Annals of Botany
Volume 121: 143-151, 2018
doi:10.1093/aob/mcx138

The role of rhizosphere pH in regulating rhizosphere priming effect and implications for the availability of soil-derived nitrogen to plants

Running title: The role of rhizosphere pH in regulating rhizosphere priming effect

Xiaojuan Wang[1], Caixian Tang[1,*]

[1]Department of Animal, Plant and Soil Sciences, Centre for AgriBioscience, La Trobe University, Melbourne Campus, Bundoora, VIC 3086, Australia

*Author for correspondence:
Caixian Tang
Telephone: +61 3 9032 7416
Email: C.Tang@latrobe.edu.au

Abstract

- *Aims and Background* A comprehensive understanding of rhizosphere priming effects (RPE) on the decomposition of soil organic carbon (SOC) requires an integration of many factors. It is unclear how N form-induced change in soil pH affect RPE and SOC sequestration.
- *Methods* This study compared the change in RPE under supply of NO_3-N and NH_4-N. The effect of RPE on the mineralization of soil N and hence its availability to plant and microbes was also examined using ^{15}N-labelled N source.
- *Key Results* The supply of NH_4-N decreased rhizosphere pH by 0.16-0.38 units, and resulted in decreased or negative RPE. In contrast, NO_3-N nutrition increased rhizosphere pH by 0.19-0.78 units, and led to persistently positive RPE. The amounts of rhizosphere-primed C were positively correlated with rhizosphere pH. Rhizosphere pH affected RPE mainly through influencing microbial biomass, activity, and utilization of soluble organic C, and the availability of SOC to microbes. Furthermore, the amount of rhizosphere primed C correlated negatively with microbial biomass atom% ^{15}N (R^2 0.77-0.98, n=12), suggesting that microbes in the rhizosphere acted as the immediate sink for N released from enhanced SOC decomposition via RPE.
- *Conclusion* N form was an important factor affecting the magnitude and direction of RPE via its effect on rhizosphere pH. Rhizosphere pH needs to be considered in SOC and RPE modelling.

Key words: ^{13}C natural abundance, microbial N immobilization, N form, ^{15}N, rhizosphere acidification

Introduction

Rhizosphere effects can induce an increase or decrease in the decomposition of soil organic carbon (SOC), which is frequently referred to as positive or negative rhizosphere priming effect (RPE). These rhizosphere effects include root release of organic C and N substances, depletion of nutrients and water, or root-induced chemical changes such as soil pH (Kuzyakov, 2002; Hinsinger *et al.*, 2003). Particularly, root exudates can stimulate microbial growth and activity, generally leading to an increase in SOC turnover in the rhizosphere (Cheng and Kuzyakov, 2005; Dijkstra and Cheng, 2007; Phillips *et al.*, 2011). Plant traits such as plant phenology, shoot and root biomass (Fu and Cheng, 2002; Cheng *et al.*, 2003), and environmental factors such as light intensity (Kuzyakov and Cheng, 2001), temperature (Zhu and Cheng, 2011) and CO_2 concentration (Carney *et al.*, 2007) could have a great impact on the RPE through affecting the quantity or quality of root exudates.

Root-mediated changes in rhizosphere pH are a well-documented interaction between soil and root interface (Hinsinger *et al.*, 2003; Tang *et al.*, 2004), but its impact on RPE has been seldom studied. The release of protons is believed to be largely influenced by plant N nutrition. In general, ammonium (NH_4-N) addition leads to rhizosphere acidification because of excess uptake of cations over anions while nitrate (NO_3-N) nutrition causes rhizosphere alkalization (Hinsinger *et al.*, 2003). Ramirez *et al*. (2010) found that soil microbial respiration was closely correlated with changes in soil pH following application of different N source. Under field conditions, long-term SOC sequestration could be favoured by the acidity generated during NH_4-N oxidation (Malhi *et al.*, 1997; Zhang and Wang, 2012). Soil pH can affect the decomposition of SOC directly through affecting SOC solubility or indirectly via changes in microbial activity (Andersson *et al.*, 2000; Briedis *et al.*, 2012). This follows that the N-form-induced change in rhizosphere pH may also

have an impact on the root-induced decomposition of SOC, namely RPE. Addition of N either increases (Cheng and Johnson, 1998), or decreases (Liljeroth *et al.*, 1994) or has no effect on RPE (Cheng *et al.*, 2003). Contradictory mechanisms have been proposed to explain the observed effects of N on RPE (Kuzyakov *et al.*, 2000; Fontaine *et al.*, 2003). So far, it is not clear whether the influence of N addition on RPE also depends on the source of N applied due to its role in regulating rhizosphere pH.

While factors affecting RPE have been extensively examined, few studies have looked at how RPE may affect the mineralization of soil N and hence its availability for plant uptake. Owing to the close link of SOC decomposition with N mineralization, increased N availability has been expected in the rhizosphere following enhanced RPE (Murphy *et al.*, 2015; Rousk *et al.*, 2016). Under N-limited conditions, positive RPEs have been proposed as an adaptive behaviour of plants to gain N through microbial mineralization of N-rich SOC (Kuzyakov, 2002; Luo *et al.*, 2006; Dijkstra *et al.*, 2013). However, enhanced N immobilization has also been detected in the rhizosphere characterized by continuous supply of labile C via rhizodeposits (Zak *et al.*, 2000; Dijkstra *et al.*, 2013). Therefore, positive RPEs have resulted in both increased and decreased net N mineralization (Dijkstra *et al.*, 2009; Phillips *et al.*, 2011; Bengtson *et al.*, 2012). Even in the case of increased N mineralization, rhizosphere-primed C did not correlate with rhizosphere-primed N (Cheng, 2009; Dijkstra *et al.*, 2009). The effect of RPE on the contribution of soil-derived N to plant uptake remains largely unknown.

This study aimed to examine the change in RPE under supply of NO_3-N and NH_4-N. The application of different N sources was expected to produce a major difference in rhizosphere pH. The study also used ^{15}N-labelled N source to examine the fact of soil-derived N following enhanced RPE. We hypothesized that the plants supplied with NH_4-N would have lower RPEs than those fed with NO_3-N due to NH_4-induced rhizosphere acidification, in comparison to the alkalization caused by NO_3-N supply.

Materials and Methods

Soil

Topsoil (0-10 cm) was collected from a C_4 Kangaroo grassland (*Themeda triandra*) in Gulgong, New South Wales (32º 11' S, 149º 33' E). The soil was air-dried and sieved through a 2-mm sieve. The soil was a granite-derived sandy loam. It 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 $\delta^{13}C$ value of the SOC was -18.0 ‰.

