Improving soil nutrient availability increases carbon rhizodeposition under maize and soybean in Mollisols

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ABSTRACT

Rhizodeposited carbon (C) is an important source of soil organic C, and plays an important role in the C cycle in the soil-plant-atmosphere continuum. However, interactive effects of plant species and soil nutrient availability on C rhizodeposition remain unclear. This experiment examined the effect of soil nutrient availability on C rhizodeposition of C4 maize and C3 soybean with contrasting photosynthetic capacity. The soils (Mollisols) were collected from three treatments of no fertilizer (Control), inorganic fertilizer only (NPK), and NPK plus organic manure (NPKM) in a 24-year long-term fertilization field trial. The plants were labelled with ^{13}C at the vegetative and reproductive stages. The ^{13}C abundance of shoots, roots and soil were quantified at 0, 7 days after ^{13}C labelling, and at maturity. Increasing soil nutrient availability induced the C rhizodeposition due to the higher C fixation and its distribution to roots and soil. The higher amount of averaged below-ground C allocated to soil resulted in greater specific rhizodeposited C from soybean than maize. Additional organic amendment further enhanced them. As a result, higher soil nutrient availability increased total soil organic C under both maize and soybean system though there was no significant difference between the two crop systems. All these suggested that higher soil nutrient availability favors C rhizodeposition. Mean 80, 260 and 300 kg fixed C ha^{-1} were estimated to transfer into soil in the Control, NPK and NPKM treatments, respectively, during one growing season.

Keywords: Assimilates; C allocation; ^{13}C labelling; Long-term fertilization; Organic amendment; Soil organic carbon

1. Introduction

Rhizodeposition is an important process of the exchange at the plant-soil interface by releasing organic compounds to soil and enhancing nutrient availability for plants (Nguyen, 2003). The organic C input to soil from released organic compounds was up to 30% of net plant-fixed C (Jones et al., 2009). It modifies the community and activity of soil microorganisms, and impacts on soil carbon (C) and nutrient cycling, and plant growth (Mwafulirwa et al., 2016). Clearly, C rhizodeposition plays an important role in the soil C cycling in the plant-soil system (Kuzyakov, 2002).

It is well known that plant photosynthetically fixed C is the primary source of rhizodeposited C, thus its quantity and quality would be controlled by various factors that affect plant growth, such as plant species and genotypes (Mwafulirwa et al., 2016), fertilizer application (Baptist et al., 2015), photosynthetic capacity of the plant (Kuzyakov and Cheng, 2001), atmospheric CO_2 concentration (Li et al., 2004), soil tillage (Huggins et al., 2007) and water supply (Lucero et al., 2002). Among these factors, plant biomass and the allocation of photosynthetically fixed C directly drive the rhizodeposited C (Baptist et al., 2015). Kuzyakov and Schneckenberger (2004) showed that the amount of rhizodeposited C allocated to soil was less than 5% of total assimilated C for wheat, pasture plants and maize after reviewing 28 experiments using C isotope pulse-labeling. In later studies, there was 10.8% of assimilated C in rice (Tian et al., 2013) and 2.7% in maize (He et al., 2008) were estimated to transfer to soil. Jin et al. (2011) indicated that soybean allocated 12.4% of net assimilated C to the soil at the four-node stage and only 2.1% at maturity. Clearly, the amount of rhizodeposited C varies across plant species and growth stage. The variation of species, such as between C3 and C4 plants, might be ascribed to the difference in photosynthetic capacity. The C4 plants have the higher photosynthetic capacity and nutrient-use efficiency by means of the C4 photosynthetic pathway compared to C3 photosynthetic pathway in C3 plants (Hatch, 1987). Both photosynthetic capacity and C rhizodeposition depend on the C requirement for plant growth at different growth stages (Anten et al., 1995). A clear understanding of the dynamics of C rhizodeposition at different growth stages between C3 and C4 plants would improve our understanding of how plant growth influences the below-ground C input from roots and rhizodeposits.

Fertilizer application is another important factor affecting C rhizodeposition. It can affect the amount of assimilated C through changes in plant biomass (Saggar et al., 1997), and the partitioning in shoots, roots and soil (Hill et al., 2007). It is generally accepted that application of N fertilizers increases plant biomass (Ge et al., 2014), and increases the rate of organic C released by plant roots (Henry et al., 2005). However, the application of N fertilizers was shown to decrease the proportion of assimilated C allocated to soil under lettuce (Kuzyakov et al., 2002). Nutrient limitation facilitated the distribution of photo-assimilated C to roots while nutrient-rich soils favored the production of above-ground biomass (Chowdhury et al., 2014). Furthermore, organic amendment increased the root C input in the winter wheat-spring barely-potato crop rotation system (Chirinda et al., 2012). The shift of crop assimilate distribution in favor of the root system (Gregory, 2006) would benefit C rhizodeposition. Although there are many studies showing changes of the allocation of assimilated C following fertilization, it is not clear how soil nutrient availability and plant growth interactively affect C rhizodeposition.

This study aimed to examine the effect of soil nutrient availability on rhizodeposited C under two contrasting plant species (C4 maize and C3 soybean) in Mollisols. We hypothesized that higher soil nutrient availability would lead to higher photosynthetic capability of the plants which in turn would increase the amount of newly fixed C translocated to plant roots, and enhance

rhizodeposition. The soils were collected from plots of different fertilizer treatments in a long-term fertilizer trial. A pulse ^{13}C-labelling technique was used to differentiate newly incorporated C derived from plants and that from the decomposition of native SOC.

