Linking microbial community dynamics to rhizosphere carbon flow in a wetland rice soil

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Abstract

Photosynthesis by terrestrial vegetation is the driving force of carbon cycling between soil and the atmosphere. The soil microbiota, the decomposers of organic matter, is the second player carrying out carbon cycling. Numerous efforts have been made to quantify rhizodeposition and soil respiration to understand and predict the carbon cycling between the soil and atmosphere. However, there have been few attempts to link directly the soil microbial community to plant photosynthesis. We carried out a pulse-chase labeling experiment in a wetland rice system in which rice plants of various ages were labeled with 13CO2 for 6 h and the distribution of the assimilated 13C to soil microorganisms was estimated by analyzing the 13C profile of microbial phospholipid fatty acids (PLFAs). The results showed that total PLFA increased with plant growth, indicating an increase of microbial biomass. But the mono-unsaturated PLFAs increased faster than the branched chain fatty acids. The 13C was incorporated into PLFAs immediately after the plant 13CO2 assimilation, proving the tight coupling of microbial activity to plant photosynthesis. In line with the finding of seasonal change in total PLFAs, more of 13C was distributed to the straight chain fatty acids (16:0, 16:1ω7, 18:1ω7 and 18:1ω9) than to the branched chain fatty acids. The total plant carbon incorporation estimated from 13C labeling roughly corresponded to the increase in total PLFAs over the growing season of plants. Our study suggests that microbial populations in rice soil differ greatly in their responses to plant photosynthate input.

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Keywords: Rice soil; Plant photosynthesis; Microbial community; Stable isotope labeling; Phospholipid fatty acids

1. Introduction

Rhizodeposition occurs continuously during the life of plants in the form of water-soluble exudates, secretions, lysates, mucilages, sloughed-off cells and decaying roots [1]. A large fraction of the carbon allocated below ground is rapidly returned to the atmosphere in the form of root and microbial respiration [2–4]. In wetland soils, plant-derived organic substances additionally serve as an important carbon source for CH4 production and emission [5–8]. Beside the microbial production of CO2 and CH4, a fraction of photosynthesized carbon is retained in the soil microbial biomass after the growing season of plants [9–12]. This new carbon could amount to 28% of total microbial biomass in a rice soil [9]. The microbial dynamics, particularly the rhizoplane [13–15] and rhizosphere populations [16–19], are thus believed to be significantly affected by photosynthetic inputs. However, there have been few direct attempts to link microbial community response to plant photosynthesis. Boschker and colleagues [20,21], by using phospholipid fatty acids (PLFAs)-based stable isotope analysis, showed that there was only a limited coupling between microbial activity and plant photosynthesis in marine sediment systems. More recently, Butler et al. [22], using a similar technique with 13C pulse-chase labeling,
showed that the microbial community had different responses to rhizodeposition in association with different ages of annual ryegrass plants. We performed a $^{13}$C pulse-chase labeling experiment in a wetland rice system, in which rice plants were pulse labeled with $^{13}$CO$_2$ at different growth stages of plants and the seasonal pattern of the photosynthate distribution to dissolved organic carbon [23], microbial biomass [9], and total soil organic matter [24] was monitored. Here we report the incorporation of rice photosynthate into microbial phospholipid fatty acids, which reveals a significant effect of plant photosynthesis on the activity and structure of the microbial community in rice soil.

2. Materials and methods

2.1. Rice-soil microcosm preparation and $^{13}$C labeling

The details of soil characteristics, microcosm preparation, fertilizer application, plant growth and management have been previously described [9,24]. Briefly, 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). The rice plants (*Oryza sativa* L. cv. Aoino-Kaze) were grown in 1-L pots with 1 kg soil for a complete season from 13 June to 10 October in 2000. The soil was irrigated with deionized water maintaining a 3- to 5-cm water layer overlying the soil surface throughout the growing season. In total, 45 microcosms were prepared: 36 microcosms were used for pulse chase labeling and 9 microcosms used as controls without labeling. The accumulation of plant biomass and the labeling of tillers indicated that the plant growth in the microcosms was similar to that under field conditions [9]. The $^{13}$C-pulse labeling was performed six times with three microcosms being destructively sampled immediately (within 1 h) after the pulse labeling (Day 0). The remaining three microcosms were returned outdoors until sampling at the end of the season (harvest). The separation of soil and roots consisted of two steps. First, soil was separated from roots by washing through a 2-mm sieve to remove the coarse root debris, and then, the soil slurries were mixed and centrifuged at 13,000 g for 15 min to remove the fine roots with light density. This two-step separation procedure was efficient for removing most of the fine living roots for the reliable measurement of soil microbial biomass [25]. The soil samples were collected and stored at −20 °C until the analysis for total PLFAs and $^{13}$C-PLFAs.

