Fate of rice shoot and root residues, rhizodeposits, and microbe-assimilated carbon in paddy soil: I. Decomposition and priming effect

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Abstract. The input of recently photosynthesized C has significant implications on soil organic C sequestration, and in paddy soils, both plants and soil microbes contribute to the overall C input. In the present study, we investigated the fate and priming effect of organic C from different sources by conducting a 300-d incubation study with four different ^{13}C-labelled substrates: rice shoots (Shoot-C), rice roots (Root-C), rice rhizodeposits (Rhizo-C), and microbe-assimilated C (Micro-C). The efflux of both $^{13}CO_2$ and $^{13}CH_4$ indicated that the mineralization of C in Shoot-C-, Root-C-, Rhizo-C-, and Micro-C-treated soils rapidly increased at the beginning of the incubation and then decreased gradually afterwards. The highest cumulative C mineralization was observed in Root-C-treated soil (45.4%), followed by Shoot-C- (31.9%), Rhizo-C- (7.90%), and Micro-C-treated (7.70%) soils, which corresponded with mean residence times of 39.5, 50.3, 66.2, and 195 d, respectively. Shoot and root addition increased C emission from native SOC up to 11.4 and 2.3 times higher than that of the control soil by day 20 and decreased thereafter. Over the whole incubation period the priming effect of Shoot-C on CO_2 and CH_4 emission was strongly positive over the entire incubation, however, Root-C did not exhibit a significant positive priming effect. Although the total C contents of Rhizo-C (1.89%) and Micro-C-treated soils (1.90%) were higher than those of untreated soil (1.81%), no significant differences in cumulative C emissions were observed. Given the fact that about 0.3% and 0.1% of the cumulative C emission derived from the labeled Rhizo-C and Micro-C, this indicates that the soil organic C-derived emissions were lower in Rhizo-C and Micro-C treated soils than in untreated soil. This indicates that rhizodeposits and microbe-assimilated C could be used to reduce the mineralization of native soil organic carbon and to effectively improve soil C sequestration. The contrasting behaviour of the different photosynthesized C substrates suggests first, that recycling rice roots in paddies is more

beneficial than recycling shoots and, second, reveals the importance of increasing rhizodeposits and microbe-assimilated C in paddy soils *via* nutrient management.

Keywords: Paddy soil; Rice; Plant residues; Rhizodeposits; Microbe-assimilated carbon; CO_2 and CH_4 emission; Priming effect

1 Introduction

The soils of rice paddies, which cover an area of ~165 million ha worldwide, hold great potential for expanded C sequestration (Conrad et al., 2012; Ge et al., 2012; Lal, 2004), and the soil organic carbon (SOC) pools in agricultural systems, of which plant C is the primary substrate, are significantly affected by the input of crop residues (Weintraub et al., 2007). For example, after crops are harvested or die, aboveground biomass, such as straw, stubble, and other surface debris, contribute to annual C inputs (Lu et al., 2003), and photosynthesized C substrates are continuously released by rice plants as rhizodeposits throughout the growing season (Lu et al., 2002, 2003). Autotrophic soil microbes that assimilate CO_2 contribute to C sequestration in paddy soil, as well (Ge et al., 2013; Yuan et al., 2012a). As C inputs promoting microbial activity and native SOC decomposition (Ye et al., 2015), their quantity and quality influence microbe-mediated decomposition processes (Brant et al., 2006; Creamer et al., 2015). Therefore, the quantification of different C substrates allocated to paddy soils and their respective effects on native SOC require further investigation.

The aboveground biomass and root systems of rice plants represent one of the most important inputs of available organic C to paddy SOC (Johnson et al., 2006), the quantity and quality of which has been reported previously (Chen et al., 2014; Kisselle et al., 2001; Zhang et al., 2015). However, although aboveground biomass has been shown to make significant contributions to SOC sequestration (Lu et al., 2003), rice roots have been reported to contribute 1.5–3-fold more C to SOC than shoots (Hooker et al., 2005). Similarly, Molina et al. (2001) emphasized that the stalks and leaves of corn contribute 50% less C to SOC than the roots and rhizodeposits. The predominant contribution of crop roots to SOC can partly be explained by the chemical composition of roots, which includes cellulose and lignin, as well as by residue–soil interactions, such as aggregate formation, which physically protect organic C from biodegradation (Baumann et al., 2009; Johnson et al., 2006; Lu et al., 2003).

Previous studies have also reported that the rhizodeposits of rice account for ~17% of the photo-assimilates (Nguyen, 2003) that enter paddy soil, and that rice rhizodeposits include soluble exudates, root border cells, dead debris, and insoluble mucilage (Lu et al., 2003). In cereal crops, 10–25% of root exudates are incorporated into SOC, and rhizodeposits are thought to play a key role in C cycling and sequestration in plant–soil–microbe systems (Kuzyakov, 2002; Kuzyakov et al., 2003). In addition to the photosynthesized C substrates of plants, soil microbes are also able to assimilate CO_2

via the Calvin-Benson-Bassham cycle and, thus, can significantly contribute to the net uptake and assimilation of atmospheric CO_2 as well (Ge et al., 2013; Yuan et al., 2012b). In fact, the CO_2 uptake by phototrophic soil microbes has been reported to account for up to 0.36% of the total C fixed in rice paddy soils and 0.19% of the total C fixed in upland soils (Ge et al., 2013; Yuan et al., 2012b).

