Blackburn et al. 2016). The containers were sealed and incubated at 25 °C in dark for 72 h. Fifteen ml of supernatant was collected by pipetting at 24, 48 and 72 h after the reaction. The supernatant was then filtered through 2–µm Whatman Puradisc 30 syringe filter (Whatman, Parramatta, Australia) and stored at 4 °C prior to pH and element analyses. The concentrations of total Fe and Al in solutions were determined by ICP-OES and thus termed as ICP-Fe and ICP-Al, respectively. The solutions were also analysed for inorganic monomeric Al using a Varian Cary 50 Bio UV-Visible Spectrophotometry (Agilent Technologies, Santa Clara, USA) according to Conyers et al. (1991).

6.3.7 Below-ground CO_2 partitioning and calculation of rhizosphere priming effect

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

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

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

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

where $\delta^{13}C_{root}$ is the $\delta^{13}C$ value of root materials (Table 6.1). $\delta^{13}C_{total}$ is the measured $\delta^{13}C$ value of total below-ground CO_2 respiration in planted treatments (Table 6.1). $\delta^{13}C_{soil}$ is the $\delta^{13}C$ value of CO_2 evolved from unplanted controls. f is the contribution of soil-derived CO_2-C to total below-ground CO_2 release. $C_{control}$ is the basal soil respiration from the unplanted controls. Root-specific RPE was calculated based on per unit of root mass and root length, respectively.

6.3.8 Statistical analysis

A two-way ANOVA was performed to examine the effects of P supply, wheat line and their interactions on all observations using Genstat (v17; VSN International, Hemel Hempstead, UK). A two-tailed Pearson's correlation analysis was conducted for RPE with all soil, plant, and microbial parameters using SPSS (v22; IBM Corp, Armonk, USA) with Pearson's correlation coefficients being tabulated in Table 6.3 when $P < 0.05$. All figures were plotted using means of four replicates with positive standard errors in Excel (v16; Microsoft Corp, Redmond, USA).

6.4 Results

6.4.1 Plant growth

By comparison to its near-isogenic line EGA-Burke, wheat line EGA-Burke *TaMATE1B* had 12% higher shoot biomass but similar root biomass, root-to-shoot ratio and total root length (Table 6.1). High P increased shoot biomass, root biomass and the root-to-shoot ratio by 32%, 57% and 20%, respectively (Table 6.1). The EGA-Burke had more negative (0.4‰) root $\delta^{13}C$ value than EGA-Burke *TaMATE1B*. High P increased the root $\delta^{13}C$ value by an average of 3.1% (Table 6.1).

6.4.2 Shoot N, P concentration and uptake

The two near-isogenic lines had similar shoot N concentrations and high P level decreased shoot N concentration by an average of 16% (Fig. 6.1A). By comparison to EGA-Burke, EGA-Burke *TaMATE1B* had 6% lower shoot P concentration under low P supply but had 18% higher shoot P concentration under high P (Fig. 6.1B), leading to a wheat line × P availability interaction.

EGA-Burke *TaMATE1B* had more shoot N and P uptakes than EGA-Burke (Fig. 6.1C, D). Specifically, EGA-Burke *TaMATE1B* took up 13% more N (Fig. 6.1C) when compared to EGA-Burke. It also took up 7% and 22% more P in shoots under low and high P supplies, respectively, leading to a wheat line × P availability interaction (Fig. 6.1D). High P enhanced N and P uptake by 12% and 38%, respectively (Fig. 6.1C, D).

Table 6.1 Shoot and root dry mass (DM), root-to-shoot (R:S), total root length, root $\delta^{13}C$ value and $\delta^{13}C$ value of below-ground CO_2 efflux of wheat line EGA-Burke (Mat.) and its near-isogenic line EGA-Burke *TaMATE1B* (Mcit.) grown for 44 days with either low P (10 µg g^{-1} soil) or high P (40 µg g^{-1} soil) supply

Wheat line	P supply (µg g^{-1} soil)	Shoot DM (g $column^{-1}$)	Root DM (g $column^{-1}$)	R:S	Root length (m $column^{-1}$)	$\delta^{13}C$ (‰)	
						Root	CO_2 released
Mat.	10	2.42±0.13	0.83±0.01	0.34±0.02	67.2±2.8	–29.1±0.2	–24.6±0.0
Mcit.	10	2.84±0.08	0.91±0.05	0.32±0.02	64.4±1.3	–28.5±0.1	–24.8±0.1
Mat.	40	3.34±0.14	1.39±0.03	0.42±0.02	68.1±5.9	–28.0±0.0	–23.7±0.1
Mcit.	40	3.62±0.12	1.34±0.05	0.37±0.02	66.9±2.5	–27.8±0.2	–23.7±0.2
Two-way ANOVA (*P*-value)							
Line		0.011	0.718	0.094	0.394	0.043	0.457
P		0.001	0.001	0.009	0.434	0.002	0.001
Line × P		0.598	0.135	0.549	0.807	0.269	0.432

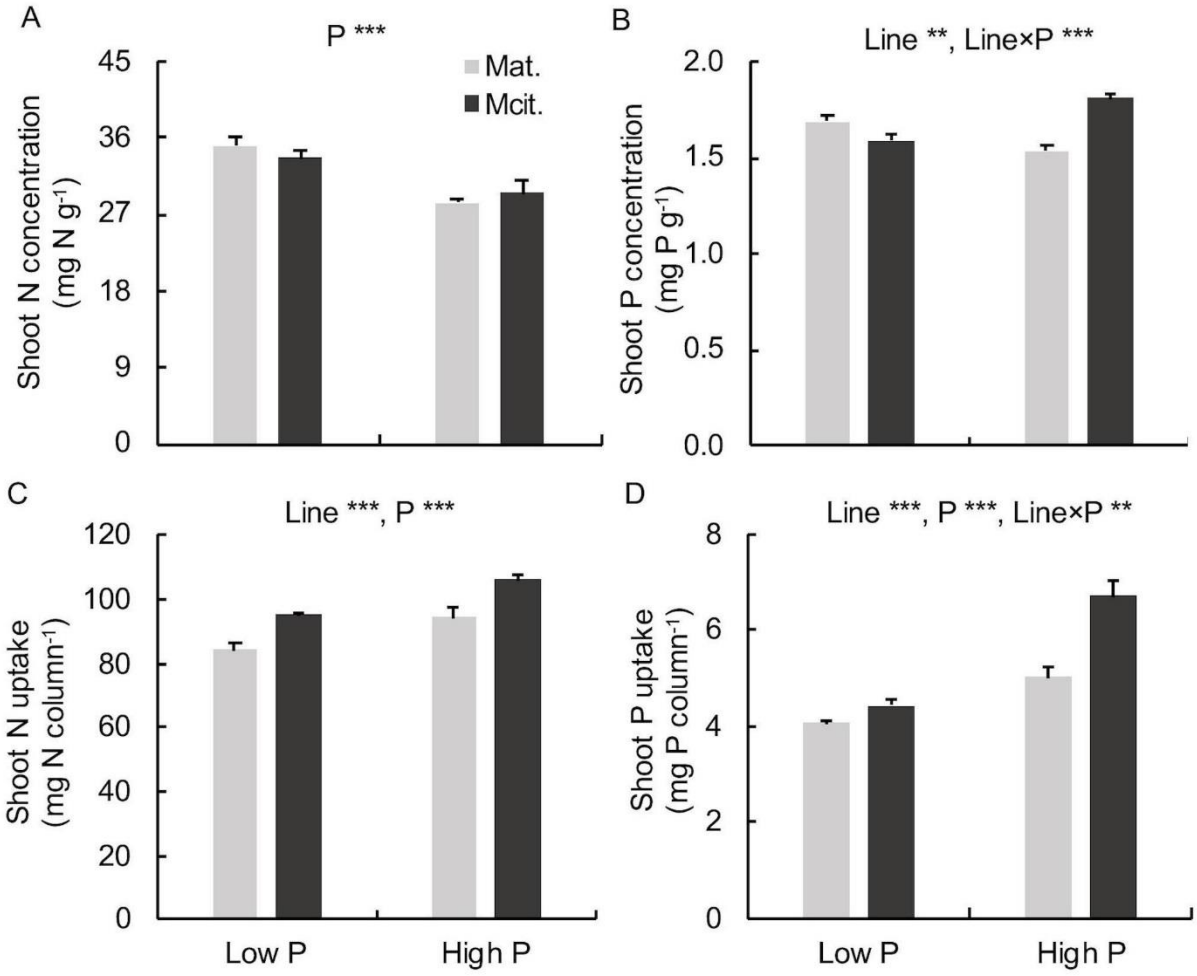

Figure 6.1 Shoot N and P concentrations (A, B; mg g^{-1}), and N and P uptake (C, D; mg $column^{-1}$) of wheat line EGA-Burke (Mat.) and its near-isogenic line EGA-Burke *TaMATE1B* (Mcit.) supplied with either low P (10 µg g^{-1} soil) or high P (40 µg g^{-1} soil). Line, wheat line; P, phosphorus supply. ** $P < 0.01$, *** $P < 0.001$

6.4.3 Below-ground CO_2 respiration and its $\delta^{13}C$ value

Basal soil respiration was 10.8 µg CO_2-C g^{-1} soil d^{-1} (dash line in Fig. 6.2) as measured from unplanted columns. Growing plants enhanced both soil-derived and root-derived CO_2 respirations. Compared to EGA-Burke, EGA-Burke *TaMATE1B* increased the root-derived CO_2 respiration by 36% under low P and 13% under high P, leading to a significant wheat line × P availability interaction (Fig. 6.2). EGA-Burke *TaMATE1B* also increased the soil-derived CO_2 respiration by an average of 9% (Fig. 6.2). High P supply stimulated root-derived and soil-derived CO_2 respirations by 20% and 35%, respectively. The two wheat lines generated similar $\delta^{13}C$ values of total below-ground CO_2 which were –24.7‰ and –23.7‰ at low P and high P, respectively (Table 6.1).

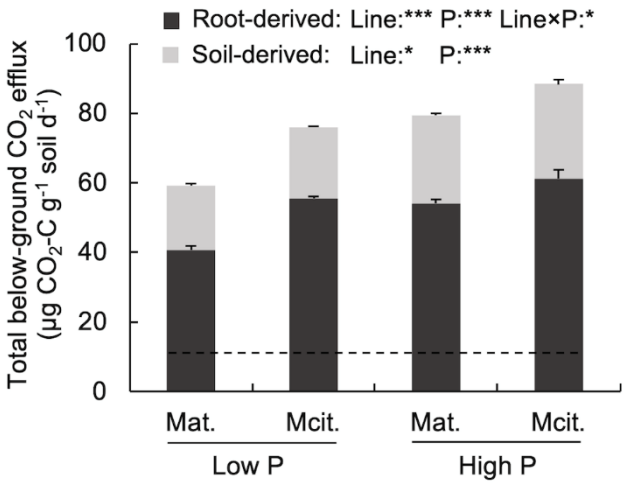

Figure 6.2 Total below-ground CO_2 efflux (soil-derived + root-derived, μg CO_2-C g^{-1} soil d^{-1}) under wheat line EGA-Burke (Mat.) and its near-isogenic line EGA-Burke *TaMATE1B* (Mcit.) supplied with either low P (10 μg g^{-1} soil) or high P (40 μg g^{-1} soil). Dash line denotes basal soil respiration. For the soil-derived CO_2, the main effects of wheat line ($P < 0.05$) and P supply ($P < 0.001$) but not their interaction are significant. For the root-derived CO_2, the main effects of wheat line ($P < 0.001$) and P supply ($P < 0.001$), and their interaction ($P < 0.05$) are significant

6.4.4 Soil inorganic N in the rhizosphere

Soil inorganic N amounted at 134 μg N g^{-1}soil in the unplanted control with NH_4^+ being 128 μg g^{-1} soil and NO_x^- 5.6 μg g^{-1} soil. Growing plants decreased soil NO_x^- but increased soil NH_4^+. Compared to EGA-Burke, growing EGA-Burke *TaMATE1B* decreased soil NH_4^+ under high P by 41% but had no effect on soil NH_4^+ under low P, leading to a significant wheat line × P availability interaction (Fig. 6.3). Growing EGA-Burke *TaMATE1B* decreased soil NO_x^- by 57% under low P, but did not affect soil NO_x^- under high P (Fig. 6.3). Growing plants at high P decreased the concentrations of NH_4^+ and NO_x^- in the rhizosphere by 61% and NO_x^- by 61% and 91%, respectively, when compared to low P (Fig. 6.3).

6.4.5 Soil microbial biomass C, N and C-to-N ratio

Microbial biomass C and N were 39.8 μg C g^{-1} soil and 4.1 μg N g^{-1} soil, respectively, in the unplanted controls but increased in the rhizosphere (Fig. 6.4). Soil microbial biomass C and N were 16% and 30% lower in the rhizosphere of EGA-Burke *TaMATE1B* than EGA-Burke (Fig. 6.4A, B). High P increased rhizosphere MBC and MBN by averages of 28% and 100%, respectively (Fig. 6.4A, B). Growing EGA-Burke *TaMATE1B* yielded 37% higher microbial

biomass C-to-N ratio in the rhizosphere when compared to EGA-Burke (Fig. 6.4C) but this was only observed at low P, leading to a significant wheat line × P availability interaction (Fig. 6.4C). High P decreased microbial biomass C-to-N ratio in the rhizosphere by an average of 36% (Fig. 6.4C).