Experiment set up

The experiment consisted of three plant treatments, two N forms and three replicates. The three plant treatments were wheat, white lupin and the no-plant control. Wheat (*Triticum aestivum* L. cv. Yitpi) and white lupin (*Lupinus albus* L. cv. Kiev) were grown in PVC columns (diameter 10 cm, height 40 cm) containing 2.8 kg air-dried C_4 soil. To prevent anaerobic conditions, a 3-cm layer of plastic beads sealed in nylon bags (mesh size 50 μm) was placed at the bottom portion of each column. Prior to planting, basal nutrients were mixed into soil at following rates (mg kg^{-1}): KH_2PO_4, 180; K_2SO_4, 120; $CaCl_2.2H_2O$, 180; $MgSO_4 \cdot 7H_2O$, 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; $Na[FeEDTA] \cdot 3H_2O$, 5.5. The two N forms were NH_4-N and NO_3-N, applied as $Ca(^{15}NO_3)_2$ (2 atom% ^{15}N) and $(^{15}NH_4)_2SO_4$ (2 atom% ^{15}N), respectively, at a rate of 30 mg N kg^{-1} at sowing. To minimize possible nitrification and maximise NH_4-N uptake by plants as the NH_4-N form, all N sources were applied twice weekly at rate of 15 mg N

kg^{-1} from week 4. The total amount of N supplied before each harvest was 90 and 240 mg N kg^{-1} at day 42 and 80, respectively. They were applied one day before the total CO_2-trapping, and three days before the destructive harvest. Soil columns without plants but with the two N forms (30 mg N kg^{-1} once at the start of the experiment) were included as no-plant controls. To test whether possible nitrification affected soil pH and consequently total amount of soil-derived CO_2 released from the control columns with N applied once, compared with planted columns with regular N additions, another set of control columns were set up to include five rates of NH_4-N (10, 30, 50, 70 and 100 mg N kg^{-1}). The columns were destructively harvested at the vegetative (day 42) and flowering stage (day 80) (three replicates at each time). An additional 8 pots (two species × two N form × two replicates) were filled with washed fine sand plus 10 g of C_3 soil as an inoculant, to account for isotopic fractionation between the root tissue and root-respired CO_2.

Planting and CO_2 trapping

The experiment was conducted in a controlled environmental room with 20 ºC and 18 ºC during the day and night, respectively, a light intensity of 400 µmol $m^{-2}s^{-1}$ and a day length of 14 h. Twelve germinated seeds of wheat and eight seeds of white lupin were sown in a row in the centre of each column. After emergence, plants were thinned to 6 per column for wheat, and 4 for white lupin. All the columns were weighed and maintained at 80% water-holding capacity by watering with reverse osmosis (RO) water for the soil columns and with the Hoagland solution (Hoagland and Arnon, 1950) for the sand columns. The sand columns had been watered with Hoagland solution twice daily to meet the requirement for water and nutrient by plants. A modified CO_2-trapping system (Wang *et al.*, 2016) was used to trap total below-ground CO_2 produced in each column three days before each harvest. Briefly, CO_2-free air was introduced through an air inlet at the top of each column after being pumped through 1 M NaOH via an air stone. The outlet, on the opposite side of the soil column, 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. The CO_2 was trapped for 48 h in 150 ml of 0.5 M NaOH at each harvest.

Harvest and measurements

At harvest, plant shoots were cut off at the soil surface for one column at a time. The rhizosphere soil was collected by gently shaking off soil adhered to the roots. All rhizosphere soil was sieved through 2-mm, and sub-samples were immediately put into a plastic bag and stored at 4 ºC for determination of soil microbial biomass C (MBC), microbial biomass N (MBN) and soil respiration. Another set of samples were air-dried for determination of soil pH, total organic C and N. Plant roots were carefully washed with water, and root length was quantified using WinRHIZO Pro 2003b (Regent Instruments, Quebec City, Canada) and an EPSON EU-35 scanner (Seiko Epson Corp, Suwa, Japan). All plant materials were oven-dried at 70 ºC, weighed and ground in a ball mill.

The $\delta^{13}C$ in the shoot and root samples was analysed using an isotope ratio mass spectrometer (IRMS) (Sercon 20-22, Crewe, UK). The C and N concentrations of shoot, root and rhizosphere soil were determined by dry combustion using a CHNS analyser (PerkinElmer EA2400, Shelton, USA). Soil pH was measured in 0.01 M $CaCl_2$ (1:5 soil solution ratio after end-over-end shaking for 1 h and centrifuging at 700 *g* for 10 min).

Microbial respiration in the rhizosphere soil was determined after 12-h incubation at 25ºC to give an indication of the quantity of substrates (e.g. root exudates) and microbial activity(Wang *et al.*, 2016). The CO_2 concentration within the headspace of jars was measured using an infrared gas analyser (Servomex 4210 Industrial Gas Analyser, Cowborough, UK) (Rukshana *et al.*, 2012). The MBC and MBN were determined following the chloroform fumigation-extraction procedure according to Vance et al. (Vance *et al.*, 1987a). Briefly, 8 g of fumigated or non-fumigated moist soil was extracted using 40 ml of 0.5 M K_2SO_4. Total soluble organic C in the extracts was determined colorimetrically following wet digestion with dichromate-sulfuric acid at 135 ºC for 30 min (Cai *et al.*, 2011). Total soluble N was determined by persulphate oxidation of both fumigated and non-fumigated extracts (Cabrera and Beare, 1993), and the final concentrations of inorganic N (NH_4^+ + NO_3^-) were measured using a QuickChem 8500 flow injection analyser (Lachat Instruments, Loveland, CO, USA). The MBC and MBN were calculated as the difference in concentrations of total organic C and total N in extracts between fumigated and non-fumigated soils, adjusted by a proportionality coefficient of 0.45 (Jenkinson *et al.*, 2004). Soil inorganic N was measured on the non-fumigated and non-oxidized 0.5 M K_2SO_4 extracts using the flow injection analyser. Before microbial biomass ^{13}C and ^{15}N determination by mass spectrometry, both fumigated and un-fumigated K_2SO_4 extracts were freeze-dried, and the dried salts were finely ground using mortar and pestle (Dijkstra *et al.*, 2006b).

The amount of CO_2 trapped in 0.5 M NaOH was determined by titrating 10 ml of NaOH solution with 0.5 M HCl after addition of 5 ml 1 M $BaCl_2$. To produce $SrCO_3$ precipitates for $\delta^{13}C$ analysis, 5 ml of 1 M $SrCl_2$ was added to another 10 ml of NaOH solution. The suspension pH was then adjusted to 7.0 using 0.3 M HCl. The $\delta^{13}C$ of the $SrCO_3$ was analysed by mass spectrometry.

Calculations and statistical analysis

To partition the total CO_2 efflux using the ^{13}C natural abundance method, the following equation was applied(Mary *et al.*, 1992):

$$C_4 = C_t \times (\delta_t - \delta_3)/(\delta_4 - \delta_3)$$

$$C_{primed}=C_4-C_{control}$$

where C_t is the total C from below-ground CO_2, C_4 is the amount of CO_2-C derived from C_4 soil, δ_t is the $\delta^{13}C$ value of the C_t, δ_3 is the $\delta^{13}C$ value of the root-derived C (CO_2 trapped from the sand columns), and δ_4 is the $\delta^{13}C$ value of the C_4 soil C (CO_2 trapped from the no-plant control columns). $C_{control}$ is the amount of CO_2-C evolved from the column without plants. C_{primed} is the total primed C.

The isotope signature of microbial biomass was calculated as follows (Dijkstra *et al.*, 2006b):

$$\delta^{13}C_{MB} = (\delta^{13}C_F*C_F - \delta^{13}C_{UF}*C_{UF})/C_{MB}$$

$$Atom\%^{15}N_{MB} = (atom\%\ ^{15}N_F*N_F - atom\%\ ^{15}N_{UF}*N_{UF})/N_{MB}$$

where MB, F and UF are microbial biomass, fumigated and un-fumigated fractions, respectively. C_F, N_F, C_{UF} and N_{UF} are total dissolved organic C and N in the fumigated and un-fumigated extracts, respectively.

