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Contribution of plant photosynthates to dissolved organic carbon in a flooded rice soil

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Abstract. Dissolved organic C (DOC) plays important roles in nutrient cycling and methane production in flooded rice ecosystem. The microcosm experiment was carried out to measure directly the contribution of photosynthates to DOC by using a ^{13}C pulse-chase labeling technique. DOC was operationally divided into water-extractable organic C (WEOC) and salt-extractable organic C (SEOC) by successive extraction firstly with deionized water and then with 0.25 M K_2SO_4. Total WEOC increased with plant growth, whereas SEOC concentration did not change significantly over the growing season. About 0.037–0.36% (mean 0.16%) of the assimilated ^{13}C was incorporated into WEOC immediately after $^{13}CO_2$ assimilation (Day 0), but only 0–0.025% (mean 0.01%) was incorporated into SEOC. At the end of the growing season, the ^{13}C amounts of WEOC substantially decreased, while those of SEOC slightly increased. The estimated net plant C contribution was 21 mg C plant^{-1} to WEOC and 6 mg C plant^{-1} to SEOC, corresponding to 33.8% of total WEOC and 20.2% of total SEOC at the end of the growing season, respectively. The results suggest that the incorporation and decomposition of the photosynthesized C occurred rapidly in rice soil which significantly affected the WEOC dynamics, but SEOC appeared to be in equilibrium with the native soil organic matter, receiving less effect from the plant growth.

Introduction

The function and dynamics of dissolved organic C (DOC) in soils are determined by its quality and quantity, which largely depend on its origins (McDowell and Liken 1988; Moore 1998; Kalbitz et al. 2000). Root-derived materials are an important DOC source in soil. Roughly 30 to 60% of the net photosynthesized C is allocated below ground, and as much as 40 to 90% of this fraction enters the soil in the forms of root exudates, mucilage, sloughed-off cells and decaying roots (Lynch and Whipps 1990; Whipps 1990). Recent researches have revealed that the root-derived DOC plays a crucial role in C dynamics of the flooded rice soil. Firstly, the root-derived DOC serves as a major C source for methanogenic activity (Wassmann and Aulakh 2000). Lu et al. (2000a, b) found that DOC in a small volume of soil surrounding the rice roots (the rhizosphere) increased with plant growth, whereas DOC in the bulk soil was not affected by plant growth. CH_4 production and emission were positively correlated to DOC concentration in the rhizosphere, and the difference in CH_4 emissions among rice varieties could be explained by the differences in the rhizosphere DOC concentrations (Lu et al. 2000a). Apparently,

DOC in the rhizosphere soil was enriched by the root-derived C, but the 'new' DOC was decomposed to CH_4 rapidly. Secondly, the growth of soil microorganisms is likely limited by C availability in soils (Smith and Paul 1990). An increase of root-derived DOC with plant growth, therefore, caused an increase of soil microbial biomass (Lu et al. 2002a). Thirdly, a fraction of the root-derived DOC is likely to be resistant to decomposition. The leaching of this fraction with percolation water was reported to promote the subsoil humus formation (Maie et al. 1997, 1998). Quantification and characterization of the root-derived DOC are, therefore, essential in order to better understand and predict microbial activity, CH_4 production, CH_4 emissions and soil humus chemistry in the flooded soil systems.

Most compositions of DOC are, in principle, capable of being sorbed on soil surface to a certain extent (Zsolnay et al. 1996). DOC pool, therefore, can be divided into two subpools: those sorbed on soil particles and those dissolved in the interstitial pore water (Zsolnay et al. 1996; Tao and Lin 2000). It was suggested that water could extract organic matter in the free form and the weakly adsorbed forms (i.e., through hydrogen bonding), while a sulfate solution could be used to extract the organic matter which was adsorbed through ionic bonding (Jardine et al. 1989; Xu and Yuan 1995; Maie et al. 1997, 1998). In this experiment, DOC was operationally divided into two fractions: water extractable organic C (WEOC) and salt extractable organic C (SEOC) through successive extraction of soil samples firstly with deionized water and then with 0.25 M K_2SO_4. Our objectives were to: (a) determine whether the dynamics of the two fractions was controlled by the release of organic substances from rice roots; (2) quantify the contribution of the photosynthates to DOC over a rice growing season by using a ^{13}C pulse-chase labeling technique.

