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REGULAR ARTICLE

# Tracking the photosynthesized carbon input into soil organic carbon pools in a rice soil fertilized with nitrogen

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**Abstract**
*Aims* Replenishment of soils with carbon (C) produced during photosynthesis plays an important role in global C cycling. Nitrogen (N) fertilization is critical for rice production, but its effects on the deposition of photosynthesis-derived C into soil C pools is poorly understood. To address this, we used continuous $^{14}C$-labeling to quantify the deposition of photosynthesis-derived C into various soil organic pools in a rice-soil system.
*Methods* Rice (*Oryza sativa* L.) was continuously supplied with $^{14}C$-labeled $CO_2$ ($^{14}C$-$CO_2$) for 36 days, with increasing N fertilizer rates (0 [$N_0$], 10 [$N_{10}$], 20 [$N_{20}$], or 40 mg N $kg^{-1}$ soil [$N_{40}$], respectively).
*Results* Rice shoot and root biomass significantly increased following N fertilization. The amount of photosynthesis-derived C converted into soil organic carbon ($^{14}C$-SOC) was proportional to the soil N concentration, and accounted for 8.0–19.3 % of rice biomass C. The $^{14}C$-SOC content was positively correlated with the rice root biomass, suggesting that N increased root exudation of photosynthesis-derived C. The amounts of $^{14}C$-labeled C in the dissolved organic carbon ($^{14}C$-DOC) and in the microbial biomass carbon ($^{14}C$-MBC), as proportions of $^{14}C$-SOC, were 3.9–7.8 and 6.6–24.0 %, respectively. The $^{14}C$-DOC, $^{14}C$-MBC, and $^{14}C$-SOC as proportions of total DOC, MBC, and SOC were 9.7–11.6, 6.9–10.6, and 0.37–1.71 %, respectively.
*Conclusions* Nitrogen fertilization promotes deposition of photosynthesis-derived C into SOC pools in a rate-dependent manner. However, the $^{14}C$-MBC as a proportion of both $^{14}C$-SOC ($^{14}C$-MBC/$^{14}C$-SOC) and MBC ($^{14}C$-MBC/MBC) increase during rice growth at lower N concentrations.

**Keywords** Soil organic C · Rice · N application rate · $^{14}C$ labeling · Photo-assimilated C

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## Introduction

Plant photosynthate is the primary source of soil organic C, which is essential for the cycling of soil organic C (SOC), for C sequestration and as a substrate-C source for microorganisms in the rhizosphere (Weintraub et al. 2007; Ge et al. 2012; Tian et al. 2013a). Therefore,

quantification of this flux as it relates to the SOC pool will make an important contribution toward our understanding of the global C cycling process. The extent of root-derived C depends on the input from photosynthesized C. It comprises root exudates that are dominated by low molecular weight compounds such as carbohydrates, amino acids and organic acids (Nguyen 2003; Kuzyakov and Jones 2006). These compounds are important sources of dissolved organic carbon (DOC) and are readily available for soil microorganisms (Lu et al. 2002a; Ge et al. 2012). Part of the root-derived C is used for growth of the roots themselves (Farrar et al. 2003; Boddy et al. 2007), whereas another fraction is biodegraded by soil microorganisms (Kaštovská and Šantrůčková 2007). Nevertheless, below-ground C cycling remains poorly understood, due to the complexity of root exudate composition, the high degree of spatial heterogeneity in the rhizosphere, and a lack of available methods for its measurement within the rhizosphere (Walker et al. 2003; He et al. 2004; Kuzyakov and Jones 2006). Therefore, quantification of root-derived DOC and microbial biomass carbon (MBC) are essential for understanding processes such as microbial activity and soil respiration, providing further insight into C cycling in the plant-soil system.

The fate of photosynthesized C can be affected by N fertilizer application (Zagal et al. 2001; Kuzyakov et al. 2002a). However, the contribution of photosynthesized C to SOC pools varies with the N application rates, depending on experimental conditions, such as light, temperature, $CO_2$ concentration, moisture, nutrition status, and soil texture (Zagal et al. 1993; van Ginkel et al. 2000), and on plant factors such as plant species, variety, and growth stage (Swinnen et al. 1994a, b; Kuzyakov et al. 2002b).

Upland crop plants such as maize, ryegrass, and barley are often used to determine the amount of plant-derived C entering soil and its subsequent metabolism. Kuzyakov et al. (1999) found that the total amount of $^{14}C$-labeled C in the soil plus roots ranged from 8.2 to 27.7 % of total ryegrass-assimilated C after 8 days of $^{14}C$-pulse labeling. Johansson (1992) reported that at low N levels, 32 % of the net assimilated $^{14}C$ was translocated below ground, whereas at high N levels, 27 % was translocated. In the paddy ecosystem, N supply affects both rice C assimilation (as indicated by grain yield) and soil C sequestration (Cai and Qin 2006).

Rice is harvested annually from 165 million hectares of land worldwide and provides the basic food for nearly half the world's population (FAO 2011). In addition, the paddy ecosystem can sequester more C than upland soils, slowing the increase in atmospheric $CO_2$ concentration (Wang et al. 2007; Wu 2011). Therefore, potential changes in paddy soil C storage in response to climate change are of considerable interest (Ge et al. 2012). Hence, studying the processes of rice C distribution and transformation, and quantifying the amount of photosynthesis-derived C inputs into the soil are critical activities required to increase our understanding of global C cycling and the ecological functions of paddy ecosystems. However, there have been few direct studies of the interactions between C assimilation by photosynthesis and C cycling in rice-soil systems. Rice-derived C inputs can be partitioned into MBC- and DOC-derived sources, and N fertilizer can affect this partition of rice-derived C inputs during growth. This information will also facilitate studies of the amount and fate of plant-derived C that enters the soil; these processes are currently unclear, particularly with regard to root exudation.