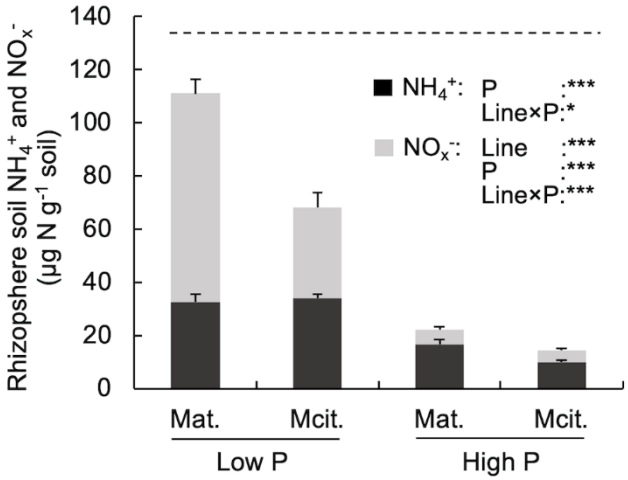

Figure 6.3 Concentrations of soil ammonium (NH_4^+, μg N g^{-1} soil), nitrate and nitrite (NO_x^-, μg N g^{-1} soil) in the rhizospheres of wheat line EGA-Burke (Mat.) and its near-isogenic line EGA-Burke *TaMATE1B* (Mcit.) supplied with either low P (10 μg g^{-1} soil) or high P (40 μg g^{-1} soil). Dash line denotes soil inorganic N ($NH_4^+ + NO_x^-$) concentration in the unplanted control. The data of NO_x^- were log_{10}–transformed before statistical analysis. For NO_x^-, the main effects of wheat line and P supply and their interaction are all highly significant ($P < 0.001$). For NH_4^+, the main effects of wheat line ($P < 0.001$) and the interaction between wheat line and P supply ($P < 0.05$) are significant

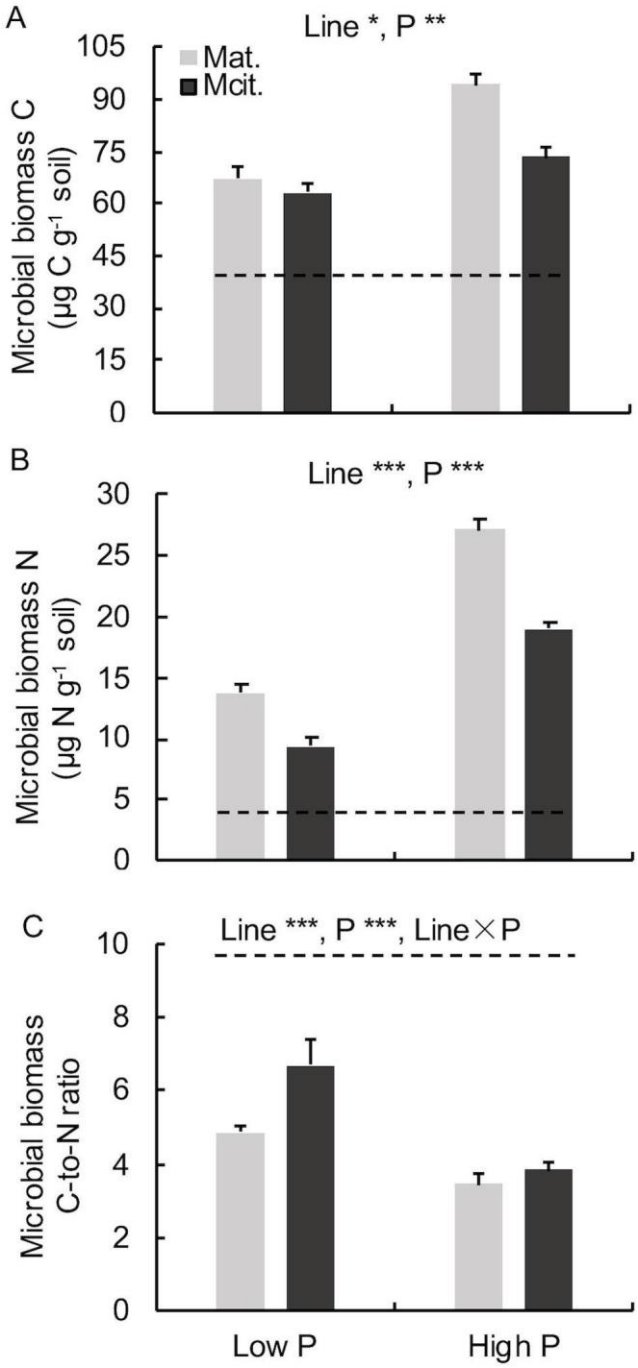

Figure 6.4 Microbial biomass C (a), N (b) (μg C/N g^{-1} soil) and C-to-N ratio (c) in the rhizospheres of wheat lines EGA-Burke (Mat.) and EGA-Burke EGA-Burke *TaMATE1B* (Mcit.) supplied with either low P (10 μg g^{-1} soil) or high P (40 μg g^{-1} soil). Dash lines denote the values of the unplanted control. Line, wheat line; P, phosphorus supply. * $P < 0.05$, ** $P < 0.01$; *** $P < 0.001$

Table 6.2 Rhizosphere soil respiration ($Rh_{resp.}$) and water-extractable P, Fe and Al in the rhizosphere of wheat line EGA-Burke (Mat.) and its near-isogenic line EGA-Burke *TaMATE1B* (Mcit) after grown for 44 days with either low P (10 µg g^{-1} soil) or high P (40 µg g^{-1} soil) supply

Wheat line	P supply (µg g^{-1} soil)	pH	$Rh_{resp.}$ (µg CO_2 g^{-1} soil)	Water-extractable elements (µg g^{-1})		
				P	Fe	Al
Control		4.69±0.00	41.5±4.7	1.18±0.12	1.89±0.27	10.9±1.7
Mat.	10	4.79±0.01	79.0±2.4	1.21±0.09	10.1±1.76	53.7±10.7
Mcit.	10	4.79±0.03	65.9±5.4	1.86±0.28	16.8±4.6	83.2±31.0
Mat.	40	4.78±0.01	89.5±4.9	2.92±0.09	32.2±1.3	167±32.2
Mcit.	40	4.76±0.01	83.8±3.4	3.51±0.39	43.5±7.3	230.3±41.7
Two-way ANOVA (*P*-value)						
Line		0.201	0.052	0.094	0.083	0.100
P		0.030	0.008	0.001	0.001	0.004
Line × P		0.115	0.403	0.582	0.571	0.614

Table 6.3 Pearson's correlation coefficients among rhizosphere priming effect, shoot and root dry mass (DM), shoot N and P uptake, mineral N ($NH_4^+ + NO_x^-$), water-extractable P, Fe and Al concentrations, and microbial biomass N (MBN) in rhizosphere soil and root-derived CO_2-C

	Shoot DM	Root DM	Shoot nutrient uptake		Mineral N	Water-extractable elements			MBN	Root derived-CO_2-C
			N	P		P	Fe	Al		
RPE	0.86**	0.95**	0.77**	0.80**	–0.95**	0.96**	0.90**	0.94**	0.64*	0.80**
Shoot DM		0.81**	0.86**	0.91**	–0.90**	0.85**	0.81**	0.82**	0.50*	0.77**
Root DM			0.54*	0.69**	–0.91**	0.91**	0.84**	0.87**	0.77**	0.69*
Shoot N uptake				0.84**	–0.77**	0.69**	0.68*	0.67*	n.s.	0.84**
Shoot P uptake					–0.74**	0.81**	0.79**	0.84**	n.s.	0.69*
Mineral N						–0.92**	–0.87**	–0.84**	–0.68**	–0.86**
Water-extractable P							0.98**	0.98**	n.s.	0.90**
Water-extractable Fe								0.99**	n.s.	0.91**
Water-extractable Al									n.s.	0.91**
MBN										n.s.

n.s. $P > 0.05$ (2–tailed), * $P < 0.05$ (2–tailed), ** $P < 0.01$ (2–tailed)

6.4.6 Rhizosphere soil pH, respiration and water-extractable P, Fe and Al

Growing plants for 44 days increased rhizosphere soil pH by around 0.1 unit. High P supply decreased the rhizosphere soil pH by 0.02 unit when compared to low P (Table 6.2).

Compared to that in the no-plant control, soil respiration in the rhizosphere was 75% and 109% higher under low P and high P, respectively (Table 6.2). Soil respiration tended to be lower in the rhizosphere of EGA-Burke *TaMATE1B* (P = 0.052, Table 6.2) when compared to EGA-Burke. High P increased rhizosphere soil respiration by an average of 20%.

The concentrations of extractable P, Fe and Al were higher in the rhizosphere than in the no-plant control. When compared to low P, high P supply increased the concentrations of extractable P, Fe and Al by 30%, 43% and 190%, respectively (Table 6.2). Compared to EGA-Burke, growing EGA-Burke *TaMATE1B* tended to increase the concentrations of water-extractable P, Fe and Al in the rhizosphere ($P \leq 0.10$) (Table 6.2).

6.4.7 Rhizosphere priming effect

Rhizosphere priming effect depended on both soil P availability and wheat line with no interaction between them. By comparison to its near-isogenic EGA-Burke, wheat line EGA-Burke *TaMATE1B* increased RPE by an average of 18%, RPE per unit root weight by 13% and RPE per unit root length by 16% (Fig. 6.5). High P resulted in 81% higher RPE, 14% higher RPE per unit root weight and 79% higher per unit root length than low P supply (Fig. 6.5). The RPE was positively correlated with plant biomass, shoot N and P uptakes, microbial biomass N, water-extractable P, Fe and Al, and root-derived CO_2 respiration, but negatively with soil mineral N in the rhizosphere (Table 6.3).

6.4.8 Results of mineral dissolution experiment

Citric acid and potassium citrate had different effects on solution pH and element release from soil (Fig. 6.6). The solution pH decreased to 2.6 with increasing citric acid concentration but increased to 7.7 with increasing potassium citrate concentration as compared to pH 6.7 for the control (Fig. 6.6A). The concentration of Fe and Al in solution rapidly increased with 24 h of ligand addition and increased further with time. Citric acid was more effective than potassium citrate in mineral dissolution (Fig. 6.6B, C). In general, the concentrations of metal elements in solution increased with increasing ligand concentrations except for potassium citrate at 24 h

where the highest metal concentrations were found at 0.5 mM. The highest monomeric Al concentrations were found in solutions at concentrations of citrate ligand ≤ 1 mM. Specifically, the relative contribution of monomeric Al to ICP-Al was 27% and 64%, respectively, for potassium citrate and citric acid respectively. In comparison, the contributions were 4% and 3%, respectively, for potassium citrate and citric acid of ≥ 5 mM.

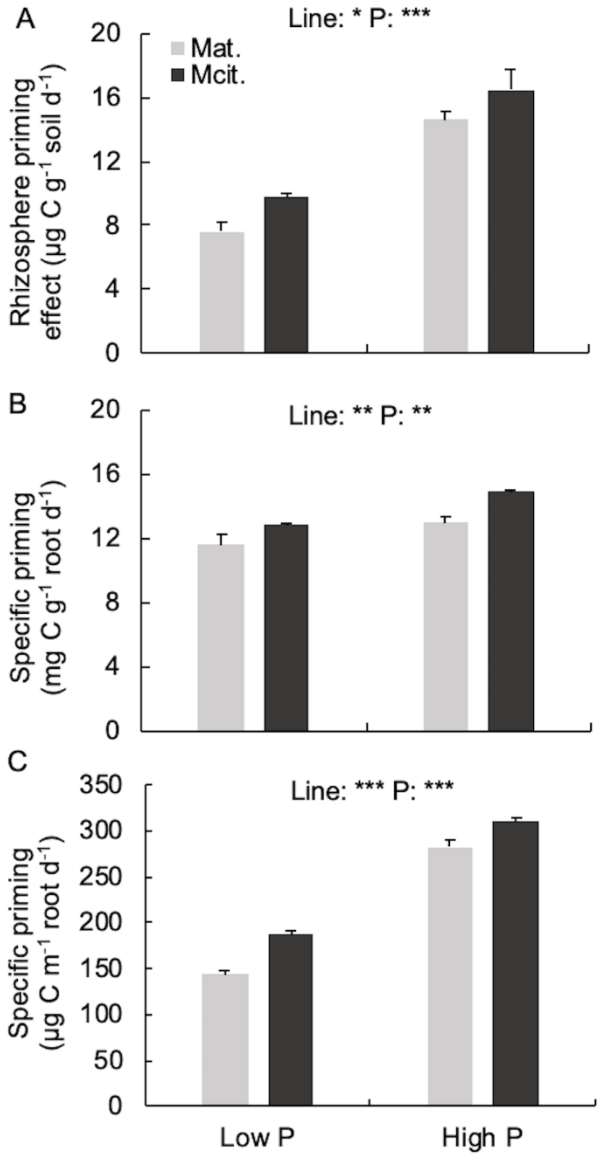

Figure 6.5 Rhizosphere priming effect (A, µg C g^{-1} soil d^{-1}) and root-specific priming (B, mg C g^{-1} root d^{-1}; C, µg C m^{-1} root d^{-1}) under wheat line EGA-Burke (Mat.) and its near-isogenic line EGA-Burke *TaMATE1B* (Mcit.) supplied with either low P (10 µg g^{-1} soil) or high P (40 µg g^{-1} soil). The main effects of wheat line ($P < 0.05$) and P supply ($P < 0.001$) but not their interaction are significant

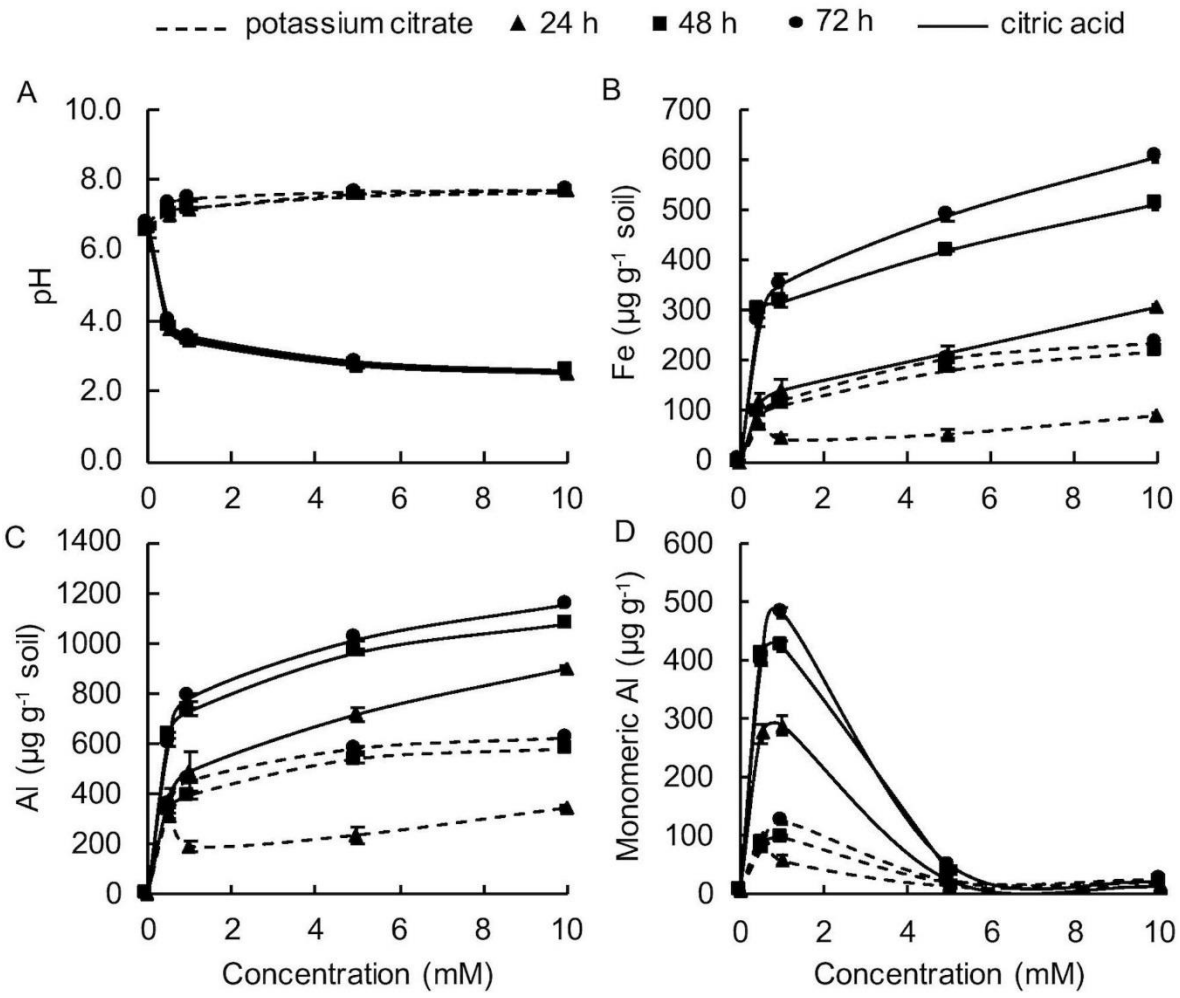

Figure 6.6 General trends of solution pH and element release data (concentration in µg g^{-1}) from the mineral dissolution experiment where ~1 g C4 soil reacted with 200 ml either potassium citrate or citric acid at concentrations of 0, 0.5, 1, 5 and 10 mM for 24, 48 and 72 h: (A) solution pH, (B) ICP-Fe, (C) ICP-Al and (D) monomeric Al. Means and standard errors of triplicates were denoted by symbols and vertical bars

6.5 Discussion

6.5.1 Microbial mechanisms of rhizosphere priming effect

High P availability increased RPE and root-specific RPE, which rejects our first hypothesis. Although low P in this study may have favoured the secretion of citrate as discovered by Wang et al. (2017), the plants under low P conditions might have secreted proportionally fewer total root exudates due to the smaller root systems (Bengtson et al. 2012) when compared to high P supply. The RPE was positively correlated with both shoot and root biomass (Table 6.3) which

is consistent with previous studies (Fu and Cheng 2002; Dijkstra et al. 2006). Root exudation is regulated by photosynthesis with around 2–5% of photosynthesised-C being exported proportionally by plants as root exudates (Jones et al. 2004; Pausch and Kuzyakov 2018). High P increased total root exudation as indicated by the greater the root-derived CO_2-C (Fig. 6.2) through impacts on photosynthesis and biomass production (Table 6.1).