Materials and methods

Soil preparation and rice growth

The details of soil preparation, plant growth and ^{13}C labeling have been described previously (Lu et al. 2002a, b). A yellow soil (Oxiaquic Dystrochrepts) was collected from the plow layer (0–15 cm) of a rice field at Aichi-ken Anjo Research and Extension Center, Central Japan (34°48′N, 137°30′E). Bulk soil was sieved (<4 mm) under moist conditions (water content, 16.7%) to remove the coarse plant residues. The soil sample had the following characteristics: pH of 6.3 (1:1, soil/water ratio), CEC of 14.4 cmol kg^{-1}, organic C of 13.1 g kg^{-1}, total N of 1.62 g kg^{-1}, and clay content of 23%.

Soil was mixed with $(NH_4)_2SO_4$, calcium super-phosphate and KCl at the rates of 0.1 g N, 0.04 g P and 0.08 g K kg^{-1} soil prior to the rice planting. One kilogram of the fertilized soil sample (equivalent to 857 g oven-dry soil) was filled into 1 l pots and submerged with deionized water. Two 35-day-old rice seedlings (*Oryza sativa* L. cv. Aoino-Kaze) were transplanted to each pot on June 13, 2000. The pots were placed outdoors and irrigated with deionized water maintaining a 3–5 cm water layer on the soil surface throughout the growing season. Ammonium sulfate was topdressed on 12

and 29 July, and on 6 and 13 August at a rate of 0.1 g N kg^{-1} soil, respectively. Total applied N was 0.5 g N per pot, corresponding to 150 kg N ha^{-1}. Weeds were removed manually.

Carbon-13 labeling

The $^{13}CO_2$ labeling was performed six times on 4 and 19 July, 4 and 21 August and 4 and 20 September, respectively, covering the major development stages of rice plant. At the labeling date, plants were transferred to an artificially lit growth chamber (area 100 cm × 60 cm, height 100 cm) with the pot surface covered by a black plastic sheet to avoid algal photosynthesis in the floodwater and to allow the rice shoots to be exposed to $^{13}CO_2$. No specific caution such as a leak check was done to ensure the isolation of shoots from roots. Minoda and Kimura (1994) and Minoda et al. (1996) used the similar plant labeling system to determine the contribution of photosynthates to methane emissions from rice soil. By feeding plant with $^{13}CO_2$ under dark condition (without photosynthesis), they found no detectable ^{13}C in soil, evidencing that direct diffusion of $^{13}CO_2$ through flooded water layer was negligible. Moreover, if there was any $^{13}CO_2$ leaked into flooded water, they were excluded from DOC measurements because the solutions were acidified prior to the analysis of ^{13}C (see measurement details below). The $^{13}CO_2$ was generated through reaction between $Ba^{13}CO_3$ (99 at.% ^{13}C) and lactic acid in a beaker placed inside the chamber. Mean $^{13}CO_2$ concentrations were between 180 and 270 ppm, which accounted for 24–57% of the total CO_2 concentration inside the chamber. Plants were labeled for 6 h (0900–1500). Nine pots were kept as controls without labeling and placed 5 m away from labeling pots outdoors.

Sampling

At the end of each labeling, three pots were destructively sampled immediately (Day 0) and three pots were returned outdoors until they were sampled at the end of the growing season (Harvest) (10 October). The sampling of the control pots was conducted on 17 July, 30 August and 10 October.

During sampling, shoots were cut off at the root base. Roots and soil were separated by gently shaking in 1.2 l deionized water at 25 °C. Soil slurries were passed through a 2 mm sieve to remove the course root debris. An appropriate amount of water was added to obtain a final water to soil ratio of about 2:1. Soil slurries were then gently shaken for 30 min at 25 °C. The samples were centrifuged at 13,000 *g* for 15 min. The supernatants were filtered through a 0.45 μm fiber glass filter, acidified to <pH 3.0 with 10% HCl, and stored at −20 °C. The organic C contained in these solutions were taken as WEOC. The soil residues after the above centrifugation were re-suspended in 0.25 M K_2SO_4 at a solution to soil ratio of 5:1. The slurries were shaken for 30 min at 25 °C. The samples were then processed through centrifugation, filtration, acidification and storage similarly. The organic C extracted by 0.25 M K_2SO_4 were taken as SEOC.

Measurement of total C and ^{13}C

The total C contents of WEOC and SEOC were measured by a total carbon analyzer (TOC-500, Shimadzu Co., Japan). Prior to analysis for ^{13}C, about 50 ml of WEOC and SEOC solution samples were freeze-dried and ground to fine powders. The stable C isotope ratios were measured using an isotope ratio mass spectrometer (Delta^{plus}, Finnigan MAT GmbH, Bremen, Germany) coupled to an elemental analyzer (NC 2500, ThermoQuest Italia S.p.A., Milan, Italy) by an interface (ConFlo II, Finnigan MAT GmbH, Bremen, Germany). The natural abundance of heavy isotopes was expressed as parts per thousands relative to the international standard PDB using delta units (δ).

