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Nitrogen fertilization increases rice rhizodeposition and its stabilization in soil aggregates and the humus fraction`

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Abstract

Background and aims Rhizodeposited-carbon (C) plays an important role in regulating soil C concentrations and turnover, however, the distribution of rhizodeposited-C into different soil organic carbon (SOC) pools and how regulated by nitrogen (N) fertilization still remains elusive. *Methods* We applied five N fertilization rates (0, 10, 20, 40, and 60 mg N kg^{-1} soil) to rice (*Oryza sativa* L.) with continuously labeled $^{13}CO_2$ for 18 days, to measure ^{13}C allocation into plant tissues and soil C fractions. *Results* Relative to the unfertilized controls, the ratio of ^{13}C in plant aboveground shoot /belowground root increased as a result of N fertilization, and the contribution of rhizodeposited-C to SOC was increased by N fertilization, presumably due to the relatively high root biomass and exudates. Also, N fertilization increased ^{13}C incorporation into large aggregates (0.25–2.0 mm) and the humic acid fraction. Biological C immobilization might occur and preserve rhizodeposition following high rates of N addition, which regulates rhizodeposits and C cycling, thus determining the stabilization of rhizodeposits in the different SOC pools. *Conclusion* Rhizodeposited-C from rice plants and its distribution within SOC pools strongly depend upon N fertilization, thus determines C sequestration potential from the rice plants.

Keywords Rice rhizodeposited-C · N fertilizer application · Continuous $^{13}CO_2$ labeling · Carbon allocation · SOC fractions

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Abbreviations

SOC	Soil organic carbon
TN	Soil total nitrogen
DOC	Dissolved organic carbon
MBC	Microbial biomass carbon

Introduction

Plant carbon (C) is the primary source of soil organic carbon (SOC), delivered to the soil as aboveground shoot residues and belowground rhizodeposits, which drive C cycling in terrestrial ecosystems (Ge et al. 2015; Weintraub et al. 2007). Rhizodeposited-C accounts for approximately 11% of all net photo-assimilates, mainly comprising water-soluble compounds with low molecular weight, e.g. organic acids, and insoluble compounds with greater molecular weight, such as mucilage (Jones et al. 2009; Kuzyakov and Schneckenberger 2004). Rhizodeposited-C strongly influences C sequestration in soil by affecting the mineralization and formation of SOC (Kuzyakov et al. 2003). Enhancing C sequestration and stabilization is especially important in paddy soils, due to its large contributions (up to 0.52 Gt of CO_2 equivalents) to atmosphere C (Ge et al. 2012, 2015). It was reported that C transferred remaining belowground and rhizodeposition were 1065 kg C ha^{-1} and 200 kg C ha^{-1} for flooded rice, respectively (Lu et al. 2002b). Therefore, understanding rhizodeposited-C flow and its ecological implications for paddy soils requires investigation into the fate of this C within SOC pools.

Isotopic labeling allow to distinguish rhizodeposited C released by roots from the C already present in soil (SOM, microbial biomass etc.) (Kuzyakov et al. 2003). Pulse labeling, which label period is much shorter than the plant growth and C flow process, allows to investigate the dynamics of C fluxes in the plant organs (shoots, leafs, roots, etc.) and in soil compartments (e.g. microbial biomass, DOM, SOM pools, etc.). Based on the dynamics of the ^{14}C or ^{13}C in any of the pools after the pulse labeling, the rates of the inputs and outputs of root released C in the respective pool can be calculated. The alternative is the labeling period corresponding to the whole duration of the process under study, termed as continuous labeling, which allows direct estimation of total C input by roots into the soil over the whole growth period.

The fractionation of SOC based on physically defined fractions play various roles in understanding SOC stabilization (Six et al. 2002). Studies on the distribution of rhizodeposited-C are crucial for understanding its C sequestration effects as the spatial arrangement largely determines SOC bio-accessibility to microorganisms (Brookes et al. 2017; Schmidt et al. 2011; von Lützow et al. 2007). The dynamics of soil aggregates has gained increasing attention because of their role in C sequestration thus maintaining SOC levels. It is estimated that 90% of surface SOC is associated with aggregates, and 20–40% of this large subset is intra-microaggregate SOC (Carter 2002; Six et al. 2002). The formation of microaggregates through biotic glues and abiotic clay flocculation makes them more stable, with longer turnover times than macroaggregates formed by more transient biogenic processes (Rillig and Mummey 2006; Six et al. 2002).