As microbial energy and C source, primary metabolites of root exudates such as sugars, amino acids and organic acids (Jones et al. 2004) could stimulate microbial growth and/or activity (Kuzyakov et al. 2000; Cheng and Kuzyakov 2005; Nannipieri et al. 2008), for example, the up-regulation of extracellular enzyme activity (Brzostek et al. 2012). In this study, both microbial biomass C (Fig. 6.4A) and rhizosphere soil respiration were higher under high P than under low P supply (Table 6.2). The larger microbial population and stimulated activity increased native SOC decomposition probably due to the 'co-metabolism' mechanism by which the extracellular enzymes synthesised specifically catalyse substrate degradation driving SOM decomposition (Kuzyakov et al. 2000).

Microbial N mining is also previously proposed to explain the enhanced RPE since C inputs from roots normally increase microbial N requirement, leading to a greater microbial decomposition of SOM for N (Kuzyakov et al. 2000; Chen et al. 2014). However, this mechanism was possible in this present study although the microbial N demand was greater under high P. In this present study, soil microorganisms would primarily rely on external supply for their N nutrition, considering that N was supplied periodically and that albeit smaller, soil inorganic N under high P was still sufficient for microbial growth when compared to low P (Figs 6.3, 6.4C). In an incubation study with boreal forest soils, Wild et al. (2017) also found that substrate addition increased microbial growth and N demand but not microbial mining of soil N as there was no increase in protein depolymerisation. Besides, microorganisms immobilised more soil available N under high P than under low P as shown by the greater MBN (Fig. 6.4B), lower MBC-to-N ratio (Fig. 6.4C) and lower soil inorganic N (Fig. 6.3) under high P. This might have also contributed to the absence of microbial N mining (Wild et al. 2017). The greater root exudation under high P could also shift microbial structure composition with more dominance of fungi (Griffiths et al. 2012) because fungi, as heterotrophic organisms, depend largely on exogenous C for growth. Certain components of root exudates could also serve as chemical signals to structure fungal community (Broeckling et al. 2008). Rather than stabilising SOC (Schmidt et al. 2011), fungi have been recently found

to increase priming effect and SOC decomposition (Fontaine et al. 2011; Phillips et al. 2012; Shahzad et al. 2015), probably due to their greater capacity of accessing soil C via hyphal exploration (Fontaine et al. 2011). These findings suggest that high P could increase RPE through boosting biomass production and the associated stimulation of microbial growth, activity and fungal abundance.

6.5.2 Chemical mechanism of rhizosphere priming effect

Apart from the above microbial mechanisms, root exudates (organic acid ligands, in particular) could also affect RPE by liberating mineral-associated organic C via chemical dissolution and/or complexation (Finley et al. 2018). In this study, high P could have potentially increased mineral dissolution as evidenced by the higher water-extractable Fe and Al concentrations in rhizosphere soil (Table 6.2) when compared to low P. The mineral dissolution might have released previously mineral-protected organic C which became more vulnerable to microbial decomposition, leading to an increase in RPE (Table 6.4).

Soil organic C is protected by adsorption on short-range-order (SRO) minerals through their large reactive surface area and/or formation of metal-containing (Fe, Al) complexes due to their various binding sites (Kleber et al. 2015; Finley et al. 2018). Carboxylate components of root exudates could increase soil C release (Jones et al. 2004) by physiochemically breaking down organic-mineral associations and/or displacing of SOC on mineral surfaces (Kleber et al. 2015). In a study by Naveed et al. (2017), barley root exudates with large amounts of organic acid anions are thought to be capable of dispersing soil particles by increasing the net negative charges of clay when adsorbed on surfaces of soil mineral particles (Shanmuganathan and Oades 1983). Keiluweit et al. (2015) also found that oxalic acid (a common component of root exudates) has the ability to liberate protected C from mineral phases via the following processes. Firstly, organic acid anions can release SOC absorbed on SRO surfaces by complexation and dissolution. Secondly, they can solubilise SOC from metal-organic ligand complexes by binding with metal cations (Keiluweit et al. 2015). These processes facilitate microbial access to SOC, and could induce a high priming effect as revealed recently (Keiluweit et al. 2015; Finley et al. 2018).

This present study further validated the above assumption by using a pair of wheat near-isogenic lines which differ only in citrate efflux from the roots. The differences in citrate efflux

have been quantified previously with line EGA-Burke *TaMATE1B* (Ryan et al. 2014). Wheat line EGA-Burke *TaMATE1B* induced higher RPE and root-specific RPE than line EGA-Burke under both P levels (Fig. 6.5). However, the up-regulation of citrate efflux in EGA-Burke *TaMATE1B* as indicated by more shoot biomass and greater root-derived CO_2-C (Table 6.1, Fig. 6.2) did not stimulate either microbial growth (Fig. 4A) or activity in the rhizosphere (Table 6.2) probably because citrate is similar to oxalic acid which is energetically non-favourable to microorganisms (Keiluweit et al. 2015).

In addition to microbial mechanisms, the enhanced RPE might be induced by chemical dissolution and/or displacement of mineral-associated organic C as stated above. It was supported by the higher concentrations of water-extractable P, Fe and Al in the rhizosphere of line EGA-Burke *TaMATE1B* than EGA-Burke though only significant at $P \leq 0.10$ (Table 6.2). The mineral dissolution experiment provided further evidence that citrate ligand could facilitate mineral dissolution by forming citrate-Fe/Al complexes (Fig. 6.6) and thus may liberate previously-protected C through this process. The results are consistent with previous findings. For example, organic acid anions (e.g citrate and malate) secreted from plant roots have been found to increase Fe-dissolution (Jones et al. 1996) and release of P from Al and Fe oxides (Sanyal and Datta 1991) in acid soils. The release of minerals by chemical dissolution is important especially in nutrient-scarce soils, which could potentially alleviate plant and/or microbial nutrient limitation and consequently affect rhizosphere priming effect. The results indicate that root exudates could contribute to rhizosphere priming through the release of mineral-protected organic C.

6.6 Conclusions

This study suggests that both microbial and chemical mechanisms are accountable for the enhanced RPE when high P is supplied. Rhizosphere priming effect is more likely caused by microbial activation and co-metabolism of both easily available SOC and mineral-protected SOC released via chemical dissolution. Further studies are needed to validate the relative contributions of microbial mechanism and mineral dissolution to the rhizosphere priming effect.

Chapter 7 General Discussion

Adopting the natural ^{13}C-tracing technology, this thesis examined the effects of short-term atmospheric CO_2 enrichment on priming decomposition of organic matter in soils (either native soil organic carbon (SOC) or crop residues) in the rhizospheres of legume (white lupin) and/or cereal (wheat) plants. The thesis also investigated the dynamics and stability of SOC after long-term CO_2 enrichment in three major soils from South-eastern Australian croplands. The thesis further examined the mechanism of rhizosphere priming effect (RPE) from an abiotic perspective (mineral dissolution). The key findings are:

i. Short-term CO_2 enrichment can either increase or decrease RPE, depending on plant species and growth stages.

ii. The negative effects of elevated CO_2 (eCO_2) on RPEs are found under wheat plants at a late growth stage but not under white lupin, suggesting that limited N availability is a key factor limiting root exudation of wheat and microbial degrading capacities.

iii. Reducing N supply increases RPEs due to microbial N mining in an early growth stage when the competition for N between plants and microorganisms is weak but decreases RPEs at a late growth stage under wheat due to severe N limitation.

iv. Except for native SOC, CO_2 enrichment enhances residue decomposition under white lupin due to microbial activation and/or metabolism decomposition by greater root exudation.

v. The presence of plants does not always have a positive effect on residue decomposition and the negative effects at a late growth stage are caused by rhizosphere acidification, microbial N limitation and less labile C remaining in the residue.

vi. Eight years of CO_2 enrichment does not increase SOC stock in the topsoil (0–5 cm) of all three cropland soils (Chromosol, Vertosol and Calcarosol) but results in more C distributed in coarse fractions and more priming decomposition of the low-C sandy Calcarosol soil induced by external substrate addition.

vii. A wheat line with citrate efflux induced a greater RPE than its near-isogenic line but with no increases in microbial population and activity. The higher RPE could

be caused by a two-step process: 1) root exudates (i.e. citrate) mobilise mineral-bounded organic carbon and 2) microorganisms utilise of the mobilised carbon.

Elevated CO_2 affects soil C and N availabilities, soil moisture and pH in plant rhizosphere by changing root physiology (quantity and quality of root exudates) and morphology (root length and diameter). Compared to aCO_2, eCO_2 generally enlarges root system with which plants could explore a larger volume of the soil. It could also stimulate root exudation as indicated by higher K_2SO_4-extractable organic carbon (EOC) in rhizosphere soil via enhanced root growth and/or specific exudation (Cheng and Johnson 1998). Besides, eCO_2 may also change the chemical composition of root exudates. For example, more phenolic compounds are found in the rhizosphere of mature rice under eCO_2 (Goufo et al. 2014). De Graaff et al. (2007) reported that eCO_2 could also enhance N rhizodeposition. Elevated CO_2 could intensify plant N demands and also reduce soil N mineralisation (Berntson and Bazzaz 1997; Hungate et al. 1999), especially for non-legumes, due to enhanced plant growth and root exudation. Elevated CO_2 could also acidify rhizosphere soil via enhanced efflux of carboxylates (Jin et al. 2015) and/or unbalanced cation-anion uptake by plant roots (Haynes 1990). All these changes in rhizosphere by eCO_2 could potentially influence rhizosphere microbial growth and activity, leading to changed decomposition of native SOC and other organic matters (e.g. plant residues).

Plant species intrinsically differ in their root physiology and morphology. Cereal plants such as wheat and legumes such as white lupin used in this thesis have contrasting root systems. Wheat has a finer and longer root system which can explore a much larger volume of soil when compared to white lupin. However, white lupin has a greater capacity than wheat in secreting low-molecular organic anions (e.g. citrate) (Weisskopf et al. 2008). Root physiology and morphology further differ at distinctive growth stages. As a result, plant traits (e.g. species and growth stage) are also important factors regulating RPE and its responses to eCO_2.

Elevated CO_2 increased the RPEs under white lupin at both growth stages, decreased the RPEs under wheat at a late growth stage irrespective of the amount of N supplied (Chapters 2 and 3). The increased RPEs under eCO_2 were associated with greater EOC, microbial biomass C (MBC) and/or rhizosphere soil respiration. The results indicate that eCO_2 might have activated soil microorganism and/or their biosynthesis of extracellular enzymes to degrade soil organic matter for N when microbial N competition with the plant is weak. With plant ages, soil N was depleted quickly under eCO_2, leading to a severe N deficiency. The limitation of N under eCO_2

may potentially constrain microbial growth and their degradation of SOC (e.g. under wheat in Chapter 2). However, microorganisms in the rhizosphere of legume – white lupin, compared to wheat, were less or not limited by C and N, probably due to enhanced N_2 fixation by eCO_2 as found by others (de Graaff et al. 2006b; Jin et al. 2012; Lam et al. 2012a). The absence of microbial C and N limitation accounts for the increased responses of RPEs to eCO_2 under white lupin even at the late growth stage. These assumptions were confirmed by the findings that low N supply decreased RPEs under wheat but not under white lupin at the late growth stage (Chapter 3).

Due to the importance in soil C and N cycling, RPE has become the priority of research in soil ecology. However, the eCO_2 effect on RPE has less been studied. To the best of my knowledge, there are only three publications (Cheng and Johnson 1998; Nie et al. 2015; Nie and Pendall 2016) which directly addressed the responses of RPE to eCO_2 before this PhD project had commenced. However, more studies have reported the effect of eCO_2 on the decomposition of soil organic matter (soil-derived CO_2-C). The results from Chapters 2 and 3 of this thesis were synthesised with previous studies by a meta-analysis (supplementary material, Fig. 7.1) to give a general picture of the eCO_2 effect on RPE and native SOC decomposition. In general, eCO_2 increased RPE in all five studies (Fig. 7.1a). Although not statistically significant, the eCO_2 effect on RPE tended to be higher in systems with higher N (> 100 kg N ha^{-1}) supply when compared to low N. The eCO_2 effects were also similar in cropland and grassland soils. The literature synthesis also suggested that eCO_2 enhanced decomposition of native SOC with no significant differences between N levels or vegetation types (Fig. 7.1b). The difference in eCO_2 effects on RPE and SOC decomposition could be caused by different number of studies and different basal soil respiration in the two scenarios. These results confirmed the general hypothesis that eCO_2 could prim soil microorganisms to decompose native SOC by providing them with labile C substrates and energy in forms of rhizodeposits.

Soil organic C is mainly formed from microbial transformation of organic matter from plant residues. Some parts of plant residues are under microbial processing once returned to soils. During these processes, nutrients are released back to increase soil productivity with quite a large amount of C being lost to the environment. Residue return is an important agricultural practice to improve both soil quality and productivity and its decomposition in soil has been a vital research topic. This thesis also quantitatively examined the effect of higher CO_2 concentration on residue decomposition at the presence of plant roots (Chapter 4). The presence

of plant roots accelerated residue decomposition with large amounts of residues being decomposed in the first five weeks. Afterwards, the decomposition of residue (wheat straw and field pea residue) was decreased by growing plants and the decrease in residue decomposition was associated with less labile residue C, rhizosphere acidification and microbial N limitation.