Calculation and statistical analysis

The ^{13}C incorporation into DOC was expressed as the increase of $\delta^{13}C$ value ($\Delta\delta^{13}C$) relative to the control without labeling and as the percent of total ^{13}C assimilated by rice plants and of total ^{13}C retained in soil.

Atom% ^{13}C was calculated according to Boutton (1991) as follows:

$$\text{Atom\% } ^{13}\text{C} = \frac{(\delta^{13}\text{C} + 1000) \times R_{\text{PDB}}}{(\delta^{13}\text{C} + 1000) \times R_{\text{PDB}} + 1000} \times 100 \quad (1)$$

where R_{PDB} was the $^{13}C/^{12}C$ ratio of the standard PDB (=0.0112372) and $\delta^{13}C$ is the ^{13}C natural abundance of the DOC sample.

The ^{13}C amounts of DOC were estimated based on the difference in atom% ^{13}C of DOC of the labeled sample and that of the non-labeled control as follows:

$$^{13}\text{C of DOC} = [(\text{atom } ^{13}\text{C\%})_{\text{DOC, labeled}} - (\text{atom } ^{13}\text{C\%})_{\text{DOC, non-labeled}}] \times \text{DOC} \quad (2)$$

The total plant-assimilated ^{13}C and the ^{13}C fraction that was retained in soil have been reported previously (Lu et al. 2002a,b).

The experiment was carried out with three replications and arranged in a completely randomized design. The data were subjected to analysis of variance (ANOVA). Duncan Multiple Range Test (DMRT) was used to test differences in the measured variables among labeling events (or plant ages). Student's *T*-test was used to test the differences between sampling times (at the end of labeling and at the end of growing season) and between DOC types (WEOC and SEOC).

Results

Total concentrations of WEOC and SEOC

Rice plants reached the maximum tillering (MT) on 13 July (30 days after transplanting (DAT)) and started heading (corresponds to beginning of plant flowering)

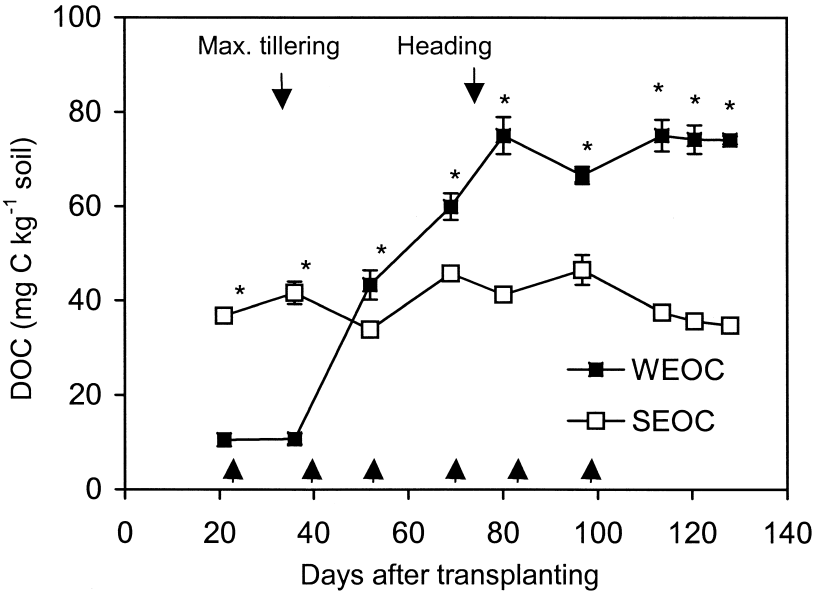

Figure 1. The concentrations of two forms of dissolved organic carbon (DOC) in a flooded rice soil: water-extractable organic C (WEOC); and salt-extractable (extracted by 0.25 M K_2SO_4) organic C (SEOC). The downward arrows denote the time of the maximum tillering and heading (corresponding to the beginning of plant flowering) of rice plants, respectively. The upward arrows denote the time at which the ^{13}C labeling was performed. Bars represent the standard errors. Asterisks indicate a significant difference between WEOC and SEOC ($P < 0.05$).

on 25 August (74 DAT). At the end of the growing season, the plants yielded 40.1 g dry weight of shoots, 10.0 g dry weight of roots and 15.5 g grains per pot. More details of the plant growth were referred to Lu et al. (2002a). The seasonal pattern of total DOC concentrations differed greatly between WEOC and SEOC (Figure 1). At the beginning of the experiment, SEOC concentration was greater than WEOC. However, starting from 36 DAT (6 days after MT), WEOC sharply increased, reached maximal at 77 DAT (4 days after rice heading), and maintained at a high level until Harvest. On the other hand, SEOC did not change significantly throughout the growing season. Consequently, the concentration of WEOC at Harvest was about twice greater than that of SEOC (Figure 1).