We studied the effects of N fertilizer rates on the allocation and fate of assimilates by labeling rice with $^{14}CO_2$ during vigorous growth (including the entire tillering stage). The objectives of this study were to (1) quantify the photosynthesized C inputs to SOC pools in the rice-soil system, and (2) evaluate the contribution of photosynthesized C to DOC and MBC at various N application rates.

## Materials and methods

### Soil

A typical stagnic anthrosol (Gong et al. 2009), developed from a granitic red soil, was selected from a rice field (113°19′52″E, 28°33′04″N, 80 m a.s.l.) located at Changsha Research Station for Agricultural and Environmental Monitoring. The station is in a subtropical region of China, with an annual mean temperature of 17.5 °C, rainfall of 1,300 mm, sunshine of 1,663 h, and frost-free period of 274 days. Soil samples were collected from the plow layer (0–20 cm) and sieved field moist (<4 mm) (water content, 14.8 %) to remove coarse plant residues. The soil contained 7.51 % clay, 68.36 % silt, 24.13 % sand, 18.1 g organic C $kg^{-1}$ soil, and 1.80 g total N $kg^{-1}$ soil, and had a pH of 5.56 (1:2.5, soil/water ratio).

### Treatments

Four treatments, each with four replicates, were established as follows: No N supplied ($N_0$), 10 mg N $kg^{-1}$ soil ($N_{10}$), 20 mg N $kg^{-1}$ soil ($N_{20}$), and 40 mg N $kg^{-1}$ soil with rice growth ($N_{40}$). The prepared soil (1.50 kg on an oven-dry basis) was mixed with $(NH_4)_2SO_4$ to obtain the appropriate N levels, and each treatment sprayed with the same concentrations of $NaH_2PO_4$ and KCl (20 mg P $kg^{-1}$ soil and 80 mg K $kg^{-1}$ soil, respectively). The soils were then placed into plastic pots (20 cm diameter and 5 cm height) and sufficient deionized water was added to maintain a 1 to 2 cm depth of water covering the soil surface throughout the rice cultivation period.

### $^{14}CO_2$ labeling of rice

The plant-growth chamber for uniform $^{14}CO_2$ labeling of rice was described by Ge et al. (2012, 2013), with some minor modifications. Briefly, three 25-day-old rice seedlings (*Oryza sativa* L., two-line hybrid rice 'Zhongzao 39') were transplanted to each pot on May 29, 2012. All rice plant pots were transferred to an automatically controlled gas-tight growth chamber. The experiment spanned a $^{14}CO_2$ labeling period from June 4 to July 11, 2012, in the growth chamber (area 110 cm×250 cm, height 180 cm). The soil surface was covered by a black plastic sheet to avoid algal photosynthesis on the surface soils and the rice shoots exposed to $^{14}CO_2$ for 36 days. The $^{14}CO_2$ was supplied to all pots and irrigation water was introduced through a nylon tube (5 mm inner diameter) connected to each pot.

The $^{14}CO_2$ was generated from the reaction between $^{14}C$-$Na_2CO_3$ ($1.6\times10^4$ μg $mL^{-1}$ and $16.5\times10^3$ Bq $mL^{-1}$) and HCl (2 M) in plastic beakers placed inside the chamber, giving a concentration between 360 and 380 μL $^{14}CO_2$ $L^{-1}$ (Shsen-QZD, Qingdao, China). When the $CO_2$ concentration in the chamber fell below 360 μL $L^{-1}$, more $^{14}CO_2$, generated by the above reaction, was introduced into the chamber. Conversely, when the $^{14}CO_2$ concentration in the chamber was higher than 380 μL $L^{-1}$, a switch diverted the gas flow to pass through $CO_2$ traps (1 M NaOH solution) to absorb excess $^{14}CO_2$. Two temperature humidity sensors (SNT-96S, Qingdao, China) were installed: one inside the chamber, and another in the surrounding rice field in the open air. Two fans continuously circulated the air in the growth chamber. When the temperature/humidity sensor inside the chamber recorded a temperature 1 °C higher than the external value, a corresponding script in the data-logger triggered the relay to activate air-conditioning until the temperature inside the chamber was 1 °C lower than that outside. The entire growth chamber system was placed outdoors in order to maintain natural exposure to sunlight.

### Sampling and harvesting

Plants were harvested after 36 days of labeling. The shoots were cut off at the stem base, allowing for separation of the roots, shoots, and soil. Any soil adhering to the root was removed by gentle agitation in 0.01 M $CaCl_2$ (pH 6.2) for 1 min and any adhering soil was then thoroughly washed away under running tap water to remove soil absorbed on the surfaces. All roots, shoots, and a small soil sub-sample were dried to a constant weight in an oven at 70 °C for total C and $^{14}C$ analyses. The remaining soil was stored at 4 °C until required.

### Analytical methods

Soil pH was determined using a pH meter (Delta 320; Mettler-Toledo Instruments Co., Ltd., China) with a soil/water ratio of 1:2.5, and soil particle composition using a laser particle size analyzer (Mastersizer 2000; Malvern Instruments Ltd., UK). Organic C and total N were measured by dry combustion using an element analyzer (Vario MAX, Elementar Analysensysteme GmbH, Germany). Soil DOC was extracted with $K_2SO_4$ (0.5 M) according to Bolan et al. (1996), and subsequently analyzed using a total carbon analyzer (Phoenix-8000). Soil MBC was determined using fumigation-extraction (Wu et al. 1990).

