New Phytologist
Volume 211: 864-873, 2016
doi: 10.1111/nph.13966

Rhizosphere priming effect on soil organic carbon decomposition under plant species differing in soil acidification and root exudation

X. Wang[1], C. Tang[1*], J. Severi[1], C. R. Butterly[1], J. A. Baldock[2]

[1] Department of Animal, Plant and Soil Sciences, La Trobe University, Melbourne Campus, Bundoora, VIC 3086, Australia
[2] CSIRO Agriculture, Glen Osmond, SA 5064, Australia

* Corresponding author:
C. Tang
Department of Animal, Plant and Soil Sciences, La Trobe University, Melbourne Campus, Bundoora, Vic 3086, Australia
Telephone: +61 3 9032 7416
Fax: +61 3 9032 7605
Email: C.Tang@latrobe.edu.au

Summary

- The effect of rhizosphere properties such as pH on the rhizosphere priming effect (RPE) is unknown. This study aimed to link species variation in RPE with plant traits such as root exudation and H^+ extrusion.
- Four C_3 species (chickpea, field pea, white lupin and wheat) differing in soil acidification and root exudation, were grown in soil collected from a C_4 grassland. The CO_2 released from soil was trapped using a newly-developed NaOH-trapping system.
- White lupin and wheat showed greater positive RPEs, in contrast to the negative RPE produced by chickpea. The high RPE of white lupin was in line with its capacity to release root exudates, while the negative RPE of chickpea was attributed to its great ability to acidify rhizosphere soil. The enhanced RPE of field pea at maturity might result from high N deposition and release of structural root C components following root senescence. Root biomass and length played a minor role in the species variation in RPE.
- For the first time, rhizosphere acidification was shown as an important factor affecting the magnitude and direction of RPE. Future studies on RPE modelling and mechanistic understanding of the processes that regulate RPE should consider the effect of rhizosphere pH.

Key words: CO_2-trapping system, ^{13}C natural abundance, rhizosphere acidification, root exudates, species variation

Introduction

Priming effect is a strong short-term change in microbial mineralization of soil organic carbon (SOC) in response to labile carbon (C) inputs (Kuzyakov *et al*., 2000). When the source of labile organic C is rhizodeposits derived from living roots and a change in decomposition of SOC is induced in the direct vicinity of roots, it is termed a rhizosphere priming effect (RPE). Rhizodeposits consist of soluble and insoluble forms of organic C such as sugars, organic acids, mucilage, sloughed cell walls and root hairs, but can also include nitrogen-containing organic compounds such as amino acids (Hütsch *et al*., 2002). Owing to the ongoing supply of these root-derived materials, the rhizosphere is characterized by a surplus of C and either an increase (a positive RPE) or decrease (a negative RPE) in SOC decomposition (Kuzyakov *et al.*, 2000). Measured RPEs on the decomposition of SOC have been ranged from -70% to 380% (Cheng *et al.*, 2014; Zhu & Cheng, 2011).

Several mechanisms have been proposed to explain different RPEs. Positive RPEs are believed to be driven by co-metabolism of SOC with rhizodeposits (Kuzyakov *et al*., 2000), the activation of microbes able to access and degrade SOC (Fontaine *et al.*, 2003) or N mining from N-rich SOC when N is limited (Murphy *et al*., 2015). In contrast, preferential substrate utilization involving the switch from SOC to labile C substrate by microbes, and N constraints on SOC decomposition resulting from competition for N between plants and microorganisms, have been proposed for the negative RPEs (Schimel *et al*., 1989). The microbial activation theory is supported by the fact that positive RPEs are often associated with increased microbial biomass (Kuzyakov, 2010). On the other hand, the N competition and N mining theory indicates a basic N requirement of microbes for biomass accumulation and exoenzyme synthesis in response to the continuous supply of root-derived C, predominantly root exudates. All of these mechanisms are believed to act alone, or

together, or the dominant process may change from one to another over time, accounting for either positive or negative RPEs in many studies (Blagodatskaya & Kuzyakov, 2008). The switch between these mechanisms is possibly triggered by changes in the size and community composition of microbial community (Dorodnikov *et al*., 2009; Talbot *et al*., 2008) or in nutrient availability, especially N (Dijkstra *et al*., 2013).

Plant species has been widely recognized for its importance in regulating the magnitude of RPE. For example, leguminous crop species consistently show higher RPEs than non-legumes (Cheng *et al*., 2003; Cheng & Kuzyakov 2005; Fu & Cheng, 2002), and some tree species such as Ponderosa pine produced a more prominent RPE than other species such as Fremont cottonwood (Dijkstra & Cheng 2007b). Species variations in RPEs are frequently attributed to differences in the quantity and quality of rhizodeposits (Cheng *et al*., 2014). Other plant traits such as shoot and root biomass, and plant phenology can also affect RPEs, but the proposed mechanisms are inevitably referred to plant growth-induced change in rhizodeposits (Cheng *et al*., 2003; Dijkstra *et al*., 2006; Fu & Cheng, 2002). Positive relationships between the amount of rhizodeposits and RPEs have been observed in some tree species (Bengtson *et al*., 2012; Dijkstra & Cheng, 2007a; Phillips *et al*., 2011). Also, higher RPEs of soybean than wheat or sunflower are believed to be attributed to its N-rich rhizodeposits (Cheng *et al*., 2003; Cheng & Kuzyakov 2005; Fu & Cheng, 2002).

Plant species also differ greatly in their capacity to change rhizosphere properties such as rhizosphere pH. Some species such as chickpea are capable of acidifying their rhizosphere to a greater extent than others such as white lupin and field pea, due to greater excess cation uptake over anion (Tang *et al*., 1997; 1999). There is ample evidence suggesting that soil pH can regulate SOC transformation directly through its effect on SOC solubility or indirectly by altering microbial activity (Andersson *et al*., 2000; Kemmitt *et al*., 2006; Rousk *et al*., 2009). The greatest priming effects are detected in the pH range of 6 to 8 (Blagodatskaya & Kuzyakov, 2008). Nevertheless, the effect of root-induced rhizosphere acidification on root-induced change in the decomposition of SOC, namely RPE, is unknown. Furthermore, little attention has been given to the integration of multiple factors and their relative importance in affecting RPE.

The objectives of this study were to: (1) examine RPEs of crop species with contrasting rhizosphere effect in terms of rhizosphere acidification and root exudation, and (2) link the species variation in RPE with rhizosphere properties including pH, microbial biomass C and N and plant traits such as root exudates, shoot and root biomass. We hypothesized that (1) crop species differ in RPE with the species of greater root exudation resulting in greater RPE, and (2) RPEs correlate negatively with rhizosphere acidification.

Materials and methods

Soil

The experimental soil was collected from the top 10-cm layer of a C_4 Kangaroo grassland (*Themeda triandra*) at a property, 20 km north of Gulgong, New South Wales, Australia (32° 11′ S, 149° 33′ E). The soil was a granite-derived sandy loam and had the following basic properties: pH 5.0 (0.01 M $CaCl_2$), organic C 27 g kg^{-1}, total N 1.6 g kg^{-1}, clay 130 g kg^{-1} and soil pH buffer capacity 30 $mmol_c\ kg^{-1}\ pH^{-1}$. The

soil was air-dried and sieved through a 2-mm sieve. The $\delta^{13}C$ value of the total SOC was -18‰. The $\delta^{13}C$ signature of different SOC pools was also analysed. Briefly, 20 g of dry soil was sieved and divided into pools with different particle sizes: <53, 53-250, 250-500, 500-1000, 1000-2000 µm. To collect particulate organic carbon, another 20 g of soil was added into 80 ml water and the soil suspension was passed through a 53-µm sieve. The coarse organic materials retained on the 53-µm sieve were rinsed, separated from sand and air-dried. All SOC fractions were ball-milled before being analysed for $\delta^{13}C$ by isotope ratio mass spectrometry (IRMS) (Sercon 20-22, Crewe, UK).