2.2. PLFA analysis

Total PLFA was extracted from 5 g wet soil samples following the method of White et al. [26] with minor modification [27]. The extracted lipids were separated on a solid phase extraction column (Sep-Pack® Cartridges Silica, Waters) and the fatty acid components were released and methylated by the reaction with Hydrogen Chloride–Methanol Reagent 10 (Tokyo Chemical Industry, Japan) for 2 h at 80 °C. The fatty acid methyl esters (FAME) were determined using an HP 5890 serials II gas chromatograph equipped with an Ultra 2 capillary column (50 m long, 0.2 mm inner diameter, 0.33 μm film thickness; He carrier gas). The stable carbon-isotope compositions of individual compounds were determined using a Finnigan MAT delta plus isotope ratio mass spectrometer coupled to HP 6890 serials II gas chromatograph (column as above) with a Finnigan Standard GC Combustion Interface III. The stable isotope ratios of individual FAME were corrected for the isotope composition of the exogenous methyl derivative group to obtain the actual ratios of PLFAs [28]. The PLFAs 18:1ω7 and 18:1ω9 were not clearly separated under the chromatographic conditions used for $^{13}$C measurements and hence the $^{13}$C results of these PLFAs were combined as 18:1ω7&9. The $^{13}$C incorporation into PLFAs was calculated as $^{13}$C of PLFAs in the labeled samples in excess of those in the non-labeled controls

$$^{13}\text{C-PLFA} = \left( \frac{\text{atom } ^{13}\text{C}^%_{\text{PLFA, labeled}}}{\text{atom } ^{13}\text{C}^%_{\text{PLFA, non-labeled}}} - 1 \right) \times \text{PLFA},$$

(1)

where $^{13}$C-PLFA was the $^{13}$C amount incorporated into individual PLFAs (μg $^{13}$C kg$^{-1}$ soil). The (Atom $^{13}$C$^%_{\text{PLFA, labeled}}$ and (atom $^{13}$C$^%_{\text{PLFA, non-labeled}}$ were atom $^{13}$C$^%$ of individual PLFAs in labeled and non-labeled samples, respectively. PLFA was the content of individual PLFAs in the labeled sample (μg C kg$^{-1}$ soil). The relative abundance of $^{13}$C in individual PLFAs was calculated as

$$^{13}\text{C}^% = \frac{^{13}\text{C-PLFA}_{\text{individual}}}{\sum ^{13}\text{C-PLFA}_{\text{individual}}} \times 100.$$  

(2)

Plant C contribution to PLFA formation was estimated by relating $^{13}$C-PLFAs to plant $^{13}$C assimilation.
and plant growth rates (Eq. (3)), and integrated to the complete season according to the six pulse-chase labeling events

$$\text{PLFA}_{\text{plant}} = \frac{(^{13}\text{C-PLFA})_{\text{harvest}}}{(\text{plant}^{13}\text{C assimilation}) \times \text{plant\ growth\ rate}}, \quad (3)$$

where PLFA_{plant} indicated PLFAs (µg C d^{-1} kg^{-1} soil) formed from plant photosynthates. (^{13}\text{C-PLFA})_{harvest} was ^{13}\text{C-PLFA} (µg ^{13}\text{C} kg^{-1} soil) measured at harvest. The plant ^{13}\text{C assimilation} (µg ^{13}\text{C} kg^{-1} soil) and plant growth rates (µg C d^{-1} kg^{-1} soil) have been previously reported [9,24].