Nitrogen (N) is one of the most limiting nutrients for plants, and its availability can affect the quantity and quality of rhizodeposition (Atere et al. 2017; Ge et al. 2015). A recent review found conflicting results and different methodological approaches among existing studies concerning N effects on the fate of rhizodeposition (Bowsher et al. 2017). For example, Ge et al. (2015) found enhanced assimilation of rice C into SOC at high rates of N fertilizer in paddy ecosystems, while, with barley, the opposite results were observed. More photosynthesis-derived C in barley systems was incorporated into SOC without N fertilization (Johansson 1992). Although previous research has gained knowledge of N fertilization effects on the above/belowground distribution and fate of plant-assimilated C in soils, whether N promotes deposition of photosynthesis-derived C into different soil organic C pools, e.g. aggregates still remains largely unknown (Ge et al. 2015).

Thus, we performed ^{13}C-CO_2 continuous labeling (18 days) of rice plants grown in a paddy system under a range of N fertilization rates during the entire tillering stage during the rice vegetative growth period, to quantify rice rhizodeposited-C inputs into the aboveground plant, belowground root and SOM fractions (by both physically separated aggregation and as chemical extraction approaches), at different N fertilization rates. We hypothesized that (1) N fertilization increased total plant biomass and, as a result, increased belowground C allocation through the release of C from rice roots into the soil, (2) the distribution of rhizodeposited-C in particle-size and humus fractions was shifted by N fertilization.

Materials and methods

Soil

Soil samples were collected from the Ap horizon (0–20 cm) from a rice field (113°19′52″E, 28°33′04″N, 80 m a.s.l.) in the subtropical region of China, with a

mean annual temperature of 17.5 °C; rainfall of 1300 mm; sunshine of 1663 h; and frost-free period of 274 days per year (Shen et al. 2014). Soil samples were then sieved (<4 mm) and the coarse plant residues were removed. The soil was classified as an Hydragric Anthrosol (Eutric, Siltic, Gleyic), Ge et al. (2017) developed from a granite parent material after subtropical weathering (Ge et al. 2017). The soil texture was 7.5% clay, 68.4% silt, and 24.1% sand. The soil contained 18.1 g kg^{-1} organic C, 1.8 g kg^{-1} total N, and 0.4 g kg^{-1} total P, and had a pH of 5.6 (1:2.5, soil to water ratio).

Experimental layout

Nitrogen fertilizer as $(NH_4)_2SO_4$ was applied to the soils with five levels: N-free (N0); 10 mg N kg^{-1} soil (N10); 20 mg N kg^{-1} soil (N20); 40 mg N kg^{-1} soil (N40); and 60 mg N kg^{-1} soil (N60). The soils were then placed into plastic pots (17.2 cm diameter and 16.7 cm height) with NaH_2PO_4 (20 mg P kg^{-1} soil) and KCl (80 mg K kg^{-1} soil) and deionized water, which was maintained throughout the rice growing season at 3- to 5-cm above the soil surface. Three replicates for each N fertilization regime resulted in 6 pots: 3 pots were used for continuous ^{13}C labeling (see below) and 3 pots were used as controls without labeling. Soils were sprayed with NaH_2PO_4 and KCl (20 mg P kg^{-1} soil and 80 mg K kg^{-1} soil, respectively), and placed into plastic pots with 20 cm diameter and 5 cm height. Sufficient deionized water was added to maintain a 1 to 2 cm depth of water covering the soil surface throughout the rice cultivation period. More information about the experimental design is given by Ge et al. (2017).

$^{13}CO_2$ continuous labeling

Rice plants were subjected to $^{13}CO_2$ continuous labeling between May 14th and 31st, 2013, which was during the rice vegetative growth period (including the entire tillering stage). During the labeling period, plants were transferred to an automatically controlled gas-tight growth chamber system (area 110 cm × 250 cm, height 180 cm). Growth chambers were placed in a rice field so that environmental conditions would be identical for labeled plants and unlabeled controls. Pot surfaces were covered with black plastic sheets to prevent algal photosynthesis in the flood water so that only the rice shoots were be exposed to $^{13}CO_2$. The $^{13}CO_2$ (99 atom% ^{13}C) was supplied during rice growth, and the pots were watered every few days to maintain a water depth of 3–5 cm above the soil surface until harvest. Irrigation water was introduced through a nylon tube (inner diameter, 5 mm) connected to each pot. More information about $^{13}CO_2$ continuous labeling is given by Ge et al. (2017).