Experimental set up

This column experiment used a ^{13}C natural abundance approach whereby C_3 plants were grown in the C_4 soil, and the ^{13}C was used as a tracer to separate plant-derived CO_2-C from soil-derived CO_2-C. Common crop species, chickpea (*Cicer arietinum* L. cv. Slasher), field pea (*Pisum sativum* L. cv. Kospa), wheat (*Triticum aestivum* L. cv. Yitpi) and white lupin (*Lupinus albus* L. cv. Kiev), selected for their contrasting effect on soil acidification and root exudation, were grown in PVC columns (diameter 10 cm, height 40 cm) containing 2.8 kg air-dried C_4 soil with the following basal nutrients (mg kg^{-1}): KH_2PO_4, 180; K_2SO_4, 120; $CaCl_2.2H_2O$, 180; $MgSO_4.7H_2O$, 50; $MnSO_4.H_2O$, 15; $ZnSO_4.7H_2O$, 9; $CuSO_4.5H_2O$, 6; $Na_2MoO_4.2H_2O$, 0.4; FeEDTA, 5.5. Four replicates of each species were included. Columns without plants (+/- N) were also included as controls. Plants were destructively harvested at 35, 56 and 84 days, which represent the early vegetative, late vegetative and flowering stages, respectively, for wheat and white lupin, and late vegetative, flowering and grain-filling/maturity stage, respectively, for chickpea and field pea. Since isotopic fractionation between root tissue and root-respired CO_2 has been increasingly recognized, another set of columns filled with coarse river sand were also included for each species with two replicates. These columns were set up as previously described except that the washed river sand, free of organic C, was inoculated with 1% (w/w) of C_3 soil before planting columns for each species with two replicates. The sand-filled columns were watered with Hoagland solution (Hoagland & Arnon, 1950) to replenish both water and nutrients.

Planting

The experiment was conducted in a controlled environment room (CER), with temperatures set at 20 ºC day / 18 ºC night and light intensity at 400 µmol $m^{-2}s^{-1}$ with a day length of 14 h. Twelve pre-germinated seeds of wheat and eight seeds of chickpea, field pea and white lupin were sown in a row into each column. All the legumes were inoculated with an appropriate rhizobium inoculant (EasyRhiz, New-Edge Microbials, Albury, Australia). For wheat, urea was applied at a rate of 30 mg N kg^{-1} weekly from week 2. In order to prevent possible anaerobic conditions, 250 g of plastic beads sealed in nylon bags (mesh size 50 µm) were placed at the bottom of each column. After emergence, plants were thinned to 7 plants per column for wheat, and 4 for other species. The soil water content was monitored gravimetrically and maintained at ~80% field capacity daily.

CO_2 trapping

The CO_2 trapping system was modified based on the closed-circulation system of Cheng *et al.* (2003). The modified system consisted of an inlet at the top of each column where CO_2-free air was introduced after being pumped through 1 M NaOH

via an air stone (Fig. 1). On the opposite side of the soil column, at the base, the outlet was fitted with a vacuum line passing through two CO_2 traps containing 150 ml of 0.5 M NaOH. The simultaneous operation of the pump and vacuum facilitated airflow through the soil columns, reduced the pressure within the headspace and stopped possible leaking around the plant stems.

Prior to trapping, two pieces of halved PVC sheet with pre-drilled holes for plant stems were fitted around the plant base and sealed using Blu-Tack (Bostik, Thomastown, Australia) (Fig. 1). The system was checked for possible air leaks by vacuuming the CO_2-free air (no pumping) through the soil column, before it was connected to the trap of 0.5 M NaOH (Fig. 1a). A complete seal at plant base was indicated by bubbles in the trap of 1 M NaOH at moderate vacuum force, since low pressure could be easily built up in a sealed system. All previously accumulated CO_2 was then removed by circulating CO_2-free air for each column for 30 min. Total belowground CO_2 was trapped for 30 min every 6 h for 2 d (Fig. 1). The air-flow rate was controlled at 80-100 ml min^{-1} to maximize the trapping efficiency (Cheng & Coleman, 1989). Although two traps were included to guarantee that all CO_2 was captured, no CO_2 was detected in the second trap. Only the first trap was used for quantifying and sampling CO_2-C.

Harvest and measurements

Plants were harvested straight after each CO_2 collection. Plant shoots were cut off at the soil surface for one column at a time, in order to minimize the decomposition of root exudates. The rhizosphere soil was collected by quickly shaking off soil adhered to the roots and passed through a 2-mm sieve to remove broken roots. Before moving on to the next column, the collected rhizosphere soil was immediately stored at 4 ºC for later determination of soil microbial biomass carbon (MBC) and soil respiration. After harvest, plant roots were carefully washed with tap water and root length and diameter were quantified using WinRHIZO Pro 2003b (Regent Instruments, Quebec City, Canada) and an EPSON EU-35 scanner (Seiko Epson Corp, Japan). All plant materials were oven-dried at 70 ºC and weighed. After the shoot and root samples were ground using a ball mill, the ^{13}C in the samples was analysed using a mass spectrometer (Sercon 20-22, Crewe, UK). A sub-sample of homogenised rhizosphere soil was air-dried for the determination of soil pH, $\delta^{13}C$, total organic C and N content. Soil pH was measured in 0.01 M $CaCl_2$ (1:5 soil solution ratio, 1-hour end-over-end shaking, centrifuging at 700 *g* for 10 min). The C and N contents of shoots, roots and soil samples were determined by dry combustion using a CHNS Analyser (PerkinElmer EA2400, Shelton, USA).

Soil respiration was determined during a 12-h incubation of rhizosphere soil at 25 ºC (Rukshana *et al*., 2012). This could provide a good measure of root exudates as microbial utilization of root exudates required only a few hours in contrast to a few days for other rhizodeposits such as sloughed-off cell walls and root hairs (Gregory, 2006; Fischer *et al*., 2010). Briefly, 10 g of fresh soil was placed in a glass incubation jar and the CO_2 concentration within the headspace of incubation jars was measured using an infrared gas analyser (Servomex 4210 Industrial Gas Analyser, Cowborough, UK). The MBC was determined using chloroform fumigation-extraction according to Vance *et al*. (1987). Total organic C in the extracts (0.5 M K_2SO_4) was determined colorimetrically after wet digestion with $K_2Cr_2O_7$ and concentrated H_2SO_4 at 135 ºC for 30 min (Cai *et al*. 2011). Total soluble N was measured using a QuickChem 8500

flow injection analyser (Lachat Instruments, Loveland, CO, USA) after persulfate oxidation of both fumigated and non-fumigated extracts (Cabrera & Bear, 1993). The MBC and microbial biomass N (MBN) were calculated as the difference in total organic C and N concentration between fumigated and non-fumigated soils, adjusted by a proportionality coefficient (0.45) for both C and N (Jenkinson *et al*., 2004). Soil inorganic N (NH_4^+ + NO_3^-) was also measured by flow injection analyser (Lachat Instruments, Loveland, CO, USA) on non-fumigated 0.5 M K_2SO_4 extracts.

The amount of CO_2 in the alkali traps was determined by titrating 10 ml of 0.5 M NaOH solution with 0.5 M HCl after adding 5 ml 1 M $BaCl_2$. To form pure $SrCO_3$ precipitates to quantify the $\delta^{13}C$ of the trapped CO_2, 5 ml of 1 M $SrCl_2$ was added to another 10 ml of the 0.5 M NaOH solution. The suspension pH was adjusted to 7.0 by adding 0.3 M HCl at an amount based on the titration. The $\delta^{13}C$ of the $SrCO_3$ was analysed by IRMS.