So far, the effect of C input from different C sources on the balance and stability of SOC has received limited attention. For example, roots and shoots are particulate and must be first depolymerized before taken up by microorganisms. In contrast, rhizodeposits can be efficiently taken up by microorganisms and converted to microbial biomass, which is, according to Kuzyakov (2002) and Kuzyakov et al. (2003), an important step in the formation of stable organic matter. Also low molecular weight substances such as rhizodeposits are protected from mineralization *via* sorption onto soil particles (Jones and Edwards, 1998; Saidy et al. 2012; Sodano et al. 2016), which contributes to the stability and sequestration of SOC (Ge et al., 2012). In addition, different C substrates can also have stimulating or restraining effects on the mineralization of native SOC, which are known as positive or negative priming effects (PEs), respectively (Kuzyakov, 2010). Priming is often caused by the addition of substrates with relatively high C availability and nutrient contents, which results in increased microbial activity (Blagodatsky et al., 2010; Huo et al., 2013). Hence, such easily degradable compounds greatly enhance the decomposition of native SOC (Blagodatsky et al., 2007; Qiao, et al., 2014), compared with the effects of ryegrass, cellulose, or wheat straw, which have complex structures that are less available to microbes (Kuzyakov and Bol, 2006; Kuzyakov et al., 2000). However, as easily available low-molecular substrates like rhizodeposits can be easily immobilized by microbial metabolism (Lu et al., 2002; Gunina et al., 2014) or sorption (Jones and Edwards, 1998; Saidy et al. 2012; Sodano et al. 2016), their PE can be small or even negative (Ge et al., 2012).

Accordingly, the quantity and quality of different C inputs, as well as their fate and PE in paddy soils, are globally important (Bastida et al., 2013; Johnson et al., 2006; Wang et al., 2015). There were only limited studies of estimating the fate of plant residues and rhizodeposits in paddy soils and, to our knowledge, there is no comparative information on (1) the decomposition of different organic C sources, such as rice shoots and roots, rhizodeposits, and microbe-assimilated C; or (2) the effects of different organic C sources on the mineralization of native SOC. We hypothesized that depending of the type of the primer both, the decomposition of the primer itself and with that the PEs on native soil

organic matter vary. We assume that shoots and roots, entering the soil as unprotected particulate organic residues, are well available for microorganisms and thus also stimulate native organic matter decomposition. In contrast, rhizodeposits and microbial carbon reflect a carbon sources that are rather stabilized and contribute less to priming. We investigated these hypotheses by quantifying the contribution of different organic C sources to CO_2 and CH_4 emission and by analysing their PE, in a 300-d incubation study using ^{13}C-labelled rice plant residues, rhizodeposits, and microbe-assimilated C in paddy soils.

2 Materials and methods

2.1 Study site and soil sampling

The experimental rice field was located at the Changsha Research Station for Agricultural and Environmental Monitoring, Hunan, China (113°19′52″ E, 28°33′04″ N; 80 m above sea level), where the climate is subtropical, with a mean annual temperature and rainfall of 17.5 ℃ and 1300 mm, respectively. The soil developed from highly weathered granite and is classified as a typical Stagnic Anthrosol, Moist soil samples were collected from the plough layer (0–20 cm) and sieved (<4 mm) to remove visible plant residues. The soil contained 18.1 g kg^{-1} organic C with a $\delta^{13}C$ value of -26.7‰, 1.8 g kg^{-1} total N, 0.4 g kg^{-1} total K and had a pH of 5.6 at a soil: water ratio (w/v) of 1: 2.5.

2.2 Production of ^{13}C-labelled substrates

Rice cultivation and $^{13}CO_2$ labelling were performed as described by Ge et al. (2012; 2013), with some modifications. Briefly, 60 pots were filled with 1 kg dry soil, and of these, 40 pots were planted with three 30-d-old rice seedlings (*Oryza sativa* L. 'Zhongzao 39') each, whereas the remaining 20 pots were unplanted.

For ^{13}C labelling, 20 planted and 10 unplanted pots were transferred to an automatically controlled gas-tight growth chamber (110 cm length, 250 cm width, 180 cm height) and exposed to $^{13}CO_2$-fumigation for 18 d (May 14–31, 2013), during the vegetative growth period (including the

entire tillering stage). The growth chambers were placed in a rice field to ensure that the environmental conditions of the labelled and control plants would be identical for labelled plants and unlabelled controls. The remaining 30 pots (20 planted, 10 unplanted), which served as controls for measuring natural ^{13}C abundance, were placed 10–15 m from the growth chambers. The surface of each planted pot was covered with black plastic sheeting, to prevent algal photosynthesis in the floodwater and to ensure that only the rice shoots were exposed to $^{13}CO_2$, whereas the unplanted pots were left uncovered, so that the soils were directly exposed to $^{13}CO_2$ and so phototrophic soil microbes could assimilate atmospheric $^{13}CO_2$. All the pots were watered every few days, in order to maintain a water depth of 2–3 cm above the soil surface, until harvest. Weeds were removed manually.