Rice growth conditions and sampling

The temperature and CO_2 concentrations in the growth chamber were measured and controlled according to our previous study (Ge et al. 2012, 2017). The rice plants were harvested and soils were sampled after 18 days of continuous ^{13}C labeling. Shoots were cut off at the stem base to separate the components of root, shoot, and soil. Soil adhering to the roots was removed by gentle agitation with 0.01 M $CaCl_2$ (pH 6.2) for 1 min and thorough washing with water, then air-dried for aggregate size separation and SOM fractionation. Then, the shoots and roots were dried in an oven at 70 °C for 72 h, weighed, and pulverized.

Aggregate size fractionation

Aggregate size fractionation was performed by a wet sieving method according to Gale et al. (2000). Briefly, soil was sieved <8 mm and gently crumbled manually to approximately 2-mm pieces. Soil sample (100 g) was transferred to two sieves (< 0.25 and < 0.053 mm) and shaken for 5 min. Subsequently, macro-aggregates (2–0.25 mm) and micro-aggregates (0.25–0.053 mm) were collected from the <0.25 mm and < 0.053 mm sieves, respectively. The remaining material that passed through the <0.053 mm sieve was classified as silt and clay (< 0.053 mm). All size fractions were dried at 70 °C, weighed, and stored for ^{13}C analysis.

Chemical fractionation of soil organic matter

The extraction of SOC pools from air-dried soil was performed using the methodology suggested by the International Humic Substances Society (IHSS), using $NaOH-Na_4P_2O_7\cdot10H_2O$ (0.1 M, pH = 13) as the extraction agent. Three fractions were separated from 5 g soil samples based on their solubility in alkaline and acid solutions: (a) alkali- and acid-extractable fulvic acids (FAs); (b) alkali-extractable, acid non-extractable humic acids (HAs); and (3) alkali and acid non-extractable humin (Hu).

Measurement of C_{tot} and $\delta^{13}C$

The C contents in the soils (including aggregate size fractions), shoots, roots, FA, and HA extracts were analyzed using an automated C/N analyzer (vario MAX; Elementar Analysensysteme GmbH, Germany). The stable C isotope ratios ($^{13}C/^{12}C$) of shoots, soils, FA, and HA extract samples were measured using an isotope ratio mass spectrometer (MAT253) coupled with an elemental analyzer (FLASH 2000; Thermo Fisher Scientific, USA). The natural ^{13}C abundance is expressed as parts per thousand relative to an international standard Peedee Belemnite (PDB) using delta units (δ‰).

Calculations of rice-derived C (^{13}C) amount in soil

The ^{13}C incorporation into plant and soil samples was calculated according to the following equation:

$$^{13}C_s = \left[\left(Atomic^{13}C\%\right)_{s,L} - \left(Atomic^{13}C\%\right)_{s,UL}\right] \times C_s/100 \qquad (1)$$

where $(Atomic\ ^{13}C\%)_{s,L}$ and $(Atomic\ ^{13}C\%)_{s,UL}$ are the atomic $^{13}C\%$ in labeled and unlabeled samples, respectively, and C_s is the total C content of the samples.

The ^{13}C incorporation into shoots, roots, and soil pools was expressed as a percentage of ^{13}C recovery. The total ^{13}C recovery after sampling was calculated as the sum of ^{13}C in the shoots, roots, and soil (Tian et al. 2012).

Statistics

All data are expressed as the mean of three replicates ± standard error. Differences between means were evaluated using least significant difference analysis at the 5% level and were calculated using a one-way analysis of variance (ANOVA) and SPSS (Version 10.5, SPSS Inc., Chicago, IL, USA). Correlation analyses between ^{13}C-SOC in physical fractionation (>0.25; 0.25–0.053 mm; <0.053 mm) and chemical fractionation pools (FA, HA and Hu) and N fertilization rates were conducted using SPSS 10.5 (SPSS Inc., Chicago, IL), with significance defined at $p < 0.05$.

## Results

^{13}C incorporation into plant shoot, root and soil

Total ^{13}C incorporated into shoots, roots, and soils increased linearly with N fertilization rate ($p < 0.01$; Fig. 1). The amount of ^{13}C incorporated into shoots (range, 44.0 to 157.6 mg pot^{-1}) was approximately 3.2 to 5.2 fold higher than that in roots and soil. The highest ratio of ^{13}C-aboveground to ^{13}C-belowground was observed in the N10 treatment, and the ratios gradually decreased from 2.4 (N10) to 1.9 (N60).