A two-way ANOVA was conducted using Genstat (11th Version) (VSN International, Hemel Hempstead, UK) to assess the effect of N form and plant species at each harvest. While necessary, square-root transformation of data was performed to obtain a normal distribution. Significant ($P \leq 0.05$) differences between means were identified using the Tukey's HSD test. Single linear regression analyses were used to determine the relationship between rhizosphere primed C and soil pH or atom% ^{15}N in microbial biomass.

Results

Nitrogen form did not affect the shoot dry weight of either species. However, compared with NO_3-N, NH_4-N nutrition decreased root dry weight and total root length of wheat by 16% and 28%, respectively, at day 80 (Table 1). The concentration of N in the shoot or root did not differ between the two N forms for both species. The $\delta^{13}C$ value of CO_2 trapped from the sand column (root-derived C) receiving NO_3-N was 1.2 to 2.7‰ more enriched than that of the root tissues, indicating an isotopic fractionation between the root tissue and root-respired CO_2 (Table 1). Considering that $\delta^{13}C$ value of root tissue was not affected by N forms, the $\delta^{13}C$ value of CO_2 trapped from the sand columns with NO_3-N addition was used to represent the root-derived C for both N treatments. Shoot biomass of both species in the sand columns was lower when N was applied as NH_4-N compared with NO_3-N (data not shown), probably due to low rhizosphere pH in this poorly-buffered system.

When compared with the no-plant control, the rhizosphere pH was 0.37 units lower at both harvests of wheat fed with NH_4-N, but was 0.19 and 0.78 units higher for wheat fed with NO_3-N at days 42 and 80, respectively (Fig. 1). The supply of NH_4-N decreased soil pH by 0.16-0.39 units in the rhizosphere of white lupin, relative to the no-plant control, in contrast to alkalization of 0.2-0.4 units under NO_3-N supply. Both soil pH and total CO_2 production from the control columns were not affected by the rate of NH_4-N (data not shown) or N form (Figs. 1, 2A). Little change in soil pH following addition of NH_4-N at different rates was mainly attributed to the relatively high pH buffer capacity of the C4 soil used in this study.

Total below-ground CO_2 efflux was 17-18% lower under NH_4-N than NO_3-N addition in all planted columns, except that N form did not affect the CO_2 efflux under wheat at day 42 (Fig. 2A). The $\delta^{13}C$ value of CO_2 showed no difference between two N sources for white lupin and the no-plant control, but decreased by 0.9‰ for wheat fed with NH_4-N relative to NO_3-N (Fig. 2B). The CO_2 collected from the control column had consistent $\delta^{13}C$ value of -13.2‰ during the whole experiment. Regardless of plant species, N form had a significant impact on the decomposition of SOC and RPE. Decreased or negative RPE was observed for the plants fed with NH_4-N while consistently higher and positive RPE was shown for the plants fed with NO_3-N (Fig. 2C). The amounts of rhizosphere-primed C were positively correlated with rhizosphere pH (P<0.05) (Fig. 3A), and negatively correlated with the atom% ^{15}N in the microbial biomass (P<0.05) (Fig. 3B, C). Other soil properties such as extractable organic C, soil respiration and microbial C and N were not correlated with primed C, when the regression analysis was performed for all data. The SOC concentration in the rhizosphere of plant fed with NH_4-N was 3-5% higher (P<0.05) than that of NO_3-N (Table 2).

The K_2SO_4-extractable organic C (EOC) was higher in the rhizosphere of plants fed with NH_4-N than NO_3-N, except for wheat at day 42 (Table 2). Noticeably, EOC in the rhizosphere of white lupin was 72 and 83% higher under NH_4-N nutrition than the no-plant control at days 42 and 80, respectively. Wheat showed little effect on EOC, except 51% decrease at day 80 when fed with NO_3-N. The rhizosphere soil respiration during 12-h incubation was invariably higher in the planted columns than the no-plant controls, and in the rhizosphere of white lupin than wheat. When compared between two N forms, soil respiration was lower under NH_4-N than NO_3-N nutrition at day 80 for both species (Table 2).

The effect of N form on MBC and MBN in the rhizosphere soil differed between wheat and white lupin (Table 2). Microbial biomass C and MBN in the rhizosphere of white lupin supplied with NO_3-N increased by 46% and 68%, respectively, relative to the no-plant control, but remained less changed for wheat. The addition of NH_4-N showed no effect on MBC, but decreased MBN in

the rhizosphere of wheat, compared to the control. For both species, MBC and MBN were invariably lower under NH_4-N than NO_3-N supply.

The ^{15}N abundance in the extractable inorganic N in the rhizosphere did not differ between two N sources except for white lupin at day 80 (Fig. 4A). Nitrogen form greatly affected the ^{15}N enrichment in microbial biomass and plants (Fig. 4B, C). Microbial biomass was less enriched with ^{15}N when NO_3-N was applied, in comparison with NH_4-N (Fig. 4B). Nevertheless, an opposite trend was shown in the plant shoot at day 42, although no effect of N form was detected at day 80 (Fig. 4C). In the no-plant control and bulk soil, N form did not affect microbial atom%^{15}N.

Discussion

This study revealed that the supply of NO_3-N led to enhanced RPEs in contrast to decreased or negative RPEs caused by NH_4-N addition. The higher SOC concentration in the rhizosphere of plant fed with NH_4-N than NO_3-N provided further evidence of reduced SOC decomposition by NH_4-N nutrition. This was also consistent with the enhancement of SOC sequestration due to NH_4-N fertilization reported by other studies (McAndrew and Malhi, 1992; Malhi *et al.*, 1997; Zhang and Wang, 2012). Furthermore, the C_4 soil used in this study was a mixture of different SOC pools with different $\delta^{13}C$ values, e.g. the easily decomposable C was more ^{13}C enriched (-13.2‰) than non-labile C fractions associated with soil minerals (-18.8‰) (Wang *et al.*, 2016). If the persistent positive RPE resulted in the decomposition of more stable SOC at the late stage, NO_3-induced increase in RPE might have been underestimated due to the use of $\delta^{13}C$ values of soil-derived CO_2 for the non-planted control columns (-13.2‰). Decreased or negative RPE following NH_4-N addition should be less affected by the non-uniformity of the ^{13}C signature among different SOC fractions. As indicated by the constant $\delta^{13}C$ values of CO_2 evolved from the control columns with time, decomposition of SOC in the absence of plant was mainly from labile C pools. Low or negative RPEs mean that the quantity of soil-derived CO_2 from the planted columns was similar to or less than that from the control. Overall, N form had a great impact on both the magnitude and direction of RPE, and future evaluation of the effect of N addition on RPE should also consider the form of N sources.