The CO_2 concentrations of the growth chambers were measured using an infrared analyser (Shsen-QZD, Qingdao, China) and maintained at 360–380 μl L^{-1}. The $^{13}CO_2$ was generated by acidifying $Na_2{}^{13}CO_3$ (1.0 M, 99 atom % ^{13}C; Cambridge Isotope Laboratories, Tewksbury, MA, USA) with H_2SO_4 (0.5 M) in beakers that were placed inside the growth chambers. During the labelling period, $^{13}CO_2$ was only released when CO_2 concentrations fell below 360 μl L^{-1}, and at CO_2 concentrations >380 μl L^{-1}, the gas flow was diverted and passed through CO_2 traps (NaOH solution). An air-conditioning system was used to control the temperature inside the chamber within 1 ℃ of the ambient temperature in the rice field. Two fans continuously circulated the air in the growth chamber.

2.3 ^{13}C-labelled substrate collection

All the rice plants and soils were sampled destructively after 18 d of $^{13}CO_2$ labelling. Rice shoots were removed at their bases, whereas rice roots were separated from the soil by washing with deionized water, and both shoots and roots were dried at 60 ℃ for 48 h and then cut into <5 mm pieces. ^{13}C-labelled rhizodeposits were obtained by gently shaking moist soil from the rice roots, and the soil adhering to the roots was washed by distilled water, then the soil slurries were mixed well and centrifuged at 13,000 g for 15 min. The fine roots with light density were removed together with supernatants while the rhizodeposits were collected with the soil. To obtain microbe-assimilated ^{13}C, we collected soil from ^{13}C-treated, unplanted pots and mixed it thoroughly.

2.4 Soil incubation

To determine the PEs of different C sources and the effect of different C substrates on CO_2 and CH_4 emission, we conducted a 300-d incubation study of paddy soils that had been supplemented with ^{13}C-labelled shoots, roots, rhizodeposits, or microbe-assimilated C. Five treatments were used: (1) unlabelled and unplanted paddy soil supplemented with ^{13}C-labelled shoot residue (Shoot-C), (2) unlabelled and unplanted paddy soil supplemented with ^{13}C-labelled root residue (Root-C), (3) soil containing ^{13}C-labelled rhizodeposits (Rhizo-C), (4) ^{13}C-labelled soil containing ^{13}C-labelled microbe-assimilated C (Micro-C), and (5) unlabelled and unplanted soil without supplementation (CK). Three additional treatments were used to determine the natural occurrence of ^{13}C: (1) unlabelled and unplanted paddy soil with unlabelled shoot residue, (2) unlabelled and unplanted paddy soil with unlabelled root residue, and (3) unlabelled and unplanted paddy soil with unlabelled rhizodeposits.

For the Shoot-C and Root-C treatments, 150 g (100 g dry weight equivalent) unlabelled, unplanted soil with a water content of 50% was homogenized with 0.6 g of labelled and dried shoot and root residue, respectively, with a final residue content of 6 g kg^{-1}. Subsequently, the samples were transferred to 500 ml serum bottles with 100 ml deionized water, to ensure a water layer of 2–3 cm, and the bottles were sealed with butyl rubber stoppers. For the Rhizo-C and Micro-C treatments, 150 g fresh soil containing either ^{13}C-labelled rhizodeposits (from rice roots) or ^{13}C-labelled microbe-assimilated C (from labelled, unplanted pots) were directly weighed into 500 ml serum bottles, respectively. Incubation was conducted at 25 ℃ in the dark for 300 d, with four replicates for each treatment. CH_4 and CO_2 concentrations of the headspace samples were collected at 1, 3, 5, 10 d and then every 10 d after sealing, the gas was collected using a gas-tight syringe and stored in pre-evacuated Exetainer glass bottles (Labco, High Wycombe, UK). After each sampling point, the serum bottle was ventilated for 10 min, and then sealed with butyl rubber stoppers.

2.5 Analytical methods

The C content of the soil and plant residues (shoots and roots) was determined using dry combustion with an elemental analyser (vario MAX; Elementar Analysensysteme GmbH, Hanau, Germany), whereas the CH_4 and CO_2 concentrations of the headspace samples were measured using a gas chromatographer (Agilent 7890A, Agilent Technologies, Alto Palo, California, USA) equipped with a thermal conductivity detector for measuring CO_2 and a flame ionization detector for measuring CH_4. In

addition, the stable C isotope composition of soils and plant residues were analysed using an isotope ratio mass spectrometer coupled with an elemental analyser (FLASH 2000; Thermo Fisher Scientific, USA), whereas the stable C isotope composition of CO_2 and CH_4 in the headspace samples were analysed using the isotope ratio mass spectrometer coupled with a GasBench (Thermo Fisher Scientific, USA).

2.6 Calculations and statistical analysis

The $\delta^{13}C$ values of plant residues, rhizodeposits, microbe-assimilated C, soils, CO_2, and CH_4 were converted in δ (‰) relative to the Pee Dee Belemnite (PDB, 0.0111802) standard and further expressed in atom% as following

$$\text{atom\%} = \frac{100 * 0.0111802 * (\frac{\delta}{1000} + 1)}{1 + 0.0111802 * (\frac{\delta}{1000} + 1)} \quad (1)$$

and the incorporation of ^{13}C (^{13}C excess) in plant residues, rhizodeposits, microbe-assimilated C, bulk soils, CO_2, and CH_4 was calculated as follows:

$$\text{excess } {}^{13}C_{sample} = [(\text{atom\%}\,{}^{13}C)_L - (\text{atom\%}\,{}^{13}C)_{UL}] \times \text{Csample} / 100 \quad (2)$$

Where $(\text{atom\%}\ {}^{13}C)_L$ and $(\text{atom\%}\ {}^{13}C)_{UL}$ are the *atom% ^{13}C* in labelled and unlabelled samples, respectively, and C_{sample} are the C contents of each sample.