Calculations
The $\delta^{13}C$ values measured in the CO_2 traps were corrected for contamination from air using the equations of mass conservation (Mary *et al*., 1992);
$C_t = C_1 + C_2$
$\delta_t C_t = \delta_1 C_1 + \delta_2 C_2$
$\delta_1 = (\delta_t C_t - \delta_2 C_2) / (C_t - C_2)$

Where C_t is the total amount of C in the sample solution including C_2, C_1 is the amount of C in the sample solution without C_2, C_2 is the amount of C in blank solutions. δ_t is the $\delta^{13}C$ value of a sample before correction, δ_1 is the $\delta^{13}C$ value of a sample after correction, δ_2 is the $\delta^{13}C$ value of the air (-8‰).

The total CO_2 efflux from planted soil was partitioned using the following equations (Mary *et al*., 1992);
$C_1 = C_t \times (\delta_t - \delta_2) / (\delta_1 - \delta_2)$

Where C_t is the total C from below-ground CO_2, δ_t is the $\delta^{13}C$ value of the C_t, C_1 is the amount of C derived from the C_4 soil, δ_1 is the $\delta^{13}C$ value of the C_4 soil C [$\delta^{13}C$ value of CO_2-C released from the control soil (no plant)], C_2 is the amount of C derived from the C_3 plant, δ_2 is the $\delta^{13}C$ value of the C_3 root-derived C (CO_2 trapped from the sand column).

Statistical analysis
A two-way ANOVA was conducted to assess the effects of species and harvest time on plant biomass, plant N concentrations, root length and ^{13}C abundance of root and root-derived CO_2 (Table 1). Significant (P=0.05) differences between means were identified using Tukey's HSD test. One-way ANOVA (Tukey's test) was used to compare soil-derived CO_2, rhizosphere pH, soil respiration and microbial biomass C and N between treatments at each harvest (Fig. 3-6). The relationship between RPE and selected variables was quantified by conducting multiple regression analyses, with the most significant variables in regulating RPE determined by a forward stepwise regression procedure using Genstat (11th version).

Results
Plant growth

Chickpea produced the highest shoot biomass at day 84, followed by field pea, wheat and white lupin. Moreover, the root dry weight of chickpea was 2.2, 3.8 and 6.6 times higher than wheat, white lupin and field pea, respectively, when averaged over three harvests (Table 1). Wheat showed the greatest root length, 1.5 times higher than chickpea and 10 times higher than white lupin and field pea. The N concentration was highest in the shoot and root of white lupin, and lowest in field pea (Table 1).

Total belowground CO_2 efflux and ^{13}C abundance

The $\delta^{13}C$ value for the roots of chickpea, wheat, white lupin and field pea ranged from -28.5 to -32.8‰. The $\delta^{13}C$ value of CO_2 trapped from sand-filled columns was slightly enriched (0.3-1.8‰) compared with that in the root tissue, indicating isotopic fractionation between root tissue and root-respired CO_2 (Table 1). On the other hand, the $\delta^{13}C$ value of the particulate organic matter in the soil (0.5-2 mm) (-14.8‰) was higher than those of soil particles less than 0.5 mm (-18.8‰). This suggests that different SOC pools have different $\delta^{13}C$ value, with the easily decomposable C more ^{13}C enriched than non-labile C fractions associated with soil minerals (data not shown).

Total CO_2 efflux from the non-planted control showed a slight decline over time (Fig. 2a), and the $\delta^{13}C$ value of CO_2 evolved was constant throughout the experiment (-14.5 to -15.2‰) (Fig. 2b). Growing plants increased total CO_2 efflux by 240-430% compared with the controls. The $\delta^{13}C$ value of total CO_2 collected from planted columns (-23.2 to -26.6‰) was significantly lower than the control, and became more depleted over time (-29.7 to -30.7‰) except for field pea (Fig. 2b). The $\delta^{13}C$ value of CO_2 trapped for field pea decreased by 3.9‰ at day 56 but increased by 1.6‰ at day 84. Chickpea showed much lower $\delta^{13}C$ value in the total evolved CO_2 than other species (Fig. 2b) (P<0.05).

Soil-derived CO_2, primed C and rhizosphere soil C concentration

White lupin showed the greatest positive RPE at days 38 and 56 (Fig. 3b), with daily soil-derived CO_2 increasing by 53% relative to the control, and followed by 40% for wheat (Fig. 3a) (P<0.05). The positive RPE of wheat and white lupin showed a decrease at day 84. In contrast, no RPE was detected for field pea at day 38, but soil-derived CO_2 increased by 95% at day 84, compared to control (Fig. 3a) (P<0.05). Both chickpea and field pea showed a negative RPE at day 56, where soil-derived CO_2 decreased by 70 and 40%, respectively (Fig. 3a, b) (P<0.05). The decrease in primed C by chickpea was persistent at day 84, though to a lesser extent.

Consistently, when compared with the control, the presence of white lupin decreased SOC concentration in the rhizosphere soil by 4% (P<0.05) at the end of the experiment (Fig. 4a). In contrast, SOC concentration in the rhizosphere of chickpea was equal to or higher than the control. There was no change in SOC concentration in the rhizosphere of field pea and wheat at day 38 and 56, but SOC decreased by 4% at day 84 for field pea.

Rhizosphere pH, soil respiration and inorganic N concentration

Among the four species, only chickpea acidified its rhizosphere (by 0.23-0.65 pH units) (Fig. 4b) (P<0.05). The rhizosphere pH of other species was the same or higher than the control. For all treatments, rhizosphere pH tended to decline with growth stage (Fig. 4b).

Regardless of growth stage, the rhizosphere soil respiration during 12-hour incubation was consistently the highest in the rhizosphere of white lupin, followed by chickpea, wheat and field pea and lowest in the control (Fig. 4c).

Inorganic N (NH_4^+ + NO_3^-) concentrations were lower in the rhizosphere soil of all species than in the control, especially for field pea and chickpea (Fig. 5). For field pea, a greater inorganic N concentration was detected in the rhizosphere soil at day 84 than at days 38 and 56.

Rhizosphere soil microbial C and N

When compared with the control, the presence of white lupin increased MBC (Fig. 6a) (P<0.05). An increase in MBC in the rhizosphere of field pea was only detected at day 84. The MBN showed an apparent response to rhizosphere effect for most species. For instance, MBN was invariably higher in the rhizosphere of white lupin and wheat than in the control, whereas it was 20% lower in the rhizosphere of chickpea than in the control at days 58 and 84 (P<0.05). The change in MBN in the rhizosphere of field pea showed a distinct pattern: decreased by 30% at day 58 but increased by 25% at day 84, in comparison with the control (Fig. 6b).

Correlation between RPE and selected factors

Rhizosphere pH and MBN correlated positively with RPE (Table 2). However, rhizosphere pH and MBN only accounted for 52% of the variation in RPE. When multiple regression analysis was performed at each sampling time, soil respiration and soil pH accounted for 78% of variation in RPE at day 38. However, MBN, rhizosphere pH and soil respiration explained 96% of change in RPE at day 58. At day 84, only rhizosphere pH was shown as a significant variable, accounting for 54% of the variation in RPE.

Discussion

Species variation in RPE

The four species differed substantially in their RPE on the decomposition of SOC. Except at day 84, white lupin showed the highest positive RPE of all species, in contrast to the negative RPE produced by chickpea. This was further evidenced by an apparent loss and accumulation of SOC in the rhizosphere of white lupin and chickpea, respectively. The positive RPE of wheat did not cause a significant decrease in SOC, possibly because the amount of C loss due to RPE was equal to the C input in the forms of rhizodeposition. Using both the C budget and ^{13}C tracer method, Cheng (2009) found that the RPE of wheat plants resulted in faster C cycling in soil, but no net change in SOC. Different RPEs have also been reported among other crop species such as maize and soybean, and tree species such as Ponderosa pine and Fremont cottonwood (Fu & Cheng, 2002; Bengtson *et al*., 2012; Cheng *et al*., 2003; Dijkstra & Cheng, 2007a). Changes in SOC decomposition due to RPE have ranged from -70% to 380% (Cheng *et al*., 2014; Zhu & Cheng, 2011). Our study expanded the species range by examining RPEs of three distinct crop legumes, with the magnitude of RPE (-70% to 53%) falling within the reported range. Understanding species variation in SOC decomposition could help to define the mechanisms accounting for changes in SOC stocks under field conditions.