2. Materials and methods

2.1. Soil preparation and plant culture

Soils were collected from the 0.2-m tillage layer of a 24-year fertilization experiment located at the State Key Experimental Station of Agroecology, Hailun County, Heilongjiang, China (47°26'N, 126°38'E). The mean annual temperature is 2.2 °C, and mean annual precipitation is about 550 mm. The soil was a silty clay loam including 400 g kg^{-1} clay and 258 g kg^{-1} silt. The soil bulk density was 1.0 Mg m^{-3}. Three soil nutrient availability treatments were 1) inorganic fertilizer only (NPK), 2) organic amendment in combination with inorganic fertilizer (NPKM), and 3) no-fertilizer (Control). The annual application rates of nutrients in NPK and NPKM treatments were (kg ha^{-1}): 113 nitrogen (N), 20 phosphorus (P) and 25 potassium (K) for maize, and 20, 23 and 16 for soybean, correspondingly. The N, P and K were applied as urea, $(NH_4)_2HPO_4$ and K_2SO_4. The amount of organic amendment was 2.25 Mg ha^{-1} of pig manure, which provided 36 kg N ha^{-1}, 4.4 kg P ha^{-1} and 54 kg K ha^{-1}. The detailed information on the amount and method of fertilizer application was described by Qiao et al. (2014). Briefly, the crop management was a wheat-soybean-maize rotation, with the maize being the crop plant prior to soil collection for this study. Each year, the above-ground parts were harvested at autumn and the below-ground parts were retained in the field. The soil was tilled to 20 cm depth manually. The soil is a typical Mollisol without carbonate reaction. The basic properties of the soil are shown in Table 1, in which available N in soil was measured using the alkaline diffusion method (Black, 1965), extractable P with NaHCO$_3$ extraction (0.5 mol L^{-1}, pH 8.5) (Olsen et al., 1954), and extractable K with 1 N NH$_4$OAc (Jones, 1973). Soil pH was measured with a Mettler Toledo pH meter (S210 Switzerland) after shaking soil in water suspension (1:2.5) for 30 min and centrifuged for 10 min.

Table 1. Soil pH, soil organic carbon (SOC), total and available nitrogen, phosphorus and potassium measured prior to the experiment. The soils were collected from the no-fertilizer control, chemical fertilizer (NPK) and chemical fertilizer plus manure (NPKM) treatments of a 24-year field trial.

Treatment	pH	SOC	Total N	Total P	Total K	Available N	Available P	Available K
		(mg g^{-1})				(µg g^{-1})		
Control	6.11±0.03	24.3±0.5	1.92±0.67	0.74±0.02	19.3±0.04	141±10	13.5±1.5	148±5
NPK	5.92±0.07	26.4±1.3	2.19±0.31	0.84±0.02	20.9±0.04	192±9	40.8±2.2	168±2
NPKM	5.98±0.04	28.7±0.7	2.26±0.38	0.91±0.02	21.2±0.01	210±10	43.4±0.8	197±2

Values present means ± se of three replicates.

Every composite sample was formed by fifteen cores collected from 0-20 cm depth of each fertility treatment from three field replicates. The soil samples were gently crushed and thoroughly mixed, and the visible roots and residues were removed. All soil samples were sieved through a 4-mm sieve. The 6.5 kilograms of each composite sample was filled into each pot (20 cm in height and 20 cm in diameter). To obtain the same fertility status as that under field condition, the inorganic nutrients of NPK and NPKM treatments were applied at the following rates (g pot^{-1}): 0.37

$(NH_4)_2HPO_4$ and 0.70 K_2SO_4 for soybean, and 1.53 urea (0.51 was applied at sowing and 1.02 applied at the jointing stage), 0.70 $(NH_4)_2HPO_4$ and 0.70 K_2SO_4 for maize. The additional 70 g pot^{-1} of compost pig manure (including 2.1 g C kg^{-1} pig manure) was added to the NPKM treatment. All fertilizers were fully mixed with the soil, which was then compacted to a bulk density of 1.05 Mg m^{-3}.

A pot experiment was conducted in a naturally lit greenhouse with day/night temperatures of 24-28/16-20 °C during the experiment. It was completely factorial, and consisted of two crop species, three levels of soil nutrient availability with three replicates. Pots were destructively sampled six times. A total of 108 pots for crop species and fertilizer treatments were prepared and additional 45 pots without plants were used as no-plant control. The two crops were maize (*Zea mays* L. cv. Haiyu 6), and soybean (*Glycine max* L. cv. Heinong 35) which are C4 monocotyledon and C3 dicotyledon species, respectively. Three uniformly germinated seeds were sown to each pot, and seedlings were thinned to one plant per pot one week after emergence. Soil water content was maintained at 60% of water-holding capacity by weighing every 3 days.

2.2. Labeling process and measurements

The pulse ^{13}C-labeling was performed at the vegetative and reproductive stages (jointing stage and grain-filling stage for maize, and branching stage and grain-filling stage for soybean, respectively). On the labeling day, plants were transferred into an air-tight glass chamber (area 130×80 cm^2, height 240 cm). Pure $^{13}CO_2$ (chemical purity ≥99.9%) of 500 ml was injected with a syringe through a rubber gasket into the chamber, and CO_2 concentration in the chamber maintained at 350-400 ppm. A fan was fixed upper of the chamber to homogenise the atmosphere, and cool water was spread outside of the chamber to keep the atmosphere temperature to ambient environment. The labeling procedure was run for 6 h under ~1000 μE m^{-2} s^{-1} photosynthetically active photon flux density, with 26-28 °C temperature, and 70-90% relative humidity. Nine pots of each treatment were labeled at two growth stages, and three pots harvested 6 h after ^{13}C labeling (Day 0), at Day 7 and at the end of growing season (maturity), respectively. Otherwise, nine pots without $^{13}CO_2$ labelling were harvested as the non-labelled control at the same stage with the same sampling method.

At harvest, the plants were cut at the cotyledon mark of soybean and the first knot of maize, and fallen leaves were considered as shoot parts. All the left part was moved on a 2-mm sieve and washed carefully to collect roots (the recovery rate was about 95%). All plant samples were then dried at 70 °C for 72 h, weighed and ground.

Three soil cores with 20 mm in diameter were taken by depth from the soil surface at the positions 0, 40 and 80 mm away from the center of pot. The composite soil samples with three soil cores were manually broken down into 20-40 mm aggregates along the natural weak planes. All the samples were air-dried for aggregate screening. Three size-classes (>0.25 mm, 0.25-0.053 mm, and <0.053 mm) of aggregates were obtained by wet sieving (Six et al., 2002). Briefly, air-dried soil (~20 g) was placed in a 500-ml flask wetting fully with 100 ml distilled water drop-wise to remove trapped air. After 10 min, the soil was gently transferred to the top of a set of two sieves of 0.25- and 0.053-mm, and kept 30 mm below the water surface (Six et al., 1998). The set of sieves were then moved up and down 5 times min^{-1} for 10 min. After wet-sieving, the aggregates on each sieve were transferred into a beaker for air-drying. Soil left in the pot was mixed thoroughly, air-dried and milled before analysis of ^{13}C abundance and SOC content.

All plant tissues, bulk soil and aggregate samples were passed through a 0.25-mm sieve. Total

SOC and total N were measured using a CHN analyzer (HeraeusElementarVario EL, Germany). The ^{13}C abundance was measured by GC-IRMS (Eurppa Scientific Ltd., Cheshire, UK), with an analytical precision of 0.05%.