The amount of $^{14}C$-labeled C in soil ($^{14}C$-SOC) was measured according to Wu and O'Donnell (1997). Briefly, 1.50 g soil (<0.149 mm) was weighed into a double-necked flask containing 20 mL $K_2Cr_2O_7$ (0.2 M) and 30 ml $H_2SO_4$-$H_3PO_4$ (5: 1, v:v). The mixture was digested at 165 °C for 8 min, and then pure $O_2$ was continuously pumped into the flask to encourage digestion for a further 10 min. The evolved $CO_2$-C was trapped in 40 mL NaOH (0.4 M). The amount of labeled $CO_2$ ($^{14}CO_2$) trapped was measured by mixing 1 mL NaOH with 9 mL liquid scintillation cocktail (Beckman Coulter, Fullerton, CA, USA) and counting for 5 min using an automated liquid scintillator (LS-6500; Beckman, Germany). The amount of soil organic C

($^{14}$C-SOC) that was derived from the labeled rice plants (mg C kg$^{-1}$ soil) was calculated using the following formula:

$$^{14}\text{C-SOC} = F_1 R_s / R_p W \quad (1)$$

where $F_1$ is the converting factor for the counting volume (1 mL) to the volume of the trap solution (40 ml), $R_s$ and $R_p$, are the specific activities (Bq L$^{-1}$; blank counts subtracted) of the NaOH trap solution and $Na_2C^{14}O_3$ (Bq mg$^{-1}$ C L$^{-1}$) was used to produce $^{14}C$-$CO_2$ in the growth chamber, W, is the weight (kg) of soil on an oven-dry basis, respectively.

The amount of $^{14}$C-DOC (i.e., the amount of DOC that was $^{14}$C labeled) was analyzed in the non-fumigated soil and determined as described above. The $^{14}$C-MBC (i.e., the amount of soil microbial biomass C that was $^{14}$C labeled) was extracted from the soil and measured (Ge et al. 2012) using an automated liquid scintillator (LS-6500, Beckman, Germany). The amounts of $^{14}$C-DOC (mg C kg$^{-1}$ soil) and $^{14}$C-MBC (mg C kg$^{-1}$ soil) were calculated using the following formulas, respectively,

$$^{14}\text{C-DOC} = F_2 R_{uf} / R_p W \quad (2)$$

$$^{14}\text{C-MBC} = F_3 (R_f - R_{uf}) / R_p W k_c \quad (3)$$

where $F_2$ and $F_3$ represent the converting factor for the counting volume (1 mL) to the total volume of extract (80 mL) plus soil water (in ml); $R_f$ and $R_{uf}$, radioactivity (Bq L$^{-1}$; blank counts omitted) for the extracts of the fumigated soil and unfumigated soil, respectively; $R_p$, is the radioactivity of $^{14}C$-$Na_2CO_3$ (Bq mg$^{-1}$ C L$^{-1}$) used to produce $^{14}C$-$CO_2$ in the growth chamber; W, is the weight (kg) of soil on an oven-dry basis, and $k_c$, the factor (0.45) converting $^{14}$C determined into the biomass $^{14}$C (Wu et al. 1990).

### Statistical analysis

All data are expressed as the means of four replicates± SD. A one-way ANOVA with a Duncan test was used to identify differences between treatments, and linear regression analysis was used to identify significant relationships among $^{14}$C-SOC, $^{14}$C-MBC, and N rate ($P$<0.05, unless otherwise stated). All analyses were performed using SPSS for Windows version 14.0 software (SPSS Inc., Chicago, Illinois, USA).

## Results

### Shoots, root biomass, and shoot-to-root ratios under different N application rates

After 36 days of labeling the dry matter of rice shoots and root biomass had increased significantly with increasing N application rates (Fig. 1). Their maximum values were 4.09 and 2.12 g pot$^{-1}$, respectively, and were obtained at a rate of 40 mg N kg$^{-1}$ soil ($N_{40}$ defined as the high N level below). These values are significantly higher than the minimum values (1.90 and 1.23 g pot$^{-1}$, respectively) that were obtained without added N ($N_0$). No significant differences were observed in the yield of rice roots or total biomass ($P$>0.05) between the treatments of $N_{20}$ (defined as the medium N level) and $N_{40}$ (Fig. 1).

There were no significant differences in the root-to-shoot ratios, which ranged from 0.52 to 0.59 in the N-fertilized soils, but this ratio was significantly higher in the $N_0$ group compared to $N_{10}$ (10 mg N kg$^{-1}$ soil, defined as a low N level), $N_{20}$, and $N_{40}$ (Fig. 1).

### The amounts of rice photosynthesis-derived C deposited into SOC ($^{14}$C-SOC)

The amounts of $^{14}$C-SOC at different N application rates during rice growth ranged from 67.6 ($N_0$) to 314.8 ($N_{40}$) mg C kg$^{-1}$ after labeling for 36 days (Fig. 2). The rates of N applied had a significant effect on $^{14}$C-SOC ($P$<0.05), in the following order: $N_{40}$>$N_{20}$>$N_{10}$>$N_0$ (Fig. 2). Further, the $^{14}$C-SOC concentrations were