Plant species variation in RPE was also affected by plant growth stage. Given that each species reached a similar growth stage at different harvests, species variation in RPE at each harvest may be due, at least in part, to variations in plant phenology. For example, field pea, the only species that reached maturity at the final harvest, showed greatly increased RPE at day 84 than days 34 and 56. A higher RPE at maturity than other growth stages was also detected for a C4 plant species *Amaranthus* (Fu & Cheng, 2002). For species that did not reach maturity by day 84, RPE tended to peak at a late vegetative and declined thereafter, in line with other studies (Cheng et al. 2003; Cheng & Kuzyakov, 2005). Nevertheless, the RPE of white lupin and wheat could have possibly been underestimated at day 84, due to using of $\delta^{13}C$ value of soil-derived CO_2 for the no-plant control column (i.e. -14.8‰). The persistent positive RPE of wheat and white lupin were very likely to cause depletion of labile SOC and decomposition of stable SOC pools with lower $\delta^{13}C$ values. In order to test the sensitivity of RPE calculation to changes in $\delta^{13}C$ values of soil-derived C, we changed the $\delta^{13}C$ value from -14.8 to -18.8‰ (i.e. assuming all of CO_2 originated from the stable C pools) and found the RPE of these two species were still lower at day 86 than that at days 56 and 36. Thus, the decrease in RPE of wheat and white lupin with time was not an experimental artefact. Considering that plant traits such as root exudates and root morphology vary across plant growth stages, changes in the RPE with time were expected. However, without gaining a deep and complete understanding of all factors regulating RPE, it is difficult to explain species variation in RPE in this study.

The species variation in RPE could not be explained by the difference in root morphology. It was evident that, from this study, root biomass and total length did not correlate with RPEs across the species and growth stages. While the high RPE of wheat, lacking noticeable release of root exudates, could be partly attributed to its extensive root system, the negative/low RPE of chickpea mainly resulted from the great capacity of its large and dense root system to acidify the rhizosphere soil. Moreover, white lupin although low in total root length has a distinct cluster-root structure which releases large amounts of root exudates (Neumann & Martinoia 2002), leading to a high RPE in this study. In another study, Fu & Cheng (2002) found that the amount of primed-C correlated positively with root biomass of C_3 plant species such as soybean and sunflower. Principally, the length and morphology of plant roots are believed to affect RPEs through influencing the rhizosphere volume. Nevertheless, given that only zones immediately behind the root tips are generally active in exudation (Badri & Vivanco 2009), a high root biomass or length might not necessarily result in great root exudation in the rhizosphere. It appeared that plant root physiological traits such as root exudate release and rhizosphere acidification play a more predominant role than root morphological traits in RPEs.

Root exudates

The significant (P=0.002) correlation between rhizosphere soil respiration and RPE at day 35 suggests that the quantity of root exudates acted as an important factor affecting RPE during early stages of plant growth. In this study, white lupin was selected for its well-known high root exudation capacity in contrast to wheat and field pea (Sas *et al*., 2001; Veneklaas *et al*., 2003; Wang *et al*., 2008). As expected, higher rhizosphere soil respiration and higher RPE of white lupin were in line with its capacity to exude root exudates. Previous studies also showed that the amount of root exudates released was positively correlated with microbial growth and SOC decomposition (Bengtson *et al*., 2012; Dijkstra & Cheng, 2007a; Phillips *et al*., 2011).

Our study provides further evidence that the species of high root exudation exhibit greater RPE at early growth stages.

The magnitude of RPE could not be simply explained by changes in the quantity of root exudates, as reflected by the poor correlation between soil respiration and RPE at late growth stages. For field pea, cellular root material could contribute substantially to rhizodeposits following senescence of roots at maturity, in addition to root exudates (Arcand *et al*., 2013; Gavito *et al*., 2001; Wichern *et al*., 2007), which could account for an increased microbial growth and enhanced RPE at day 84. Moreover, the inorganic N concentration in the rhizosphere of field pea at day 84 nearly doubled that at day 56 (Fig. 5), possibly reflecting higher N deposits from the root at maturity. Nitrogen-rich substrates could stimulate microbial growth, increase the production of exoenzymes and cause greater priming effects than substrates of low or no N (Drake *et al*., 2013). On the other hand, the decrease in RPE of wheat and white lupin with time was possibly attributed to decline in the easily decomposable SOC pool, as indicated by a decreasing total CO_2 evolved from the control columns. In short-term incubation experiments, Kuzyakov *et al.* (2000) showed that priming effects mainly originated from the mineralization of labile SOC. Drake *et al.* (2013) also found that the release of low-molecular-weight compounds by plant roots enhanced the production of exoenzymes targeting low-molecular-weight SOC. Finally, other factors such as N availability and rhizosphere pH could also have accounted for the lack of positive relationship between root exudates and RPE at late growth stages.

N availability

Microbial N, but not microbial C, correlated positively with rhizosphere primed C. At the later growth stages, microbial C:N ratios were generally higher in the rhizosphere of all N_2-fixing legumes than wheat which was regularly fed with fertiliser N. In addition, higher microbial C:N ratios and negative RPEs of chickpea and field pea were in accordance with the lowest inorganic N concentration in their rhizospheres at day 56. Thus, lower microbial N and higher microbial C:N ratios possibly reflected a microbial N limitation with time. Several studies found that RPEs were constrained at extremely low N conditions (Cheng & Kuzyakov 2005; Drake *et al*., 2013). Alternatively, low microbial N might be a consequence of negative RPEs. In contrast to positive RPEs which could enhance microbial N immobilization (Kuzyakov & Xu 2013), decreased RPEs were indicted to decrease microbial uptake of soil-derived N and hence microbial biomass N. Further study is required to assign the causes and effects between microbial N and RPEs.

Rhizosphere pH

The species variation in RPE was partly attributed to the species effect on rhizosphere pH (P<0.01). The greater capacity of chickpea in rhizosphere acidification could be explained by its apparently excess uptake of cations over anions during N_2 fixation (McLay *et al*., 1997; Tang *et al*., 1997; 1999). Low pH would reduce microbial biomass, microbial activity or substrate availability (Andersson *et al*., 2000; Kemmitt *et al*., 2006; Rousk *et al*., 2009), and hence RPE. In the present study, the rhizosphere pH of chickpea reduced from 5 to 4.3, at which microbial activity could be greatly suppressed (Leifeld *et al*., 2008; Rousk *et al*., 2010). Quiquampoix (2000) found that soil priming effects decreased at low soil pH due to the sorption of enzymes on surfaces of clay particles. The greatest priming effects have often been detected in the pH range of 6 to 8 (Blagodatskaya & Kuzyakov, 2008). On the other hand, chickpea

had been shown to release higher quantity of root exudates than wheat and field pea in this study, or the same amount as white lupin by others (Veneklaas *et al*., 2003). The negative/low RPEs of this species suggest that low pH is the predominant factor affecting RPE. Therefore, rhizosphere acidification could be an important mechanism of negative RPE and rhizosphere pH should be recognized for its important role in regulating RPE. Further studies are required to identify the boundary pH value triggering the functioning of other mechanisms.

Conclusion

This study provided a simplified, low-cost and reliable CO_2 trapping system for examining RPE of different species. Species with different capacity to release root exudates had quite different RPEs. For the first time, rhizosphere pH was revealed as an important factor affecting the magnitude and direction of RPE, and could partly account for species variation in RPE along with root exudation, N availability and labile carbon pool size. Under field conditions, adoption of legumes species in the cropping system needs to consider both soil acidification and C loss or gain due to RPE. For species like white lupin with high RPE, maximizing plant residue return might be essential to compensate the total SOC loss from the system. Future studies on either RPE modelling or mechanistic understanding of RPE should also consider the effect of rhizosphere pH on SOC stability and long-term C storage.