2.3. Calculations

Total biomass was the product of dry weight per plant (g plant^{-1}) and plant density (plant ha^{-1}). In the present study, the densities of soybean and maize were 280 and 5 thousand plant per hectare.

Atomic ^{13}C % was calculated as

$$\text{Atomic } ^{13}C\% = \left[(\delta^{13}C + 1000) \times R_{PBD} \div 1000\right] \div \left[(\delta^{13}C + 1000) \times R_{PDB} \div 1000 + 1\right] \times 100 \quad (1)$$

where R_{PDB} is the $^{13}C/^{12}C$ ratio of the standard PDB (=0.0112372) and $\delta^{13}C$ is the ^{13}C natural abundance of plants from the no-labelled controls or soil sample from the no-plant controls.

The net fixed -^{13}C during labeling was calculated as the difference between ^{13}C abundance of the labeled samples and those without ^{13}C labeling using the following formula (Werner and Brand, 2001).

$$\text{Net fixed-}^{13}C \text{ (mg plant}^{-1}) = [(\text{atomic } ^{13}C\%)_l - (\text{atomic } ^{13}C\%)_{nl}] \times TC \quad (2)$$

where l and nl indicate labeling and without labeling; TC is the total C content per plant.

The sum of ^{13}C in shoots, roots and soil was considered as the total ^{13}C fixed by the plant after 6-h labeling (day 0) (Lu et al., 2002).

Relative growth rate (RGR) of shoots and roots was calculated as

$$\text{RGR (mg } g^{-1}\text{DW } d^{-1}) = \frac{lnDW_{t2} - lnDW_{t1}}{t2 - t1} \quad (3)$$

where DW_{t1} and DW_{t2} represent the dry weight of shoots or roots at t_1 and t_2 days, respectively, t_1 and t_2 are the sampling time of 0 and 7 days after ^{13}C labeling.

The C-growth rate used to present the plant growth was calculated according to Jin et al., (2011).

$$\text{C-growth rate (mg C plant}^{-1} \text{ d}^{-1}) = \frac{DW_{t2} \times CC_{t2} - DW_{t1} \times CC_{t1}}{t2 - t1} \quad (4)$$

where DW_{t1} and DW_{t2} represent the dry weight of shoots at t_1 and t_2 days, respectively; CC_{t1} and CC_{t2} are the C concentrations of shoots at t_1 and t_2 days; t_1 and t_2 are the sampling times of 0 and 7 days after ^{13}C labeling, respectively.

Total below-ground C from plant was estimated assuming respiration of fixed ^{13}C after 6 hours was negligible

$$\text{Total belowground C (g C plant}^{-1}) = \frac{^{13}C_{total} - ^{13}C_{shoot}}{^{13}C_{total}} \times C_{growth\ rate} \quad (5)$$

where $^{13}C_{total}$ is the fixed-^{13}C at the 0 day, $^{13}C_{shoot}$ means the ^{13}C in shoots at the end of growing season.

The proportion of total below-ground C allocated to soil and roots at the end of growing season was calculated as

$$\text{Proportion (\%)} = \frac{^{13}C_{\ sample}}{\text{net } ^{13}C_{\ assimilation}} \times 100 \quad (6)$$

where $^{13}C_{sample}$ represents the ^{13}C in roots or soils at the end of growing season, net ^{13}C assimilation means the sum of net ^{13}C in roots, shoots and soils as determined using Equation (2). Thus, the lost proportion equals the 100% minus the proportion in roots and soils.

The lost (%) presents the lost percent of total belowground C, and was calculated as

Lost (%)= 100% - root (%) - soil (%)

The C rhizodeposition at the vegetative and reproductive stages was calculated according to Lu et al. (2002)

$$\text{Deposited C (kg C } plant^{-1}) = \frac{13C_{soil}}{total\ fixed\ ^{13}C} \times C_{growth\ rate} \tag{7}$$

where $^{13}C_{soil}$ is the total ^{13}C in soil at maturity; total fixed ^{13}C presents the total assimilated ^{13}C at the vegetative or reproductive stage.

Plant-derived C, the proportion of newly derived C to total SOC was calculated as

$$\text{Plant– derived C (\% of total SOC)} = \frac{\text{Deposited C} \times \text{DP}}{\text{V} \times \text{BD} \times C_{soil}} \times 100 \tag{8}$$

where Deposited C was calculated using Equation (7), DP means the plant density (280 thousand plants for soybean and 46 thousand plants for maize per hectare), V presents the volume of one hectare at 20-cm depth, BD is soil bulk density, C_{soil} is the C content in soil.

2.4. Statistical analysis

All values are shown as the means ± standard error of three replicates. Two-way analysis of variances (ANOVA) was conducted to evaluate the effect of soil nutrient availability and crop species on total biomass, C-growth rate, RGR, fixed C and its allocation, amount of belowground C and its allocation, SOC, deposited-C and plant-derived C using Origin 8 SR4 v8.0951 (B951). Significant differences were reported at $p \leq 0.05$ level, among the treatments within individual component using Fisher's LSD test.

3. Results

3.1. Plant growth

The total biomass of maize grown in NPK and NPKM were 2.3 and 2.7 times, respectively, those in the Control at the vegetative stage, and 3.7 and 4.2 times those in the Control at the reproductive stage. In comparison, the total biomass of soybean plants in NPK and NPKM treatment showed 3.6 and 5.3 fold greater, respectively, at the vegetative stage, and 1.9 and 2.3 fold greater, respectively, at the reproductive stage than those in the Control (Fig. 1).
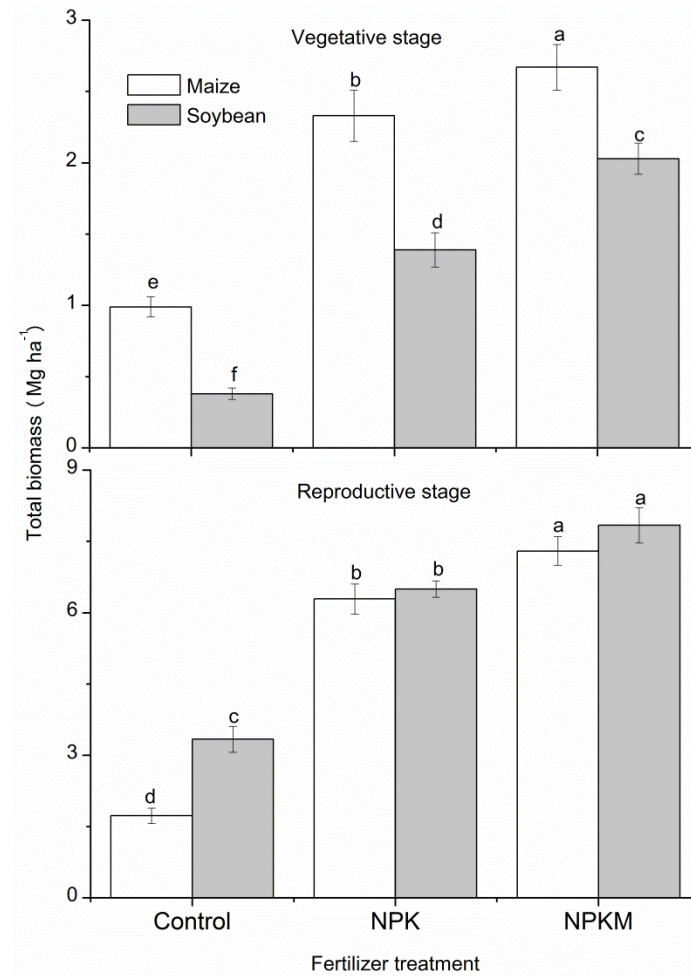
Figure 1. Total biomass of maize and soybean at 7 days after ^{13}C labelling at the vegetative and reproductive stages. Standard error bars (n=6) are shown. The soils were collected from the no-fertilizer control, chemical fertilizer (NPK) and chemical fertilizer plus manure (NPKM) treatments of a 24-year field trial. Means with a common letter are not significantly different at p=0.05 across the treatments within an individual component at the same growth stage using Fisher's LSD test.