Carbon-13 in WEOC

Labeling 1 was done before MT, Labeling 2, 3 and 4 were done between MT and heading of rice, and Labeling 5 and 6 were done during maturation of plants (indicated in Figure 1). The natural ^{13}C abundance of WEOC was -27.0 ± 0.1‰ for non-labeled control samples collected at three occasions. The $\delta^{13}C$ values increased immediately after ^{13}C labeling (Figure 2(a), Day 0). The increase was most significant for Labeling

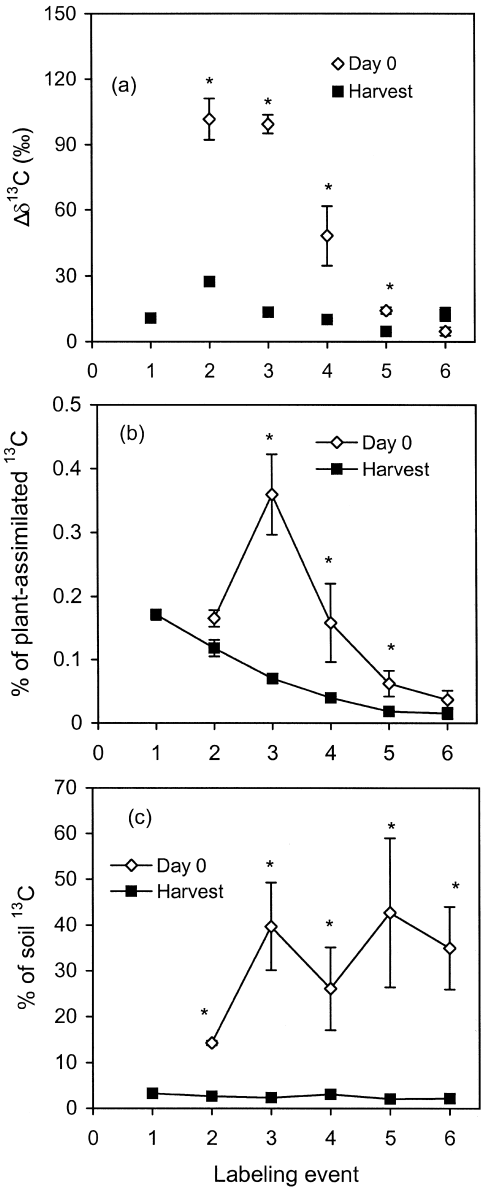

Figure 2. The ^{13}C in water-extractable organic C immediately after labeling (Day 0) and at the end of growing season (Harvest) expressed as: (a) increase of $\delta^{13}C$ values relative to control without labeling ($\Delta\delta^{13}C$); (b) proportion of the total plant-assimilated ^{13}C; (c) proportion of the total soil ^{13}C. Bars represent the standard errors. Asterisks indicate a significant difference between Day 0 and Harvest ($P < 0.05$).

2 and 3, followed by Labeling 4, and least for Labeling 5 and 6. The $\delta^{13}C$ values at Harvest were still higher than the natural ^{13}C abundance of the non-labeled control. However, they are substantially lower than those at Day 0, except for Labeling 6 where the difference between Day 0 and Harvest was not significant.

The ^{13}C amounts of WEOC as percent of the plant-assimilated ^{13}C represented the relative contribution of photosynthates to WEOC. The percentages ranged between 0.037 and 0.36% (mean 0.16%) at Day 0. They increased and reached maximal at Labeling 3, followed by a trend of gradual decline toward the last labeling event (Figure 2(b)). The percentages remaining at Harvest were between 0.015 and 0.17% (mean 0.072%), which were about half of that at Day 0. They showed a constant decrease from Labeling 1 to Labeling 6.

The ^{13}C amount of WEOC as a proportion of total soil ^{13}C ranged between 14.3 and 35.0% (mean 25.0%) at Day 0 and decreased to 2.3–3.3% (mean 2.8%) at Harvest (Figure 2(c)). There was no significant difference among the labeling events for the proportions both at Day 0 and at Harvest.