^{13}C incorporation in the aggregate-size classes and SOM fractions

The size classes were fractionated by sieving as macro-aggregates (2.00–0.25 mm), micro-aggregates (0.25–0.053 mm), and silt and clay (<0.053 mm), which represented, on average, 45%, 39%, and 16% of the bulk soil, respectively. N fertilization did not affect aggregate size distribution (data not shown), but it did affect the ^{13}C incorporated into aggregate classes (macro-aggregates, micro-aggregates, and silt and clay ($p < 0.01$; Fig. 2). The total ^{13}C incorporated into coarse sand was increased by 494% in the N60 treatment compared with that in N0. The ^{13}C incorporated into fine sand, silt and clay was enhanced approximate 2.5 and 3.0
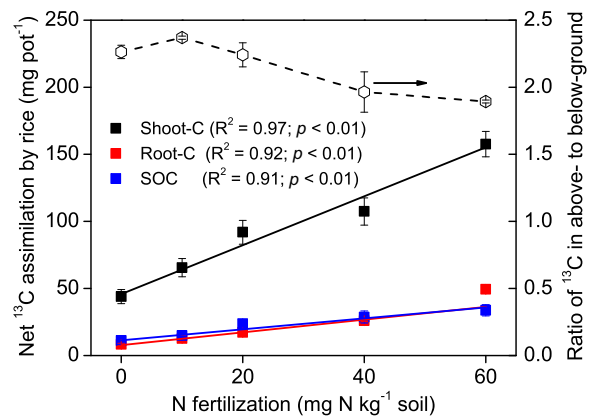

Fig. 1 Net ^{13}C assimilation (mg pot^{-1}) by rice plants and the ratio of ^{13}C distributed into above- to below-ground depending on N fertilization rates during the 18-d continuous $^{13}CO_2$ labeling period. Error bars represent standard errors of the means ($n = 3$). (Net ^{13}C assimilation by rice plants is the sum of ^{13}C content in roots, shoots and soils measured immediately after the day 18 of continuous $^{13}CO_2$ -labeling)
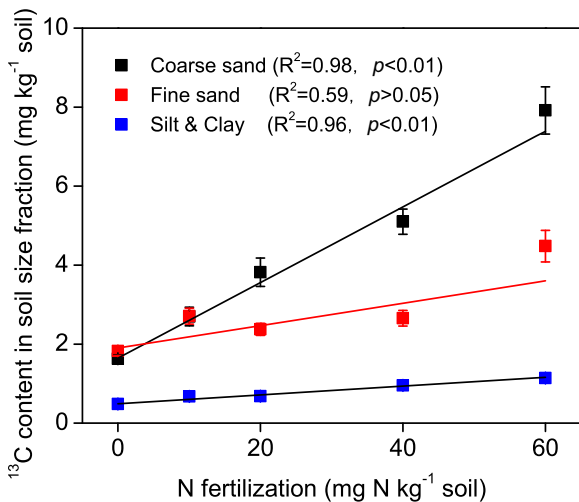
Fig. 2 ^{13}C content (mg kg^{-1}) belowground (sum of ^{13}C in soil and root) recovery in particle size fractions of coarse sand (2–0.25 mm), fine sand (0.25–0.053 mm) and silt and clay (<0.053 mm) depending on N fertilization rates. Error bars represent standard errors of the means ($n = 3$)

folds, respectively, when the N fertilization rate was increased from 0 to 60 mg N mg^{-1} soil. The ^{13}C distribution into both FA and Hu increased exponentially with N fertilizer addition ($p < 0.05$; Fig. 3), which ranged from 3.2 to 8.4 mg kg^{-1} soil and ranged from 2.2 to 8.1 mg kg^{-1} soil, respectively (Fig. 3). The ^{13}C content of HA lineally increased with N fertilization rate, however, it was only 6% of that in FA and Hu.
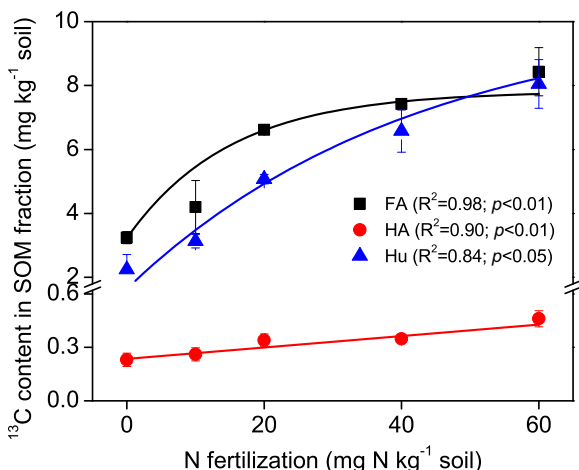
Fig. 3 ^{13}C content belowground (sum of ^{13}C in soil and root) recovery in organic matter fractions (alkali- and acid-extractable fulvic acids (FA); alkali-extractable, acid non-extractable humic acids (HA), and alkali and acid non-extractable humin (Hu)) depending on N fertilization rates. Error bars represent standard errors of the means ($n = 3$)