The $^{13}CO_2$ and $^{13}CH_4$ efflux (%) were calculated as the increases in excess of ^{13}C-CO_2 and ^{13}C-CH_4 within each sampling interval,, respectively, as percentages of the ^{13}C input. The mineralization percentage of the input ^{13}C was calculated as the sum of total ^{13}C in CO_2 and CH_4, at each sampling day, relative to the initially added total ^{13}C.

The kinetics of the mineralization were described by fitting a first order single exponential function:

$$y = a\,(1 - e^{-bx}) \quad (3)$$

where *a* describes the amount of bioavailable labelled-substrate pool; *b* is the mineralization rate of substrate; and *x* is time (d). Obtained parameters were used to calculate the mean residence time as 1/*b* and half-life as ln (2)/*b*.

The end-member mixing model was used to calculate the fractions of SOC- (C_{SOC}) and plant residue-derived C (C_{shoot} and C_{root}), as described by Phillips et al. (2005) and Wild et al. (2014). This

model allows the combination of mass spectrometric and efflux measurements. The shoot-derived $^{13}CO_2$ emission ($^{13}CO_{2shoot\text{-}derived}$) was calculated as follows:

$$^{13}CO_{2shoot-derived} = \frac{atom\%\ CO_{2shoot} - atom\%\ CO_{2CK}}{atom\%\ C_{shoot} - atom\%\ C_{soil}} \times CO_{2shoot-C} \quad (4)$$

where atom% CO_{2shoot} and atom%CO_{2CK} are the atom% ^{13}C values of CO_2 derived from shoot treated soil and untreated soil (CK), respectively; atom%C_{shoot} and atom%C_{soil} are the atom% ^{13}C values of shoot and bulk soil respectively; and $CO_{2shoot\text{-}C}$ is the total CO_2 derived from shoot treated soil; and the shoot-derived $^{13}CH_4$ emission ($^{13}CH_{4shoot\text{-}derived}$) and the root-derived $^{13}CO_2$ and $^{13}CH_4$ emission ($^{13}CO_{2root\text{-}derived}$ and $^{13}CH_{4root\text{-}derived}$, respectively) were calculated similarly (Phillips et al., 2005; Ye et al., 2015).

The PE of SOM on CO_2 and CH_4 emission was calculated as follows:

$$PE_t(\%) = \frac{Gas - Gas_{CK}}{Gas_{CK}} \times 100 \quad (5)$$

where PE_t is the PE at time t (d); *Gas* the total amount of CO_2 and CH_4 derived from native SOC mineralization in the treatment of Shoot-C and Root-C, Gas_{CK} is the SOC mineralization in the CK treatment (Hu et al., 2012).

Analysis of variance in conjunction with Duncan's multiple range test ($p < 0.05$) and correlation analysis were conducted using SPSS 17 (SPSS Inc., Chicago, IL, USA), and figures were created using Origin 8.5 (OriginLab, Northampton, MA, USA).

3 Results

3.1 CO_2 and CH_4 emission of carbon substrate-treated soils

The excess of ^{13}C per 100 g soil was 11.4, 5.75, 1.61, and 0.49 mg in the Shoot-C, Root-C, Rhizo-C, and Micro-C treatments, respectively (Table 1). The $^{13}CO_2$ efflux from the each treatment increased rapidly at the beginning of the incubation, peaked after 20 d, and then decreased gradually (Fig. 1a). The CO_2 efflux rates from Shoot-C and Root-C treated soils were 0.71 and 0.66 % of initial ^{13}C per day, respectively, which was higher than those of Rhizo-C- (0.11% of initial ^{13}C per day) and Micro-C-treated (0.06% of initial ^{13}C per day) soils. The $^{13}CH_4$ efflux rates exhibited similar patterns (Fig. 1b). The cumulative $^{13}CO_2$ and $^{13}CH_4$ emissions increased exponentially during the first 60 d of incubation, after which they increased slowlier (Fig. 2). The total $^{13}CO_2$ emissions accounted for 28.6 and 43.8% of the initial ^{13}C from Shoot-C and Root-C, respectively, and 7.90% and 7.70% of the initial

^{13}C in Rhizo-C and Micro-C (Fig. 2a). The cumulative $^{13}CH_4$ emissions only accounted for 3.3 and 1.6% of the initial ^{13}C from Shoot-C and Root-C, respectively. But the $^{13}CH_4$ was not detected in Rhizo-C and Micro-C (Fig. 2b).