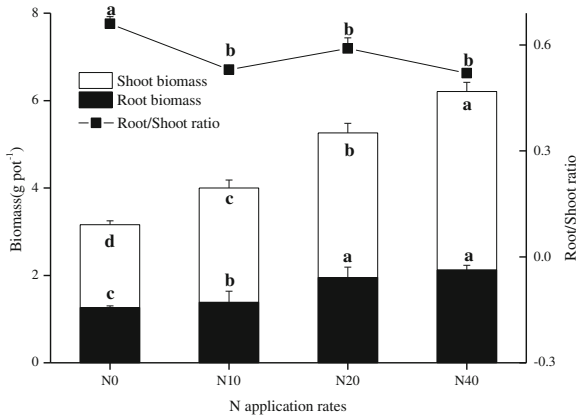


**Fig. 1** Amounts of rice shoot and root biomass and root-to-shoot ratios at different N levels after continuous labeling for 36 d in a closed chamber. Different lower case letters indicate significant differences ($P$< 0.05) among treatments. $N_0$–N free; $N_{10}$–10 mg N kg$^{-1}$ soil; $N_{20}$–20 mg N kg$^{-1}$ soil; $N_{40}$–40 mg N kg$^{-1}$ soil

positively correlated with the rice root biomass ($n$=4, $r$= 0.97, $P$=0.03) (Fig. 3).

The contribution of photosynthesized carbon to SOC ($^{14}$C-SOC) can be expressed as a percentage of the total rice biomass C (Fig. 2). The percentage with $N_{40}$ was 19.3 %, which is significantly higher ($P$<0.05) than that of $N_0$ (8.0 %), $N_{10}$ (14.1 %), or $N_{20}$ (15.2 %). There was no significant difference in $^{14}$C-SOC/rice biomass C between $N_{10}$ and $N_{20}$, but these numbers were significantly higher than for $N_0$ (Fig. 2).

### Contribution of rice photosynthesis-derived C to MBC

After labeling for 36 days, N addition increased both MBC and the labeled MBC ($^{14}$C-MBC) compared with the treatment of $N_0$ (Fig. 4a, b). The amount of total MBC in the $N_{10}$, $N_{20}$, and $N_{40}$ treatments increased by 35.5, 32, and 9.6 %, respectively, compared with the $N_0$ treatment (Fig. 4b). The effects of different N application rates on $^{14}$C-MBC contents were similar to the effects on the amounts of total MBC (Fig. 4a, b), and followed the pattern: $N_{10}$>$N_{20}$>$N_{40}$>$N_0$. The amount of $^{14}$C-MBC at $N_{10}$ was 33.79 mg C kg$^{-1}$, which was significantly higher ($P$<0.05) than that observed with the other treatments (Fig. 4a).

### Contribution of rice photosynthesis-derived C to DOC

After labeling for 36 days, the $^{14}$C incorporation into DOC ($^{14}$C-DOC) was highly dependent on N concentration, and followed the series $N_{40}$>$N_{20}$>$N_{10}$>$N_0$ (Fig. 4c), although $N_{20}$ and $N_{40}$ were not significantly different. Similarly, significant differences were observed in the amounts of DOC in different N treatment (Fig. 4d). In comparison with the $N_0$ group, the amount of DOC increased with increased addition of N. However, there was no significant difference in the DOC values between the $N_{20}$ and $N_{40}$ treatment groups (Fig. 4d). In addition, under the N treatments applied during rice growth, the amount of $^{14}$C incorporated into MBC ($^{14}$C-MBC) differed greatly from its incorporation into DOC ($^{14}$C-DOC) (Fig. 4a and c).

### Distribution of $^{14}$C in SOC pools

After labeling for 36 days, $^{14}$C-SOC as a proportion of total SOC ($^{14}$C-SOC/SOC) ranged from 0.37 to 1.71 % in the different N treatments during rice growth (Table 1). The value for $N_{40}$ was significantly higher than that with the other treatments (Table 1).

The $^{14}$C-MBC as a proportion of total $^{14}$C-SOC ($^{14}$C-MBC/$^{14}$C-SOC) ranged from 6.63 % ($N_{40}$) to 24.00 % ($N_0$) after labeling for 36 days and was inversely proportional to the N concentrations supplied (Table 1). However, there was no significant difference ($P$>0.05) in the proportion of $^{14}$C-MBC between $N_0$ and $N_{10}$, which in turn were both significantly higher than either $N_{20}$ or $N_{40}$ ($P$<0.05). Soil $^{14}$C-MBC as a proportion of total MBC ($^{14}$C-MBC/MBC) ranged from 6.9 to 10.6 %, and was significantly higher with $N_{10}$ than with treatments $N_0$, $N_{20}$, and $N_{40}$. There was no significant difference between treatments in the $N_{20}$ and $N_{40}$ groups (Table 1). After the labeling period, the soil $^{14}$C-DOC as a proportion of total DOC ($^{14}$C-DOC/DOC) ranged from 9.7 to 11.6 %, and was not significantly different among the N treatments. Furthermore, the value for $^{14}$C-MBC/MBC was lower than that for $^{14}$C-DOC/DOC at each N concentration (Table 1).

$^{14}$C-DOC as a proportion of $^{14}$C-SOC ranged from 3.92 % ($N_{40}$) to 7.78 % ($N_0$). This was significantly higher in the $N_0$ group when compared to other N treatments (Table 1). However, there were no significant differences between the $N_{10}$ and $N_{20}$ groups.

## Discussion

### Continuous $^{14}$C-$CO_2$ labeling

Continuous $^{14}$C-$CO_2$ labeling was used, which provides a considerable advantage, and is a more realistic representation of C allocation than pulse labeling, which was the method used by most previous groups (Lu et al. 2002a). In the case of continuous labeling, the rice assimilated labeled $CO_2$ constantly, over a long period, mostly between the emergence of the first leaf and the sampling time. In addition, the distribution of labeled C corresponded to the distribution of total C, as long as it was applied from first leaf emergence to harvest time (the specific $^{14}$C activity is equal in all plant parts). Therefore, continuous labeling is particularly appropriate for the estimation of the amount of total C transferred by the plants into the soil and below-ground pools during the labeling period.