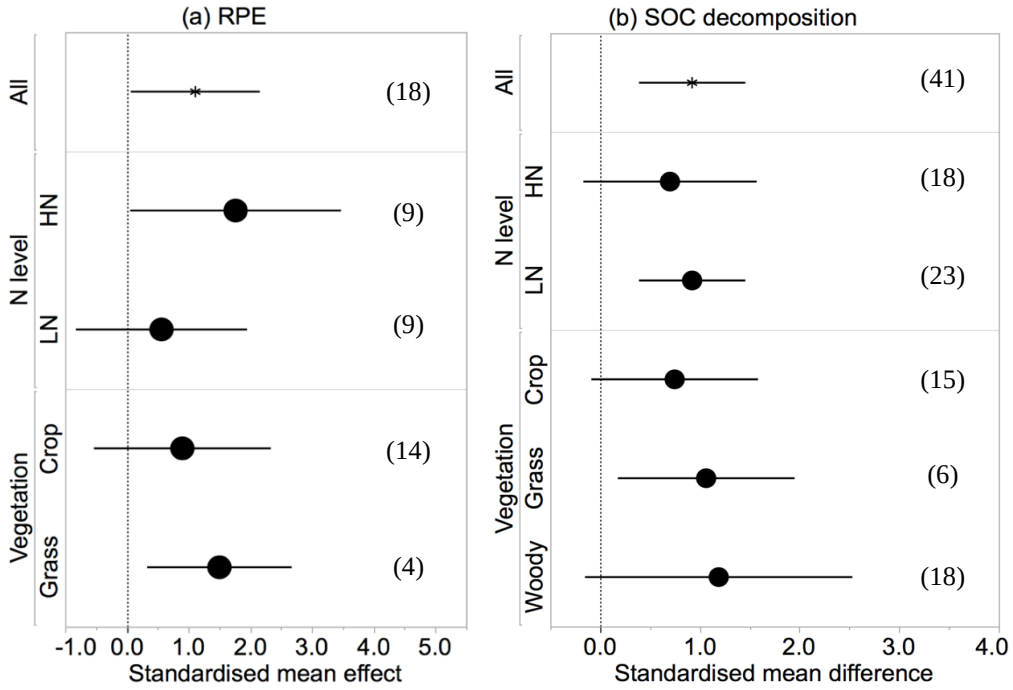

Figure 7.1 Standardised mean difference (eCO_2 – aCO_2) and 95% confidence interval of rhizosphere priming effect (a, difference in SOC decomposition between planted and unplanted soils) and SOC decomposition (b) grouped by N level (LN, low N, < 100 kg N ha^{-1} and HN, high N, > 100 kg N ha^{-1}) and vegetation type (crops, grass and woody species). Numbers in brackets are sample size for each group. Significant treatment difference is denoted if the 95% confidence interval does not overlap with the dashed line (zero effect). The results are based on Kuikman et al. (1990), van Ginkel et al. (1997), Cheng and Johnson (1998), Lin et al. (1999), Cheng et al. (2000), Cardon et al. (2001), Lin et al. (2001), Pendall et al. (2003), Søe et al. (2004), Trueman and Gonzalez-Meler (2005), Allard et al. (2006), Paterson et al. (2008), Nie et al. (2015), Nie and Pendall (2016), Xu et al. (2017) and Xu et al. (2018)

Elevated CO_2 increased residue decomposition especially when limited amounts of labile residue C were remaining. This might be caused by enhanced microbial population and synthesis of extracellular enzymes in line with the microbial activation or co-metabolism mechanism. The enhanced decomposition of residue may be accompanied with N

mineralisation and could, therefore, provide extra N for the use of non-leguminous plants. In these short-term experiments (Chapters 2, 3 and 4), eCO_2 was found to enhance the decomposition of both native SOC and newly-input residues.

Even with high RPEs, eCO_2 could still increase or have no effect on SOC content if above- and below-ground plant materials replenish and exceed the part of C lost from priming. The size of SOC pool depends on the net balance of soil C input and output. The effect of eCO_2 on the size of SOC pool has been examined in forest and grassland soils by a number of long-term Free-air CO_2 Enrichment (FACE) experiments with no consistent results being generated. Specifically, SOC stock could be increased (Luo et al. 2006), decreased (Hoosbeek et al. 2004; Carney et al. 2007; Langley et al. 2009) or unaffected (van Groenigen et al. 2006; Keiluweit et al. 2015) by CO_2 enrichment.

The effect of eCO_2 history on SOC stock in cropland soils was examined in Chapter 5 using soil samples collected from the SoilFACE (Horsham, VIC, Australia). Eight-years of eCO_2 fumigation changed SOC distribution with more C in the coarse fraction (> 53 μm) but did not increase SOC content in the topsoil (0–5 cm) of all three major cropland soils in South-western Australia. Elevated CO_2 did not build up topsoil SOC after 8 years although with more biomass production and thus more annual residue return, suggesting eCO_2 increased the decomposition of crop residues and perhaps SOC. Soil organic C stability after long-term CO_2 fumigation was also examined in Chapter 5. By comparison to aCO_2, the low-C Calcarosol fumigated with eCO_2 lost more SOC after substrate addition probably due to increased microbial biomass C and decreased soil inorganic N which stimulated microbial degradation of soil organic matter for N. However, this eCO_2 effect disappeared in the Chromosol and Vertosol soils probably because the SOC in these two soils were largely protected from microbial accessibility (Six et al. 2002; Reis et al. 2014) when compared to the sandy-textured Calcarosol. These results suggest that RPE may not a short-term effect and could affect soil SOC balance (van Groenigen et al. 2017) in the long run (van Groenigen et al. 2017).

Rhizosphere priming effect is traditionally explained by microbial responses to changes in rhizosphere conditions (e.g. quantity and quantity of root exudates, nutrient availability, pH etc.) due to plant growth. Biotic mechanisms (see Chapter 1 for details) like ‘microbial activation’, ‘co-metabolism’, ‘microbial N mining’, ‘preferential substrate utilisation’ and ‘N competition’ have been proposed and examined previously (Horvath 1972; Cheng and

Kuzyakov 2005; Mondini et al. 2006; Carney et al. 2007; Blagodatskaya et al. 2011). Labile SOC is traditionally thought to be the C pool that is the most susceptible to RPE. However, mineral-associated C might also be released through mineral dissolution by certain kinds of root exudates (e.g. citrate, oxalic acid etc.) and contributes to RPE by increasing microbial accessibility to previously-protected C (Chapter 6). The thesis, hence, has improved our understandings of RPE from both biotic and abiotic mechanisms.

Anthropogenic activities have been irreversibly increasing atmospheric CO_2, which could potentially affect soil C and N cycling by influencing both C input and output. This thesis has examined the possible factors that could alter RPE under eCO_2 and the associated mechanisms. Based on the thesis, some future studies are suggested to further the understandings of RPE from the following perspectives. Firstly, plant species and genotypes differ intrinsically in root exudation which is additionally controlled by growth stages and eCO_2. A full investigation of the changes and functions of root exudates by these factors is essential to understand the inner mechanism of RPE and to select proper crop species or to breed possible genotypes with root exudation that could lead to less RPE especially in future higher CO_2 scenario. Secondly, RPE is more like a long-term effect which has only been examined in short-term experiments in controlled environments due to methodological limitation. The combination of Free-air CO_2 Enrichment (FACE) experiments with continuous ^{13}C-labelling technique is promising to quantify RPE in field across the whole growth stage. Moreover, a three-source isotopic tracing is needed to quantitively study the dynamics of plant-, residue- and soil-derived C. Furthermore, except surface SOC, the eCO_2 effect should also be examined for SOC stored in the subsoil and more protected pools. Lastly, N mineralisation is associated with SOC decomposition but relatively less studied, which should be also be given priority in future studies.

Supplementary material

Methods of the meta-analysis in Chapter 7

Using Google scholar and the bibliographies of published works, 38 publications were accessed and targeted through June to July 2018. Search terms were 'elevated CO_2' or 'CO_2 enrichment' or 'FACE' AND 'priming' or 'isotopic' or 'isotope'. The research papers were further screened for reporting of total below-ground CO_2 efflux and its isotopic signature, as a result, those research that estimates soil-derived CO_2-C from respiration partitioning chamber (Kou et al. 2007) and changes in labile soil C pool (Shang et al. 2008) were ruled out. The remaining publications were further scrutinized to exclude research that does not report soil-derived CO_2-C and/or cannot be calculated from related work published by the same author/group. Moreover, publications that report priming effect from laboratory incubation of soils previously treated with aCO_2 and eCO_2 were excluded as we focused on rhizosphere priming effect. At last, the articles that report soil-derived CO_2-C in elevated CO_2 treatment only were also omitted. For example, in some FACE experiments, ^{13}C-depleted CO_2 was supplied to enrich the microclimatic CO_2 concentration, which allows for separation of plant- and soil-derived CO_2-C in the elevated CO_2 treatment only (Pendall et al. 2001). Overall, the references used in the meta-analysis were listed in the description of Fig. 7.1. We collected or calculated treatment means, sample size and standard deviation of each article. Data of RPE and SOC decomposition rate were recalculated and normalised as mg C kg^{-1} soil d^{-1} and g C m^{-2} respectively, for cross-comparison. Sub-treatments (e.g. N level, growth stage etc.) from one publication were considered as independent studies in the meta-analysis to increase statistical power due to the small number of publication. Studies were further excluded if their sample size were less than two. For studies that had multiple measurements across a period of time, the sample size was determined as the number of the multiple measurements. Data were extracted with Digitizer (Rohatgi 2011) if they were presented in plots. The meta-analysis and subgroup analysis (mean effect and 95% confidence interval) were conducted in OpenMEE (Wallace et al. 2016) using the Continuous Random-effects method. The forest plots (Fig. 7.1) were generated using JMP (Trial 14.0.1, SAS Institute Inc., Cary, USA). The positive treatment effect was indicated by the positive standardised mean difference with significant treatment effect being denoted by no overlap of the left 95% confidence internal range with the zero effect vertical dash line.

List of References

Adam NR, Wall GW, Kimball BA, Pinter Jr PJ, LaMorte RL, Hunsaker DJ, Adamsen FJ, Thompson T, Matthias AD, Webber AN, Leavitt SW (2000) Acclimation response of spring wheat in a free-air CO_2 enrichment (FACE) atmosphere with variable soil nitrogen regimes. 1. leaf position and phenology determine acclimation response. Photosynth Res 66:65–77

Ainsworth EA, Long SP (2004) What have we learned from 15 years of free-air CO_2 enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO_2. New Phytol 165:351–372

Ainsworth EA, Rogers A (2007) The response of photosynthesis and stomatal conductance to rising [CO_2]: mechanisms and environmental interactions. Plant, Cell Environ 30:258–270

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

Anderson T-H, Heinemeyer O, Weigel H-J (2011) Changes in the fungal-to-bacterial respiratory ratio and microbial biomass in agriculturally managed soils under free-air CO_2 enrichment (FACE) – A six-year survey of a field study. Soil Biol Biochem 43:895–904

Andersson S, Nilsson SI, Saetre P (2000) Leaching of dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) in mor humus as affected by temperature and pH. Soil Biol Biochem 32:1–10

Andrews JH, Harris RF (1986) r-and K-selection and microbial ecology. In: Marshall KC (ed) Advances in Microbial Ecology. Springer Science and Business Media, New York, USA, pp 99–147

Aye NS, Butterly CR, Sale PWG, Tang C (2016) Residue addition and liming history interactively enhance mineralization of native organic carbon in acid soils. Biol Fertil Soils 53:61–75

Bais HP, Weir TL, Perry LG, Gilroy S, Vivanco JM (2006) The role of root exudates in rhizosphere interactions with plants and other organisms. Annu Rev Plant Biol 57:233–266

Baldock JA, Sanderman J, Macdonald LM, Puccini A, Hawke B, Szarvas S, McGowan J (2013) Quantifying the allocation of soil organic carbon to biologically significant

fractions. Soil Res 51:561–576

Bardgett RD, Mommer L, Vries FTD (2014) Going underground: root traits as drivers of ecosystem processes. Trends Ecol Evol 29:692–699

Batjes NH (1996) Total carbon and nitrogen in the soils of the world. Eur J Soil Sci 47:151–163

Bengtson P, Barker J, Grayston SJ (2012) Evidence of a strong coupling between root exudation, C and N availability, and stimulated SOM decomposition caused by rhizosphere priming effects. Ecol Evol 2:1843–1852

Berntson GM, Bazzaz FA (1997) Nitrogen cycling in microcosms of yellow birch exposed to elevated CO_2: simultaneous positive and negative below-ground feedbacks. Glob Chang Biol 3:247–258

Bhattacharyya P, Roy KS, Neogi S, Manna MC, Adhya TK, Rao KS, Nayak AK (2013) Influence of elevated carbon dioxide and temperature on belowground carbon allocation and enzyme activities in tropical flooded soil planted with rice. Environ Monit Assess 185:8659–8671

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

Billings SA, Ziegler SE (2005) Linking microbial activity and soil organic matter transformations in forest soils under elevated CO_2. Glob Chang Biol 11:203–212

Billings SA, Ziegler SE (2008) Altered patterns of soil carbon substrate usage and heterotrophic respiration in a pine forest with elevated CO_2 and N fertilization. Glob Chang Biol 14:1025–1036

Black CK, Davis SC, Hudiburg TW, Bernacchi CJ, DeLucia EH (2016) Elevated CO_2 and temperature increase soil C losses from a soy-maize ecosystem. Glob Chang Biol 23:435–445

Blagodatskaya E, Blagodatsky S, Dorofnikov M, Kuzyakov Y (2010) Elevated atmospheric CO_2 increases microbial growth rates in soil: results of three CO_2 enrichment experiments. Glob Chang Biol 16:836–848

Blagodatskaya E, Kuzyakov Y (2008) Mechanisms of real and apparent priming effects and their dependence on soil microbial biomass and community structure: critical review. Biol Fertil Soils 45:115–131

Blagodatskaya E, Yuyukina T, Blagodatsky S, Kuzyakov Y (2011) Turnover of soil organic matter and of microbial biomass under C3-C4 vegetation change: Consideration of

^{13}C fractionation and preferential substrate utilization. Soil Biol Biochem 43:159–166

Bowes G (1991) Growth at elevated CO_2: photosynthetic responses mediated through Rubisco. Plant, Cell Environ 14:795–806

Brenzinger K, Kujala K, Horn MA, Moser G, Guillet C, Kammann C, Müller C, Braker G (2017) Soil conditions rather than long-term exposure to elevated CO_2 affect soil microbial communities associated with N-cycling. Front Microbiol 8, Article 1976

Broeckling CD, Broz AK, Bergelson J, Manter DK, Vivanco JM (2008) Root exudates regulate soil fungal community composition and diversity. Appl Environ Microbiol 74:738–744

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

Brzostek ER, Greco A, Drake JE, Finzi AC (2012) Root carbon inputs to the rhizosphere stimulate extracellular enzyme activity and increase nitrogen availability in temperate forest soils. Biogeochemistry 115:65–76

Burton AJ, Pregitzer KS, Crawford JN, Zogg GP, Zak DR (2004) Simulated chronic NO_3^- deposition reduces soil respiration in northern hardwood forests. Glob Chang Biol 10:1080–1091

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

Butterly CR, Armstrong R, Chen D, Tang C (2016a) Free-air CO_2 enrichment (FACE) reduces the inhibitory effect of soil nitrate on N_2 fixation of *Pisum sativum*. Ann Bot 117:177–185

Butterly CR, Baldock JA, Tang C (2013) The contribution of crop residues to changes in soil pH under field conditions. Plant Soil 366: 185–198

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

Butterly CR, Wang X, Armstrong RD, Chen D, Tang C (2016b) Elevated CO_2 induced rhizosphere effects on the decomposition and N recovery from crop residues. Plant Soil 408:55–71

Campbell CD, Sage RF (2002) Interactions between atmospheric CO_2 concentration and phosphorus nutrition on the formation of proteoid roots in white lupin (*Lupinus albus* L). Plant, Cell Environ 25:1051–1059

Cardon ZG (1996) Influence of rhizodeposition under elevated CO_2 on plant nutrition and soil organic matter. Plant Soil 187:277–288

Cardon ZG, Hungate BA, Cambardella CA, Chapin III FS, Field CB, Holland EA, Mooney HA (2001) Contrasting effects of elevated CO_2 on old and new soil carbon pools. Soil Biol Biochem 33:365–373