Rice rhizodeposition-derived C in SOM fraction per root

To provide some simple tools for assessment of rice rhizodeposition, we calculated the ratio of rice-derived C (^{13}C) amount in soil. The contribution of rice rhizodeposits to ^{13}C assimilation in the coarse sand fraction significantly increased with increasing N fertilization rate, while the largest ratios of ^{13}C assimilation in the fine sand, silt and clay fractions were observed in the N10 treatment. The rhizodeposits derived C in the SOM fractions were relatively higher than in the soil aggregates and increased with increasing N fertilizer rates. The incorporation of rhizodeposits derived C into the FA fraction increased from N0 to N20 treatment, and then decreased at higher rates. With the increase in N fertilizer application rate the amount of rhizodeposits derived C assimilated into HA was decreased, and was less than 10% of that in FA. Nitrogen fertilization significantly increased the amounts of rhizodeposits incorporated into Hu, and the highest amount of rhizodeposits derived C in Hu was 7.2 mg kg^{-1} soil at the N40 rate ($p < 0.05$; Fig. 4).

^{13}C recovery belowground

The N fertilization rate affected the ^{13}C recovery from the different aggregate classes (Fig. 5). The ^{13}C in the macro-aggregates was greater than that in the other size fractions and increased with the level of N fertilization. In contrast, the ^{13}C in micro-aggregates and silt and clay tended to decrease exponentially with N application rate (Fig. 5). The ^{13}C in the three SOM fractions (FA, HA and Hu) decreased with N fertilizer addition ($p < 0.05$; Fig. 5). The ^{13}C content was greater ($p < 0.05$) in the FA fractions than from the Hu and HA fractions, regardless of the N fertilization rate. The ^{13}C recovery from the FA fraction of the N60 treatment was 7% lower than with other N treatments, which were not different from each other (Fig. 5).

Discussion

Effect of N fertilization on allocation of assimilates in aggregates

Although changes in plant rhizodeposition induced by N fertilizer have been widely reported, its distribution
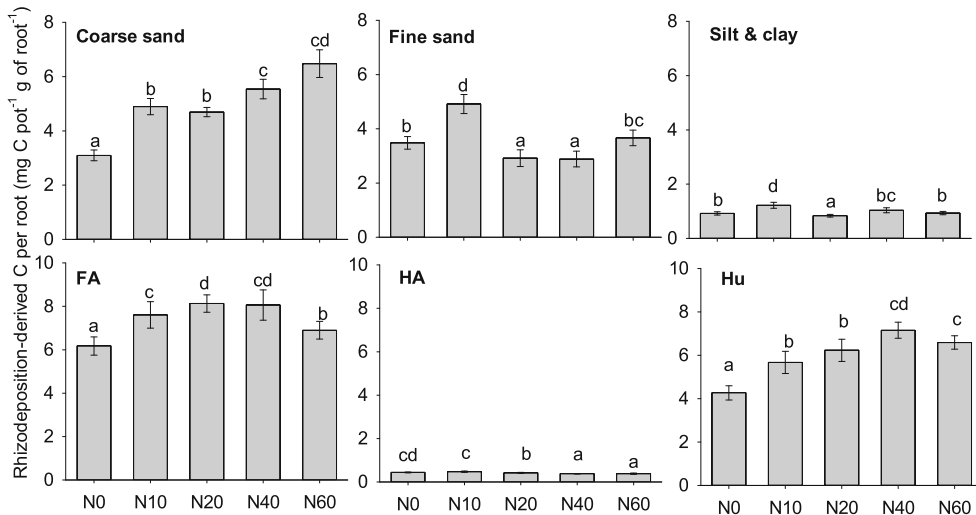
Fig. 4 Rice rhizodeposition-derived C in SOM fraction per root (mg C pot^{-1} g of root^{-1}) depending on N fertilization. Lower case letters indicate significant differences ($p < 0.05$) among treatments. Error bars represent standard errors of the means ($n = 3$)