Acknowledgments

This research was supported under Australian Research Council's Discovery Projects funding scheme (project DP120104100).

References

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 Biology & Biochemistry* **32:** 1-10.

Arcand MM, Knight JD, Farrell RE. 2013. Temporal dynamics of nitrogen rhizodeposition in field pea as determined by ^{15}N labelling. *Canadian Journal of Plant Science* **93:** 941-950.

Badri DV, Vivanco JM. 2009. Regulation and function of root exudates. *Plant, Cell and Environment* **32:** 666-681.

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. *Ecology and Evolution* **2**: 1843-1852.

Blagodatskaya E, Kuzyakov Y. 2008. Mechanisms of real and apparent priming effects and their dependence on soil microbial biomass and community structure: critical review. *Biology and Fertility of Soils* **45**: 115-131.

Cabrera ML, Beare MH. 1993. Alkaline persulfate oxidation for determining total nitrogen in microbial biomass extracts. *Soil Science Society of America Journal* **57**: 1007-1012.

Cai Y, Peng C, Qiu S, Li Y, Gao Y. 2011. Dichromate digestion–spectrophotometric procedure for determination of soil microbial biomass carbon in association with fumigation–extraction, *Communications in Soil Science and Plant Analysis* **42:** 2824-2834.

Cheng W. 2009. Rhizosphere priming effect: its functional relationships with microbial turnover, evapotranspiration, and C-N budgets. *Soil Biology & Biochemistry* **41**: 1795-1801.

Cheng W, Coleman DC. 1989. A simple method for measuring CO_2 in a continuous air-flow system: modifications to the substrate-induced respiration technique. *Soil Biology & Biochemistry* **21**: 385-388.

Cheng W, Johnson DW, Fu SL. 2003. Rhizosphere effects on decomposition: controls of plant species, phenology, and fertilization. *Soil Science Society of America Journal* **67**: 1418-1427.

Cheng W, Kuzyakov Y. 2005. Root effects on soil organic matter decomposition. In: Wright S, Zobel R, eds. *Roots and Soil Management: Interactions Between Roots and the Soil*, Agronomy Monograph No. 48, American Society of Agronomy, Madison, Wisconsin, USA, 119-143.

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

Dijkstra FA, Carrillo Y, Pendall E, Morgan JA. 2013. Rhizosphere priming: a nutrient perspective. *Frontiers in Microbiology* **4**: 1-8.

Dijkstra FA, Cheng WX. 2007a. Interactions between soil and tree roots accelerate long-term soil carbon decomposition. *Ecology Letters* **10**: 1046-1053.

Dijkstra FA, Cheng WX. 2007b. Moisture modulates rhizosphere effects on C decomposition in two different soil types. *Soil Biology & Biochemistry* **39**: 2264-2274.

Dijkstra FA, Cheng WX, Johnson DW. 2006. Plant biomass influences rhizosphere priming effects on soil organic matter decomposition in two differently managed soils. *Soil Biology & Biochemistry* **38**: 2519-2526.

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: research article. *FEMS Microbiology Ecology* **69**: 43-52.

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

Fischer H, Ingwersen J, Kuzyakov Y. 2010. Microbial uptake of low-molecular weight organic substances out-competes sorption in soil. *European Journal of Soil Science* **61**: 504-513.

Fischer H, Kuzyakov Y. 2010. Sorption, microbial uptake and decomposition of acetate in soil: transformations revealed by position-specific ^{14}C labelling. *Soil Biology & Biochemistry* **42**: 186-192.

Fontaine S, Mariotti A, Abbadie L. 2003. The priming effect of organic matter: a question of microbial competition? *Soil Biology & Biochemistry* **35**: 837-843.

Fu S, Cheng W. 2002. Rhizosphere priming effects on the decomposition of soil organic matter in C_4 and C_3 grassland soils. *Plant and Soil* **238**: 289-294.

Gavito ME, Curtis PS, Jakobsen I. 2001. Neither mycorrhizal inoculation nor atmospheric CO_2 concentration has strong effects on pea root production and root loss. *New Phytologist* **149:** 283-290.

Gregory PJ. 2006. *Plant root growth, activity and interaction with soil*. Oxford: Blackwell Publishing, 216-222.

Hoagland DR, Arnon DI. 1950. *The water-culture method for growing plants without soil.* Berkeley, Calif: University of California, College of Agriculture, Agricultural Experiment Station.

Hütsch BW, Augustin J, Merbach W. 2002. Plant rhizodeposition – an important source for carbon turnover in soils. *Journal of Plant Nutrition and Soil Science* **165**: 397-407.

Jenkinson DS, Brookes PC, Powlson DS. 2004. Measuring soil microbial biomass. *Soil Biology & Biochemistry* **36**: 5-7.

Kemmitt SJ, Wright D, Goulding KWT, Jones DL. 2006. pH regulation of carbon and nitrogen dynamics in two agricultural soils. *Soil Biology & Biochemistry* **38**: 898-911.

Kuzyakov Y. 2010. Priming effects: interactions between living and dead organic matter. *Soil Biology & Biochemistry* **42**: 1363-1371.

Kuzyakov Y, Friedel JK, Stahr K. 2000. Review of mechanisms and quantification of priming effects. *Soil Biology & Biochemistry* **32**: 1485-1498.

Kuzyakov Y, Xu X. 2013. Competition between roots and microorganisms for nitrogen: mechanisms and ecological relevance. *New Phytologist* **198**: 656-669

Leifeld J, Zimmermann M, Fuhrer J. 2008. Simulating decomposition of labile soil organic carbon: Effects of pH. *Soil Biology & Biochemistry* **40**: 2948-2951.

Mary B, Mariotti A, Morel JL. 1992. Use of ^{13}C variations at natural abundance for studying the biodegradation of root mucilage, roots and glucose in soil. *Soil Biology & Biochemistry* **24**: 1065-1072.

Murphy CJ, Baggs EM, Morley N, Wall DP, Paterson E. 2015. Rhizosphere priming can promote mobilisation of N-rich compounds from soil organic matter. *Soil Biology & Biochemistry* **81**: 236-243.

McLay CDA, Barton L, Tang C. 1997. Acidification potential of ten grain legume species grown in nutrient solution. *Australian Journal of Agricultural Research* **48**: 1025-1032

1. **Neumann G, Martinoia E. 2002**. Cluster roots: an underground adaptation for survival in extreme environments. *Trends in Plant Science* **7**: 162-167

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. *Ecology Letters* **14**: 187-194.

Quiquampoix H. 2000. Mechanisms of protein adsorption on surfaces and consequences for extracellular enzyme activity in soil. In: Bollag JM, Stotzky G, eds. *Soil Biochemistry*. Marcel Dekker, New York, 10, 171-206.

Rousk J, Brookes PC, Bååth E. 2009. Contrasting soil pH effects on fungal and bacterial growth suggests functional redundancy in carbon mineralisation. *Applied and Environmental Microbiology* **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. *The ISME Journal* **4**: 1340-1351.

Rukshana F, Butterly CR, Baldock JA, Xu JM, Tang C. 2012. Model organic compounds differ in priming effects on alkalinity release in soils through carbon and nitrogen mineralisation. *Soil Biology & Biochemistry* **51:** 35-43.

Sas L, Rengel Z, Tang C. 2001. Root morphology, excess cation uptake, and extrusion of proton and organic acid anions in *Lupinus albus* L. under phosphorus deficiency. *Plant Science* **160:** 1191-1198.

Schimel JP, Jackson LE, Firestone MK. 1989. Spatial and temporal effects on plant microbial competition for inorganic nitrogen in a California annual grassland. *Soil Biology & Biochemistry* **21:** 1059-1066.

Talbot JM, Allison SD, Treseder KK. 2008. Decomposers in disguise: Mycorrhizal fungi as regulators of soil C dynamics in ecosystems under global change. *Functional Ecology* **22**: 955-963.