Carney KM, Hungate BA, Drake BG, Megonigal JP (2007) Altered soil microbial community at elevated CO_2 leads to loss of soil carbon. Proc Natl Acad Sci 104:4990–4995

Chatterjee D, Datta SC, Manjaiah KM (2013) Clay carbon pools and their relationship with short-range order minerals: avenues to mitigate climate change? Curr Sci 105:1404–1410

Cheng L, Booker FL, Tu C, Burkey KO, Zhou L, Shew HD, Rufty TW, Hu S (2012) Arbuscular mycorrhizal fungi increase organic carbon decomposition under elevated CO_2. Science 337:1084–1087

Cheng W (1996) Measurement of rhizosphere respiration and organic matter decomposition using natural ^{13}C. Plant Soil 183:263–268

Cheng W (1999) Rhizosphere feedbacks in elevated CO_2. Tree Physiol 19:313–320

Cheng W (2009) Rhizosphere priming effect: its functional relationships with microbial turnover, evapotranspiration, and C-N budgets. Soil Biol Biochem 41:1795–1801

Cheng W, Johnson DW, Fu S (2003) Rhizosphere effects on decomposition: controls of plant species, phenology, and fertilization. Soil Sci Soc Am J 67:1418–1427

Cheng S-H, Moore BD, Seemann JR (1998) Effects of short-and long-term elevated CO_2 on the expression of ribulose-1, 5–bisphosphate carboxylase/oxygenase genes and carbohydrate accumulation in leaves of *Arabidopsis thaliana* (L.) Heynh. Plant Physiol 116:715–723

Cheng W, Gershenson A (2007) Carbon fluxes in the rhizosphere, in: Cardon ZG; Whitbeck JL (eds), The rhizosphere: An ecological perspective Academic Press, New York, USA, pp 31–56

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

Cheng W, Kuzyakov Y (2005) Root effects on soil organic matter decomposition, in: Zobel

RW, Wright SF (eds), Roots and Soil Management: Interactions between Roots and the Soil. ASA-CSSA-SSSA, Madison, Wisconsin, USA, pp 119–143

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

Cheng W, Sims DA, Luo Y, Johnson DW, Ball JT, Coleman JS (2000) Carbon budgeting in plant-soil mesocosms under elevated CO_2: locally missing carbon? Glob Chang Biol 6:99–109

Chen R, Senbayram M, Blagodatsky S, Myachina O, Dittert K, Lin X, Blagodatskaya E, Kuzyakov Y (2014b) Soil C and N availability determine the priming effect: microbial N mining and stoichiometric decomposition theories. Glob Chang Biol 20:2356–2367

Chen Y, Liu Q, Liu Y, Jia F, He X (2014a) Responses of soil microbial activity to cadmium pollution and elevated CO_2. Sci Reports 4:4287

Chesney M, Gheyssens J, Taschini L (2013) The Issue of Climate Change, in: Chesney M, Gheyssens J, Taschini L (eds), Environmental Finance and Investments. Springer Berlin Heidelberg, Heidelberg, Germany, pp 5–16

Choi I-Y, Nguyen HV-M, Choi JH (2017) Effects of controlled environmental changes on the mineralization of soil organic matter. Environ Eng Res 22:347–355

Ciais P, Sabine C, Bala G, Bopp L, Brovkin V, Canadell J, Chhabra A, DeFries R, Galloway J, Heimann M, Jones C, Le Quéré C, Myneni RB, Piao S, Thornton P (2013) Carbon and Other Biogeochemical Cycles. In: Stocker TF, Qin D, Plattner G-K, Tignor M, Allen SK, Boschung J, Nauels A, Xia Y, Bex V, Midgley PM (eds). Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, pp 465–570

Conyers MK, Poile GJ, Cullis BR (1991) Lime responses by barley as related to available soil aluminum and manganese. Aust J Agric Res 42:379–390

Cotrufo MF, Gorissen A (1997) Elevated CO_2 enhances below-ground C allocation in three perennial grass species at different levels of N availability. New Phytol 137:421–431

Cotrufo MF, Ineson P (1996) Elevated CO_2 reduces field decomposition rates of *Betula pendula* (Roth.) leaf litter. Oecologia 106:525–530

Cruz JL, Alves AAC, LeCain DR, Ellis DD, Morgan JA (2016) Elevated CO_2 concentrations

alleviate the inhibitory effect of drought on physiology and growth of cassava plants. Sci Hortic 210:122–129

Curtis PS (1996) A meta-analysis of leaf gas exchange and nitrogen in trees grown under elevated carbon dioxide. Plant, Cell Environ 19:127–137

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

Daepp M, Nösberger J, Lüscher A (2001) Nitrogen fertilization and developmental stage alter the response of *Lolium perenne* to elevated CO_2. New Phytol 150:347–358

Dalenberg JW, Jager G (1989) Priming effect of some organic additions to ^{14}C-labelled soil. Soil Biol Biochem 21:443–448

Das S, Bhattacharyya P, Adhya TK (2011) Interaction effects of elevated CO_2 and temperature on microbial biomass and enzyme activities in tropical rice soils. Environ Monit Assess 182:555–569

Davidson EA, Janssens IA (2006) Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. Nature 440:165–173

De Graaff M-A, van Groenigen K-J, Six J, Hungate B, van Kessl C (2006b) Interactions between plant growth and soil nutrient cycling under elevated CO_2: a meta-analysis. Glob Chang Biol 12:2077–2091

De Graaff M-A, van Kessel CV, Six J (2009) Rhizodeposition-induced decomposition increases N availability to wild and cultivated wheat genotypes under elevated CO_2. Soil Biol Biochem 41:1094–1103

De Graaff M-A, Six J, Blum H, van Kessel C (2006a) Prolonged elevated atmospheric CO_2 does not affect decomposition of plant material. Soil Biol Biochem 38:187–190

De Graaff M-A, Six J, van Kessel C (2007) Elevated CO_2 increases nitrogen rhizodeposition and microbial immobilization of root-derived nitrogen. New Phytol 173:778–786

De Nobili M, Contin M, Mondini C, Brookes PC (2001) Soil microbial biomass is triggered into activity by trace amounts of substrate. Soil Biol Biochem 33:1163–1170

Dijkstra FA, Bader NE, Johnson DW, Cheng W (2009) Does accelerated soil organic matter decomposition in the presence of plants increase plant N availability? Soil Biol Biochem 41:1080–1087

Dijkstra FA, Blumenthal D, Morgan JA, Pendall E, Carrillo Y, Follett RF (2010) Contrasting effects of elevated CO_2 and warming on nitrogen cycling in a semiarid grassland. New Phytol 187:426–437

Dijkstra FA, Carrillo Y, Pendall E, Morgan JA (2013) Rhizosphere priming: a nutrient

perspective. Front Microbiol 4: article 216

Dijkstra FA, Cheng W (2007a) Interactions between soil and tree roots accelerate long-term soil carbon decomposition. Ecol Lett 10:1046–1053

Dijkstra FA, Cheng W (2007b) Moisture modulates rhizosphere effects on C decomposition in two different soil types. Soil Biol Biochem 39:2264–2274

Dijkstra FA, Cheng W, Johnson DW (2006) Plant biomass influences rhizosphere priming effects on soil organic matter decomposition in two differently managed soils. Soil Biol Biochem 38:2519–2526

Dijkstra FA, Pendall E, Mosier AR, King JY, Milchunas DG, Morgan JA (2008) Long-term enhancement of N availability and plant growth under elevated CO_2 in a semi-arid grassland. Funct Ecol 22:975–982

Dilkes NB, Jones DL, Farrar J (2004) Temporal dynamics of carbon partitioning and rhizodeposition in wheat. Plant Physiol 134:706–715

Dong J, Xu Q, Gruda N, Chu W, Li X, Duan Z (2018) Elevated and super-elevated CO_2 differ in their interactive effects with nitrogen availability on fruit yield and quality of cucumber. J Sci Food Agric 98:4509–4516

Dorodnikov M, Blagodatskaya E, Blagodatsky S, Fangmeier A, Kuzyakov Y (2009) Stimulation of r- vs K-selected microorganisms by elevated atmospheric CO_2 depends on soil aggregate size. FEMS Microbiol Ecol 69:43–52

Drake JE, Darby BA, Giasson M-A, Kramer MA, Phillips RP, Finzi AC (2013) Stoichiometry constrains microbial response to root exudation- insights from a model and a field experiment in a temperate forest. Biogeosciences 10:821–838

Drake JE, Gallet-Budynek A, Hofmockel KS, Bernhardt ES, Billings SA, Jackson RB, Johnsen KS, Lichter J, McCarthy HR, McCormack ML, Moore DJP, Oren R, Palmroth S, Phillips RP, Pippen JS, Pritchard SG, Treseder KK, Schlesinger WH, DeLucia EH, Finzi AC (2011) Increases in the flux of carbon belowground stimulate nitrogen uptake and sustain the long-term enhancement of forest productivity under elevated CO_2. Ecol Lett 14:349–357

Drake JE, Macdonald CA, Tjoelker MG, Crous KY, Gimeno TE, Singh BK, Reich PB, Anderson IC, Ellsworth DS (2015) Short-term carbon cycling responses of a mature eucalypt woodland to gradual stepwise enrichment of atmospheric CO_2 concentration. Glob Chang Biol 22:380–390

Drigo B, Kowalchuk GA, van Veen JA (2008) Climate change goes underground: effects of elevated atmospheric CO_2 on microbial community structure and activities in the

rhizosphere. Biol Fertil Soils 44:667–679

Duiker SW, Rhoton F, Torrent J́, Smeck NE, Lal R (2003) Iron (hydr)oxide crystallinity effects on soil aggregation. Soil Sci Soc Am J 67: 606–611

Elliott HA, Sparks DL (1981) Electrokinetic behavior of a paleudult profile in relation to mineralogical composition. Soil Sci 132:402–409

Ellsworth DS, Reich PB, Naumburg ES, Koch GW, Kubiske ME, Smith SD (2004) Photosynthesis, carboxylation and leaf nitrogen responses of 16 species to elevated pCO_2 across four free-air CO_2 enrichment experiments in forest, grassland and desert. Glob Chang Biol 10:2121–2138

Finley BK, Dijkstra P, Rasmussen C, Schwartz E, Mau RL, Liu XA, Gestel NV, Hungate BA (2018) Soil mineral assemblage and substrate quality effects on microbial priming. Geoderma 322:38–47

Finzi AC, Moore DJP, DeLucia EH, Lichter J, Hofmockel KS, Jackson RB, Kim H-S, Matamala R, McCarthy HR, Oren R, Pippen JS, Schlesinger WH (2006) Progressive nitrogen limitation of ecosystem processes under elevated CO_2 in a warm-temperated forest. Ecology 87:15–25

Fontaine S, Henault C, Aamor a, Bdioui N, Bloor JMG, Maire V, Mary B, Revaillot S, Maron P (2011) Fungi mediate long term sequestration of carbon and nitrogen in soil through their priming effect. Soil Biol Biochem 43:86–96

Fontaine S, Barot S (2005) Size and functional diversity of microbe populations control plant persistence and long-term soil carbon accumulation. Ecol Lett 8:1075–1087

Fontaine S, Mariotti A, Abbadie L (2003) The priming effect of organic matter: a question of microbial competition? Soil Biol Biochem 35:837–843

Freeman C, Kim S-Y, Lee S-H, Kang H (2004) Effects of elevated atmospheric CO_2 concentrations on soil microorganisms. J Microbiol 42:267–277

Fu S, Cheng W, Susfalk R (2002) Rhizosphere respiration varies with plant species and phenology: a greenhouse pot experiment. Plant Soil 239:133–140

Ghildiyal MC, Sharma-Natu P (2000) Photosynthetic acclimation to rising atmospheric carbon dioxide concentration. Indian J Exp Biol 38:961–966

Goufo P, Pereira J, Moutinho-Pereira J, Correia CM, Figueiredo N, Carranca C, Rosa EAS, Trindade H (2014) Rice (*Oryza sativa* L.) phenolic compounds under elevated carbon dioxide (CO_2) concentration. Environ Exp Bot 99:28–37

Griffiths BS, Ritz K, Ebblewhite N, Paterson E, Killham K (1998) Ryegrass rhizosphere microbial community structure under elevated carbon dioxide concentrations, with

observations on wheat rhizosphere. Soil Biol Biochem 30:315–321

Griffiths BS, Spilles A, Bonkowski M (2012) C: N P stoichiometry and nutrient limitation of the soil microbial biomass in a grazed grassland site under experimental P limitation or excess. Ecol Process 1:6

Grosso F, Bååth E, Nicola FD (2016) Bacterial and fungal growth on different plant litter in Mediterranean soils: Effects of C/N ratio and soil pH. Appl Soil Ecol 108:1–7

Grover M, Maheswari M, Desai S, Gopinath KA, Venkateswarlu B (2015) Elevated CO_2: plant associated microorganisms and carbon sequestration. Appl Soil Ecol 95:73–85

Guenet B, Lenhart K, Leloup J, Giusti-Miller S, Pouteau V, Mora P, Nunan N, Abbadie L (2012) The impact of long-term CO_2 enrichment and moisture levels on soil microbial community structure and enzyme activities. Geoderma 170:331–336

Guo J, Zhang W, Zhang M, Zhang L, Bian X (2012) Will elevated CO_2 enhance mineral bioavailability in wetland ecosystems?: Evidence from a rice ecosystem. Plant Soil 355:251–263

Hagedorn F, Maurer S, Bucher JB, Siegwolf RT (2005) Immobilization, stabilization and remobilization of nitrogen in forest soils at elevated CO_2: a ^{15}N and ^{13}C tracer study. Glob Chang Biol 11:1816–1827

Hagedorn F, Maurer S, Egli P, Blaser P, Bucher JB, Siegwolf R (2001) Carbon sequestration in forest soils: effects of soil type, atmospheric CO_2 enrichment, and N deposition. Eur J Soil Sci 52:619–628

Hamer U, Marschner B (2005a) Priming effects in different soil types induced by fructose, alanine, oxalic acid and catechol additions. Soil Biol Biochem 37:445–454

Hamer U, Marschner B (2005b) Priming effects in soils after combined and repeated substrate additions. Geoderma 128:38–51

Hartmann A, Schmid M, van Tuinen D, Berg G (2009) Plant-driven selection of microbes. Plant Soil 321:235–263

Hartwig UA, Sadowsky MJ (2006) Biological nitrogen fixation: a key process for the response of grassland ecosystems to elevated atmospheric [CO_2], in: Nösberger J, Long SP, Norby RJ, Stitt M, Hendrey GR, Blum H (eds), Managed Ecosystems and CO_2 Case Studies, Processes, and Perspectives. Springer Berlin Heidelberg, Germany pp 325–336

Haynes RJ (1990) Active ion uptake and maintenance of cation-anion balance: a critical examination of their role in regulating rhizosphere pH. Plant Soil 126:247–264

Health J, Ayres E, Possell M, Bardgett RD, Black HIJ, Grant H, Ineson P, Kerstiens G (2005)

Rising atmospheric CO_2 reduces sequestration of root-derived soil carbon. Science 309:1711–1713

Hill PW, Jones DL, Marshall C, Farrar JF (2006) Temporal and spatial dynamics of soil solution C and N concentrations during *Lolium perenne* L. sward establishment and the effects of elevated CO_2 and N additions. Soil Biol Biochem 38:1290–1297