At the vegetative stage, shoot relative growth rate (RGR) of maize in NPK and NPKM treatment was 3.6 and 3.1 times higher than Control during the first week after ^{13}C labelling, respectively, while that of soybean was decreased by 65% and 32% correspondingly (Fig. 2). The RGR of maize shoots was lower than that of soybean in the Control, but reverse was true in the NPK and NPKM treatments. At the reproductive stage, though the shoot RGR in maize was higher than soybean, increasing fertilization did not affect shoot RGR of maize and decrease that of soybean. The RGR of shoots was higher at the vegetative than reproductive stage.
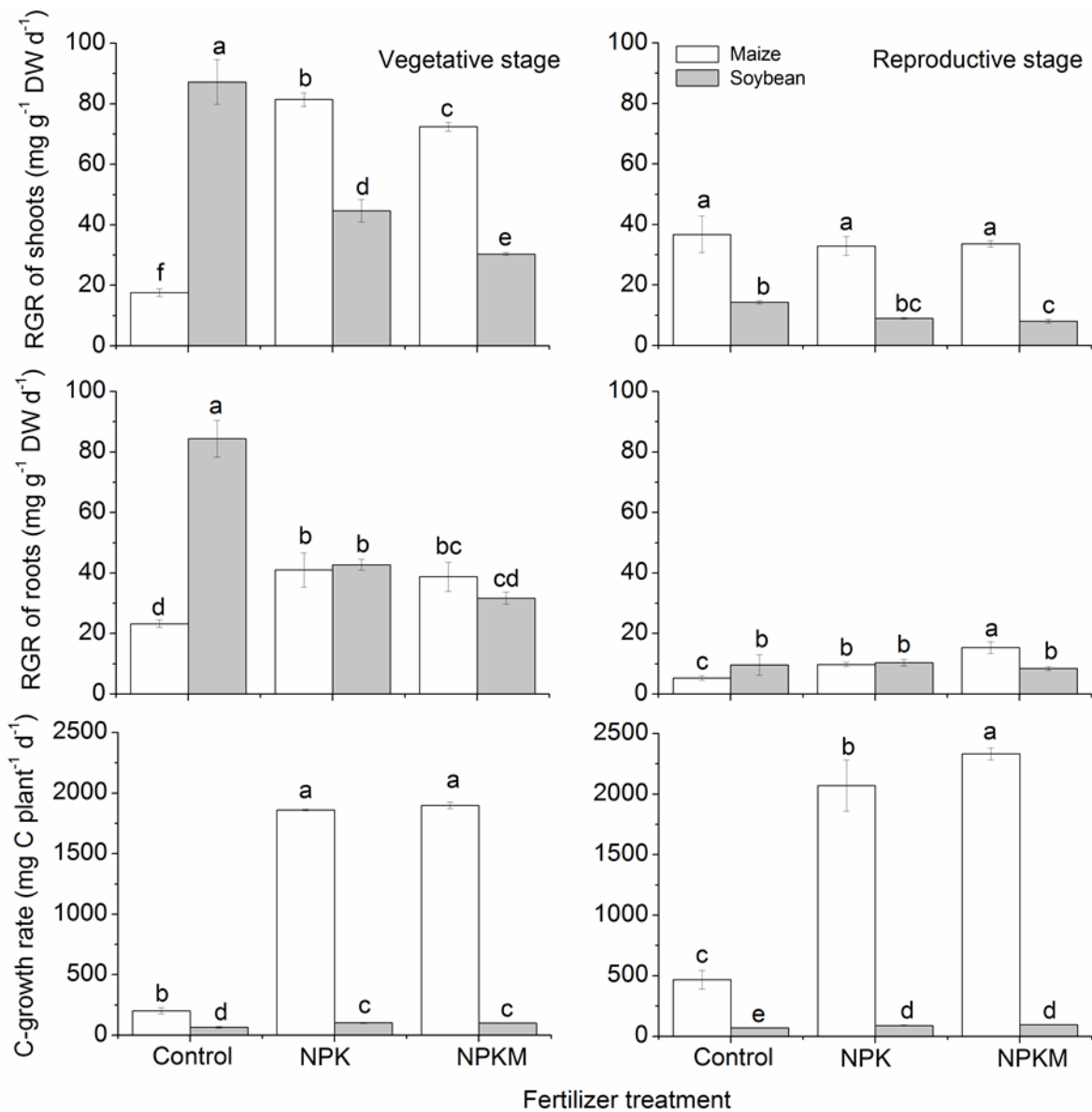
Figure 2. Relative growth rate (RGR) of shoots and roots, and C-growth rates of whole plant for maize and soybean in the first 7 days after ^{13}C labelling at the vegetative and reproductive stages. Standard error bars (n=3) are shown. The soils were collected from the no-fertilizer control, chemical fertilizer (NPK) and chemical fertilizer plus manure (NPKM) treatments of a 24-year field trial. Means with a common letter are not significantly different at $p=0.05$ across the treatments within an individual component at the same growth stage using Fisher's LSD test.