Carbon-13 in SEOC

The ^{13}C incorporation into the SEOC fraction greatly differed from that into WEOC. The increase of $\delta^{13}C$ values at Day 0 after labeling was much smaller than those of WEOC (Figures 2(a) and 3(a)); and the values increased at Harvest as compared to those at Day 0 (significantly for Labeling 3, 5 and 6) (Figure 3(a)). The ^{13}C of SEOC as percents of the plant-assimilated ^{13}C were 0–0.025% (mean 0.01%) at Day 0 and 0.006–0.036% (mean 0.02%) at Harvest (Figure 3(b)). These values were about 14 and 3.5 times lower than those of WEOC at Day 0 and Harvest, respectively. The ^{13}C of SEOC as proportions of total soil ^{13}C showed no significant differences either between Day 0 and Harvest or among the labeling events, except for Labeling 4 where a relatively greater proportion was observed at Harvest (Figure 3(c)). The mean proportions were 1.3% at Day 0 and 0.9% at Harvest, which were 19 and two times lower than those of WEOC.

Change between Day 0 and Harvest

The ^{13}C amounts of WEOC decreased, whereas those of SEOC increased over the period between Day 0 and Harvest (Table 1). The changes indicated either decomposition or accumulation of plant C in each DOC fraction. Roughly, 30–80% (mean 58%) of ^{13}C in WEOC was decomposed, while about 103% of ^{13}C in SEOC fraction was accumulated over the period from Day 0 to Harvest.

Net contribution of photosynthates to DOC

The net contribution of the plant photosynthates formed at the various growth stages of rice plant to WEOC and SEOC at the end of the growing season was

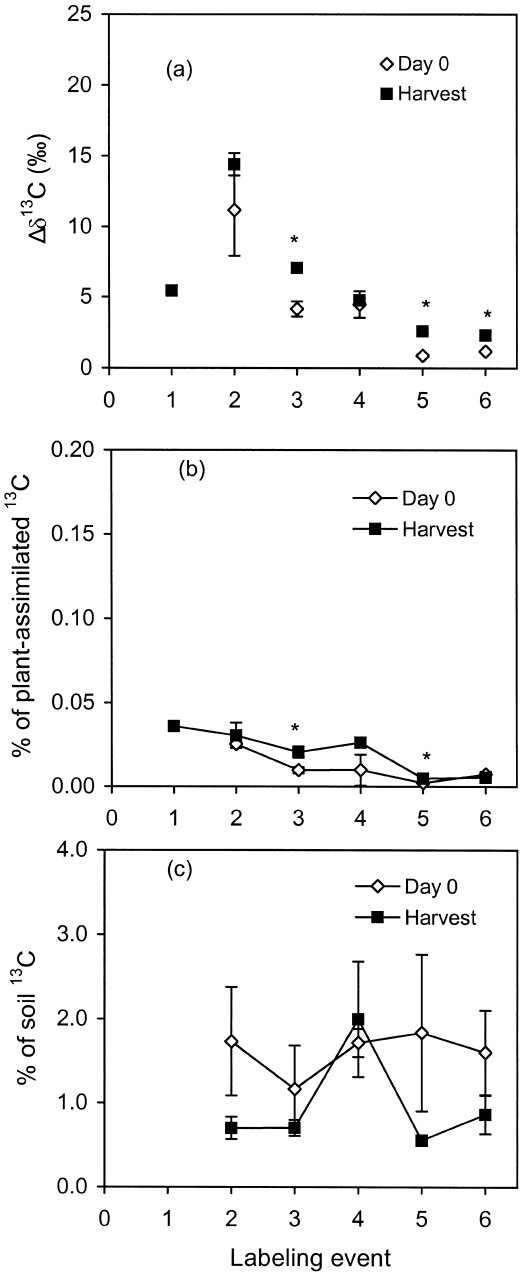

Figure 3. The ^{13}C in salt-extractable organic C immediately after labeling (Day 0) and at the end of growing season (Harvest) expressed as: (a) increase of $\delta^{13}C$ values relative to control without labeling ($\Delta\delta^{13}C$); (b) proportion of the total plant-assimilated ^{13}C; (c) proportion of the total soil ^{13}C. Bars represent the standard errors. Asterisks indicate a significant difference between Day 0 and Harvest ($P < 0.05$).

Table 1. The ^{13}C amounts incorporated into water-extractable organic C (WEOC) and salt-extractable organic C (SEOC) and their changes over the period between Day 0 (immediately after labeling) and Harvest (at the end of the growing season).