Tang C, McLay CDA, Barton L. 1997. A comparison of the potential proton excretion of twelve pasture legumes grown in nutrient solution. *Australian Journal of Experimental Agriculture* **37:** 563-570.

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 Phytologist* **143**: 513-521.

Vance ED, Brookes PC, Jenkinson DJ. 1987. An extraction method for measuring soil microbial biomass C. *Soil Biology & Biochemistry* **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 and Soil* **248**: 187-197.

Wang X, Tang C, Guppy CN, Sale PWG. 2008. Phosphorus acquisition characteristics of cotton (*Gossypium hirsutum* L.), wheat (*Triticum aestivum* L.) and white lupin (*Lupinus albus* L.) under P deficient conditions. *Plant and Soil* **312**: 117-128.

Wichern F, Mayer J, Joergensen RG, Müller T. 2007. Rhizodeposition of C and N in peas and oats after $^{13}C^{15}N$ double labelling under field conditions. *Soil Biology & Biochemistry* **39**: 2527-2537.

Zhu B, Cheng W. 2011. Rhizosphere priming effect increases the temperature sensitivity of soil organic matter decomposition. *Global Change Biology* **17**: 2172-2183.

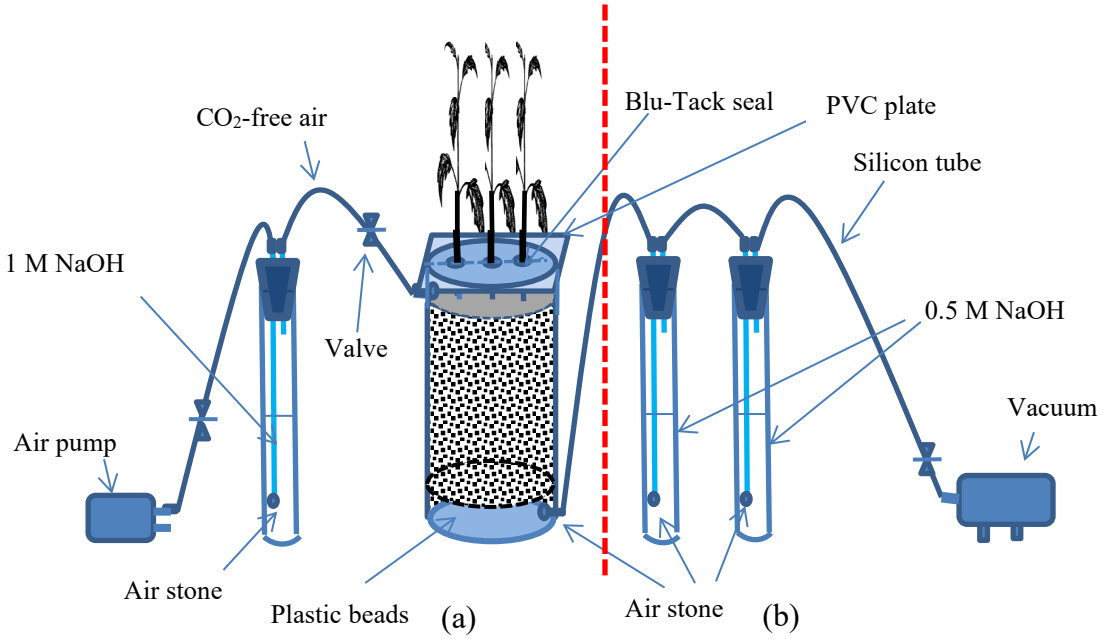

Figure 1. Trapping system of CO_2 released from belowground [(a): leak checking; (a) & (b): CO_2 trapping] (Modified from Cheng *et al.* (2003).

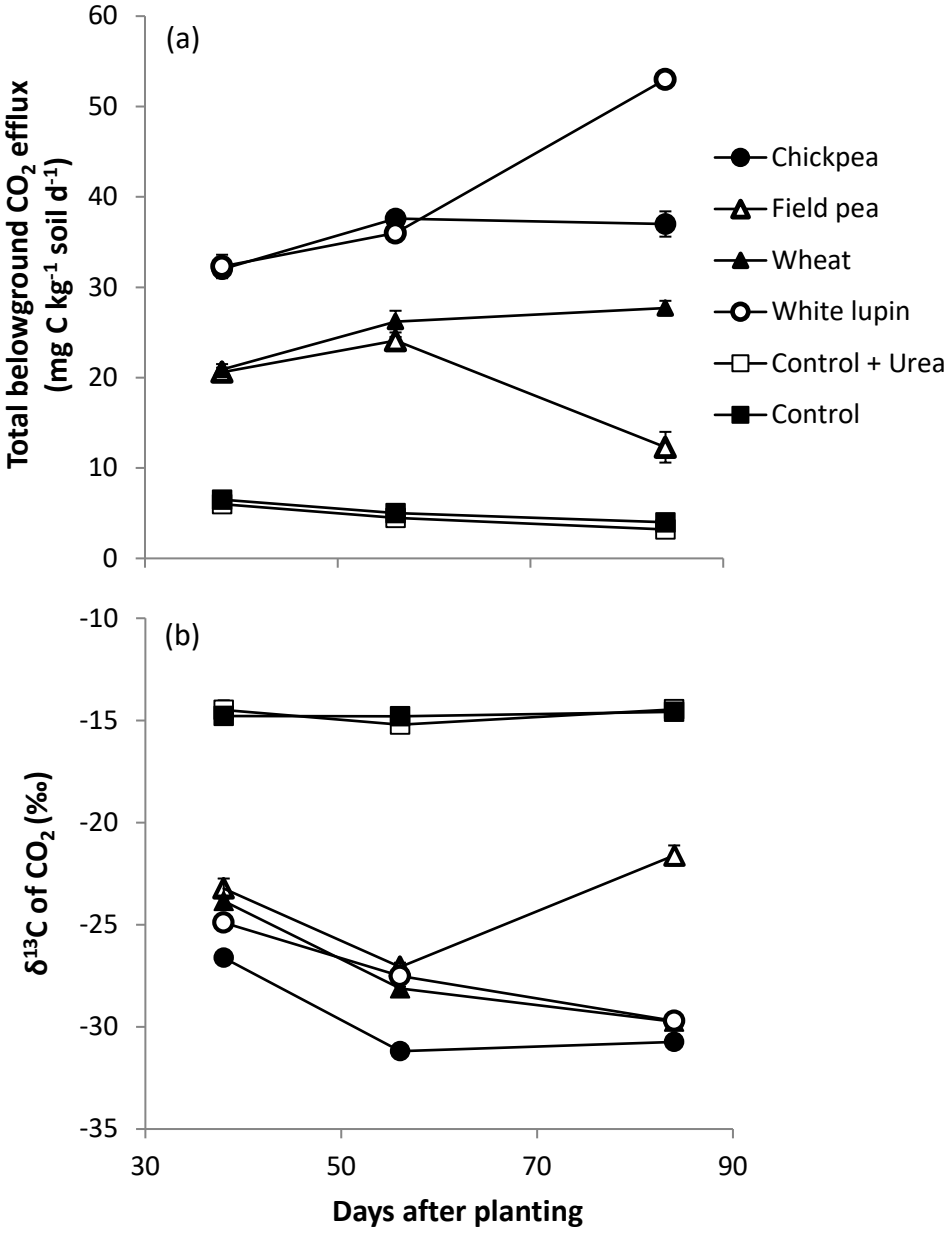

Figure 2. Total belowground CO_2 efflux (a) and $\delta^{13}C$ values of CO_2 (b) evolved from soil columns with chickpea, field pea, wheat, white lupin and non-planted controls at day 38, 56 and 84. Error bars represent standard error of means of four replicates.