Hirschel G, Körner C, Arnone III JA (1997) Will rising atmospheric CO_2 affect leaf litter quality and in situ decomposition rates in native plant communities? Oecologia 110:387–392

Hodge A, Millard P (1998) Effect of elevated CO_2 on carbon partitioning and exudate release from *Plantago lanceolata* seedlings. Physiol Plant 103:280–286

Hodge A, Paterson E, Grayston SJ, Campbell CD, Ord BG, Killham K (1998) Characterisation and microbial utilisation of exudate material from the rhizosphere of *Lolium perenne* grown under CO_2 enrichment. Soil Biol Biochem 30:1033–1043

Hofmockel KS, Zak DR, Moran KK, Jastrow JD (2011) Changes in forest soil organic matter pools after a decade of elevated CO_2 and O_3. Soil Biol Biochem 43:1518–1527

Hoosbeek MR, Lukac M, van Dam D, Godbold DL, Velthorst EJ, Biondi FA, Peressotti A, Cotrufo MF, de Angelis P, Scarascia-Mugnozza G (2004) More new carbon in the mineral soil of a poplar plantation under Free Air Carbon Enrichment (POPFACE): cause of increased priming effect? Glob Biogeochem Cycles 18, GB1040

Hoosbeek MR, Vos JM, Meinders MB, Velthorst EJ, Scarascia-Mugnozza GE (2007) Free atmospheric CO_2 enrichment (FACE) increased respiration and humification in the mineral soil of a poplar plantation. Geoderma 138:204–212

Horvath (1972) Microbial co-metabolism and the degradation of organic compounds in nature. Bacteriol Rev 36:146–155

Hovenden MJ, Newton P, Carran R, Wills K, Theobald P, Vander Schoor J, Williams AL, Osanai Y (2008) Warming prevents the elevated CO_2–induced reduction in available soil nitrogen in a temperate, perennial grassland. Glob Chang Biol 14:1018–1024

Hu S, Chapin FS III, Firestone MK, Field CB, Chiariello NR (2001) Nitrogen limitation of microbial decomposition in a grassland under elevated CO_2. Nature 409:188–191

Hu S, Firestone MK, Chapin III FS (1999) Soil microbial feedbacks to atmospheric CO_2 enrichment. Trends Ecol Evol 14:433–437

Hu S Tu C, Chen X, Gruver JB (2006) Progressive N limitation of plant response to elevated CO_2: a microbiological perspective. Plant Soil 289:47–58

Hungate BA, Dijkstra P, Johnson D, Hinkle CR, Drake B (1999) Elevated CO_2 increases

nitrogen fixation and decreases soil nitrogen mineralization in Florida scrub oak. Glob Chang Biol 5:781–789

Hungate BA, Holland EA, Jackson RB, Chapin III FS, Mooney HA, Field CB (1997a) The fate of carbon in grasslands under carbon dioxide enrichment. Nature 388:576–579

Hungate BA, Lund CP, Pearson HL, Chapin III FS (1997b) Elevated CO_2 and nutrient addition after soil N cycling and N trace gas fluxes with early season wet-up in a California annual grassland. Biogeochemistry 37:89–109

Huo C, Luo Y, Cheng W (2017) Rhizosphere priming effect: A meta-analysis. Soil Biol Biochem 111:78–84

Huyghe C (1997) White lupin (*Lupinus albus* L.). Field Crop Res 53:147–160

Isbell RF, National Committee on Soil and Terrain (2016) The Australian soil classification, second edition. CSIRO Publishing, Clayton South, Melbourne, Australia

Jackson RB, Cook CW, Pippen JS, Palmer SM (2009) Increased belowground biomass and soil CO_2 fluxes after a decade of carbon dioxide enrichment in a warm-temperate forest. Ecology 90:3352–3366

Janus LR, Angeloni NL, McCormack J, Rier ST, Tuchman NC, Kelly JJ (2005) Elevated atmospheric CO_2 alters soil microbial communities associated with trembling aspen (*Populus tremuloides*) roots. Micro Ecol 50:102–109

Jin J, Armstrong R, Tang C (2017) Long-term impact of elevated CO_2 on phosphorus fractions varies in three contrasting cropping soils. Plant Soil 419:257–267

Jin J, Tang C, Armstrong R, Sale P (2012) Phosphorus supply enhances the response of legumes to elevated CO_2 (FACE) in a phosphorus-deficient Vertisol. Plant Soil 358:91–104

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

Jin J, Tang C, Sale P (2015) The impact of elevated carbon dioxide on the phosphorus nutrition of plants: a review. Ann Bot 116:987–999

Jones DL, Darah PR, Kochian LV (1996) Critical evaluation of organic acid mediated iron dissolution in the rhizosphere and its potential role in root iron uptake. Plant Soil 180:57–66

Jones DL, Hodge A, Kuzyakov Y (2004) Plant and mycorrhizal regulation of rhizodeposition. New Phytol 163:459–480

Jones DL, Nguyen C, Finlay RD (2009) Carbon flow in the rhizosphere: carbon trading at

the soil-root interface. Plant Soil 321:5–33

Jones TH (1998) Impacts of rising atmospheric carbon dioxide on model terrestrial ecosystems. Science 280:441–443

Joos F, Leuenberger M, Chappellaz J, Brovkin V, Köhler P, Stocker TF, Fischer H, Schmitt J, Elsig J, Schneider R, Eggleston S (2015) Ice core-based isotopic constraints on past carbon cycle changes. PAGES Magazine 23:12–13

Kamh M, Horst WJ, Amer F, Mostafa H (1998) Exudation of organic anions by white lupin (*Lupinus albus* L.) and their role in phosphate mobilization from soil. In: Merbach W (ed) Pflanzenernährung, Wurzelleistung und Exsudation. Vieweg+Teubner Verlag, Wiesbaden, Germany. pp 238–245

Keiluweit M, Bougoure JJ, Nico PS, Pett-Ridge J, Weber PK, Kleber M (2015) Mineral protection of soil carbon counteracted by root exudates. Nat Clim Chang 5:588–595

Kelly JJ, Peterson E, Winkelman J, Walter TJ, Rier ST, Tuchman NC (2012) Elevated atmospheric CO_2 impacts abundance and diversity of nitrogen cycling functional genes in soil. Microb Ecol 65:394–404

Kemmitt SJ, Wright D, Goulding KWT, Jones DL (2006) pH regulation of carbon and nitrogen dynamics in two agricultural soils. Soil Biol Biochem 38:898–911

Kimball BA (2016) Crop responses to elevated CO_2 and interactions with H_2O, N, and temperature. Curr Opin Plant Biol 31:36–43

Kleber M, Eusterhues K, Keiluweit M, Mikutta C, Nico PS (2015) Mineral-organic associations: formation, properties, and relevance in soil environments. Adv Agron 130:1–140

Knnops JMH, Naeem S, Reich PB (2007) The impact of elevated CO_2 increased nitrogen availability and biodiversity on plant tissue quality and decomposition. Glob Chang Biol 13:1960–1971

Knorr M, Frey SD, Curtis PS (2005) Nitrogen additions and litter decomposition: a meta-analysis. Ecology 86:3252–3257

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

Kuikman PJ, Lekkerkerk LJA, van Veen JA (1990) Carbon dynamics of a soil planted with wheat under an elevated atmospheric CO_2 concentration. In: Wilson WS, Gray Timothy RG (eds) Advances in soil organic matter research: the impact on agriculture and the environment. The Royal Society of Chemistry, Special Publication,

Cambridge, UK, pp 267–274

Kuzyakov Y (2002) Review: Factors affecting rhizosphere priming effects. J Plant Nutr Soil Sci 165:382–396

Kuzyakov Y (2010) Priming effects: Interactions between living and dead organic matter. Soil Biol Biochem 42:1363–1371

Kuzyakov Y, Cheng W (2001) Photosynthesis controls of rhizosphere respiration and organic matter decomposition. Soil Biol Biochem 33:1915–1925

Kuzyakov Y, Friedel JK, Stahr K (2000) Review of mechanisms and quantification of priming effects. Soil Biol Biochem 32:1485–1498

Kuzyakov Y, Hill PW, Jones DL (2007) Root exudate components change litter decomposition in a simulated rhizosphere depending on temperature. Plant Soil 290:293–305

Kuzyakov Y, Xu X (2013) Competition between roots and microorganisms for nitrogen, mechanisms and ecological relevance. New Phytol 198:656–669

Lagomarsino A, Moscatelli MC, Angelis PD, Grego S (2006) Labile substrates quality as the main driving force of microbial mineralization activity in a poplar plantation soil under elevated CO_2 and nitrogen fertilization. Sci Total Environ 372:256–265

Lagomarsino A, Moscatelli MC, Hoosbeek MR, De Angelis P, Grego S (2008) Assessment of soil nitrogen and phosphorous availability under elevated CO_2 and N-fertilization in a short rotation poplar plantation. Plant Soil 308:131–147

Lal R (2004a) Soil carbon sequestration to mitigate climate change. Geoderma 123:1–22

Lal R (2004b) Soil carbon sequestration impacts on global climate change and food security. Science 304:1623–1627

Lal R (2008) Crop residues as soil amendments and feedstock for bioethanol production. Waste Manag 28:747–758

Lal R (2009) Sequestering atmospheric carbon dioxide. Crit Rev Plant Sci 28:90–96

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

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

Lam SK, Norton R, Armstrong R, Chen D (2014) Increased microbial activity under elevated [CO_2] does not enhance residue decomposition in a semi-arid cropping system in

Australia Soil. Biol Biochem 72:97–99

Langley JA, McKinley DC, Wolf AA, Hungate BA, Drake BG, Megonigal JP (2009) Priming depletes soil carbon and releases nitrogen in a scrub-oak ecosystem exposed to elevated CO_2. Soil Biol Biochem 41:54–60

Larson JL, Zak DR, Sinsabaugh RL (2002) Extracellular enzyme activity beneath temperate trees growing under elevated carbon dioxide and ozone. Soil Sci Soc Am J 66:1848–1856

Lee S-H, Kang H (2016) Elevated CO_2 causes a change in microbial communities of rhizosphere and bulk soil of salt marsh system. Appl Soil Ecol 108:307–314

Lekkerkerk LJA, Van De Geijn SC, van Veen JA (1990) Effects of elevated atmospheric CO_2 levels on the carbon economy of a soil planted with wheat, in: Bouwman AF (Ed) Soils and the greenhouse effect. John Wiley & Sons, New York, USA, pp 423–429

Li J-H, Dijkstra P, Hinkle CR, Wheeler RM, Drake BG (1999) Photosynthetic acclimation to elevated atmospheric CO_2 concentration in the Florida scrub-oak species *Quercus geminata* and *Quercus myrtifolia* growing in their native environment. Tree Physiol 19:229–234

Li W, Hartmann H, Adams HD, Zhang H, Jin C, Zhao C, Guan D, Wang A, Yuan F, Wu J (2018) The sweet side of global change-dynamic responses of non-structural carbohydrates to drought, elevated CO_2 and nitrogen fertilization in tree species. Tree Physiol 38:1706–1723

Li Q, Tian Y, Zhang X, Xu X, Wang H, Kuzyakov Y (2017) Labile carbon and nitrogen additions affect soil organic matter decomposition more strongly than temperature. Appl Soil Ecol 114:152–160

Liljeroth E, Kuikman P, Van Veen J (1994) Carbon translocation to the rhizosphere of maize and wheat and influence on the turnover of native soil organic matter at different soil nitrogen levels. Plant Soil 161:233–240

Lin G, Ehleringer JR, Rygiewicz PT, Johnson MG, Tingey DT (1999) Elevated CO_2 and temperature impacts on different components of soil CO_2 efflux in Douglas-fir terracosms. Glob Chang Biol 5:157–168

Lin G, Rygiewicz PT, Ehleringer JR, Johnson MG, Tingey DT (2001) Time-dependent responses of soil CO_2 efflux components to elevated atmospheric [CO_2] and temperature in experimental forest mesocosms. Plant Soil 229:259–270

Liu X-JA, Sun J, Mau RL, Finley BK, Compson ZG, van Gestel N, Brown JR, Schwartz E,

Dijkstra P, Hungate BA (2017b) Labile carbon input determines the direction and magnitude of the priming effect. Appl Soil Ecol 109:7–13

Liu Y, Zhang H, Xiong M, Li F, Li L, Wang G, Pan G (2017a) Abundance and composition response of wheat field soil bacterial and fungal communities to elevated CO_2 and increased air temperature. Biol Fertil Soils 53:3–8

Lloyd DA, Ritz K, Paterson E, Kirk GJD (2016) Effects of soil type and composition of root exudates on rhizosphere priming phenomena. Soil Biol Biochem 103:512–521

Loiseau P, Soussana JF (1999) Elevated [CO_2], temperature increase and N supply effects on the turnover of below-ground carbon in a temperate grassland ecosystem. Plant Soil 210:233–247

Luo Y, Su B, Currie WS, Dukes JS, Finzi A, Hartwig U, Hungate B, McMurtrie RE, Oren R, Parton WJ, Pataki DE, Shaw MR, Zak DR, Field CB (2004) Progressive nitrogen limitation of ecosystem responses to rising atmospheric carbon dioxide. BioScience 54:731–739

Luo Y, Hui D, Zhang D (2006) Elevated CO_2 stimulates net accumulations of carbon and nitrogen in land ecosystems: a meta-analysis. Ecology 87:53–63

Luo Z, Wang E, Sun OJ (2016) A meta-analysis of the temporal dynamics of priming soil carbon decomposition by fresh carbon inputs across ecosystems. Soil Biol Biochem 101:96–103

Lupwayi NZ, Clayton GW, O'Donovan JT, Harker KN, Turkington TK, Rice WA (2004) Decomposition of crop residues under conventional and zero tillage. Can J of Soil Sci 84:403–410

Lutze JL, Gifford RM (1998) Carbon accumulation, distribution and water use of *Danthonia richardsoni*i swards in response to CO_2 and nitrogen supply over four years of growth. Glob Chang Biol 4:851–861

Madhu M, Hatfield JL (2013) Dynamics of plant root growth under increased atmospheric carbon dioxide. Agron J 105:657–669

Makino A, Mae T (1999) Photosynthesis and plant growth at elevated levels of CO_2. Plant Cell Physiol 40:999–1006

Marschner M, Yang C, Lieberei R, Crowley DE (2001) Soil and plant specific effects on bacterial community composition in the rhizosphere. Soil Biol Biochem 33:1437–1445

Marx M, Buegger F, Gattinger A, Zsolnay Á, Munch JC (2007) Determination of the fate of ^{13}C labelled maize and wheat exudates in an agricultural soil during a short-term

incubation. Eur J Soil Sci 58:1175–1185

Mason-Jones K, Kuzyakov Y (2017) "Non-metabolizable" glucose analogue shines new light on priming mechanisms: Triggering of microbial metabolism. Soil Biol Biochem 107:68–7

Mau RL, Liu CM, Aziz M, Schwartz E, Dijkstra P, Marks JC, Price LB, Keim P, Hungate BA (2015) Linking soil bacterial biodiversity and soil carbon stability. ISME 9:1477–1480

McKinley DC, Romero JC, Hungate BA, Drake BG, Megonigal JP (2009) Does deep soil N availability sustain long-term ecosystem responses to elevated CO_2? Glob Chang Biol 15:2035–2048