Labeling event	Date	DAT[a]	WEOC		Change[b] (%)	SEOC		Change (%)
			^{13}C amount			^{13}C amount		
			Day 0 (μg pot^{-1})	Harvest (μg pot$^{-1)}$		Day 0 (μg pot^{-1})	Harvest (μg pot^{-1})	
1	4 July	21	ND[c]	8.8 (0.3)[d]	ND	ND	1.86 (0.1)	ND
2	19 July	36	25.1 (0.7)*[,e]	18.6 (1.2)	−26	4.16 (1.6)	4.80 (0.2)	+15
3	4 August	51	40.5 (3.8)*	8.3 (0.5)	−80	1.03 (0.2)	2.43 (0.2)*	+135
4	21 August	68	27.5 (7.9)*	7.9 (0.2)	−71	1.91 (0.4)	5.20 (1.8)*	+172
5	4 September	83	9.8 (1.1)*	3.0 (0.1)	−69	0.37 (0.03)	0.82 (0.1)*	+118
6	20 September	99	4.7 (0.8)*	2.6 (1.6)	−45	0.44 (0.01)	0.77 (0.1)*	+76
Mean					−58			+103

[a]Days after transplanting.
[b]Change% was calculated as: $\text{Change}\% = ({}^{13}C_{\text{Harvest}} - {}^{13}C_{\text{Day 0}}) / {}^{13}C_{\text{Day 0}} \times 100$.
[c]Not determined.
[d]Data in parenthesis represents the standard errors ($n = 3$).
[e]Asterisks indicate the significant difference in ^{13}C amounts between Day 0 and Harvest at $P < 0.05$.

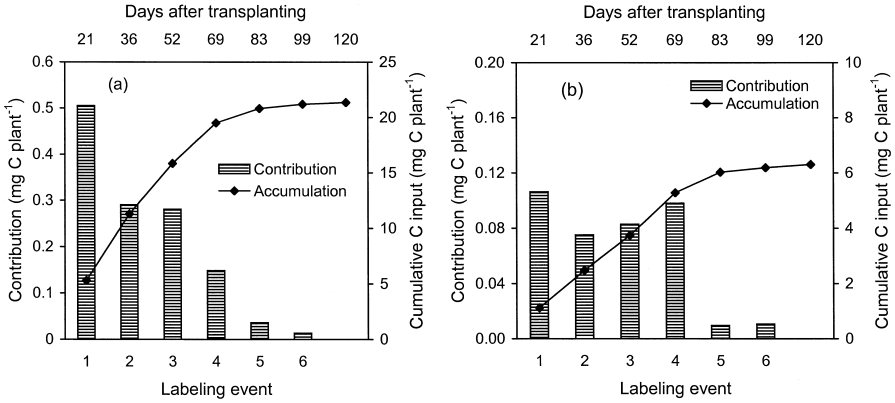

Figure 4. The estimated contribution of photosynthates formed at different ages of rice to two forms of dissolved organic C at the end of growing season (bars): (a) water-extractable organic C; (b) salt-extractable organic C. Also shown is the accumulation of the contributions over the growing season of rice (lines).

estimated by relating ^{13}C distribution in DOC at Harvest to total plant growth rates. The estimated contribution to WEOC decreased from 0.51 mg C plant^{-1} by the photosynthates of young rice (21 DAT) to 0.013 mg C plant^{-1} by the photosynthates of maturing rice (99 DAT) (Figure 4(a)). The contributions to SEOC were smaller than those to WEOC (Figure 4(b)). The cumulative contribution (mg C plant^{-1} season^{-1}), which was calculated by integrating the contributions of photosynthates at various growth stages of rice, amounted to 21.3 mg C plant^{-1} season^{-1} for WEOC and 6.3 mg C plant^{-1} season^{-1} for SEOC, corresponding to 33.8% of total WEOC and 20.2% of total SEOC at Harvest, respectively.

Across comparison among DOC, microbial biomass and soil organic C

Across comparison between two forms of DOC, microbial biomass C (MBC) and soil organic C (SOC) was made for labeling event #3 when rice plant was at the maximum tillering stage (at the fastest growth of plant biomass). The details of plant C contribution to MBC and SOC have been reported earlier (Lu et al. 2002a,b). The $\delta^{13}C$ value of WEOC was highest at Day 0 (immediately after labeling), followed by that of microbial biomass, and those of SEOC and SOC were relatively low (Figure 5). At the end of growing season (Harvest), The $\delta^{13}C$ values of WEOC and MBC relatively decreased, whereas those of SEOC and SOC slightly increased. The $\delta^{13}C$ values of MBC became greater than that of WEOC at Harvest.