The cumulative mineralization of substrate-derived ^{13}C was more rapid at the beginning of the incubation and followed a single exponential model (Fig. S1), and at the end of the incubation, we found that the percentage of substrate-derived carbon mineralized was highest in Root-C-treated soils (45.4%), followed by Shoot-C (31.9%), Rhizo-C (7.90%), and Micro-C treated (7.70%) soils. And about 0.3% and 0.1% of the cumulative C emission derived from the labelled Rhizo-C and Micro-C. In addition, the size of bioavailable labelled-substrate C pool in the Shoot-C and Root-C treated soils was 34.2 and 46.2%, respectively, which was 4–5-fold larger than that of the Micro-C- (9.7%) and Rhizo-C-treated (7.8%) soils, and the mean residence times (MRT) of the Shoot-C, Root-C, Rhizo-C, and Micro-C treated soils were 39.5, 50.3, 66.2, and 195 d, respectively (Table 2).

3.2 Priming effect of Shoot-C and Root-C on CO_2 and CH_4 emission

During incubation, the emission rates of CO_2 and CH_4 from control soils ranged from 4.7 to 15.9 mg kg^{-1} d^{-1}. Shoot and root addition increased total C emission from native SOC up to 11.4 and 2.3 times than that of the control soil by day 20, respectively, and the stimulatory effect persisted to the end of incubation period. The C emission from native SOC increased linearly at the initial 20 days, and then decreased rapidly (Fig. 3). The PE of the Shoot-C treatment peaked at 378% after 20 d of incubation and decreased to 52% by the end of the incubation, whereas the PE of the Root-C treatment peaked at 43% after 50 d of incubation and then decreased to 2.9%. Thus, the positive PE of Shoot-C was clearly stronger than that of Root-C, especially since the PE of Root-C exhibited no significant positive priming effect (Fig. 4).

3.3 Mineralization of soil organic carbon in Rhizo-C and Micro-C treated soils

The total C emissions of Rhizo-C and Micro-C treated soils increased significantly from 116 mg kg^{-1} and 81 mg kg^{-1} after 10 d of incubation, respectively, to 1754 mg kg^{-1} and 1785 mg kg^{-1} by the end of the incubation. The total C emission of Rhizo C-treated soil was significantly higher than that of the Micro-C and untreated soil during the first 200 d of incubation; however, no significant differences

were identified at the end of the incubation (Fig. 5a). Also the total ^{13}C emissions derived from the labelled substrates was significantly higher for the Rhizo-C than those for the Micro-C-treated soils ($p < 0.05$; Fig. 5b). However, the cumulative C mineralization of neither the Rhizo-C nor the Micro-C treated soils was significantly different from that of the untreated soil, which suggested that the rhizodeposits and microbe-assimilated C had no positive effect on the mineralization of native SOC.

4 Discussion

4.1 Mineralization of carbon substrates in paddy soil

The effluxes of both CO_2 and CH_4 from soils treated with ^{13}C-labelled substrates exhibited a rapid increase at the beginning of the incubation, followed by a slow decrease (Fig. 1), which indicated that microbes prefer fresh C substrates over native SOC (Yuan et al., 2012c), as has been reported by previous studies on the decomposition of fresh C substrates in both paddy and upland soils (Lu et al., 2003; Parshotam et al., 2000). In these systems, the initial rapid decomposition is due to the addition of easily degradable organic C in the added substrates, such as starch and other labile compounds. Then, after the exhaustion of labile C of the added substrates, more recalcitrant components, such as cutin and lignin from both, the added substrates and the native SOM, and native mineral-stabilized SOC are utilized (Baumann et al., 2009). The transition could also involve an alteration in species dominance, with rapidly proliferating bacteria using more available compounds during the early stages of decomposition and slower-growing fungi using the more recalcitrant components during later stages (Baumann et al., 2009; Brant et al., 2006).

Both CO_2 and CH_4 efflux are important components of the C cycle in paddy soils and represent a major proportion of the C released by microbial decomposition (Yuan et al., 2012c), and the results of the present study suggest that the mineralization of shoot- and root-derived ^{13}C was ~3-4 times higher than that of rhizodeposite- and microbe-derived ^{13}C (i.e., Root-C > Shoot-C > Rhizo-C > Micro-C; Fig. 2). The present study also found that the percentage of root-derived ^{13}C recovered from CO_2 was 1.6-fold higher than that from shoot-derived ^{13}C. This suggests that root residue was more easily decomposed, a conclusion that was also supported by the higher $^{13}CO_2$ efflux of Root-C-treated soils.

However, the C mineralization rates of Rhizo-C and Micro-C treated soils were much slower, and the MRTs of Rhizo-C and Micro-C treated soils were 2–4-fold higher than those of Shoot-C and

Root-C treated soils. Presumably this owes to the formation of mineral-associated organic matter during the labelling period that was well protected from microbial degradation and had a slow turnover rate (Basler et al., 2015; Mikutta et al., 2014; Saidy et al., 2012; Schurig et al., 2013). Another possible reason for this observation is possibly that most of the C in Rhizo-C and Micro-C treated soils was not mineralized to CO_2 but, instead, underwent intensive internal recycling (Gunina and Kuzyakov, 2015; Knowles et al., 2010). Further, the easily available substrate-derived C can be incorporated into metabolic products, such as sugars, carboxylic acids, and amino acids, which in turn are used to build up stable cell membranes, cell walls, or polymers (Apostel et al., 2015; Gunina et al., 2014). Besides that, microorganisms are associated with minerals and thus are involved in the formation of occluded particulate organic matter and mineral-associated organic matter (Basler et al., 2015, Schurig et al., 2013), with particular the latter considered being very stable.