### The contribution of rice photosynthesis-derived C to SOC

The main sources of SOC are usually from plant residue and root exudates (Lu et al. 2002a; Ge et al. 2013). The

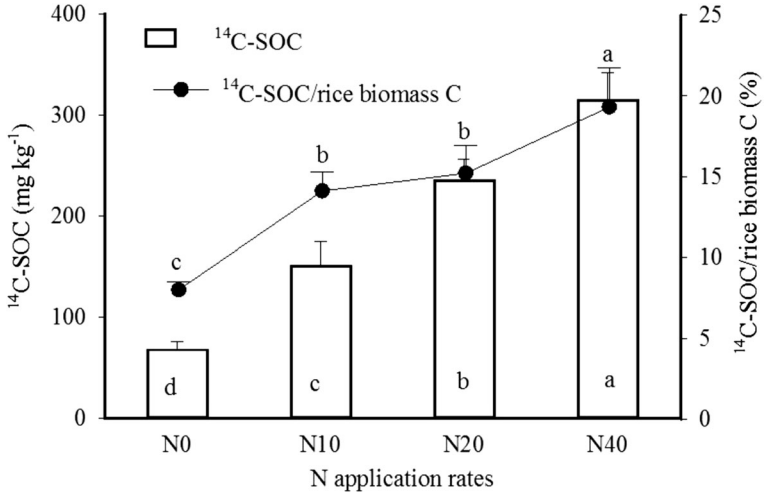


**Fig. 2** The content of $^{14}$C in soil ($^{14}$C-SOC) and $^{14}$C-SOC as a proportion of rice biomass C ($^{14}$C-SOC/rice biomass C (%)) supplemented with different N concentrations after a 36-d continuous $^{14}$C -labeling period. Bars indicate the standard error of the mean ($n$=4). See Fig. 1 for definitions of $N_0$, $N_{10}$, $N_{20}$, and $N_{40}$. Different lower case letters indicate significant differences ($P$< 0.05) between treatments

proportion of total plant C derived from photosynthesis that is deposited in soil can reach 40 %, depending on the plant species and environmental conditions (Zagal 1994). Hütsch et al. (2002) reported that photosynthesis-derived C deposits in soil reached a maximum of 20 %, and between 64 and 86 % of this was used for soil respiration, whereas only 2–5 % was incorporated into SOC. In our experiment, $^{14}$C-SOC as a proportion of rice biomass C ranged from 8.0 to 19.3 % (Fig. 2), which is much higher than the previously reported values of 1–5 % (Lu et al. 2002b; Ge et al. 2012), and is most likely due to the continuous labeling and inclusion of N fertilization in our study. Therefore, the labeling periods and crop growth conditions should be considered when make comparisons between these studies. In general, the SOC content is higher in paddy fields than in upland soils (Wu 2011). This may indicate that "new" SOC derived from roots in paddy soil is more stable (Yuan et al. 2013; Wu et al. 2014). This is plausible, since complex formation with active iron oxide in soil may stabilize root-derived organic matter (Pan et al. 2008). This organic matter can participate in soil aggregate formation (0.25–2 mm diameter) that in turn decreases the rate of decomposition of new SOC, although this process is weaker in upland soils (Pan et al. 2008). Carter (2002) showed that SOC in coarse micro-aggregate fractions (0.02–0.25 mm diameter) contains the active C that has been recently derived from the incorporation of fresh (or less decomposed) plant residues. In addition, Tian et al. (2013b) found that flooded condition resulted in the lower rhizodeposition for rice as compared in non-flooded condition.

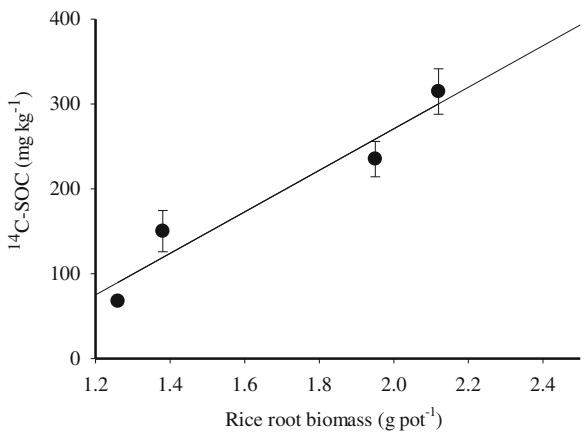


**Fig. 3** Relationship between $^{14}$C-SOC and rice root biomass at different N levels after a 36-d continuous $^{14}$C-labeling incubation. Points represent individual N treatment means. Lines indicate linear regression between $^{14}$C-SOC and rice root biomass: $^{14}$C-SOC rice root biomass=244.8×(rice root biomass) - 218.7 $r$= 0.967, $P$=0.03. Bars represent the standard errors. For data on rice root biomass and $^{14}$C-SOC see Figs. 1 and 2

## Effects of N application rates on the input of photosynthesized C into SOC pools

The amount of $^{14}$C-SOC in the $N_0$ group and the corresponding proportion of rice biomass C were both significantly lower than in the N-treated groups (Fig. 2 and Table 1). This indicates that the amount of root-derived SOC is lower in N-deficient soils, perhaps because rice roots under these conditions are more effective in their competition with soil microorganisms for root exudates when compared to roots supplied with abundant N. A relatively large