into different soil aggregate fractions remains poorly understood. Early studies reported that the stabilization of rhizodeposited-C in aggregate size fractions in the rice-soil system mainly depends on water management, with more ^{14}C distribution in macroaggregates in the non-flooded treatment, while there were no significant differences under flooded and alternate water regime after 45-day periods of $^{14}CO_2$ labeling (Tian et al. 2012). This study revealed that N fertilizer increased the incorporation of assimilates (^{13}C) into macroaggregates but not into microaggregates, silt, or clay fractions (Fig. 3). Because most of the roots are located between and within large macro-aggregates and the root biomass was strongly increased by N fertilization (Fig. 1), the formation of macro-aggregates from micro-aggregates, silt, and clay particles was stimulated, leading to the observed decrease in the incorporation of new C into the latter fractions. Also, it is likely that the roots-associated symbionts promote soil aggregation. The entangling of fine roots and fungal hyphae might be involved in the stabilization of the macro-aggregates (Jastrow et al. 2007; Rillig and Mummey 2006).

Paddy soils are regularly flooded and intermittently irrigated, which lead to unique properties and have two consequences, including: 1) water between macro-aggregates (in large pores) has much greater O$_2$ exchange with the atmosphere than water in small pores and micro-aggregates. The relatively high O$_2$ availability leads to preferential root allocation between and within macro-aggregates, and 2) regular flooding
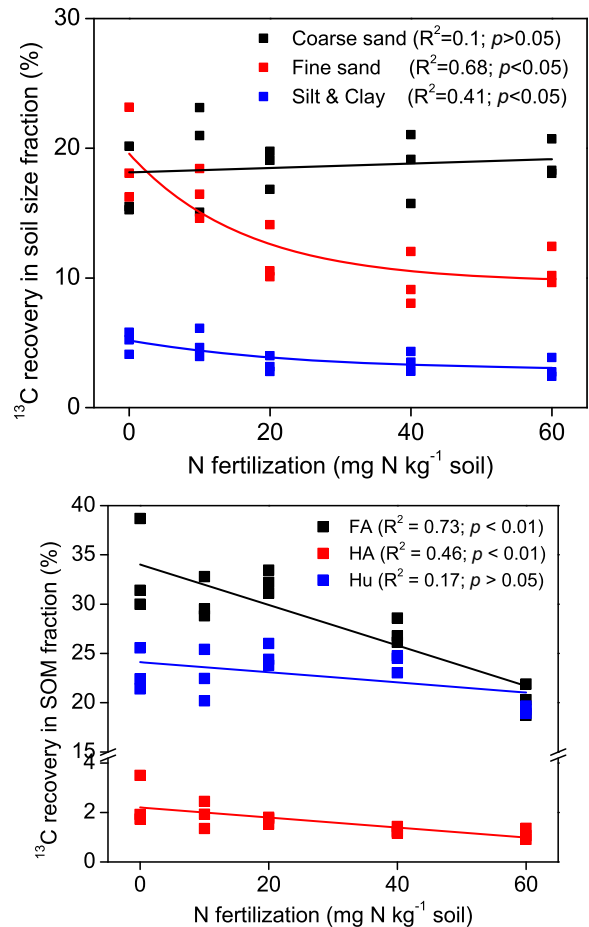
Fig. 5 ^{13}C recovery belowground (sum of ^{13}C in soil and root) in ^{13}C-SOM particle-size and humus fraction depending on N fertilization

changes the oxidizing agents in paddy fields from O_2 to low-molecular-weight organic acids (e.g., acetate), which caused the formation of complexes and stabilize the root-derived organic matter(Six et al. 2002; Yuan et al. 2016).

Allocation of rice rhizodepositions within SOM chemical fractions

Although few studies have evaluated the distribution of photosynthesized C within the soil density fractions (Comeau et al. 2013; Qiao et al. 2017) or particle size factions using isotopic techniques with either ^{14}C (Tian et al. 2012) or ^{13}C (Atere et al. 2017; Mwafulirwa et al. 2016), little information is available on the translocation of plant-assimilated C into different chemical fractions, especially with different N fertilizer rates. Previous incubation experiments showed that the greatest amount of ^{13}C was derived from rice rhizodeposition within the Hu soil extract fractions (Lu et al. 2002a). In the present study, however, more ^{13}C assimilates were incorporated into the FA fractions than the Hu and HA fractions, regardless of the N fertilizer rate (Fig. 3). Lu et al. (2002a) used $^{13}CO_2$ pulse-labeling and this might explain the differences in the ^{13}C distribution among soil extract fractions between their study and the present study which used $^{13}CO_2$ continuous-labeling. It should be noted that the Hu fraction contains not only humic substances but also particulate organic matter, such as root debris. Therefore, if plant residues are not completely removed from the soil before fractionation, the remained ^{13}C-labeled plant in soil might lead to an over-estimation of Hu. The FA and HA, in contrast, are not affected by plant residues remaining in the soil at the time of extraction.