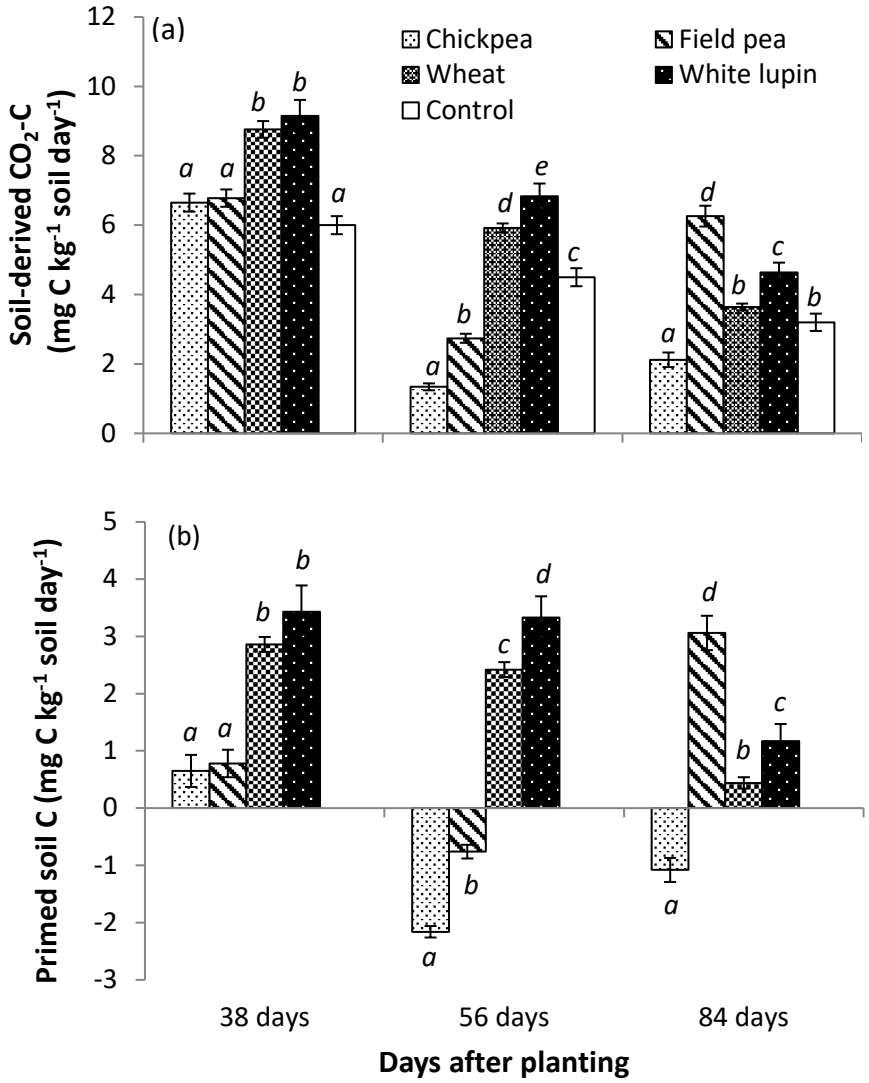

Figure 3. Soil-derived CO_2–C (a) and primed soil C (b) from soil columns under chickpea, field pea, wheat, white lupin and non-planted controls at days 38, 56 and 84. Error bars represent ± standard error of means of four replicates. For each panel, different italic letters above the bars indicate significant differences among species at each harvest time (Tukey's test, $P<0.05$).

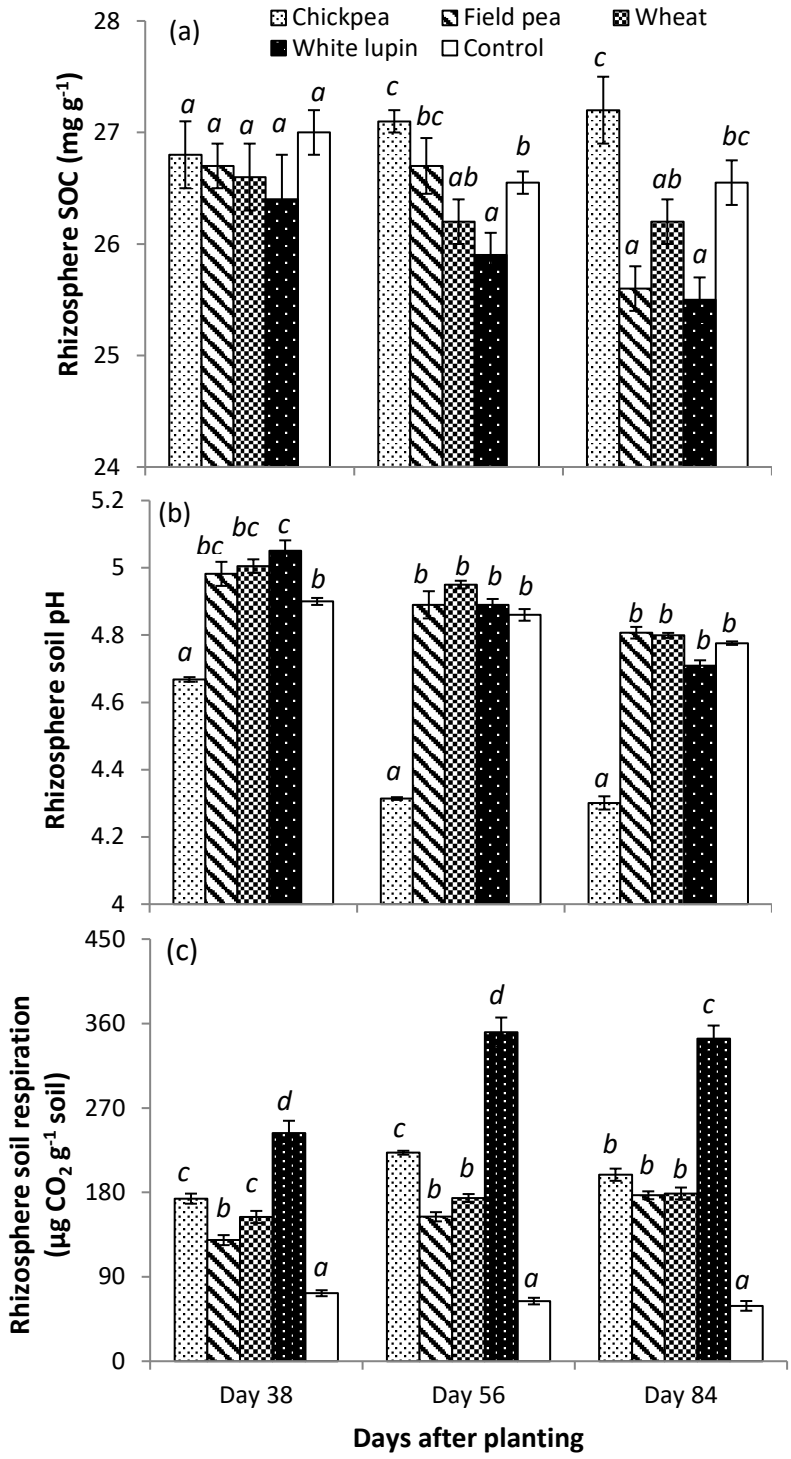

Figure 4. Soil organic C (SOC) (a), pH (b) and respiration (c) of the rhizosphere soil of chickpea, field pea, wheat, white lupin and non-planted controls at days 38, 56 and 84. Error bars represent ± standard error of means of three replicates. For each panel, different italic letters above the bars indicate significant differences among species at each harvest time (Tukey's test, $P<0.05$).