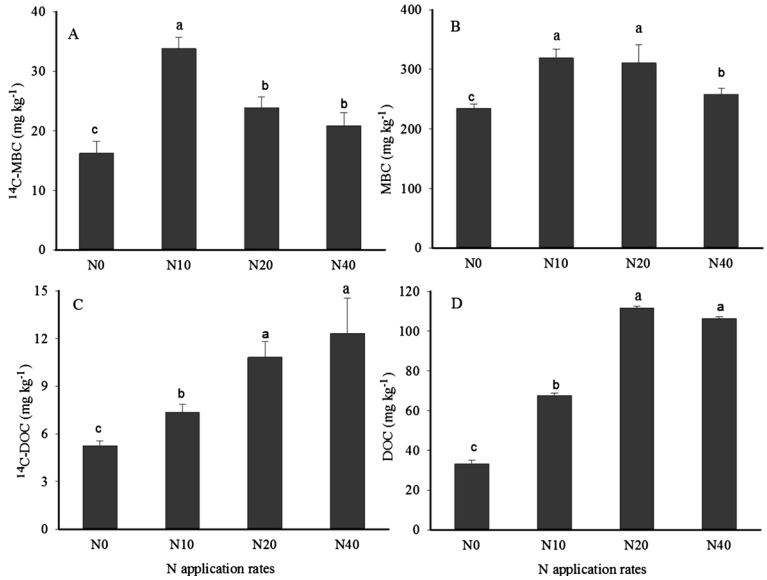


**Fig. 4** The amounts of soil $^{14}$C-MBC (**a**), MBC (**b**), $^{14}$C-DOC (**c**), and DOC (**d**) at different N levels after 36-d continuous $^{14}$C-labeling. Bars indicate the standard error of the mean ($n$=4). See Fig. 1 for $N_0$, $N_{10}$, $N_{20}$, and $N_{40}$. Different lower case letters indicate significant differences ($P$< 0.05) between treatments

quantity of photosynthesized C was incorporated into SOC when N was added at 40 mg N kg$^{-1}$ soil, which is generally consistent with the findings of Anderson (1988) and Liljeroth et al. (1994). The application of high N rates can enhance the deposition of photosynthesis-derived C into the soil, because it increases plant growth and stimulates rhizosphere respiration (Liljeroth et al. 1994; Chantigny et al. 1999), which may trigger the accumulation of "new" SOC derived from the roots. However, there is little information regarding the accumulation of new inputs of SOC derived from roots at the interface of the plant–soil system. We could not determine the dynamics of rhizosphere respiration during the growing season. However, previous studies have shown that the priming effects in the rhizosphere can be enhanced in high-fertilizer soils (Dijkstra et al. 2006). We therefore presume that, in the presence of high soil N, the release of C from the roots is increased, because rice growth was enhanced in the prophase of the growth stage. In the later period, more root residues remain in the soil and contribute to the root-derived SOC pools. Roots can also grow deeper when supplied with high rates of N fertilizers (Anderson 1988), and the root activities can enhance the physicochemical protection of SOC, particularly in deeper horizons (Rasse et al. 2005). In the $N_0$ group, root growth was constrained by the lack of N. The new input of SOC derived from the roots was also lower than in the N treatments because the root exudates were in the shallow soil layer (0–10 cm) where they decompose more readily. In contrast, Johansson (1992) showed that more photosynthesis-derived C was incorporated into SOC at low N levels. This discrepancy may be attributable to differences in the experimental conditions, such as the chemical form and absolute concentration

**Table 1** Distribution of $^{14}$C in SOC pools from rice-planted paddy soils at different N levels after continuous labeling for 36 days in a closed chamber

| Treatments | $^{14}$C-SOC/SOC (%) | $^{14}$C-MBC/$^{14}$C-SOC (%) | $^{14}$C-MBC/MBC (%) | $^{14}$C-DOC/DOC (%) | $^{14}$C-DOC/$^{14}$C-SOC (%) |
|---|---|---|---|---|---|
| $N_0$ | 0.37±0.0 d | 24.0±2.6a | 6.9±0.4 b | 10.0±0.6 a | 7.78±0.9a |
| $N_{10}$ | 0.82±0.4 c | 22.5±1.5a | 10.6±0.8 a | 10.9±1.3 a | 4.90±0.5b |
| $N_{20}$ | 1.29±0.2 b | 10.2±1.2b | 7.7±1.2 b | 9.7±2.3 a | 4.61±0.5b |
| $N_{40}$ | 1.71±0.2 a | 6.63±0.5c | 8.1±0.7 b | 11.6±1.0 a | 3.92±0.2c |

$N_0$–N free; $N_{10}$–10 mg N kg$^{-1}$ soil; $N_{20}$–20 mg N kg$^{-1}$ soil; $N_{40}$–40 mg N kg$^{-1}$ soil

Different lower case letters in the same column indicate significant differences ($P$< 0.05) among treatments

of N, the labeling periods, and crop type (Saggar et al. 1997; Gavrichkova and Kuzyakov 2008). Consequently, further work is required to reproduce our results on a wider scale.

The provision of C to roots following photosynthesis is a major contributor to soil C components, and in particular to MBC and DOC (Amiotte-Suchet et al. 2007; Ge et al. 2012). Here, the dynamics of $^{14}$C-DOC (Fig. 4) in response to increasing N concentrations were generally similar to those of the root C biomass (Fig. 1). This suggests that root-derived DOC is probably controlled by the release of organic materials originating from the roots. In rice cultivated in paddy soil, the amount of DOC is higher than that in non-cultivated soil, increasing gradually with rice growth, and is generally correlated with the root biomass (Lu et al. 2004).