Medlyn B, Rey A, Barton C, Forstreuter M (2001) Above-ground growth response of forest trees to elevated atmospheric CO_2 concentrations, in: The Impact of Carbon Dioxide and Other Greenhouse Gases on Forest Ecosystems Report no3 of the IUFRO Task Force on Environmental Change. CABI publishing and IUFRO, New Tork, USA, pp 127–146

Meier IC, Pritchard SG, Brzostek ER, McCormack ML, Phillips RP (2014) The rhizosphere and hyphosphere differ in their impacts on carbon and nitrogen cycling in forests exposed to elevated CO_2. New Phytol 205:1164–1174

Meinshausen M, Meinshausen N, Hare W, Raper S C, Frieler K, Knutti R, Frame D J, Allen M R (2009) Greenhouse-gas emission targets for limiting global warming to 2°. Nature 458:1158–1162

Meinshausen M, Smith SJ, Calvin K, Daniel JS, Kainuma MLT, Lamarque J, Matsumoto K, Montzka SA, Raper SCB, Riahi K (2011) The RCP greenhouse gas concentrations and their extensions from 1765 to 2300. Clim Chang 109:213–241

Menezes-Blackburn D, Paredes C, Zhang H, Giles CD, Darch T, Stutter M, George TS, Shand C, Lumsdon D, Cooper P, Wendler R, Brown L, Blackwell M, Wearing C, Haygarth PM (2016) Organic acids regulation of chemical-microbial phosphorus transformations in soils. Environ Sci Technol 50:11521–11531

Mikan CJ, Zak DR, Kubiske ME, Pregitzer KS (2000) Combined effects of atmospheric CO_2 and N availability on the belowground carbon and nitrogen dynamics of aspen mesocosms. Oecologia 124:432–445

Mitchell EAD, Gilbert D, Buttler A, Amblard C, Grosvernier P, Gobat J-M (2003) Structure of microbial communities in Sphagnum peatlands and effect of atmospheric carbon dioxide enrichment. Microb Ecol 46:187–199

Mollah M, Norton R, Huzzey J (2009) Australian grains free-air carbon dioxide enrichment (AGFACE) facility: design and performance. Crop Pasture Sci 60:697–707

Mondini C, Cayuela ML, Sanchez-Monedero MA, Roig A, Brookes PC (2006) Soil microbial biomass activation by trace amounts of readily available substrate. Biol Fert Soils 42:542–549

Morrissey EM, Mau RL, Schwartz E, McHugh TA, Dijkstra P, Koch BJ, Marks JC, Hungate BA (2017) Bacterial carbon use plasticity, phylogenetic diversity and the priming of soil organic matter. ISME 11:1890–1899

Nannipieri P, Ascher J, ceccherini M, Landi L, Pietramellara G, Renella G, Valori F (2008) Effects of root exudates in microbial diversity and activity in rhizosphere soils, in: Nautiyal CS, Dion P (eds), Molecular Mechanisms of Plant and Microbe Coexistence. Springer Berlin Heidelberg, Heidelberg, Germany, pp 339–365

Naveed M, Brown LK, Raffan AC, George TS, Bengough AG, Roose T, Sinclair I, Koebernick N, Cooper L, Hackett CA (2017) Plant exudates may stabilize or weaken soil depending on species, origin and time. Eur J Soil Sci 68:806–816

Neher DA, Weicht TR, Moorhead DL, Sinsabaugh RL (2004) Elevated CO_2 alters functional attributes of nematode communities in forest soils. Funct Ecol 18:584–591

Nguyen C (2003) Rhizodeposition of organic C by plant: mechanisms and controls. Agronomie 23:375–396

Nie M, Lu M, Bell J, Raut S, Pendall E (2013a) Altered root traits due to elevated CO_2: a meta-analysis. Glob Ecol Biogeogr 22:1095–1105

Nie M, Bell C, Wallenstein MD, Pendall E (2015) Increased plant productivity and decreased microbial respiratory C loss by plant growth-promoting rhizobacteria under elevated CO_2. Sci Reports 5:1–6

Nie M, Pendall E (2016) Do rhizosphere priming effects enhance plant nitrogen uptake under elevated CO_2? Agric Ecosyst Environ 224:50–55

Nie M, Pendall E, Bell C, Gasch CK, Raut S, Tamang S, Wallenstein MD (2013b) Positive climate feedbacks of soil microbial communities in a semiarid grassland. Ecol Lett 16:234–241

Niklaus PA, Alphei J, Ebersberger D, Ksmpichler C, Kandeler E, Tscherko D (2003) Six years of in situ CO_2 enrichment evoke changes in soil structure and soil biota of nutrient-poor grassland. Glob Chang Biol 9:585–600

Norby RJ (1994) Issues and perspectives for investigating root responses to elevated atmospheric carbon dioxide. Plant Soil 165:9–20

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

Norby RJ, Ledford J, Reilly CD, Miller NE, O'Neill EG (2004) Fine-root production dominates response of a deciduous forest to atmospheric CO_2 enrichment. Proc Natl Acad Sci United States Am 101:9689–9693

Norby RJ, Luo Y (2004) Evaluating ecosystem responses to rising atmospheric CO_2 and global warming in a multi-factor world. New Phytol 162:281–293

Norby RJ, O'neill EG, Hood WG, Luxmoore RJ (1987) Carbon allocation, root exudation and mycorrhizal colonization of Pinus echinata seedlings grown under CO_2 enrichment. Tree Physiol 3:203–210

Norby RJ, O'Neill EG, Luxmoore RJ (1986) Effects of atmospheric CO_2 enrichment on the growth and mineral nutrition of *Quercus alba* seedlings in nutrient-poor soil. Plant Physiol 82:83–89

Norby RJ, Warren JM, Iversen CM, Medlyn BE, McMurtrie RE (2010) CO_2 enhancement of forest productivity constrained by limited nitrogen availability. Proc Natl Acad Sci 107:19368–19373

Nuruzzaman M, Lambers H, Bolland MD, Veneklaas EJ (2006) Distribution of carboxylates and acid phosphatase and depletion of different phosphorus fractions in the rhizosphere of a cereal and three grain legumes. Plant Soil 281:109–120

Olsson P, Linder S, Giesler R, Högberg P (2005) Fertilization of boreal forest reduces both autotrophic and heterotrophic soil respiration. Glob Chang Biol 11:1745–1753

Pal M, Rao LS, Jain V, Srivastava AC, Pandey R, Raj A, Singh KP (2005) Effects of elevated CO_2 and nitrogen on wheat growth and photosynthesis. Biol Plant 49:467–470

Panikov NS, Sizova MV (1996) A kinetic method for estimating the biomass of microbial functional groups in soil. J Microbiol Methods 24:219–230

Parfitt RL, Scott NA, Ross DJ, Salt GJ, Tate KR (2003) Land-use change effects on soil C and N transformations in soils of high N status: comparisons under indigenous forest, pasture and pine plantation. Biogeochemistry 66:203–221

Partey ST, Preziosi RF, Robson GD (2013) Maize residue interaction with high quality organic materials: effects on decomposition and nutrient release dynamics. Agric Res 2:58–67

Paterson E, Hall JM, Rattray EAS, Griffiths BS, Ritz K, Killham K (1997) Effect of elevated CO_2 on rhizosphere carbon flow and soil microbial processes. Glob Chang Biol 3:363–377

Paterson E, Hodge A, Thornton B, Millard P, Killham K (1999) Carbon partitioning and rhizosphere C-flow in Lolium perenne as affected by CO_2 concentration, irradiance and below-ground conditions. Glob Chang Biol 5:669–678

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

Pausch J, Kuzyakov Y (2018) Carbon input by roots into the soil: Quantification of rhizodeposition from root to ecosystem scale. Glob Chang Biol 24:1–12

Paustian K, Andrén O, Janzen HH, Lal R, Smith P, Tian G, Tiessen H, Van Noordwijk M, Woomer, PL (1997) Agricultural soils as a sink to mitigate CO_2 emissions. Soil Use Manag 13:230–244

Pearse SJ, Veneklaas EJ, Cawthray GR, Bolland MDA, Lambers H (2006) Carboxylate release of wheat, canola and 11 grain legume species as affected by phosphorus status. Plant Soil 288:127–139

Pendall E, Del Grosso S, King JY, LeCain DR, Milchunas DG, Morgan JA, Mosier AR, Ojima DS, Parton WA, Tans PP (2003) Elevated atmospheric CO_2 effects and soil water feedbacks on soil respiration components in a Colorado grassland. Glob Biogeochem Cycles 17:1046

Pendall E, Leavitt SW, Brooks T, Kimball BA, Pinter PJ, Wall GW, LaMorte RL, Wechsung G, Wechsung F, Adamsen F (2001) Elevated CO_2 stimulates soil respiration in a FACE wheat field. Basic Appl Ecol 2:193–201

Pendall E, Mosier AR, Morgan JA (2004) Rhizodeposition stimulated by elevated CO_2 in a semiarid grassland. New Phytol 162:447–458

Peñuelas J, Estiarte M, Kimball BA, Idso SB, Pinter PJ, Wall GM, Garcia RL, Hansaker DJ, LaMorte RL, Hensrik DL (1996) Variety of responses of plant phenolic concentration to CO_2 enrichment. J Exp Bot 47:1463–1467

Peñuelas J, Estiarte M, Llusia J (1997) Carbon-based secondary compounds at elevated CO_2. Photosynthetica 33:313–319

Phillips RP (2007) Towards a rhizo-centric view of plant-microbial feedbacks under elevated atmospheric CO_2. New Phytol 173:664–667

Phillips RP, Bernhardt ES (2008) Rhizosphere priming effects on soil N availability in forests exposed to elevated atmospheric CO_2. AGU Fall Meet Abstr 8

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

Phillips DA, Fox TC, Six J (2006) Root exudation (net efflux of amino acids) may increase rhizodeposition under elevated CO_2. Glob Chang Biol 12:561–567

Phillips RP, Meier IC, Bernhardt ES, Grandy AS, Wickings K, Finzi AC (2012) Roots and fungi accelerate carbon and nitrogen cycling in forests exposed to elevated CO_2. Ecol Lett 15:1042–1049

Pianka ER (1970) On r- and K-selection. Am Nat 104:592–597

Poorter H, van Berkel Y, Baxter R, den Hertog J, Dijkstra P, Gifford RM, Griffin KL, Roumet C, Roy J, Wong SC (1997) The effect of elevated CO_2 on the chemical composition and construction costs of leaves of 27 C3 species. Plant, Cell Environ 20:472–482

Pregitzer KS, Zak DR, Loya WM, Karberg NJ, King JS, Burton AJ (2007) The contribution of root-rhizosphere interactions to biogeochemical cycles in a changing world. In: Cardon, ZG, Whitbeck JL (eds) The rhizosphere: An ecological perspective. Elsevier Academic Press, Burlington, pp 155–174

Pregitzer KS, Zak DR, Maziasz J, DeForest J, Curtis PS, Lussenhop J (2000) Interactive effects of atmospheric CO_2 and soil-N availability on fine roots of *Populus tremuloides.* Ecol Appl 10:18–33

Pritchard S, Rogers H, Prior SA, Peterson C (1999) Elevated CO_2 and plant structure: a review. Glob Chang Biol 5:807–837

Procter AC, Gill RA, Fay PA, Polley HW, Jackson RB (2015) Soil carbon responses to past and future CO_2 in three Texas prairie soils. Soil Biol Biochem 83:66–75

Qiao N, Xu X, Hu Y, Blagodatskaya E, Liu Y, Schaefer D, Kuzyakov Y (2015) Carbon and nitrogen additions induce distinct priming effects along an organic-matter decay continuum. Sci Reports 6:Article 19865

Rakshit R, Patra AK, Pal D, –Kumar M, Singh R (2012) Effect of elevated CO_2 and temperature on nitrogen dynamics and microbial activity during Wheat (*Triticum aestivum* L) growth on a subtropical Inceptisol. India J Agron Crop Sci 198:452–465

Rasche F, Kramer S, Enowashu E, Mackie K, Högy P, Marhan S (2017) Contrasting effect of elevated atmospheric CO_2 on the C/N ratio of faba bean and spring wheat residues exert only minor changes in the abundance and enzyme activities of soil proteolytic bacteria. Pedobiologia 62:9–15

Rasmussen C, Southard RJ, Horwath WR (2006) Mineral control of organic carbon mineralization in a range of temperate conifer forest soils. Glob Chang Biol 12:834–847

Rasmussen C, Southard RJ, Horwath WR (2007) Soil mineralogy affects conifer forest soil carbon source utilization and microbial priming. Soil Sci Soc Am J 71:1141–1150

Razanamalala K, Fanomezana RA, Razafimbelo T, Chevallier T, Trap J, Blanchart E, Bernard L (2018) The priming effect generated by stoichiometric decomposition and nutrient mining in cultivated tropical soils: Actors and drivers. Appl Soil Ecol 126:21–33

Reich PB, Hobbie SE, Lee TD, Pastore MA (2018) Unexpected reversal of C3 versus C4 grass response to elevated CO_2 during a 20–year field experiment. Science 360:317–320

Reis CES dos, Dick DP, Caldas J da S, Bdgettr C (2014) Carbon sequestration in clay and silt fractions of Brazilian soils under conventional and no-tillage systems. Sci Agric 71:292–301

Rodriguez JH, Klumpp A, Högy P, Fangmeier A, Maestri DM, Lamarque A, Labuckas D, Pignata ML (2015) Physiological response at different plant development stages in *Glycine max* exposed to elevated CO_2 concentrations and fly ash-amended soils. Agric Res 4:160–170

Rogers HH, Prior SA, Runion GB, Mitchell RJ (1996) Root to shoot ratio of crops as influenced by CO_2. Plant Soil 187:229–248

Rohatgi, Ankit. WebPlotDigitizer. Available online: http://arohatgi.info/WebPlotDigitizer/ (accessed July 2018)

Rousk J, Brookes PC, Bååth E (2009) Contrasting soil pH effects on fungal and bacterial growth suggest functional redundancy in carbon mineralization. Appl Environ Microbiol 75:1589–1596

Rousk J, Bååth E, Brookes PC, Lauber CL, Lozupone C, Caporaso JG, Knight R, Fierer N (2010) Soil bacterial and fungal communities across a pH gradient in an arable soil. ISME J 4:1340–1351

Rønn R, Ekelund F, Christensen S (2003) Effects of elevated atmospheric CO_2 on protozoan abundance in soil planted with wheat and on decomposition of wheat roots. Plant Soil 251:13–21

Ruiz-Dueñas FJ, Martínez ÁT (2009) Microbial degradation of lignin: how a bulky recalcitrant polymer is efficiently recycled in nature and how we can take advantage

of this. Microb Biotechnol 2:164–177

Ryan PR, James RA, Weligama C, Delhaize E, Rattey A, Lewis DC, Bovill WD, McDonald G, Rathjen TM, Wang E, Fettell NA, Richardson AE (2014) Can citrate efflux from roots improve phosphorus uptake by plants? Testing the hypothesis with near-isogenic lines of wheat. Physiol Plant 151:230–242

Ryan PR, Raman H, Gupta S, Horst WJ, Delhaize E (2009) A second mechanism for aluminum resistance in wheat relies on the constitutive efflux of citrate from roots. Plant Physiol 149:340–351

Saar S, Semchenko M, Barel JM, Deyn GBD (2016) Legume presence reduces the decomposition rate of non-legume roots. Soil Biol Biochem 94:88–93