The effects of N form on plant biomass and root exudation (indicated by EOC and rhizosphere microbial respiration) could not account for the significant changes in the RPE. Many plant species had shown a slower growth when N was applied as NH_4-N relative to NO_3-N (Raab and Terry, 1994; Britto and Kronzucker, 2002; Guo *et al.*, 2002). In this study, the negative effects of NH_4-N on plant growth were confirmed by decreases in root biomass and length for wheat plants at day 80. There is evidence to suggest that plants with higher shoot or root biomass generally produced greater RPE (Fu and Cheng, 2002; Dijkstra *et al.*, 2006a), however, RPE showed no correlation with shoot and root biomass in this study. For instance, restricted root growth by NH_4-N nutrition was detected only for wheat at day 80, but decreased or negative RPE occurred for both species at both harvests. This was in line with our previous finding that plant biomass played a minor role in accounting for RPE of species with contrasting effect on soil acidification (Wang *et al.*, 2016). On the other hand, NH_4-N rather than NO_3-N nutrition could increase root exudation by plant roots, due to NH_4-induced changes in root architecture such as increased branching (Martins-Loucao *et al.*, 2000), cell death in root cortex (Brown and Hornby, 1987) or increases membrane permeability by low pH (Yin and Raven, 1998). If N form-induced changes in the quantity of root exudates was mainly responsible for the differences in RPE between two N forms, RPE would be higher under NH_4-N than NO_3-N nutrition. Finally, no differences were detected in either 12-h soil respiration

or 48-h total CO_2 efflux between NH_4-N and NO_3-N-amended controls with no plants, suggesting N form *per se* had less impact on microbial decomposition of SOC.

It is evident that rhizosphere pH was positively correlated with the amount of rhizosphere-primed C. As expected, NH_4-N nutrition caused rhizosphere acidification with pH decreasing by up to 0.38 units. This had resulted from H^+ production during excess uptake of cations over anions by plant roots and/or, to a lesser extent, nitrification (Malhi *et al.*, 1991). In contrast, NO_3-N nutrition led to rhizosphere alkalisation (increasing pH by up to 0.78 units), due to excess uptake of anions over cations (Tang *et al.*, 1999). In decomposition studies, greater positive priming effect was detected with increasing soil pH (Luo *et al*., 2011; Perelo and Munch 2005), and the optimum pH lay between 6 to 8 (Blagodatskaya and Kuzyakov, 2008; Nang *et al*., 2017). Our previous study also found that rhizosphere pH was mainly responsible for the variation in RPE among crop species differing in rhizosphere acidification (from pH 4.1 to 5.2) (Wang *et al.*, 2016). It is expected that the addition of NH_4-N could decrease RPE more in acid soils than in the neutral or alkaline soils.

The N source-induced changes in rhizosphere pH could affect RPE through the following ways. Firstly, low rhizosphere pH greatly decreased microbial activity and growth (Figure 5), as indicated by lower soil respiration and microbial biomass C and N under NH_4-N than NO_3-N supply. In other studies, the effect of soil pH on SOC decomposition was mainly via its effect on microbial growth and activity (Andersson *et al.*, 2000; Briedis *et al.*, 2012), and the turnover of SOC correlated positively with soil pH in the range of 4.3 to 5.3 (Leifeld *et al.*, 2008). Secondly, decreased microbial biomass and activity at low pH possibly led to inefficient use of root exudates or soluble SOC, which accounted for the greater accumulation of EOC in the rhizosphere of plants fed with NH_4-N than NO_3-N (Figure 5). Negative relationships between soil pH and EOC were also observed by other studies (Vance *et al.*, 1987b; Pietri and Brookes, 2008). Finally, addition of NO_3-N might have favoured microbial utilization of SOC due to increased availability of soluble organic C at higher pH. Increases in the solubilisation or desorption of humic substances from the surface of mineral colloids were often detected following elevated soil pH by liming (Andersson *et al.*, 2000; Garbuio *et al.*, 2011). The activation of microbes by root exudates was believed to be the main mechanism responsible for the enhanced RPE (Kuzyakov *et al.*, 2000), however, low soil pH following NH_4-N addition decreased microbial activity regardless of the quantity of root exudates.

Microbes in the rhizosphere acted as an immediate sink for N released from enhanced SOC decomposition via RPE. The increase in both MBN and MBC indicated that microbial immobilization of N was enhanced by increased microbial growth. Moreover, decreased ^{15}N abundance in microbes under NO_3-N than NH_4-N supply possibly reflected a greater dilution of ^{15}N at higher RPE and an enhanced contribution of soil-derived N, relative to fertiliser-derived N. This was directly supported by the negative correlation between primed C and microbial biomass ^{15}N abundance in the rhizosphere of both species. The recovery of ^{15}N in microbial biomass in the unplanted columns (Fig. 4C) and in the bulk soil of the planted columns (data not shown) did not differ between two N sources, indicating a lack of microbial discrimination between two N forms. Norton and Firestone (1996) and Inselsbacher *et al*. (2010) also found that N form *per se* did not affect the amount of N assimilated by microbes. Immediate and rapid utilization of soil-derived N following positive RPE by microbes was possible (Figure 5), given the greater substrate affinities, larger specific surface area and greater spatial distribution of microbes than plant roots (Lipson and Näsholm, 2001; Kaštovská and Šantrůčková, 2011). Moreover, amino acids released during the decomposition of SOC could be more efficiently acquired by microbes before they were mineralized and came into soil solution for plant uptake (Kuzyakov and Xu, 2013). Little

difference in atom% ^{15}N in the extractable inorganic N in the rhizosphere between two N forms, in most cases, might also reflect a rapid immobilization of soil-derived N by microbes in the case of enhanced RPE.

Enhanced RPE under NO_3-N nutrition did not result in a greater contribution of soil-derived N to plant uptake. Higher ^{15}N atom% in white lupin fed with NO_3-N than NH_4-N at day 42 might indicate a faster uptake of NO_3-N because of its higher mobility in the soil (Norton and Firestone, 1996; Burger and Jackson, 2003; Song *et al.*, 2007). Also, plants fed with NH_4-N might have fixed more N_2 than those with NO_3-N, due to the slower uptake or less effectiveness of NH_4-N at suppressing nitrogenase activity (Silsbury *et al.*, 1986; Svenning *et al.*, 1996). On the other hand, if N mineralized via RPE under NO_3-N nutrition was rapidly captured by microbes at the first place, the availability of soil-derived N to plants would decrease. Other studies also found that enhanced RPE did not result in apparent increases in plant N uptake due to short-term N immobilization (Cheng, 2009; Dijkstra *et al.*, 2011; Kuzyakov and Xu, 2013). Nevertheless, N immobilized by microbes with a short turnover time (days to weeks) could eventually be transferred to the forms that can be utilized by plants (Hodge *et al.*, 2000; Cheng and Kuzyakov, 2005; Harrison *et al.*, 2007). Overall, the contribution of RPE to plant N uptake in this study was complicated and might have been regulated by many factors. Further studies are required to quantify the partitioning of SOC-derived N between microbes and plants in response to enhanced RPE during short and long-term periods.

Conclusion

Our results revealed that N form affected RPE mainly through its impact on rhizosphere pH. Rhizosphere acidification under NH_4-N resulted in decreased or negative RPEs and hence favoured SOC accumulation, while alkalization in the supply of NO_3-N enhanced RPE and hence SOC mineralization. Noticeably, microbes in the rhizosphere acted as the immediate sink for N released during SOC decomposition. Enhanced microbial N immobilization under NO_3-N supply might delay plant uptake of soil N under field conditions. The role of N fertilization on SOC sequestration should take into account the apparent effect of N form-induced pH change on RPE. Under field conditions, addition of organic amendments or crop residue returning is essential to replenish SOC lost via RPE under NO_3-N supply. This study also suggests that rhizosphere pH needs to be considered in SOC and RPE modelling.

Acknowledgments

This research was supported under Australian Research Council's Discovery Projects funding scheme (project DP120104100). We thank Dr Clayton Butterly and anonymous reviewers for their comments on the manuscript and Dr Anan Wang for technical support.