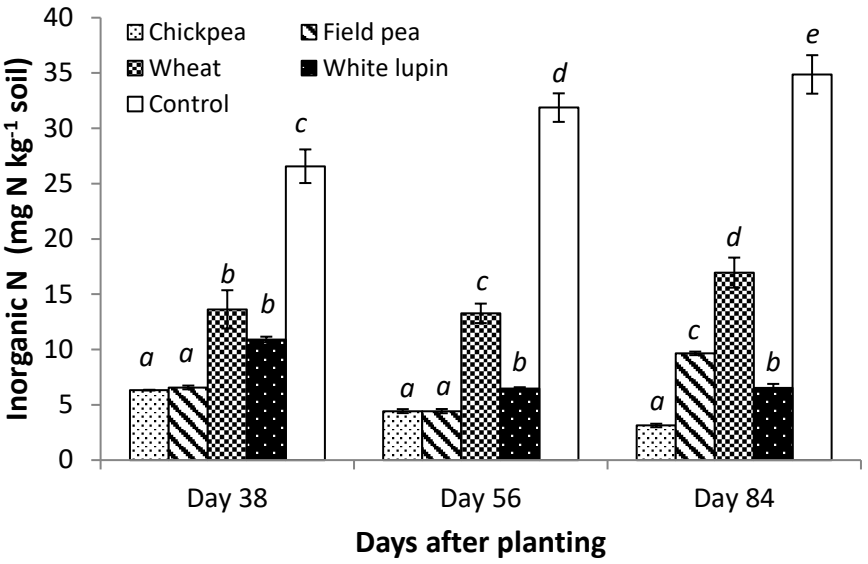

Figure 5. Inorganic N (NH_4^+ + NO_3^-) concentrations in the rhizosphere soil of chickpea, field pea, wheat, white lupin and non-planted controls at days 38, 56 and 84. Error bars represent ± standard error of means of three replicates. Different italic letters above the bars indicate significant differences among species at each harvest time (Tukey's test, $P<0.05$).

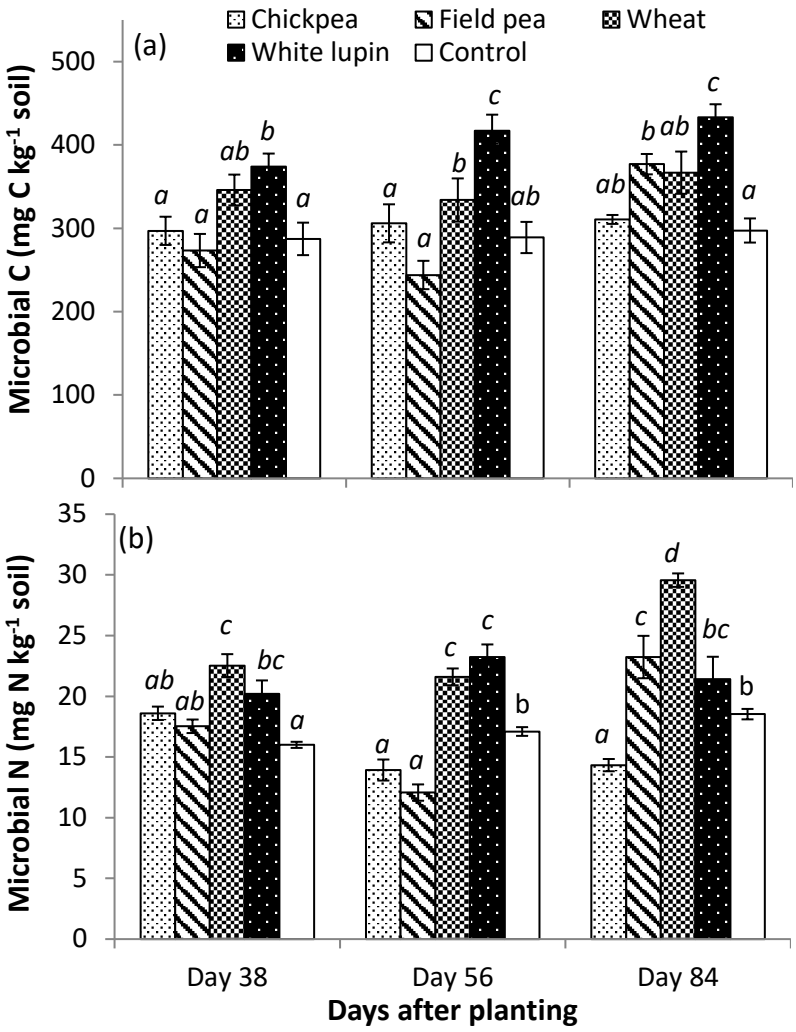

Figure 6. Microbial biomass C (a) and N (b) in the rhizosphere soil of chickpea, field pea, wheat, white lupin and non-planted controls at days 38, 56 and 84. Error bars represent ± standard error of means of three replicates. For each panel, different italic letters above the bars indicate significant differences among species at each harvest time (Tukey's test, $P<0.05$).

Table 1. Shoot and root biomass, N concentration, root length and $\delta^{13}C$ abundance of chickpea, field pea, wheat and white lupin grown for 38, 56 and 84 days. *, ** and *** represent $P<0.05$, $P<0.01$ and $P<0.001$, respectively. For each column, different letters indicate significant differences between means (two-way ANOVA, Tukey test, $P<0.05$).

Species	Growth stage	Biomass (g column^{-1})		N concentration (mg g^{-1})		Root length	$\delta^{13}C$ abundance (‰)	
		Shoot	Root	Shoot	Root	(m column^{-1})	Root	Root-derived CO_2
Day 38								
Chickpea	Late vegetation	5.00 b	2.28 e	29.5 c	27.5 de	144.0 c	-31.6 b	-29.8 bc
Field pea	Late vegetation	3.77 a	0.34 a	29.5 c	28.7 ef	30.0 ab	-28.5 e	-28.0 a
Wheat	Early vegetation	5.69 b	0.98 c	46.7 g	25.4 cd	224.2 d	-31.7 b	-30.6 c
White lupin	Early vegetation	3.15 a	0.42 ab	47.7 g	30.9 f	29.5 a	-30.4 c	-29.0 b
Day 56								
Chickpea	Flowering	12.29 d	3.19 f	26.7 b	28.6 ef	246.5 d	-32.5 a	-31.8 d
Field pea	Flowering	11.03 d	0.57 ab	22.7 a	21.7 ab	40.3 ab	-29.1 d	-28.8 ab
Wheat	Late vegetation	10.83 d	1.45 d	40.8 f	26.8 c	352.5 e	-32.6 a	-31.9 d
White lupin	Late vegetation	8.87 c	1.05 c	40.7 f	29.3 ef	33.4 ab	-31.4 b	-30.5 c
Day 84								
Chickpea	Podding	19.05 f	4.60 g	26.4 b	31.2 f	333.7 e	-32.7 a	-31.8 d
Field pea	Maturity	16.39 e	0.60 b	22.9 a	19.9 a	45.3 ab	-29.3 d	-28.8 ab
Wheat	Late flowering	15.51 e	2.29 e	33.7 d	23.6 bc	556.6 f	-32.8 a	-31.9 d
White lupin	Late flowering	11.67 d	1.46 d	37.0 e	31.1 f	55.1 b	-31.9 b	-30.8 cd
Two-way ANOVA								
Species		***	***	***	**	***	***	***
Harvest time		***	***	***	*	***	***	***
Species × Harvest time		***	***	***	***	***	***	**

Table 2. Parameters of multiple regressions of the rhizosphere priming effect (RPE) as a function of soil pH, microbial N and soil respiration.

Model	Parameter	Values	Significance level (*P* value)	Variation explained (%)
All data	Intercept	-19.26	<0.001	
	Soil pH	3.65	<0.001	44
	Microbial N	0.13	0.005	10
	r^2	0.54		
Harvest I	Intercept	-29.33	0.005	
	Soil respiration	0.02	0.002	40
	Soil pH	5.45	0.003	38
	r^2	0.78		
Harvest II	Intercept	-31.43	0.002	
	Microbial N	0.25	<0.001	77
	Soil pH	5.35	<0.001	16
	Soil respiration	0.007	0.035	3
	r^2	0.96		
Harvest III	Intercept	-27.09	0.005	
	Soil pH	6.01	0.004	
	r^2	0.54		