4.2 Effect of carbon substrates on native SOC mineralization

In the present study, the emission of CO_2 and CH_4 by Shoot-C and Root-C treated soils during the first 50 d were mainly derived from plant residue C, after which the relative contribution of native SOC increased. For Shoot-C, a positive PE was observed over the entire incubation period, while for Root-C this was significant only for early stages of the incubation (Fig. 4). These results support previous studies showing that the initial phase of rapid decomposition was the result of easily degraded organic C and other available nutrients added with the residues that promote both microbial activity and SOC decomposition (Chen et al., 2014). The compounds decomposed during the slower phase were less available for microbial growth, and as a result of C limitation, most of the available C was likely incorporated into cells and converted to storage compounds, rather than used for growth or respiration (Lu et al., 2003; Brant et al., 2006). However, the extracellular enzymes generated to degrade recalcitrant C substrates might be more effective in decomposing SOC at later stages of incubation, leading to a positive PE (Chen et al., 2014). In addition, the two phases of exogenous C decomposition and the mechanisms of PE simultaneously influence the strength and extent of native SOC mineralization (Chen et al., 2014; Ye et al., 2015).

Both, Rhizo-C and Micro-C augmented the C content of paddy soil (1.89 and 1.90%, respectively) over that of the untreated soil (1.81%). At the same time we found that the C emissions of Rhizo-C and

Micro-C treated soils were similar to those of untreated soil. As about 0.3% and 0.1% of the substrate C, respectively, were mineralized, this suggest that rhizodeposits and microbe-assimilated C input did not stimulate native SOC mineralization but rather shows a negative priming. Hence, it seems that Rhizo-C and Micro-C protects native SOC, increase the organic carbon storage of paddy soil (Ge et al., 2012; Li and Yagi, 2004; Gunina et al., 2015). Besides the lower contents of Rhizo-C and Micro-C as compared to Shoot-C and Root-C, this observation is possibly due to the different behaviour of primers in soil. Roots and shoots enter the soil as particulate and unprotected organic matter, which is to a large part well available for microorganisms; i.e. 31.9% and 45.4% where mineralized within the 300 days of incubation (Fig. S1). Rhizodeposits consist mostly of low molecular sugars and acids that are highly bioavailable (Lu et al., 2002). The relatively long MRTs of Rhizo-C (Table 2) suggests a stabilization process of this carbon, either by sorption or by microbial metabolism and recycling during the incubation (Lu et al., 2002; Gunina et al., 2014; Schurig et al., 2013). Also Micro-C hat a long MRT in the incubation (Table 2), which fits well to the observation that microbial residues are accumulating in soil (Schurig et al., 2013).

5 Conclusions

In the present study, Root-C treated soils exhibited the highest rate of C mineralization, followed by Shoot-C, Rhizo-C, and Micro-Ctreated soils, whereas the opposite trend was observed for MRT. By the end of 300-d incubation, Shoot-C treated soils exhibited higher total mineralization and positive PEs, while Root-C failed to exhibit a significant priming effect. Although plant residues are widely used for improving soil fertility, their contribution to SOC assimilation is inefficient, and their use also contributes to the emission of greenhouse gasses. However, the present study demonstrates that both, rhizodeposits and microbe-assimilated C can reduce native SOC decomposition and may more effectively contribute to the stability and sequestration of soil C.

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Tables

Table 1. The carbon (C) content, atom ^{13}C, and amount of ^{13}C in added with four labelled photosynthesized C substrates input to 100 g bulk soil

	Bulk soil	**Shoot-C**	**Root-C**	**Rhizo-C**	**Micro-C**
C content (%)	1.80 ± 0.12	40.50 ± 2.13	28.60 ± 1.15	1.89 ± 0.12	1.90 ± 0.11
Atom ^{13}C (%)	1.08 ± 0.02	5.78 ± 0.09	4.43 ± 0.07	1.16 ± 0.03	1.11 ± 0.02
Total excess of ^{13}C (mg)	-	11.43 ± 0.52	5.75 ± 0.41	1.61 ± 0.06	0.49 ± 0.05

Bulk soil, unplanted control soil; Shoot-C, paddy soil supplemented with ^{13}C-labelled shoot residue; Root-C, paddy soil supplemented with ^{13}C-labelled root residue;Rhizo-C, paddy soil supplemented with ^{13}C-labelled rhizodeposits; Micro-C, paddy soil supplemented with ^{13}C-labelled microbe-accumulated C.

514 **Table 2.** The size of bioavailable labelled-substrate C pool, mean residence time (MRT), and half-life
515 of cumulative ^{13}C recovery in CO_2 and CH_4 in four different incubation treatments.