Saiya-Cork KR, Sinsabaugh RL, Zak DR (2002) The effects of long term nitrogen deposition on extracellular enzyme activity in an *Acer saccharum* forest soil. Soil Biol Biochem 34:1309–1315

Sanyal SK, De Datta SK (1991) Chemistry of phosphorus transformations in soil. In: Stewart BA (ed) Advances in Soil Science. Springer, New York, USA, pp 1–120

Scharlemann JP, Tanner EV, Hiederer R, Kapos V (2014) Global soil carbon: understanding and managing the largest terrestrial carbon pool. Carbon Manag 5:81–91

Schmidt MW, Torn MS, Abiven S, Dittmar T, Guggenberger G, Janssens IA, Kleber M, Kögel-Knabner I, Lehmann J, Manning DA (2011) Persistence of soil organic matter as an ecosystem property. Nature 478:49–56

Schmitt J, Schneider R, Elsig J, Leuenberger D, Lourantou A, Chappellaz J, Köhler P, Joos F, Stocker TF, Leuenberger M (2012) Carbon isotope constraints on the deglacial CO_2 rise from ice cores. Science 336:711–714

Shahzad T, Chenu C, Genet P, Barot S, Perveen N, Mougin C, Fontaine S (2015) Contibution of exudates, arbuscular mycorrizal fungi and litter depositions to the rhizosphere priming effect induced by grassland species. Soil Biol Biochem 80:146–155

Shang W, Shu W, Li Y, Marcel RH (2008) Influence of FACE experiment on soil organic matter pools and priming effect. Ecol Environ Ecol Environ 17:1518–1522

Shanmuganathan RT and Oades JM (1983) Modification of soil physical properties by addition of calcium compounds. Aust J Soil Res 21: 285, 300

Shi S (2009) Influence of root exudates on soil microbial diversity and activity. PhD thesis, Lincoln University

Six J, Conant RT, Paul EA, Paustian K (2002) Stabilization mechanisms of soil organic matter: implications for C-saturation of soils. Plant Soil 241:155–176

Smith P, Davies CA, Ogle S, Zanchi G, Bellarby J, Bird N, Boddey RM, McNamara NP, Powlson D, Cowie A, Noordwijk M, Davis SC, Richter DD, Kryzanowski L, Wijk MT, Stuart J, Kirton A, Eggar D, Newton-Cross G, Adhya TK, Braimoh AK (2012) Towards an integrated global framework to assess the impacts of land use and management change on soil carbon: current capability and future vision. Glob Change Biol 18:2089–2101

Søe ARB, Giesemann A, Anderson T-H, Weigel H-J, Buchmann N (2004) Soil respiration under elevated CO_2 and its partitioning into recently assimilated and older carbon sources. Plant Soil 262:85–94

Stocker TF, Qin D, Plattner G-K, Tignor M, Allen SK, Boschung J (2013) Intergovernmental Panel on Climate Change (IPCC) Summary for policymakers: Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change, in: Climate Change 2013: The Physical Science Basis. Cambridge University Press, Cambridge & New York, USA

Sugiyama A, Yazaki K (2012) Root exudates of legume plants and their involvement in interactions with soil microbes. In: Vivanco JM, Baluška F (eds) Secretions and exudates biological systems, signaling and communication in plants. Springer, Heidelberg, pp 27–48

Sulman BN, Phillips RP, Oishi AC, Shevliakova E, Pacala SW (2014) Microbe-driven turnover offsets mineral-mediated storage of soil carbon under elevated CO_2. Nat Clim Change 4:1099–1102

Sun J, Dai W, Peng B, Liu J, He T, Jiang P, Han S, Bai E (2018) Does the accelerated soil N cycling sustain N demand of *Quercus mongolica* after decade-long elevated CO_2 treatment? Biogeochemistry 139:197–213

Suter D, Frehner M, Fischer BU, Nösberger J, Lüscher A (2002) Elevated CO_2 increases carbon allocation to the roots of *Lolium perenne* under free-air CO_2 enrichment but not in a controlled environment. New Phytol 154:65–75

Tabatabai MA (1994) Soil enzymes. In: Weaver RW, Angel JS, Bottomley PS (eds) Methods of soil analysis, part 2. Microbiological and biochemical properties. Soil Sci Soc Am Book Series no. 5, SSSA, Madison, USA, pp 775–833

Tang C, Unkovich MJ, Bowden JW (1999) Factors affecting soil acidification under legumes. III. Acid production by N_2–fixing legumes as influenced by nitrate supply. New Phytol 143:513–521

Tarnawski S, Aragno M (2006) The influence of elevated [CO_2] on diversity, activity and

biogeochemical functions of rhizosphere and soil bacterial communities, in: Managed Ecosystems and CO_2 Case Studies, Processes, and Perspectives. Springer-Verlag Berlin Heidelberg, Germany, pp 393–412

Tobita H, Yazaki K, Harayama H, Kitao M (2016) Responses of symbiotic N_2 fixation in Alnus species to the projected elevated CO_2 environment. Trees 30:523–537

Tovkach A, Ryan PR, Richardson AE, Lewis DC, Rathjen TM, Ramesh S, Tyerman SD, Delhaize E (2012) Transposon-mediated alteration of *TaMATE1B* expression in wheat confers constitutive citrate efflux from root apices. Plant Physiol 161:880–892

Trueman RJ, Gonzalez-Meler MA (2005) Accelerated belowground C cycling in a managed agriforest ecosystem exposed to elevated carbon dioxide concentrations. Glob Chang Biol 11:1258–1271

Uddin S, Löw M, Parvin S, Fitzgerald GJ, Tausz-Posch S, Armstrong R, O'Leary G, Tausz M (2018) Elevated [CO_2] mitigates the effect of surface drought by stimulating root growth to access sub-soil water. Plos One 13:101371/journalpone0198928

Uselman S, Qualls R, Thomas R (2000) Effects of increased atmospheric CO_2, temperature and soil N availability on root exudation of dissolved organic carbon by a N-fixing tree (*Robinia pseudoacacia* L). Plant Soil 222:191–202

Van Hees PA, Jones DL, Finlay R, Godbold DL, Lundström US (2005) The carbon we do not see-the impact of low molecular weight compounds on carbon dynamics and respiration in forest soils: a review. Soil Biol Biochem 37:1–13

Van Kessel C, Boots B, de Graaff M-A, Harris D, Blum H, Six J (2006) Total soil C and N sequestration in a grassland following 10 years of free air CO_2 enrichment. Glob Chang Biol 12:2187–2199

Van Kessel C, Nitschelm J, Horwath WR, Harris D, Walley F, Lüscher A, Hartwig U (2000) Carbon-13 input and turn-over in a pasture soil exposed to long-term elevated atmospheric CO_2. Glob Chang Biol 6:123–135

Van Ginkel JH, Gorissen A, van Veen JA (1996) Long-term decomposition of grass roots as affected by elevated atmospheric carbon dioxide. J Environ Qual 25:1122–1128

Van Ginkel JH, Gorissen A, van Veen JA (1997) Carbon and nitrogen allocation in *Lolium perenne* in response to elevated atmospheric CO_2 with emphasis on soil carbon dynamics. Plant Soil 188:299–308

Van Groenigen K, Harris D, Horwath WR, Hartwig UA, Van Kessel C (2002) Linking sequestration of ^{13}C and ^{15}N in aggregates in a pasture soil following 8 years of elevated atmospheric CO_2. Glob Chang Biol 8:1094–1108

Van Groenigen K-J, de Graaff M-A, Six J, Harris D, Kuikman P, van Kessel C (2006) The impact of elevated atmospheric [CO_2] on soil C and N dynamics: A meta-analysis. In: Nösberger J, Long S, Norby R, Stitt M, Hendrey G, Blum H (eds) Management Ecosystems and CO_2 Case Studies, Processes, Perspectives. Springer Berlin Heidelberg, Heidelberg, Germany, pp 373–391

Van Groenigen K-J, Gorissen A, Six J, Harris D, Kuikman PJ, van Groenigen JW, van Kessel C (2005) Decomposition of ^{14}C-labeled roots in a pasture soil exposed to 10 years of elevated CO_2. Soil Biol Biochem 37:497–506

Van Groenigen KJ, Qi X, Osenberg CW, Luo Y, Hungate BA (2014) Faster decomposition under increased atmospheric CO_2 limits soil carbon storage. Science 344:508–509

Van Groenigen KJ, Osenberg CW, Terrer C, Carrillo Y, Dijkstra FA, Health J, Nie M, Pendall E, Phillips RP, Hungate BA (2017) Faster turnover of new soil carbon inputs under increased atmospheric CO_2. Glob Chang Biol 23:4420–4429

Van Groenigen KJ, Six J, Harris D, Van Kessel C (2007) Elevated CO_2 does not favor a fungal decomposition pathway. Soil Biol Biochem 39:2168–2172

Vance ED, Brookes PC, Jenkinson DS (1987) An extraction method for measuring soil microbial biomass C. Soil Biol Biochem 19:703–707

Veneklaas EJ, Stevens J, Cawthray GR, Turner S, Grigg AM, Lambers H (2003) Chickpea and white lupin rhizosphere carboxylates vary with soil properties and enhance phosphorus uptake. Plant Soil 248:187–197

Vestergård M, Reinsch S, Bengtson P, Ambus P, Christensen S (2016) Enhanced priming of old, not new soil carbon at elevated atmospheric CO_2. Soil Biol Biochem 100:140–148

Viswanath T, Pal D, Purakayastha TJ (2010) Elevated CO_2 reduces rate of decomposition of rice and wheat residues in soil. Agric Ecosyst Environ 139:557–564

Waldrop MP, Zak DR (2006) Response of oxidative enzyme activities to nitrogen deposition affects soil concentrations of dissolved organic carbon. Ecosystems 9:921–933

Wallace BC, Lajeunesse MJ, Dietz George, Dahabreh IJ, Trikalinos TA, Schmid CH, Gurevitch J (2016) OpenMEE: Intuitive, open-source software for meta-analysis in ecology and evolutionary biology. Methods in Ecology and Evolution (2016). Available at http://brown.edu/academics/public-health/research/evidence-base-medicine/

Wand SJE, Midgley GF, Jones MH, Curtis PS (2001) Responses of wild C4 and C3 grass (*Poaceae*) species to elevated atmospheric CO_2 concentration: a meta-analytic test of

current theories and perceptions. Glob Chang Biol 5:723–741

Wang D, Heckathorn SA, Wang X, Philpott SM (2012) A meta-analysis of plant physiological and growth responses to temperature and elevated CO_2. Oecologia 169:1–13

Wang D, Lin W (1999) Effects of elevated CO_2 on root exudates in rice Acta Ecol Sin 19:570–572

Wang H, Boutton TW, Xu W, Hu G, Jiang P, Bai E (2015) Quality of fresh organic matter affects priming of soil organic matter and substrate utilization patterns of microbes. Sci Reports 5:10102

Wang X, Tang C (2018) The role of rhizosphere pH in regulating rhizosphere priming effect and implications for the availability of soil-derived nitrogen to plants. Ann Bot 121:143–151

Wang X, Butterly CR, Baldock JA, Tang C (2017) Long-term stabilization of crop residues and soil organic carbon affected by residue quality and initial soil pH. Sci Total Environ 587–588:502–509

Wang X, Tang C, Severi J, Butterly CR, Baldock JA (2016) Rhizosphere priming effect on soil organic carbon decomposition under plant species differing in soil acidification and root exudation. New Phytol 211:864–873

Wasaki J, Rothe A, Kania A, Neumann G, Römheld V, Shinano T, Osaki M, Kandeler E (2005) Root exudation, phosphorus acquisition, and microbial diversity in the rhizosphere of white lupine as affected by phosphorus supply and atmospheric carbon dioxide concentration. J Environ Qual 34:2157–2166

Watt M, Evans JR (1999) Linking development and determinacy with organic acid efflux from proteoid roots of white lupin grown with low phosphorus and ambient or elevated atmospheric CO_2 concentration. Plant Physiol 120:705–716

Weisskopf L, Le Bayon R-C, Kohler F, Page V, Jossi M, Gobat J, Martinoia E, Aragno M (2008) Spatio-temporal dynamics of bacterial communities associated with two plant species differing in organic acid secretion: A one-year microcosm study on lupin and wheat. Soil Biol Biochem 40:1772–1780

Werth M, Kuzyakov Y (2010) ^{13}C fractionation at the root-microorganisms-soil interface: A review and outlook for partitioning studies. Soil Biol Biochem 42:1372–1384

West JB, Hobbie SE, Reich PB (2006) Effects of plant species diversity, atmospheric [CO_2], and N addition on gross rates of inorganic N release from soil organic matter. Glob Chang Biol 12:1400–1408

Wild B, Alaei S, Bengtson P, Bodé S, Boeckx P, Schnecker J, Mayerhofer W, Rütting T (2017) Short-term carbon input increases microbial nitrogen demand, but not microbial nitrogen mining, in a set of boreal forest soils. Biogeochemistry 136:261–278

Williams MA, Rice CW, Owensby CE (2000) Carbon dynamics and microbial activity in tallgrass prairie exposed to elevated CO_2 for 8 years. Plant Soil 227:127–137

Xie Z, Cadisch G, Edwards G, Baggs EM, Blum H (2005) Carbon dynamics in a temperate grassland soil after 9 years exposure to elevated CO_2 (Swiss FACE). Soil Biol Biochem 37:1387–1395

Xu JM, Tang C, Chen ZL (2006) The role of plant residues in pH change of acid soils differing in initial pH. Soil Biol Biochem 38:709–719

Xu Q, Wang X, Tang C (2017) Wheat and white lupin differ in rhizosphere priming of soil organic carbon under elevated CO_2. Plant Soil 421:43–55

Xu Q, Wang X, Tang C (2018) The effects of elevated CO_2 and nitrogen availability on rhizosphere priming of soil organic matter under wheat and white lupin. Plant Soil 425:375–387

Xu Z, Jiang Y, Zhou G (2015) Response and adaptation of photosynthesis, respiration, and antioxidant systems to elevated CO_2 with environmental stress in plants. Front Plant Sci 6:Article 701

Yu G, Xiao J, Hu S, Polizzotto ML, Zhao F, McGrath SP, Li H, Ran W, Shen Q (2017) Mineral availability as a key regulator of soil carbon storage. Environ Sci Technol 51:4960–4969

Zang H, Wang J, Kuzyakov Y (2016) N fertilization decreases soil organic matter decomposition in the rhizosphere. Appl Soil Ecol 108:47–53

Zak DR, Pregitzer KS, King JS, Holmes WE (2000) Elevated atmospheric CO_2, fine roots and the response of soil microorganisms: a review and hypothesis. New Phytol 147:201–222

Zak DR, Ringelberg DB, Pregitzer KS, Randlett DL, White DC, Curtis PS (1996) Soil microbial communities beneath *Populus Grandidentata* grown under elevated atmospheric CO_2. Ecol Appl 6:257–262

Zheng J-Q, Han S-J, Zhou Y-M, Ren F-R, Xin L-H, Zhang Y (2010) Microbial activity in a temperate forest soil as affected by elevated atmospheric CO_2. Pedosphere 20:427–435

Zhu B, Cheng W (2011a) ^{13}C isotope fractionation during rhizosphere respiration of C3 and

C4 plants. Plant Soil 342:277–287

Zhu B, Cheng W (2011b) Rhizosphere priming effect increases the temperature sensitivity of soil organic matter decomposition. Glob Chang Biol 17:2172–2183

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