Discussion

The DOC pool in this study was divided into two fractions: WEOC presumably representing the DOC fraction of dissolved and weakly adsorbed forms and SEOC

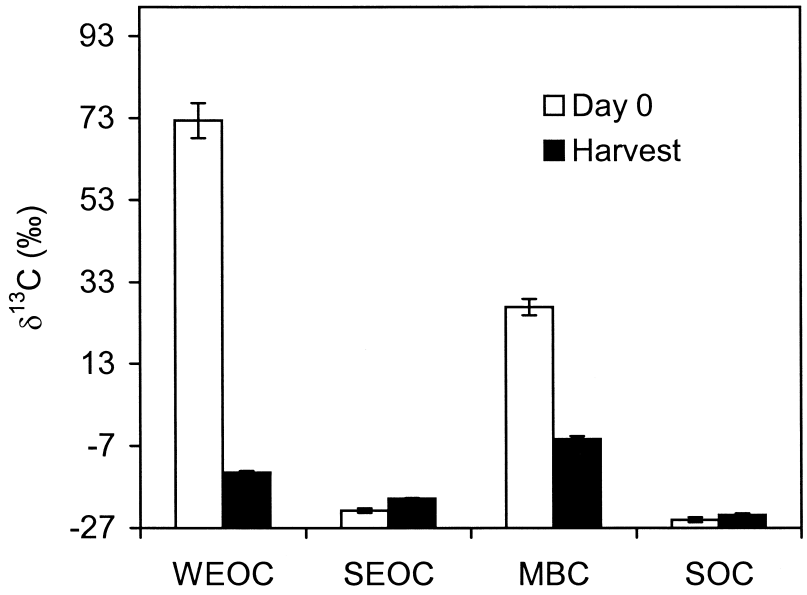

Figure 5. Across comparison of $\delta^{13}C$ values of water-soluble organic C (WEOC), salt-extractable C (SEOC), microbial biomass C (MBC) and soil organic C (SOC) after 6 h labeling of rice plants on August 4 (labeling event #3). White and black bars are values at the end of labeling (Day 0) and at the end of the growing season (Harvest), respectively. Means of three replications are shown. The *X*-axis crosses at $\delta^{13}C$ value of −27‰, which corresponds to the ^{13}C background of various soil organic pools without labeling.

presumably representing more strongly adsorbed forms. The results showed that the plant impacts on seasonal dynamics of DOC were greatly different between WEOC and SEOC. WEOC increased significantly with plant growth. Its seasonal dynamics coincided with previous observations of DOC in the rhizosphere of rice plants (Lu et al. 2000a,b), which suggested that DOC in the rhizosphere of rice plants was controlled by the release of organic materials from roots. The rapid and significant incorporation of ^{13}C into WEOC in this experiment confirmed this suggestion. However, SEOC in the present experiment was only marginally affected by plant growth. The increase of ^{13}C in SEOC over the period from Day 0 to Harvest (Table 1) was similar to the pattern of ^{13}C in whole soil organic C (Figure 5) (Lu et al. 2002b). The small and stable proportion of soil ^{13}C in SEOC fraction over the entire season (Figure 3(c)) suggested that SEOC was actually in equilibrium with soil organic C. Alternatively, some of fine root residues might remain in soil and contribute to the measured label of both SEOC and SOC at Harvest. Nevertheless, the two DOC fractions appear to be controlled by different C sources under the conditions of the present experiment. The large effect of plant C on WEOC suggests that soluble root exudates are mainly composed of neutral and weakly adsorbed molecules. For example, it was reported that rice root exudates consisted of 43–65% sugar and 31–54% organic acids (Aulakh et al. 2001).