The maximum $^{14}$C incorporated into MBC was 33.8 mg C kg$^{-1}$ soil for the $N_{10}$ group (Fig. 4), which accounted for 10.6 % of the total MBC (Table 1). This finding suggests that rice-photosynthesized C inputs into soil having an effect on the dynamics of MBC, as found by Lu et al. 2004. In our work, the application of N significantly influenced the amount of $^{14}$C-MBC and its proportion in the total MBC in the soil (Table 1 and Fig. 4). From these observations, the overall process can be described by five linked processes: (1) decomposition of organic C in native soil is stimulated at low N levels during rice growth; (2) soil microorganisms initially use C from the native soil organic C pool to a greater degree than they use root-derived C, and this stimulates their activities; (3) the population of soil microorganisms increases with increasing available C sources, which in turn maintains the decomposition of organic C in the native soil; (4) because the C sources derived from native soil cannot sustain the increased growth of soil microorganisms, about 8 % of the microorganisms use C derived from roots, whereas the rest continue to use the original soil organic C; and (5) the amount of soil MBC depends on an increased availability of root-derived C sources. Furthermore, soil $^{14}$C-MBC as a proportion of $^{14}$C-SOC ($^{14}$C-MBC/$^{14}$C-SOC) declined significantly with increasing N concentration during the vigorous growing season of rice (Table 1). This suggests that root-derived MBC is a major sink of photosynthesized C within new SOC pools, and is dependent on root-mediated C deposition in paddy soil under N-deficient conditions.

## Conclusions

Our results show that fertilizer N enhances rice photosynthesis-derived C deposition into the SOC pools, which is then incorporated into SOC. However, the amounts of both $^{14}$C-MBC and $^{14}$C-MBC as a proportion of total MBC ($^{14}$C-MBC/MBC) were significantly increased at low N levels during rice growth. This information increases our understanding of the role of below-ground biomass in the storage of SOC in flooded rice-soil systems.

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## References

Amiotte-suchet P, Linglois N, Leveque J, Andreux F (2007) $^{13}$C composition of dissolved organic carbon in upland forested catchments of the Morvan Mountains (France): influence of coniferous and deciduous vegetation. J Hydrol 335:354–363

Anderson EL (1988) Tillage and N fertilization effects on maize root growth and root: shoot ratio. Plant Soil 108:245–251

Boddy E, Hill PW, Farrar J, Jones DL (2007) Fast turnover of low molecular weight components of the dissolved organic carbon pool of temperate grassland field soils. Soil Biol Biochem 39:827–835

Bolan NS, Baskaran S, Thiagarajan S (1996) An evaluation of the methods of measurement of dissolved organic carbon in soils, manures, sludges, and stream water. Commun Soil Sci Plant Anal 27:2723–2737

Cai ZC, Qin SW (2006) Dynamics of crop yields and soil organic carbon in a long-term fertilization experiment in the Huang-Huai-Hai Plain of China. Geoderma 136(3):708–715

Carter MR (2002) Soil quality for sustainable land management: organic matter and aggregation interactions that maintain soil functions. Agron J 94:38–47

Chantigny MH, Angers DA, Prévost D, Simarda RR, Chalifourb FP (1999) Dynamics of soluble organic C and C mineralization in cultivated soils with varying N fertilization. Soil Biol Biochem 31(4):543–550

Dijkstra FA, Cheng WX, Johnson DW (2006) Plant biomass influences rhizosphere priming effects on soil organic matter decomposition in two differently managed soils. Soil Biol Biochem 38:2519–2526

FAO (2011) Rice market monitor. April 2011. XIV(2). P2. http://www.fao.org/docrep/014/am491e/am491e00.pdf

Farrar J, Hawes M, Jones D, Lindow S (2003) How roots control the flux of carbon to the rhizosphere. Ecology 84:827–837

Gavrichkova O, Kuzyakov Y (2008) Ammonium versus nitrate nutrition of Zea mays and Lupinus albus: effect on root-derived $CO_2$ efflux. Soil Biol Biochem 40:2835–2842

Ge TD, Yuan HZ, Zhu HH, Wu XH, Nie SA, Liu C, Tong CL, Wu JS, Brookes PC (2012) Biological carbon assimilation and dynamics in a flooded rice–soil system. Soil Biol Biochem 48:39–46

Ge TD, Wu XH, Chen XJ, Yuan HZ, Zou ZY, Li BZ, Zhou P, Liu SL, Tong CL, Brookes PC, Wu JS (2013) Microbial phototrophic fixation of atmospheric $CO_2$ in China subtropical upland and paddy soils. Geochim Cosmochim Acta 113: 70–78

Gong ZT, Zhang GL, Chen ZC (eds) (2009) Pedogenesis and soil taxonomy. Science Press, Beijing, pp 613–626, in Chinese

He JS, Wang ZQ, Fang JY (2004) Issues and prospects of below-ground ecology with special reference to global climate change. Chin Sci Bull 49:1226–1233

Hütsch BW, Augustin J, Merbach W (2002) Plant rhizodeposition-an important source for carbon turnover in soils. J Plant Nutr Soil Sci 165:397–407

Johansson G (1992) Release of organic C from growing roots of meadow *fescue Festuca pratensis*. Soil Biol Biochem 24(5): 427–433

Kaštovská E, Šantrůčková H (2007) Fate and dynamics of recently fixed C in pasture plant–soil system under field conditions. Plant Soil 300:61–69

Kuzyakov Y, Jones DL (2006) Glucose uptake by maize roots and its transformation in the rhizosphere. Soil Biol Biochem 38: 851–860

Kuzyakov Y, Kretzschmar A, Stahr K (1999) Contribution of Lolium perenne rhizodeposition to carbon turnover of pasture soil. Plant Soil 213:127–136