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 and Biochemistry* **32**: 1-10.

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.

Briedis C, de Moraes Sá JC, Caires EF, *et al.* 2012. Soil organic matter pools and carbon-protection mechanisms in aggregate classes influenced by surface liming in a no-till system. *Geoderma* **170**: 80-88.

Britto DT, Kronzucker HJ. 2002. NH_4^+ toxicity in higher plants: a critical review. *Journal of Plant Physiology* **159**: 567-584.

Brown ME, Hornby D. 1987. Effects of nitrate and ammonium on wheat roots in gnotobiotic culture: amino acids, cortical cell death and take-all (caused by *Gaeumannomyces graminis* var. *tritici*). *Soil Biology and Biochemistry* **19**: 567-573.

Burger M, Jackson LE. 2003. Microbial immobilization of ammonium and nitrate in relation to ammonification and nitrification rates in organic and conventional cropping systems. *Soil Biology and Biochemistry* **35**: 29-36.

Cabrera M, Beare M. 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.

Carney KM, Hungate BA, Drake BG, Megonigal JP. 2007. Altered soil microbial community at elevated CO_2 leads to loss of soil carbon. *Proceedings of the National Academy of Sciences* **104**: 4990-4995.

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

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

Cheng W, Johnson DW, Fu S. 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: S W, R Z, eds. *Roots and Soil Management: Interactions Between Roots and the Soil, Agronomy Monograph No. 48,* . Madison, Wisconsin, USA: American Society of Agronmy.

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 Biology and Biochemistry* **41**: 1080-1087.

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

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

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

Dijkstra FA, Hutchinson GL, Reeder JD, LeCain DR, Morgan JA. 2011. Elevated CO_2, but not defoliation, enhances N cycling and increases short-term soil N immobilization regardless of N addition in a semiarid grassland. *Soil Biology and Biochemistry* **43**: 2247-2256.

Dijkstra P, Ishizu A, Doucett R, *et al.* 2006b. ^{13}C and ^{15}N natural abundance of the soil microbial biomass. *Soil Biology and Biochemistry* **38**: 3257-3266.

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

Fu S, Cheng W. 2002. Rhizosphere priming effects on the decomposition of soil organic matter in C4 and C3 grassland soils. *Plant and Soil* **238**: 289-294.

Garbuio FJ, Jones DL, Alleoni LR, Murphy DV, Caires EF. 2011. Carbon and nitrogen dynamics in an Oxisol as affected by liming and crop residues under no-till. *Soil Science Society of America Journal* **75**: 1723-1730.

Guo S, Brück H, Sattelmacher B. 2002. Effects of supplied nitrogen form on growth and water uptake of French bean (*Phaseolus vulgaris* L.) plants. *Plant and Soil* **239**: 267-275.

Harrison KA, Bol R, Bardgett RD. 2007. Preferences for different nitrogen forms by coexisting plant species and soil microbes. *Ecology* **88**: 989-999.

Hinsinger P, Plassard C, Tang C, Jaillard B. 2003. Origins of root-mediated pH changes in the rhizosphere and their responses to environmental constraints: a review. *Plant and soil* **248**: 43-59.

Hodge A, Robinson D, Fitter A. 2000. Are microorganisms more effective than plants at competing for nitrogen? *Trends in plant science* **5**: 304-308.

Inselsbacher E, Umana NH-N, Stange FC, *et al.* 2010. Short-term competition between crop plants and soil microbes for inorganic N fertilizer. *Soil Biology and Biochemistry* **42**: 360-372.

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

Kaštovská E, Šantrůčková H. 2011. Comparison of uptake of different N forms by soil microorganisms and two wet-grassland plants: A pot study. *Soil Biology and Biochemistry* **43**: 1285-1291.

Kuzyakov Y. 2002. Review: factors affecting rhizosphere priming effects. *Journal of Plant Nutrition and Soil Science* **165**: 382.

Kuzyakov Y, Cheng W. 2001. Photosynthesis controls of rhizosphere respiration and organic matter decomposition. *Soil Biology and Biochemistry* **33**: 1915-1925.

Kuzyakov Y, Friedel J, Stahr K. 2000. Review of mechanisms and quantification of priming effects. *Soil Biology and 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 and Biochemistry* **40**: 2948-2951.

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 and Soil* **161**: 233-240.

Lipson D, Näsholm T. 2001. The unexpected versatility of plants: organic nitrogen use and availability in terrestrial ecosystems. *Oecologia* **128**: 305-316.

Luo Y, Field CB, Jackson RB. 2006. Does nitrogen constrain carbon cycling, or does carbon input stimulate nitrogen cycling? *Ecology* **87**: 3-4.

Luo Y, Durenkamp M, De Nobili M, Lin Q, Brookes P. 2011. Short term soil priming effects and the mineralisation of biochar following its incorporation to soils of different pH. *Soil Biology and Biochemistry* **43**: 2304-2314.

Malhi S, Nyborg M, Harapiak J, Flore N. 1991. Acidification of soil in Alberta by nitrogen fertilizers applied to bromegrass. *Plant-Soil Interactions at Low pH*. The Netherlands: Kluwer Academic.

Malhi S, Nyborg M, Harapiak J, Heier K, Flore N. 1997. Increasing organic C and N in soil under bromegrass with long-term N fertilization. *Nutrient Cycling in Agroecosystems* **49**: 255-260.

Martins-Loucao MA, Cruz C, Correia PM. 2000. New approaches to enhanced ammonium assimilation in plants. *Nitrogen in a Sustainable Ecosystem–From the Cell to the Plant. Eds. MA Martins-Loução and SH Lips*. Leiden: Backhuy.

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

McAndrew D, Malhi S. 1992. Long-term N fertilization of a solonetzic soil: effects on chemical and biological properties. *Soil Biology and Biochemistry* **24**: 619-623.

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 and Biochemistry* **81**: 236-243.

Nang SA, Butterly CR, Sale PWG, Tang C. 2017. Residue addition and liming history interactively enhance mineralization of native organic carbon in acid soils. *Biology and Fertility of Soils* **53**: 61-75.

Norton JM, Firestone MK. 1996. N dynamics in the rhizosphere of Pinus ponderosa seedlings. *Soil Biology and Biochemistry* **28**: 351-362.

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.

Perelo LW, Munch JC. 2005. Microbial immobilisation and turnover of ^{13}C labelled substrates in two arable soils under field and laboratory conditions. *Soil biology and Biochemistry* **37**: 2263-2272.

Pietri JA, Brookes P. 2008. Relationships between soil pH and microbial properties in a UK arable soil. *Soil Biology and Biochemistry* **40**: 1856-1861.

Raab TK, Terry N. 1994. Nitrogen source regulation of growth and photosynthesis in *Beta vulgaris* L. *Plant Physiology* **105**: 1159-1166.

Ramirez KS, Craine JM, Fierer N. 2010. Nitrogen fertilization inhibits soil microbial respiration regardless of the form of nitrogen applied. *Soil Biology and Biochemistry* **42**: 2336-2338.

Rousk K, Michelsen A, Rousk J. 2016. Microbial control of soil organic matter mineralization responses to labile carbon in subarctic climate change treatments. *Global change biology*.

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

Silsbury J, Catchpoole D, Wallace W. 1986. Effects of nitrate and ammonium on nitrogenase (C_2H_2 reduction) activity of swards of subterranean clover, Trifolium subterraneum L. *Functional Plant Biology* **13**: 257-273.