At the vegetative stage, root RGR in response to soil nutrient availability followed a similar trend with soybean having a greater RGR in the Control (Fig. 2). At the reproductive stage, higher soil nutrient availability increased root RGR of maize but not soybean. On average, the root RGR was 4.6 times greater at the vegetative than reproductive stage.

The C-growth rates of maize in the NPK and NPKM treatments were increased by 5.8 and 6.2 times compared to the Control treatment, respectively. On average, the C-growth rate at the reproductive stage was 22% higher than at the vegetative stage (Fig. 2). The C-growth rates of soybean in NPK and NPKM were 43 and 45% higher than Control treatment, respectively. In comparison, the C-growth rate of maize was 16.7-fold higher than that of soybean across soil

nutrient availability and growth stages.

3.2. Net assimilated-C and its allocation

Compared to those in the Control, maize plants grown in the NPK and NPKM treatments fixed 35% and 43% more ^{13}C at the vegetable stage, and 1.6 and 1.8 times more ^{13}C at the reproductive stage (Table 2). The amount of ^{13}C fixed in NPKM was 7% higher than NPK treatment at the reproductive stage. For soybean, the amounts of ^{13}C fixed in the NPK and NPKM treatments were 2.2 and 2.9 times greater at the vegetative stage, and 67% and 120% greater compared to the Control at the reproductive stage, respectively. In comparison, the amount of ^{13}C fixed in maize was 3.4-fold higher than soybean (Table 2), mainly due to the greater C-growth rate of maize.

Table 2. The net assimilation of maize and soybean during 6-h labeling and percentage distribution of the fixed ^{13}C in the plant-soil system at 0 and 7 d, and at maturity after labeling at the vegetative and reproductive stages. The soils were collected from the no-fertilizer control, chemical fertilizer (NPK) and chemical fertilizer plus manure (NPKM) treatments of a 24-year field trial.

Fertilizer Treatment	Fixed ^{13}C (mg ^{13}C plant^{-1})		Shoot		Root (%)		Soil		Lost	
	Maize	Soybean	Maize	Soybean	Maize	Soybean	Maize	Soybean	Maize	Soybean
^{13}C labelling at the vegetative stage										
Day 0 (6 h) after labeling										
Control	26.1b	2.2d	79.4b	68.6c	11.3c	18.9a	9.3b	12.5a	--	--
NPK	35.2a	7.0c	94.0a	78.5b	3.9d	13.2b	2.1d	8.3c	--	--
NPKM	37.3a	8.6c	95.2a	79.0b	3.1e	11.7c	1.7d	9.3b	--	--
Day 7 after labeling										
Control	--	--	71.8b	45.9c	13.3b	16.8a	10.7b	10.9b	4.2d	26.4b
NPK	--	--	78.5a	38.4d	5.0e	9.3c	3.3d	7.8c	13.2c	44.5a
NPKM	--	--	80.4a	36.5d	4.2f	6.7d	3.3d	13.6a	12.1c	43.2a
The end of the growing season (maturity)										
Control	--	--	52.4c	5.8f	10.7a	6.7b	2.7d	10.6b	34.2c	76.9a
NPK	--	--	58.5b	8.8e	4.7c	5.6c	5.5c	10.5b	31.3c	75.1a
NPKM	--	--	66.7a	13.3d	5.3c	5.1c	5.1c	14.8a	22.9d	66.8b
^{13}C labelling at the reproductive stage										
Day 0 (6 h) after labeling										
Control	17.1d	9.0f	89.8e	93.5c	8.8a	5.5b	1.4c	1.0d	--	--
NPK	45.1b	15.0e	96.8a	92.5d	2.5c	5.5b	0.7d	2.0b	--	--
NPKM	48.3a	19.7c	96.1b	92.1d	2.8c	5.1b	1.1c	2.8a	--	--
Day 7 after labeling										
Control	--	--	83.5b	77.5c	8.8a	3.6d	1.7c	1.7c	6.0b	17.2a
NPK	--	--	91.9a	74.8cd	2.7e	5.1b	2.2b	2.4b	3.2c	17.7a
NPKM	--	--	92.6a	73.8d	2.9e	4.4c	2.5b	3.1a	2.0c	18.7a
The end of the growing season (Maturity)										
Control	--	--	63.7bc	61.5c	6.5a	1.8d	1.0e	4.1c	28.8b	32.6a
NPK	--	--	69.2b	58.1c	2.6c	2.1d	1.2e	5.0b	27.0b	34.8a
NPKM	--	--	76.4a	58.6c	2.6c	3.2b	2.3d	5.6a	18.7c	32.6a

--, not applicable.

Values present means of three replications. The data followed by a common letter are not significantly different within an individual component at the same labelling stage (*p*=0.05) using Fisher's LSD test.

Six hours after ^{13}C labeling (Day 0), 79.4-96.8% of total fixed C in maize, and 68.6-93.5% in soybean were stored in shoots (Table 2). Soil nutrient availability greatly affected the translocation of fixed C into the roots and soils. When ^{13}C was labeled at the vegetative stage, the proportion of fixed C translocated into roots and soil was 1.3-5.5 times greater in the Control than NPK and NPKM for maize, and 0.8-2.5 times greater for soybean. The lower proportion of fixed C translocated into roots and soil was observed when ^{13}C was labeled at the reproductive stage. The proportion of fixed C translocated into roots in the Control was higher than NPK and NPKM, and which into soil in the Control was 100% greater than NPK in maize. In contrast, the proportion of fixed C released into soil under soybean was highest in NPKM and lowest in the Control.

The proportion of fixed C translocated into shoots, roots and soil generally decreased, while that lost via respiration/decomposition increased over time after labeling (Table 2). At maturity (the end of growing season), the proportion of fixed C lost was highest in the Control and lowest in the NPKM, and was higher in soybean than maize system. The opposite was true for the proportion of fixed C in shoots. The proportion of fixed C to roots in the Control was higher than those in NPK and NPKM treatments except that the opposite was the case for soybean when ^{13}C was labeled at the reproductive stage (Table 2). The proportion of fixed C released into soil was highest in NPKM and lowest in the Control, and higher when ^{13}C was labeled at the vegetative stage than reproductive stage. Soybean released 2.4- to 4.1-fold more fixed C into soil than maize (Table 2).