Treatment	Size (%)	MRT (d)	Half-life (d)	R^2
Shoot-C	32.4 ± 0.56	39.5 ± 0.63	27.4 ± 0.55	0.99
Root-C	44.9 ± 1.12	50.3 ± 0.71	34.7 ± 0.62	0.99
Rhizo-C	7.3 ± 0.38	66.2 ± 0.92	46.4 ± 1.31	0.98
Micro-C	9.1 ± 0.44	195 ± 1.52	136 ± 1.66	0.98

516 The size of bioavailable labelled-substrate C pool (% initial ^{13}C), MRT, and R^2 were calculated based
517 on Fig. 1S. Shoot-C, paddy soil supplemented with ^{13}C-labelled shoot residue; Root-C, paddy soil
518 supplemented with ^{13}C-labelled root residue; Rhizo-C, paddy soil supplemented with ^{13}C-labelled
519 rhizodeposits; Micro-C, paddy soil supplemented with ^{13}C-labelled microbe-accumulated C.

Figures captions

Figure 1. $^{13}CO_2$ (**c**) and $^{13}CH_4$ (**d**) efflux (% initial ^{13}C) d^{-1} over the 300-d incubation period. Values and error bars represent means ± SE (n = 4). Shoot-C, unlabelled paddy soil supplemented with ^{13}C-labelled shoot residue; Root-C, unlabelled paddy soil supplemented with ^{13}C-labelled root residue; Rhizo-C, paddy soil containing ^{13}C-labelled rhizodeposits; Micro-C, paddy soil containing ^{13}C-labelled microbe-accumulated C.

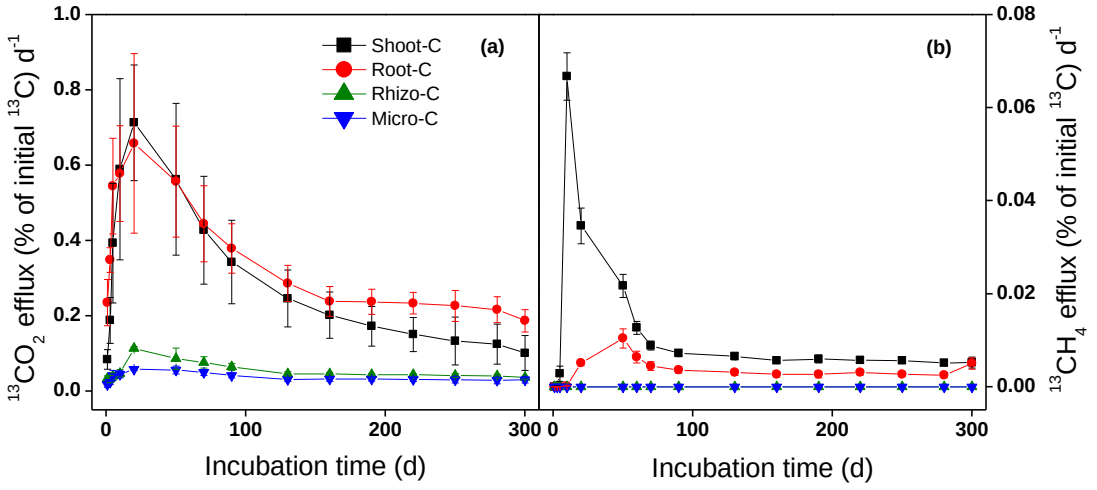
Figure 2. Cumulative $^{13}CO_2$ (**a**) and $^{13}CH_4$ (**b**) emissions (% of initial ^{13}C) over the 300-d incubation period. Values and error bars represent means ± SE (n = 4). Shoot-C, unlabelled paddy soil supplemented with ^{13}C-labelled shoot residue; Root-C, unlabelled paddy soil supplemented with ^{13}C-labelled root residue; Rhizo-C, paddy soil containing ^{13}C-labelled rhizodeposits; Micro-C, paddy soil containing ^{13}C-labelled microbe-accumulated C.

Figure 3. C (CO_2-C and CH_4-C) emissions by Shoot-C- (**a**) and Root-C-treated (**b**) soils over the 300-d incubation period. Values and error bars represent means ± SE (n = 4). Shoot-C, ^{13}C-labelled shoot residue; Root-C, ^{13}C-labelled root residue; SOC, soil organic carbon; CK, unlabelled and unplanted soil without supplementation.

Figure 4. Priming effect (%) of ^{13}C-labelled plant residues over the 300-d incubation period. Values and error bars represent means ± SE (n = 4). Shoot-C, unlabelled paddy soil supplemented with ^{13}C-labelled shoot residue; Root-C, unlabelled paddy soil supplemented with ^{13}C-labelled root residue.

Figure 5. Total C (**a**) and ^{13}C (**b**) emission by ^{13}C-labelled rhizodeposit- and microbe-accumulated C-treated soils over the 300-d incubation period. Values and error bars represent means ±SE (n = 4). Different letters indicate significant differences at $p < 0.05$ (Duncan multiple range test). Rhizo-C, paddy soil containing ^{13}C-labelled rhizodeposits; Micro-C, paddy soil containing ^{13}C-labelled microbe-accumulated C; CK, unlabelled and unplanted soil without supplementation.