Song M, Xu X, Hu Q, Tian Y, Ouyang H, Zhou C. 2007. Interactions of plant species mediated plant competition for inorganic nitrogen with soil microorganisms in an alpine meadow. *Plant and Soil* **297**: 127-137.

Svenning MM, Junttila O, Macduff JH. 1996. Differential rates of inhibition of N_2 fixation by sustained low concentrations of NH_4^+ and NO_3^- in northern ecotypes of white clover (Trifolium repens L.). *Journal of Experimental Botany* **47**: 729-738.

Tang C, Drevon J, Jaillard B, Souche G, Hinsinger P. 2004. Proton release of two genotypes of bean (*Phaseolus vulgaris* L.) as affected by N nutrition and P deficiency. *Plant and Soil* **260**: 59-68.

Tang C, Unkovich M, Bowden J. 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 E, Brookes P, Jenkinson D. 1987a. An extraction method for measuring soil microbial biomass C. *Soil biology and Biochemistry* **19**: 703-707.

Vance E, Brookes P, Jenkinson D. 1987b. Microbial biomass measurements in forest soils: the use of the chloroform fumigation-incubation method in strongly acid soils. *Soil Biology and Biochemistry* **19**: 697-702.

Wang X, Tang C, Severi J, Butterly C, Baldock J. 2016. Rhizosphere priming effect on soil organic carbon decomposition under plant species differing in soil acidification and root exudation. *New Phytologist*.

Yin Z-H, Raven JA. 1998. Influences of different nitrogen sources on nitrogen-and water-use efficiency, and carbon isotope discrimination, in C3 Triticum aestivum L. and C4 Zea mays L. plants. *Planta* **205**: 574-580.

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 Phytologist* **147**: 201-222.

Zhang W, Wang S. 2012. Effects of NH_4^+ and NO_3^- on litter and soil organic carbon decomposition in a Chinese fir plantation forest in South China. *Soil Biology and Biochemistry* **47**: 116-122.

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

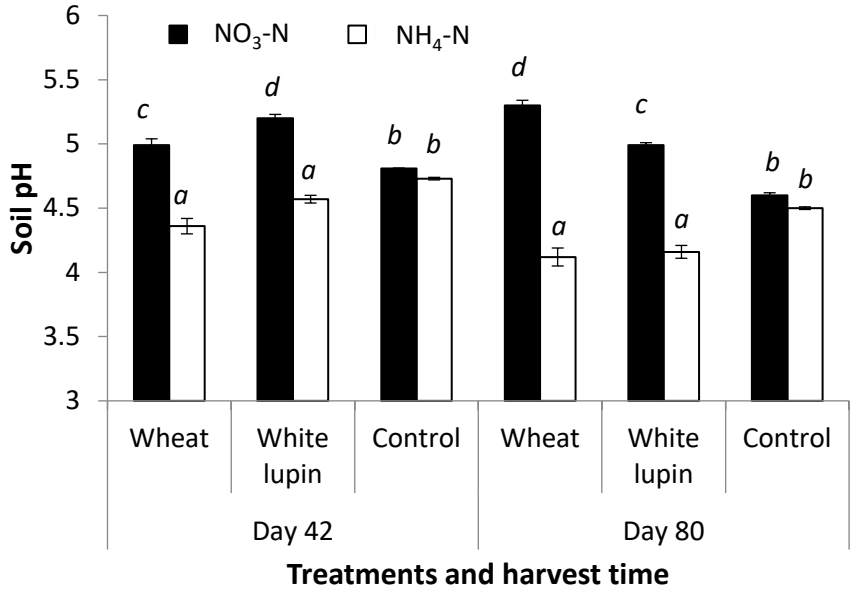

Figure 1. Soil pH in the no-plant control and rhizosphere soil of wheat and white lupin supplied with NO_3-N or NH_4-N at days 42 and 80. Error bars represent ± standard error of means of three replicates. Different italic letters above the bars indicate significant differences among treatments at each harvest time (Tukey's test, $P<0.05$).

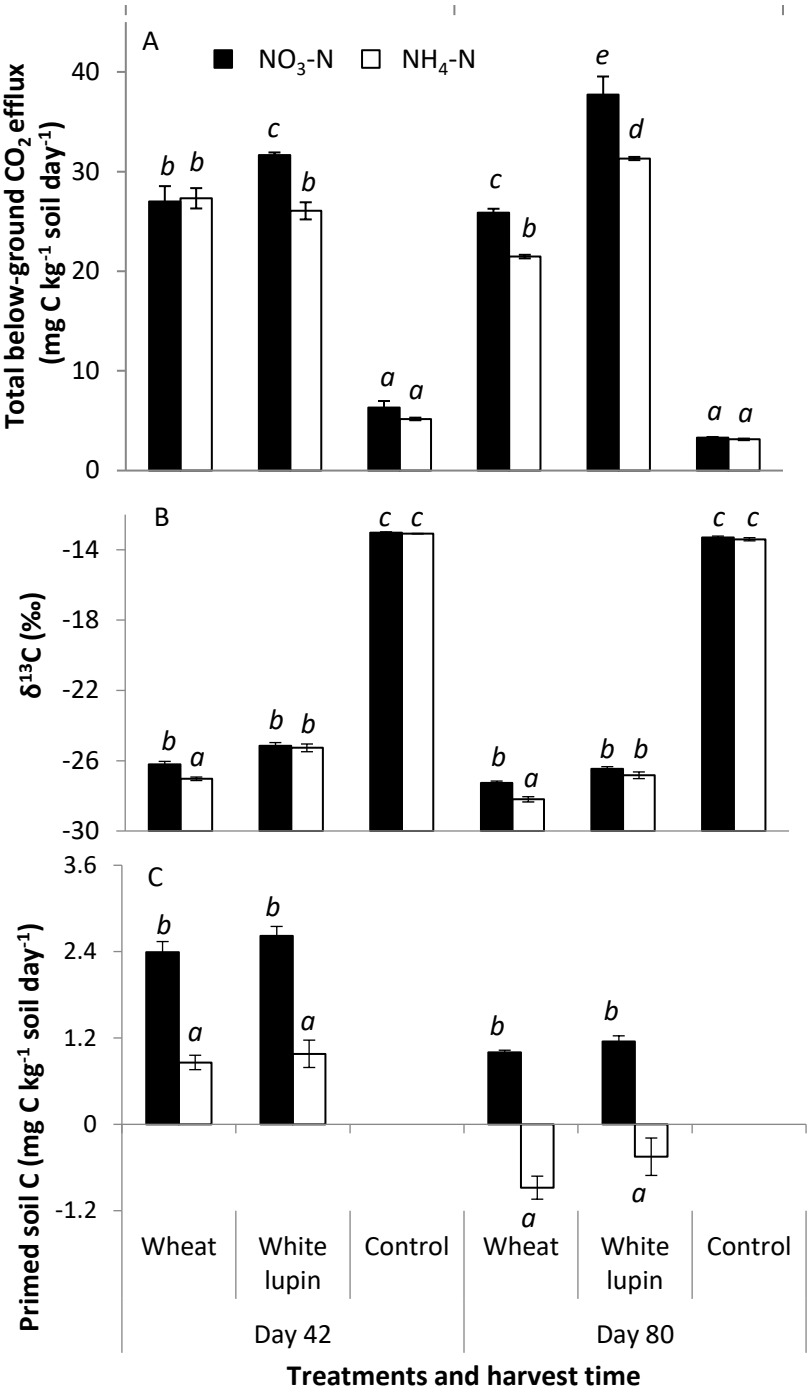

Figure 2. Total below-ground CO_2 efflux (A), $\delta^{13}C$ values of CO_2 (B), and primed soil C (C) under wheat and white lupin fed with NO_3-N and NH_4-N or no-plant control at days 42 and 80. Error bars represent standard error of means of four replicates. Different italic letters above the bars indicate significant differences among treatments at each harvest time (Tukey's test, $P<0.05$).