3.3. Below-ground C and its allocation

Compared to the Control, the amounts of total below-ground C in maize system were about 4.7 and 4.0 times higher in NPK and NPKM; those in soybean system were 1.8 and 1.9 times higher in NPK and NPKM, respectively (Fig. 3). On average, the amount of total below-ground C in maize was 5.9 fold higher than soybean, mainly due to the greater biomass and greater amount of fixed C translocated into roots (Table 2).
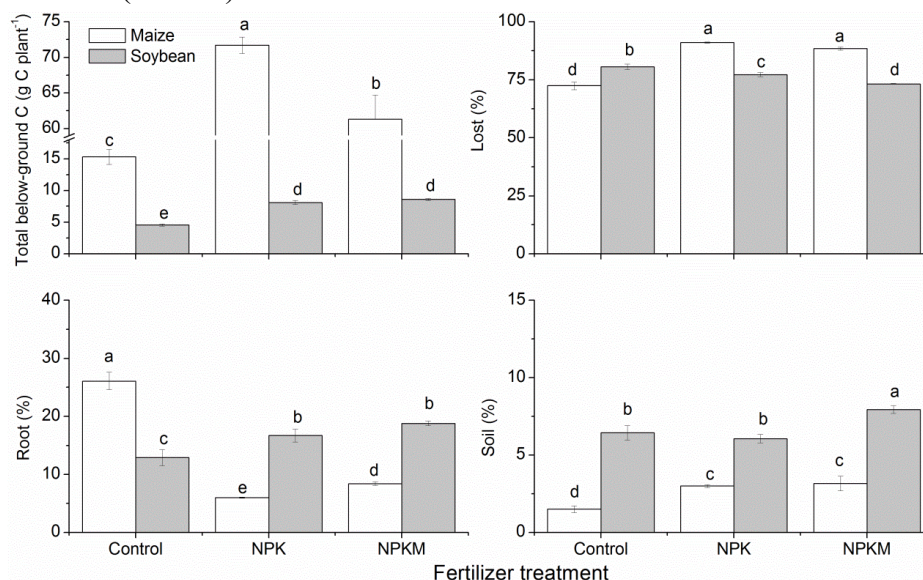

Figure 3. Total amounts of C distributed to below-ground and its distribution in roots and soil, and % lost under maize and soybean at the end of growing season. The soils were collected from the no-fertilizer control, chemical fertilizer (NPK) and chemical fertilizer plus manure (NPKM) treatments of a 24-year field trial. Means with a common letter are not significantly different at p=0.05 across the treatments within an individual component using Fisher's LSD test. Standard error bars (n=3) are shown.

The highest proportion of total below-ground C (>70%) was lost, and the lowest (<8%) was stored in soil (Fig. 3). Soil nutrient availability greatly affected the distribution of below-ground C. The proportion of lost C in NPK and NPKM were 25% and 22% higher than the Control treatment for maize, respectively, while that was 4% and 9% lower in NPK and NPKM, respectively, for soybean (Fig. 3). The proportion of lost in soybean was 11% higher than maize in the Control, and 15-17% lower in the higher soil nutrient availability treatments.

The proportion of total below-ground C stored in roots was highest in the Control and lowest in the NPK for maize, which was 77% and 67% lower in NPK and NPKM compared to the Control (Fig. 3). In soybean, the proportion of total below-ground C in NPK and NPKM were 29% and 45% higher than Control, respectively. In contrast, the proportion of total below-ground C stored in soil increased with soil nutrient availability, and was on average 2.7-fold higher in soybean than maize system (Fig. 3).

3.4. Contribution of C rhizodeposition to SOC

There were some interactive effects between crop species and soil nutrient availability on the SOC, plant-derived C and deposited C (Fig. 4). Though no statistically significant difference, soybean cultivation tended to increase the concentration of SOC as compared with the initial values (Fig. 4). Improving soil nutrient availability increased the amounts of plant-derived C and deposited C as % total SOC for both crop species with these amounts being greater under soybean than maize. For example, both NPK and NPKM increased maize-derived C (% total SOC) by 5 and 8 fold, and the deposited C by 6.2 and 9.5 times, respectively, compared to the Control.
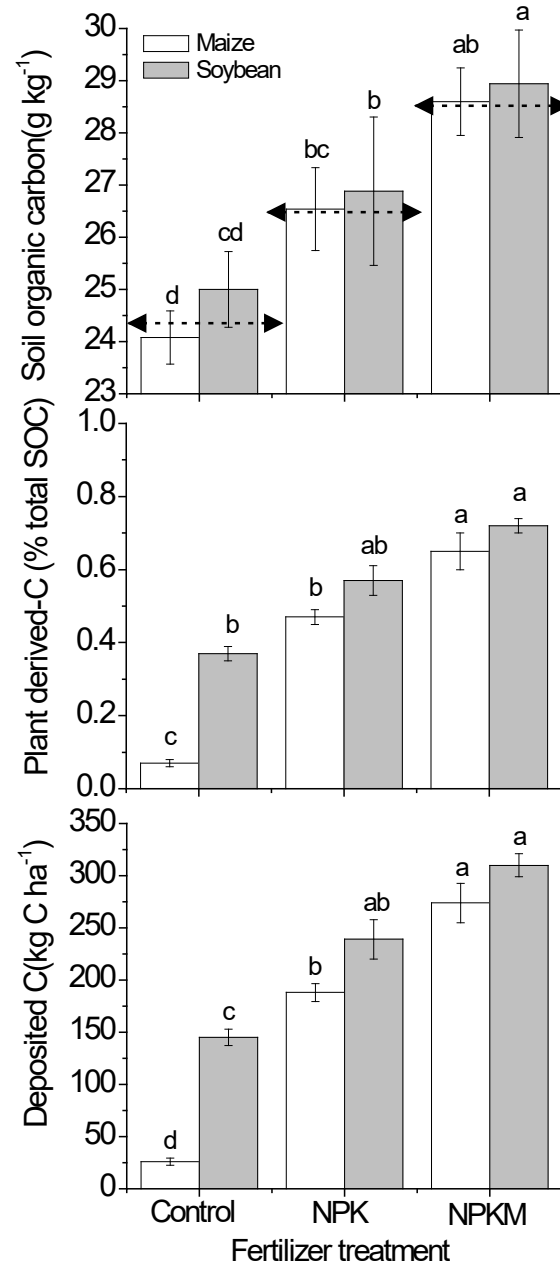
Figure 4. Soil organic C, deposited C and plant-derived C (% total SOC) under maize and soybean grown across soil fertility treatments at the end of growing season. The soils were collected from the no-fertilizer control, chemical fertilizer (NPK) and chemical fertilizer plus manure (NPKM) treatments of a 24-year field trial. Means with a common letter are not significantly different at p=0.05 across the treatments within an individual component using Fisher's LSD test. Standard error bars (n=3) are shown. The arrow-ended short lines represent the original value prior to the study.