548 **Figure 1**



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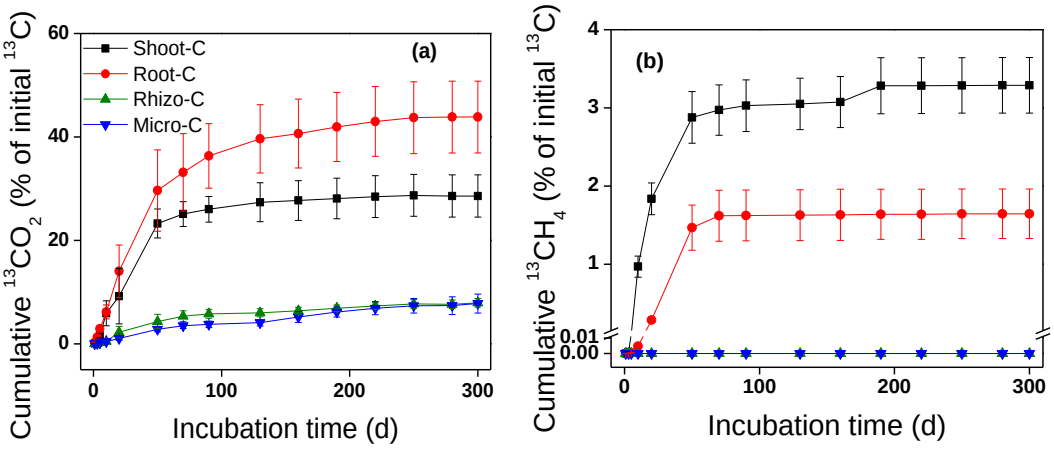
551 **Figure 2**



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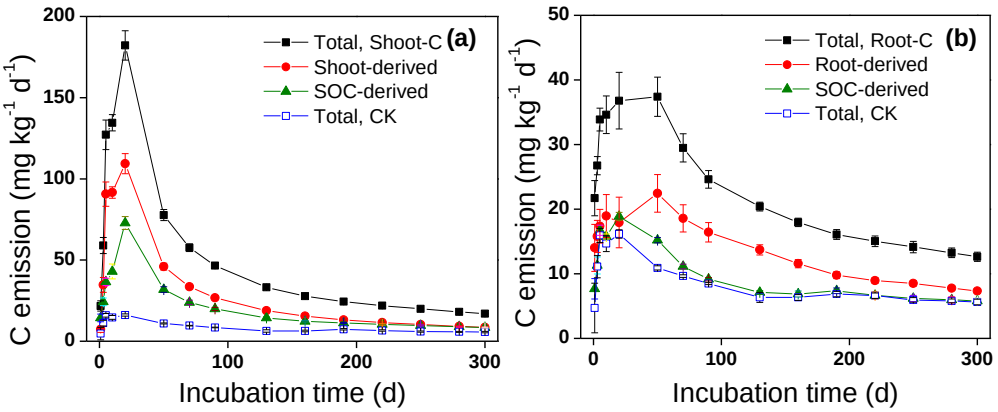
554 **Figure 3**



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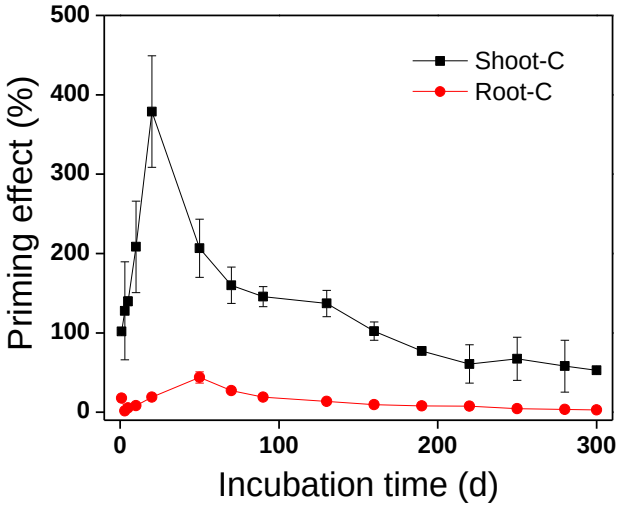
557 **Figure 4**



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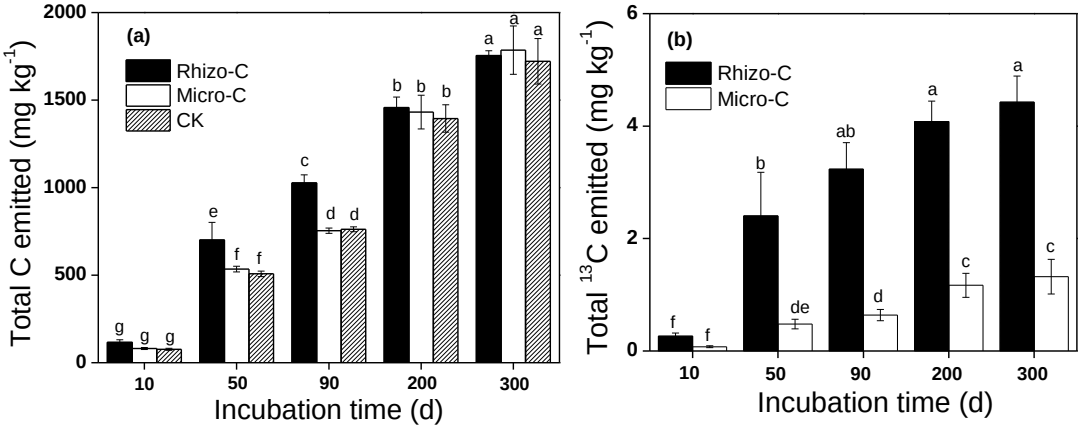
560 **Figure 5**



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