The contribution percentage of photosynthates to DOC averaged 0.16% for WEOC and 0.01% for SEOC at Day 0. The amounts remaining at Harvest averaged 0.072% for WEOC and 0.02% for SEOC, respectively. In pooling two fractions, the contribution was about 0.17% at Day 0 with 0.10% remaining at Harvest. The contribution based on such calculation was possibly underestimated, because ^{13}C at Day 0 did not necessarily represent the maximal ^{13}C incorporation. In an additional labeling experiment performed on 29 August, we found that the maximal ^{13}C in WEOC appeared at Day 3 after labeling. The ^{13}C at Day 0 was about 84% of the maximal for this particular labeling (data not shown). Taking into account this difference, the actual contribution was estimated to be 0.20% of the total assimilated C. Comparable data are not available on DOC in rice soils. However, previous experiments using ^{14}C labeling showed that the contribution of the assimilated ^{14}C to CH_4 emitted into the atmosphere was 3–6% in a rice soil (Dannenberg and Conrad 1999) and 0.1–0.5% in natural wetland soils (Megonigal et al. 1999; King and Reeburgh 2002). The contribution of photosynthates to DOC in our experiment appears to be relatively low, provided that DOC serves as a reservoir for root exudates and a supply of C for CH_4 production (Sigren et al. 1997; Lu et al. 2000a,b). The reason for this discrepancy is currently unknown. Probably, H_2/CO_2-dependent methanogenesis accounts for a large amount of CH_4 production in the vicinity of rice roots and root surface (Conrad and Klose 1999; Lemann-Richter et al. 1999; Conrad et al. 2000). However, notwithstanding the small contribution percentages, the accumulation of the contribution over a growing season amounted to 33.8% of total WEOC and 20.2% of total SEOC at the end of the growing season, respectively. These percentages agreed with the previous observation that recent photosynthates contributed 22–29% of total CH_4 emitted into the atmosphere from a similar soil (Minoda et al. 1996). These percentages were also greater than the estimates in upland soils under long-term experimental conditions (Gregorich et al. 2000; Hagedorn et al. 2002).

Root-derived DOC is considered to be highly biodegradable (Yano et al. 1998, 2000). Based on the difference in ^{13}C amounts of WEOC between Day 0 and Harvest, we estimated that at least 30–80% (mean 58%) of the root-derived DOC was decomposed. The remaining amounts at Harvest represented the net contribution of photosynthates to DOC, which was estimated to be 0.1% of the total assimilates. This fraction could be derived from original root exudates which were soluble but resistant to decomposition, and/or derived from re-cycling of C through microbial biomass (Guggenberger et al. 1994; Moller et al. 1999; Ogawa et al. 2001; Park et al. 2002). The across comparison revealed that the root-released C at Day 0 was largely water-soluble, a fraction of which was assimilated quickly by soil microbial biomass, and only a small fraction was recovered as SEOC and SOC (Figure 5). From Day 0 to Harvest, a large proportion of ^{13}C-WEOC was lost, presumably due to decomposition or assimilation by soil microorganisms. The ^{13}C-MBC also decreased (Lu et al. 2002a), indicating quick turnover of plant C through this pool. In contrast, the ^{13}C amounts of SEOC and SOC increased slightly from Day 0 to Harvest. The fine and decaying root residues probably contributed to increases of ^{13}C in SEOC and SOC (Lu et al. 2002b). The net contribution of photosynthates to DOC showed a seasonal pattern: higher during younger stages

than during maturing stages of rice (Figure 4). This pattern was consistent with the seasonal distribution of photosynthates to soil microbial biomass (Lu et al. 2002a) and to soil organic C (Lu et al. 2002b). The seasonal pattern of the photosynthate distribution appeared to be controlled by the root activity. The young plants, with active root development, tended to transfer more of its photosynthates to the belowground and release them into soil (Keith et al. 1986; Martin and Kemp 1986).

The total WEOC content increased from $10\,mg\,C\,kg^{-1}$ soil at the beginning of the experiment to $74\,mg\,C\,kg^{-1}$ soil at the end of the growing season. Plant C accounted for 39.4% of this increase. Because no other organic materials were applied to soil, the remainder (61.6%) was considered to be from the degradation of the native soil organic matters. Collins et al. (2000) classified soil organic matters into labile, less-labile and refractory organic substances. The mineralization of labile fraction of SOC, which was probably accelerated with the increased microbial activity and plant growth (Helal and Sauerbeck 1989; Kuzyakov et al. 2000, 2001; Lu et al. 2002a), may be responsible for part of the WEOC increase.

Conclusions

The results of this experiment demonstrated that significant incorporation of photosynthates into DOC occurred in flooded rice soil. A large portion (at least 60%) of the plant-derived WEOC, however, was decomposed over the growing period, which probably promoted soil microbial activity and enhanced CH_4 production. The remaining part, which constituted 34% of total WEOC at the end of the growing season, was likely to be relatively resistant to further decomposition. The ecological function of this 'new' WEOC in flooded soil deserves further investigation. For example, does leaching of this 'new' DOC with percolation water during the fallow season promote the humus formation in subsoil or enhance nutrient loss from soil? A seasonal pattern of photosynthate contribution to DOC was revealed in the present study. This information shall be useful in the construction of a predictive model of DOC, CH_4 production, and microbial dynamics in flooded rice–soil systems.

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