Kuzyakov Y, Biryukova O, Kuznetzova T, Mölter K (2002a) Carbon partitioning in plant soil, carbon dioxide fluxes and enzyme activities as affected by cutting ryegrass. Biol Fertil Soils 35:348–358

Kuzyakov Y, Siniakina SV, Ruehlmann J, Domanski G, Stahr K (2002b) Effect of nitrogen fertilization on below-ground carbon allocation in lettuce. J Sci Food Agric 82:432–1441

Liljeroth E, Kuikman P, Van Veen JA (1994) Carbon translocation to the rhizosphere of maize and wheat and influence on the turnover of native soil organic matter at different soil nitrogen levels. Plant Soil 161:233–240

Lu YH, Watanabe A, Kimura M (2002a) Input and distribution of photosynthesized carbon in a flooded rice soil. Global Biogeochem Cy 16:1085

Lu YH, Watanabe A, Kimura M (2002b) Contribution of plant-derived carbon to soil microbial biomass dynamics in a paddy rice microcosm. Biol Fertil Soils 36:136–142

Lu YH, Watanabe A, Kimura M (2004) Contribution of plant photosynthates to dissolved organic carbon in a flooded rice soil. Biogeochemistry 71:1–15

Nguyen C (2003) Rhizodeposition of organic C by plants: mechanisms and controls. Agronomie 23:375–396

Pan GX, Wu LS, Li LQ, Zhang XH, Gong W, Yvonne W (2008) Organic carbon stratification and size distribution of three typical paddy soils from Taihu Lake region, China. J Environ Sci 20:456–463

Rasse DP, Rumpel C, Dignac MF (2005) Is soil carbon mostly root carbon? Mechanisms for a specific stabilization. Plant Soil 269:341–356

Saggar S, Hedley C, Mazchzy AD (1997) Partitioning and translocation of photosynthetically fixed $^{14}C$ in grazed hill pastures. Biol Fertil Soils 25:152–158

Swinnen J, van Veen JA, Merckx R (1994a) $^{14}C$ pulse-labeling of field growth spring wheat: an evaluation of its use in rhizosphere carbon budget estimations. Soil Biol Biochem 26: 161–170

Swinnen J, van Veen JA, Merckx R (1994b) Rhizosphere carbon fluxes in field-grown spring wheat: model calculations based on $^{14}C$ partitioning after pulse-labelling. Soil Biol Biochem 26:171–182

Tian J, Dippold M, Pausch J, Blagodatskay E, Fan M, Li XL, Kuzyakov Y (2013a) Microbial response to rhizodeposition depending on water regimes in paddy soils. Soil Biol Biochem 65:195–203

Tian J, Pausch J, Fan MS, Li XL, Tang QY, Kuzyakov Y (2013b) Allocation and dynamics of assimilated carbon in rice-soil system depending on water management. Plant Soil 363: 273–285

van Ginkel JH, Gorissen A, Polci D (2000) Elevated atmospheric carbon dioxide concentration: effects of increased carbon input in a Lolium perenne soil on microorganisms and decomposition. Soil Biol Biochem 32:449–456

Walker TS, Bais HP, Grotewold E, Vivanco JM (2003) Root exudates and rhizosphere biology. Plant Physiol 132:44–51

Wang HJ, Liu QH, Shi XZ, Yu DS, Zhao YC, Sun WX, Darilek JL (2007) Carbon storage and spatial distribution patterns of paddy soils in China. Front Agric China 1:149–154

Weintraub MN, Scott-Denton LE, Schmidt SK, Monson RK (2007) The effects of tree rhizodeposition on soil exoenzyme activity, dissolved organic carbon, and nutrient availability in a subalpine forest ecosystem. Oecologia 154(2):327–338

Wu JS (2011) Carbon accumulation in paddy ecosystems in subtropical China: evidence from landscape studies. Eur J Soil Sci 62:29–34

Wu JS, O'Donnell AG (1997) Procedure for the simultaneous analysis of total and radioactive carbon in soil and plant materials. Soil Biol Biochem 29:199–202

Wu JS, Joergensen RG, Pommerening B, Chaussod R, Brookes PC (1990) Measurement of soil microbial biomass C by fumigation-extraction–an automated procedure. Soil Biol Biochem 22:1167–1169

Wu XH, Ge TD, Yuan HZ, Li BZ, Zhu HH, Zhou P, Sui FG, O'Donnell AG, Wu JS (2014) Changes in bacterial $CO_2$ fixation with depth in agricultural soils. Appl Microbiol Biotechnol 98:2309–2319

Yuan HZ, Ge TD, Zou SY, Wu XH, Liu SL, Zhou P, Chen XJ, Brookes P, Wu JS (2013) Effect of land use on the abundance and diversity of autotrophic bacteria as measured by ribulose-1, 5-biphosphate carboxylase/oxygenase (RubisCO) large-subunit gene abundance in soils. Biol Fertil Soils 49:609–616

Zagal E (1994) Carbon distribution and nitrogen partitioning in a soil-plant system with barley (Hordeum vulgare L.), ryegrass (Lolium perenne) and rape (Brassica napus L.) grown in a $^{14}CO_2$-atmosphere. Plant Soil 166:63–74

Zagal E, Bjarnason S, Olsson U (1993) Carbon and nitrogen in the root-zone of barley (Hordeum vulgare L.) supplied with nitrogen fertilizer at two rates. Plant Soil 157:51–63

Zagal E, Rydberg I, Martensson A (2001) Carbon distribution and variations in nitrogen-uptake between catch crop species in pot experiments. Soil Biol Biochem 33:523–532