Direct N effects on root biomass released rhizodeposition content

Nitrogen fertilization affects the partitioning and allocation of plant photosynthates (Atere et al. 2017). A recent review also examined N effects on rhizodeposition in soils, and found that N additions increased nearly all rhizodeposited-C pools per plant (Bowsher et al. 2017; Xu et al. 2018). The observed greater rhizodeposition occurs in rice plants grown under high rates of N addition might be due to the enhanced plant root biomass and exudates (Tian et al. 2012). Our previous studies also confirmed that rice

shoot and root biomass increased with N fertilizer rate (Ge et al. 2015). N additions increased nearly all rhizodeposition-C pools when expressed on a per plant basis, while no rhizodeposition-C pools were significantly altered when expressed per unit root mass (Bowsher et al. 2017). This is consistent with our results that the N fertilizer effects on rhizodeposition were not apparent (apart from the course fraction, Fig. 4) when expressed as per unit root mass, indicating the larger root biomass due to higher N inputs is the main reason explaining the observed higher rhizodepositd C. The N increased root biomass can be the direct and first driving force for C sequestration in traditionally flooded paddy soil, via the pathway of physical transfer (Cotrufo et al. 2015).

Indirect microbial mechanisms

Apart from the enhanced root biomass, N addition might affect soil microbial processes by changing soil C:N ratios. It was reported that the amended N exerted certain effects of nutrients availability to soil microbial biomass and enzymes, thus further modifying organic C turnover in soil (Li et al. 2017; Zhu et al. 2018). Based on the ^{14}C labeling within 16 days, it was estimated that 62% of total rhizodeposition was mineralized, 7% was incorporated into microbial biomass C (MBC) and 31% was recovered in the soil (Pausch et al. 2013). Much smaller photosysthesized ^{13}C inputs (0.15–0.94%) were incorporated into MBC in the early pulse labeling periods and 0.18–0.75% still remained in the end (Lu et al. 2002a), suggesting 1) the important role of the biomass pool in regulating rhizodeposition turnover in soil, and 2) that agriculture practices and other managements influence the microbial biomass pool size (Li et al. 2013; Zhang et al. 2016; Wei et al. 2017), which might further affect the fate of rhizodeposited-C. The N fertilization caused allocation of photosynthates belowground which might be regulated by microbial pool size, which determines the distribution of rhizodeposits within soil organic C pools. How N addition adjusts the microbial utilization of rhizodeposited C, determines its distribution in soil organic C fractions spatially and temporally is the key question towards understanding its C sequestration potential (Fig. 6).

Nitrogen enhanced rhizodeposition alters C availability to microbes which, in turn, switch substrate type from more recalcitrant SOM to root released C, which

Fig. 6 Conceptual graph of N mediated rice rhizodeposition and its stabilization (SOC: Soil organic carbon, FA: Fulvic acids, Hu: humin, HA: Humic acids, Root C: Root derived Carbon)
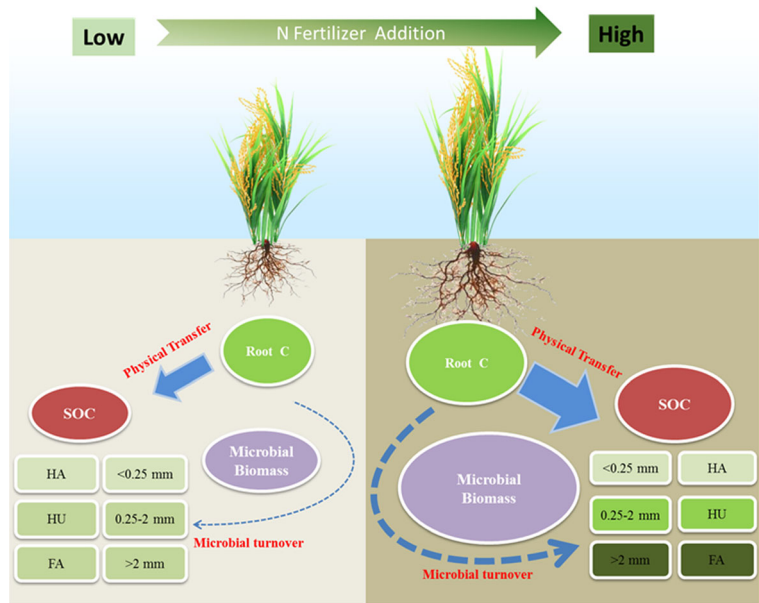
lead to less allocation within soil aggregates or respiratory processes associated with N acquisition (Jones et al. 2013; Philippot et al. 2013). When labile rhizodeposits are preferentially utilized by microbes, rhizodeposition also retards the decomposition of more recalcitrant native SOM (Johnson et al. 2006; Zhu et al. 2016). However, due to the reduction in rhizodeposition in the later stage of plant growth, irrespective of N addition, the accumuled rhizodeposited-C and N within the microbial biomass pool might be released and finally incorporated into SOM (Jones et al. 2013). To maintain the large biomass size, a switch in microbial substrate utilization (from relative available C to more recalcitrant SOM) and microbial community (r-strategist to K-strategist)