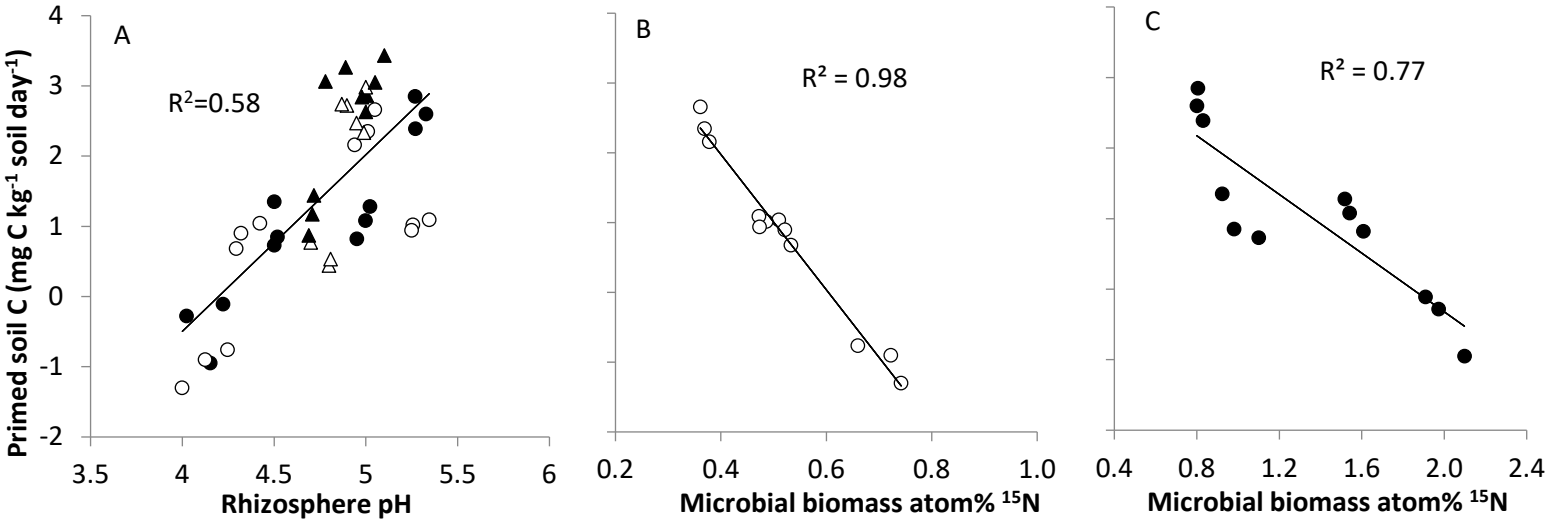

Figure 3. Relationships between rhizosphere primed soil C and rhizosphere pH (A) and microbial biomass atom% ^{15}N for wheat (B) and white lupin (C) fed with NO_3-N and NH_4-N at days 42 and 80. Open (○) and closed (●) circles denote data for wheat and white lupin, respectively. Open (∆) and closed (▲) triangles denote the data for urea-fed wheat and N_2-fixing white lupin, respectively, from Wang et al. 2016.

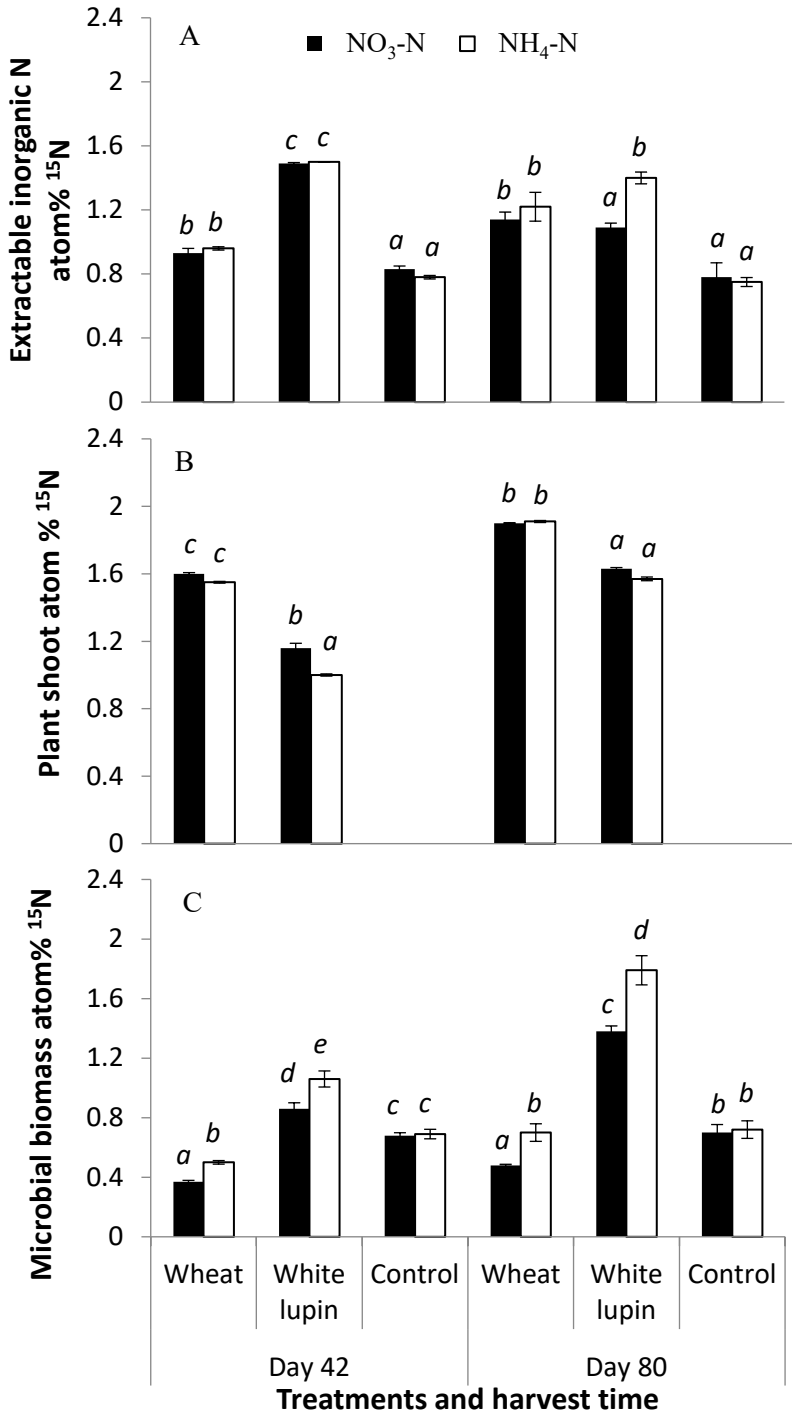

Figure 4. Atom% ^{15}N in the rhizosphere inorganic N (A), plant shoots (B) and rhizosphere microbial biomass (C) of wheat and white lupin supplied with NO_3-N or NH_4-N at days 42 and 80. Error bars represent standard error of means of three replicates. Different italic letters above the bars indicate significant differences among treatments at each harvest time (Tukey's test, $P<0.05$).