3.5. SOC and the proportion of rhizodeposited C in aggregates

Improving soil nutrient availability greatly increased the concentration of SOC in all size aggregates except of the <0.053 mm aggregates (Fig. 5). In the >0.25-mm size aggregates, only the SOC in NPKM treatment was 6% higher in maize than soybean (Fig. 5). Little difference in other soil nutrient availability and the 0.053-0.25 mm and <0.053 mm sizes were observed between the two crop species.
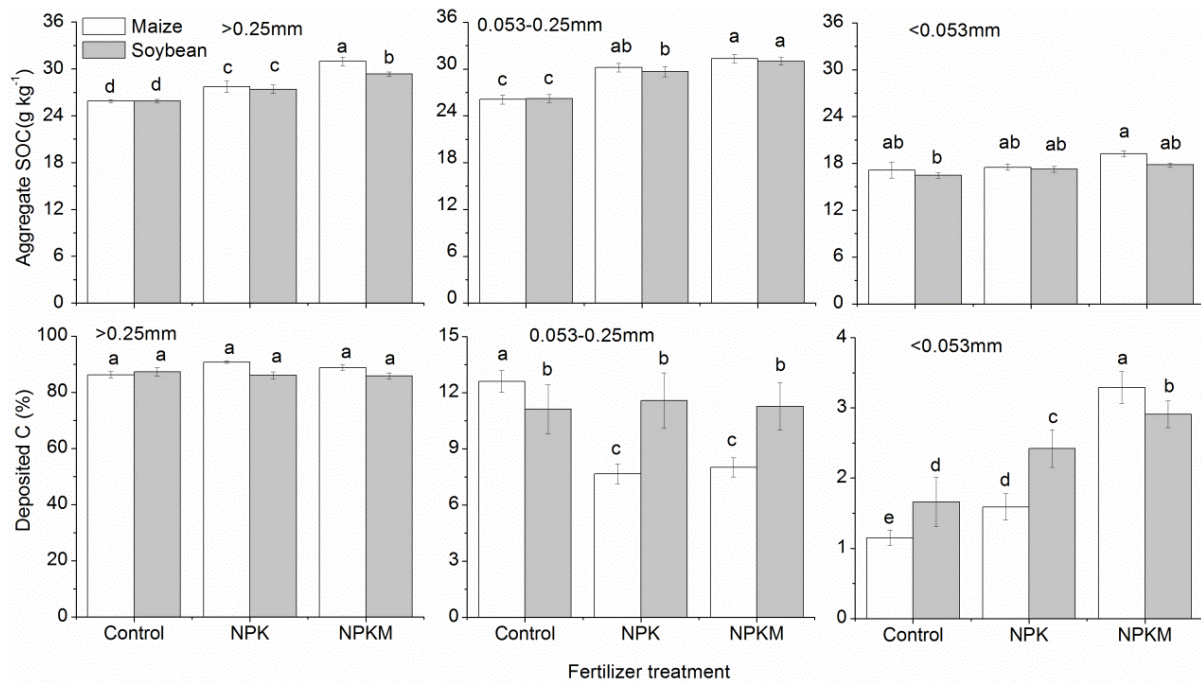

Figure 5. Soil organic carbon (SOC) concentration (top) and distribution of rhizodeposited C (bottom) in various aggregate classes under maize and soybean grown across three fertility treatments at the end of growing season. The soils were collected from the no-fertilizer control, chemical fertilizer (NPK) and chemical fertilizer plus manure (NPKM) treatments of a 24-year field trial. Means with a common letter are not significantly different at p=0.05 within an aggregate class using Fisher's LSD test. Standard error bars (n=3) are shown.

There were interactive effects of crop species and soil nutrient availability on the distribution of deposited C in aggregates except the >0.25-mm aggregates (Fig. 5). Improving soil nutrient availability decreased the proportion of deposited C to the 0.053-0.25 mm size aggregates for maize (Fig. 5). In contrast, the proportion in maize was 13% higher than soybean in the Control, and 34% and 29% lower in maize than soybean for the NPK and NPKM treatments. Improving soil nutrient availability increased the proportion of deposited C in the <0.053 mm size aggregates for two crop species (Fig. 5). The proportion of deposited C was 31% and 34% lower in maize than soybean for the Control and NPK treatments, but 13% higher for the NPKM treatment.

4. Discussion

4.1. Effect of soil nutrient availability on rhizodeposition C

This study demonstrated that improving soil nutrient availability increased the total rhizodeposited C. This result could be explained by the following reasons. First, improving soil

nutrient availability increased C fixation via photosynthesis, and increased the distribution of fixed C to soil towards maturity. It is evident that the amount of net ^{13}C fixed was up to 4 fold higher, and the distribution of fixed ^{13}C to the soil was up to 2.3 fold higher at the maturity in the higher soil nutrient availability treatments compared to the Control (Table 2). The results are consistent with those of previous studies (Lu et al., 2002; Chirinda et al., 2010; Jin et al., 2011; Ge et al., 2014; An et al., 2015). For example, An et al. (2015) found that application of N fertilizer (100 kg N ha^{-1}) increased C assimilation of maize crop by 14% in a Hapli-Udic Cambisol. Second, improving soil nutrient availability increased the production of shoot and root biomass of both species although it decreased the relative growth rate of soybean (Fig. 2). Greater root biomass favors translocation of the assimilated C to soil. It has been shown that the amount of photoassimilates released from roots to soil correlated positively with root biomass production in wheat (Henry et al., 2005). In another study, Ge et al. (2014) showed that N fertilization promoted biomass accumulation, which was accompanied by an increase in rhizodeposited C in rice. The third reason for the increased rhizodeposition under high soil nutrient availability is related to the nutrient status in plants. Although this study did not measure nutrient concentration in plant tissues, the field trial where the soil used in this study were collected, showed higher nutrient concentration in plant tissues of fertilized treatments compared to the no-fertilizer Control (Song et al., 2011). Higher soil nutrient availability can induce root branching and consequently the number of root apices (Bloom, 1997) for accumulating root biomass, where more fixed C would be released as mucilage, root border cells, extracellular enzymes, and simple and complex organic compounds (Freixes et al., 2002). For the whole growing period, this is not consistent with the generally opinion that nutrient deficiency enhanced root exudation (Paterson and Sim, 1999), but there was the similar case at early growth stages in the present study. All these results clearly demonstrated that total root growth was a major factor regulating C rhizodeposition during the whole growth season, associated with specific root exudation.