might occur after rhizodeposited-C decreased at late stages (Jones et al. 2013; Luo et al. 2011; Zhu et al. 2017). Our previous study found that 60 mg N kg^{-1} soil resulted in a lower ^{14}C recovery in MBC, than addition of 0, 10, 20, or 40 mg N kg^{-1} soil (Ge et al. 2015), suggesting the dominant microbial group might use SOM, instead of rhizodeposited-C. High N addition was also reported to increase the ^{13}C incorporation into fungal PLFAs (Ge et al. 2017), which, being regarded as oligotrophic, was able to adapt to the low substrate C inputs and utilize more recalcitrant C (SOC) as a substrate (Fontaine et al. 2011; Luo et al. 2017a), thus mediate relative long-term sequestration of carbon in soil (Luo et al. 2017b).

Table 1 Pearson correlations between root biomass (g kg^{-1} soil) and ^{13}C-SOM pools (mg pot^{-1}) at five N fertilization rates

	Root biomass	^{13}C-Root	^{13}C-SOC	^{13}C-SOC (2–0.25 mm)	^{13}C-SOC (0.25–0.053 mm)	^{13}C-SOC (<0.053 mm)	^{13}C-FAs	^{13}C-Hu
Root biomass								
^{13}C-Root	0.99							
^{13}C-SOC	0.96	0.95						
^{13}C-SOC (2–0.25 mm)	0.95	0.98	0.87					
^{13}C-SOC (0.25–0.053 mm)	0.85	0.89	0.75	0.96				
^{13}C-SOC (<0.053 mm)	–	–	–	–	–			
^{13}C-FA	0.97	0.98	0.99	0.92	0.80	–		
^{13}C-Hu	0.84	0.80	0.94	0.66	–	–	0.90	
^{13}C-HA	0.99	1.00	0.96	0.97	0.88	–	0.98	0.82

"-" signify not significant, SOC signify Soil organic carbon; FA signify Fulvic acids, Hu signify humin; HA signify Humic acids

We believe that, compared with low N treatments, plant-microbe interactions in the soil with high N additions are more complex, involving microbial community changes (bacteria to fungi) and ecology strategist shift (r-strategist to K-strategist), which further mediate decomposition of organic C (rhizoseposits and SOC) and stabilization of SOM. Also, the decomposition of rhizodeposition and its induced SOM turnover not only depends on the amount but also its form in the rhizodeposits (Cheng et al. 2014). However, we do not know the N-mediated quality of root released C and the microbial process within spatial (aggregates fractions) and temporal scales (growth stages) (Philippot et al. 2013). This needs further research by adopting DNA-SIP to examine functional microbial groups within soil aggregates during different plant growth stages (Table 1).

Conclusions

Nitrogen fertilization affected rice (*Oryza sativa* L.) growth and its rhizodeposition distribution into soil. The ratio of ^{13}C in shoot, root and SOM was enhanced with higher N fertilization rate. Also, N addition with higher rates resulted in higher ^{13}C incorporation into soil fractions (physically separated aggregations and chemical extraction forms), and the ^{13}C incorporation into macroaggregates was positively correlated with N application rate. Relative to the lower N, the higher application rate also increased ^{13}C incorporation into the humic acid fraction. This might be due to the relatively high root biomass, or microbial immobilization that lead to an accumulation of rhizodeposition in the microbial biomass pool, which was released at a later stage to contribute to the stabilization within SOM pools. We, therefore, conclude that sufficient N fertilization enhances rice rhizodeposition and leads to SOM stabilization in the paddy soil.

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