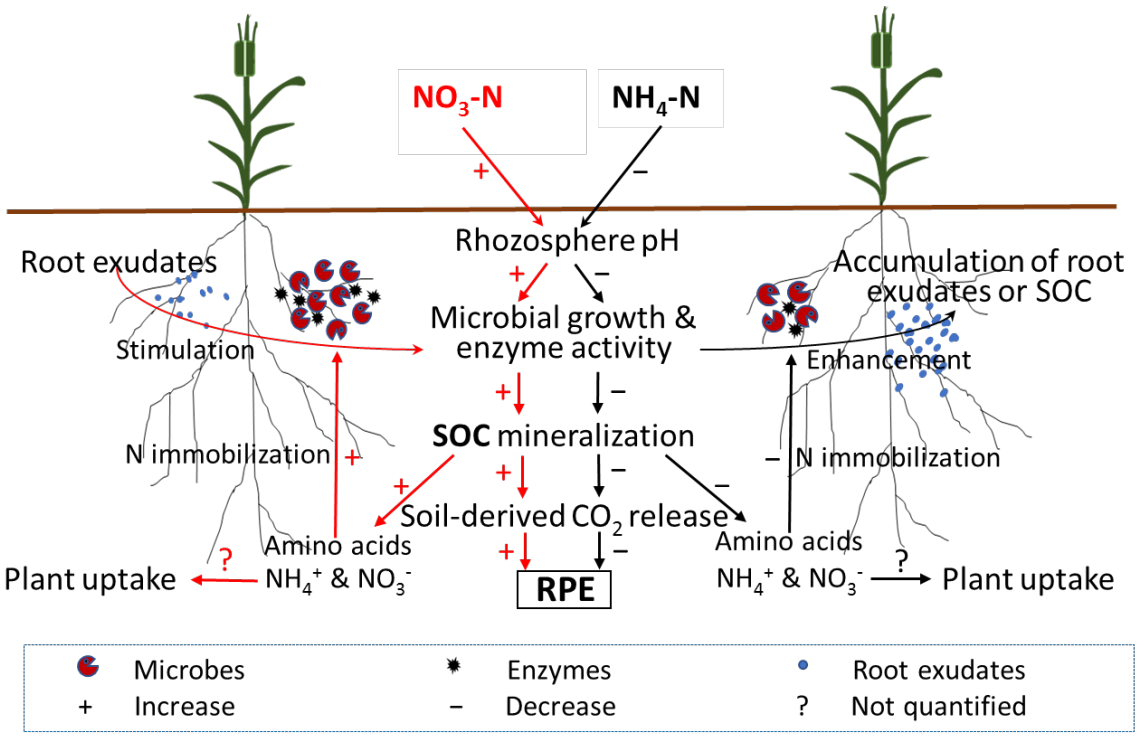

Figure 5. A conceptual diagram of the effects of N form (NO_3-N vs NH_4-N)-induced pH changes on RPE and involved N immobilization mechanisms.

Table 1. Dry biomass and N concentration of shoot and roots, root length and ^{13}C abundance in roots of wheat and white lupin supplied with NO_3-N and NH_4-N at days 42 and 80. ns, *, ** and *** represent $P>0.05$, $P<0.05$, $P<0.01$ and $P<0.001$, respectively. For each column, different letters indicate significant differences between means for a given harvest time (two-way ANOVA, Tukey test, $P<0.05$). At day 42, the effect of N form and interactions between N from and species were not significant and thus only the main effect of species (average of two N forms) was presented.

Harvest time	Species	N form	Biomass (g column^{-1})		N concentration (%)		Root length	^{13}C abundance (‰)	
			Shoot	Root	Shoot	Root	(m column^{-1})	Root	Root-derived CO_2
Day 42	Wheat	NO_3/NH_4-N	4.04 b	0.87 b	5.22 b	2.90 a	183 b	-31.89 a	-30.6 a
	White lupin	NO_3/NH_4-N	3.45 a	0.55 a	4.70 a	2.98 a	22 a	-30.30 b	-28.7 b
Day 80	Wheat	NO_3-N	15.47 b	2.50 c	3.41 a	2.01 a	437 c	-32.24 a	-30.7 a
		NH_4-N	14.90 b	2.11 b	3.32 a	1.93 a	314 b	-32.26 a	
	White lupin	NO_3-N	11.23 a	1.05 a	4.19 b	3.00 b	30 a	-31.74 b	-29.0 b
		NH_4-N	10.19 a	1.13 a	4.23 b	3.17 b	32 a	-31.62 b	
Two-way ANOVA									
Day 42	Species		**	**	*	ns	***	***	***
	N form		ns	ns	ns	ns	ns	ns	
	Species × N form		ns	ns	ns	ns	ns	ns	
Day 80	Species		***	***	***	***	***	**	***
	N form		*	*	ns	ns	***	ns	
	Species × N form		ns	*	ns	ns	***	ns	

Table 2. Extractable soil organic C, soil respiration, rhizosphere SOC, microbial C and N in the no-plant control and rhizosphere of wheat and white lupin supplied with NO_3-N or NH_4-N at days 42 and 80. ns, *, ** and *** represent $P>0.05$, $P<0.05$, $P<0.01$ and $P<0.001$, respectively. Different letters indicate significant differences among treatments at each harvest time (Tukey's test, $P<0.05$).

Harvest time	Treatment	N form	Extractable organic C (μg C g^{-1} soil)	Soil respiration (μg CO_2 g^{-1} soil)	Rhizosphere SOC (mg g^{-1})	Microbial C (μg C g^{-1} soil)	Microbial N (μg N g^{-1} soil)
Day 42	Wheat	NO_3-N	84 a	161 b	27.6 ab	256 b	20.6 b
		NH_4-N	96 a	186 b	28.2 b	216 a	15.2 a
	White lupin	NO_3-N	107 a	387 c	26.8 a	376 c	41.1 c
		NH_4-N	151 b	363 c	28.1 b	234 ab	24.6 b
	Control	NO_3-N	90 a	88 a	27.4 ab	248 ab	24.1 b
		NH_4-N	86 a	79 a	27.3 ab	266 b	23.3 b
Day 80	Wheat	NO_3-N	39 a	247 c	27.4 ab	366 bc	33.8 b
		NH_4-N	87 b	166 b	28.2 c	256 a	23.3 a
	White lupin	NO_3-N	105 b	520 e	26.5 a	416 c	46.0 c
		NH_4-N	146 c	371 d	27.9 bc	305 ab	29.0 b
	Control	NO_3-N	77 b	69 a	27.0 a	283 a	29.3 b
		NH_4-N	82 b	76 a	27.1 a	280 a	27.7 b
Two-way ANOVA							
Day 42	Treatment		**	**	ns	*	**
	N form		*	ns	*	*	*
	Treatment × N form		**	ns	*	**	*
Day 80	Treatment		***	***	*	*	***
	N form		***	***	*	***	***
	Treatment × N form		ns	***	*	*	**