The increase in total C rhizodeposition under higher soil nutrient availability is reflected by plant-derived C as % total SOC at the maturity (Fig. 4). The positive effect of fertilizer application on plant C rhizodeposition has been reported by Tanja et al. (2001) when studied the effect of N fertilization on rhizodeposition of grass species. Gong et al. (2012) also observed that chemical fertilizer application contributed to SOC sequestration by favoring wheat-derived C input in a Phaeozem soil in a pot experiment. However, these results were not consistent with the finding of Kuzyakov et al. (2002) that the proportion of assimilated C released to the belowground decreased with N application in lettuce. Differences between the studies might be due to differences in species, stage of labelling and soil nutrient availability.

In the nutrient-rich soil environment, the organic amendment in combination with inorganic fertilizer further increased the rhizodeposited C compared to inorganic fertilizer-only treatment. Organic amendments provided additional available C as energy source to soil microbes (Lazcano et al., 2013) to reduce their dependence on rhizodeposited C. Though the change in community and activities of soil microbes was beyond the scope of the present study, the significantly higher C lost in NPKM in contrast to NPK might indicate an increase in soil microbial activity. This result was well in line with Li et al. (2016) who observed that an increased N availability with organic amendment increased soil microbial biomass, and decreased microbial reliance on rhizodeposited C in a ryegrass system. The greater rhizodeposition in soil favored soil aggregation, while stable aggregates protect the fixed C from microbial degradation (McVay, 2006). Additionally, organic C from animal manures is rich in recalcitrant compounds which persist as stable SOC (Fließbach et

al., 2007). Organic amendments also improve the aggregate stability by its combination with soil particles (Hütsch et al., 2002), which favors the physical protection of rhizodeposited C. This was evidenced by the higher proportion of deposited C in the silt/clay (<0.053 mm) fraction of the NPKM than NPK treatment (Fig. 5). In an Entic Haplorthod, Kaiser and Guggenberger (2000) found that application of organic manures enhanced the stabilization of rhizodeposited C from tree species in various soils. All these indicated that the net C input induced by organic amendment was the result of comprehensive effects of the higher rhizodeposition rate and physical protection of SOC in aggregates.

4.2. Crop species differ in C rhizodeposition

The greater amount of rhizodeposited C (kg C ha^{-1}) in soybean than maize system is not consistent with total below-ground C (g C plant^{-1}) between the two crops, which can be explained in the following reasons. First, soybean has higher plant density than maize in the field. Second, compared to maize, soybean system had the lower proportion of the below-ground C that was lost as CO_2, but the higher proportion retained in soil (Fig. 3). The result was supported by Mazzilli et al. (2015), who confirmed that soybean contributed four times more plant-derived C to soil than maize in a Typic Argiudol soil. This could be explained by the greater proportion of root-derived C associated with occluded particulate organic matter, silt and clay in legume systems (Puget and Drinkwater, 2001). Differences in plant-derived C in soil between the two crop species had probably resulted from the difference in their root morphology (Mengel, 1983). It was reported that differences in the amount of C rhizodeposition among maize, wheat and barley were positively correlated with root-hair density, number of root apices, root length and root surface area when grown in sterile nutrient solution (Nguyen, 2003). Mengel (1983) elucidated that monocots (C4 maize) and dicots (C3 soybean) differed considerably in their root morphology; maize developed a fibrous root system with numerous slender long roots, while soybean possessed a tap root with laterals. Greater root/shoot ratio in soybean has potential to increase plant-derived C in soil. This is consistent with the report of Russell et al. (2009). In addition, the amount of rhizodeposited C from soybean would be greater if there was similar decomposed rate between soybean and maize. This is because the faster decomposition rate of higher quality soybean rhizodeposits compared to maize (Shah et al., 2003; Qiao et al., 2015). The higher quality rhizodeposits includes N-rich compounds (Cheng et al., 2003). The N-rich compound induces C to stabilize in organo-mineral associations to further protect the rhizodeposited C (Mazzilli et al., 2014). Third, the higher proportion of plant-derived C to soil under soybean was ascribed to the great amount of C demand for N_2 fixation in the nodules, which induced more C and N released into soil at the mature stage (Voisin et al., 2010). This more C requirement in soybean could also be explained by the overlap of the vegetative and reproductive stages in soybean, which favors continuous accumulation of assimilated C during the reproductive stage (Jin et al., 2011).

4.3. Effect of growth stage on C rhizodeposition

The greater C rhizodeposition was observed at the vegetative stage than the reproductive stage. This might be attributed to the C return to soil from dead old roots before ^{13}C labeling. Furthermore, in this study, the proportion of fixed C allocated to roots and soil was approximate 155% higher at the vegetative stage than the reproductive stage for both plant species. This was supported by previous studies. For example, Gregory and Atwell (1991) reported that around 50% of C transferred from shoots remained in the roots of young plants, while only 10% of C was recovered

in the roots of mature wheat. Swinnen (1995) found that the amount of C partitioned to below-ground was negatively related with plant age of spring barley and winter wheat grown in a calcareous silt loam soil. The change of C allocation processes between different growth stages could be attributed to C-sink strength (Warembourg et al., 2003). The roots of young plants had a greater C-sink strength compared to mature roots (Keith et al., 1986). In addition, at the reproductive stage, the fixed C would mainly supply to seed development because the developing grains become the strongest C sink. For instance, grains demanded 43% of the fixed C in soybean (Jin et al., 2011) and 80% in rice (Lu et al., 2002). It appears that the C requirement of plant growth was the driver for assimilated-C translocation to the roots and hence the rhizodeposition.

5. Conclusions

This study demonstrated that improving soil nutrient supply increased total C rhizodeposition. Higher soil nutrient availability led to more allocation of photo-assimilated C to the roots and then to the soil. The application of organic amendments in combination with chemical fertilizers further enhanced C rhizodepostion, probably through impacting on microbial activity/function, and aggregate protection. Furthermore, the C rhizodeposition, as % total C fixed, was greater in soybean than in maize. Over one growing season, mean 80, 260 and 300 kg C ha^{-1} in the Control, NPK and NPKM treatments, respectively, were estimated to be transferred into soil through rhizodeposition. These results suggest that combined application of fertilizers and organic manure, and crop selection could maintain or improve SOC content to overcome the C decline in Mollisols due to inappropriate farming practices. However, because pulse labeling does not uniformly label all plant C fractions, more ^{13}C was incorporated into movable-C than structure-C fraction after the 6-h pulse labeling. The C rhizodeposition was over-estimated. Due to the limitation of pulse labeling to study C rhizodeposition, it is required to further study with continuous labeling in the future.

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