3. Results and discussion

The photosynthates released from plant roots serve as a major energy and carbon source for soil biological activity [1]. Yet, little is known about the microbial community involving in plant-derived carbon cycling and the influence of plant-derived carbon on microbial community structure [22]. Methodological limitations have traditionally hampered the development of the knowledge in this direction [29,30]. In the present study, we conducted the PLFA-based pulse chase labeling, which linked directly the microbial community to plant photosynthesis in a rice-soil system.

Total PLFAs decreased slightly during the first month after rice planting, and then increased significantly during the later periods of growth (Fig. 1(a)). Similar dynamics of microbial biomass were obtained in the previous studies using the chloroform-fumigation-extraction method [9] and PLFA-methods [31]. It was proposed that this decrease was due to the development of anoxic conditions that might depress the aerobic populations at the beginning of the experiment, and the increase was due to the stimulation of microbial growth by organic substrates released from plant roots [9,31]. The composition of total PLFAs at the beginning of the experiment was dominated by PLFAs 16:0, 16:1ω7c, 18:1ω7, 18:1ω9, i15:0, i16:0, i17:0, a15:0, 10Me17:0 and cy19:0. Grouping of PLFAs showed that total PLFAs consisted of 23.9% saturated PLFAs (Sa PLFAs), 24.6% mono-unsaturated PLFAs (Mono PLFAs), 2.5% poly-unsaturated PLFAs (Pu PLFAs) and 49% branched chain PLFAs (Br PLFAs). Apparently, the bacteria with the characteristics of Br PLFAs were predominant in the soil.

The composition of total PLFAs (expressed as mol%) showed no significant difference either among the early or later four measurements (Fig. 1(b)). However, there was a significant difference between early and late seasons. The mol% of Mono PLFAs significantly increased, whereas the mol% of Br PLFAs significantly decreased during the late season (Fig. 1(b)). The mol% of Sa

PLFAs and Pu PLFAs also increased during the late season but to a lesser extent compared to Mono PLFAs. Cluster analysis indicated that PLFA composition during the early season was clearly separated from those during the late season (data not shown). Rice plants reached the maximal tillering on 13 July (6 days before the second labeling) and started panicle initiation on 25 August (4 days after the fourth labeling). The time of change in the PLFA composition was, thus, at the period when plant growth was most active.

The ^{13}\text{C} pulse-chase labeling was performed six times. A typical picture of the ^{13}\text{C} distribution to PLFAs was shown for the fourth labeling event in Fig. 2. For this
labeling event, plants assimilated a total of 19.8 mg $^{13}$C after 6 h exposure to $^{13}$CO$_2$, 98% of $^{13}$C was recovered in the aboveground mass, 1.4% was retained in roots and 0.7% was released into soil [24]. The $\delta^{13}$C values of PLFAs increased immediately (Day 0), indicating that recent photosynthates were incorporated into microbial biomass rapidly after plant assimilation (Fig. 2(a)). Together with previous finding that photosynthesized carbon was detected as CH$_4$ emitted into the atmosphere within a short period [5,7,32], our results proved...
that microbial activity in rice soil was tightly coupled to plant photosynthesis.

The $^{13}$C, however, was not evenly distributed among PLFAs. The most significant increase of $\delta^{13}$C values was observed for PLFAs 16:0 and 18:1ω7&9, followed by PLFAs 14:0, 16:1ω7c, 15:0, 18:0, 20:0 and ai15:0. Most of the Br PLFAs showed a smaller increase in the $\delta^{13}$C values compared to Mono PLFAs and Sa PLFAs. The percentage distribution of $^{13}$C showed that 35% and 28% of the total $^{13}$C in PLFAs were incorporated into PLFAs 16:0 and 18:1ω7&9, respectively (Fig. 2(b)). The second group with high $^{13}$C incorporation included PLFAs 16:1ω7c, ai15:0 and i15:0, each containing about 5–7% of total $^{13}$C. The difference in $^{13}$C incorporation among PLFAs indicated that the microbial populations differed in their responses to photosynthetic input. The pattern of $^{13}$C distribution at harvest remained similar except that $^{13}$C-PLFA 16:0 significantly decreased and $^{13}$C-PLFAs 16:1ω7c significantly increased (Fig. 2(c)). The difference between Day 0 and harvest implied either a different response of microbial community to the later released $^{13}$C substances (possibly more recalcitrant compared to those released at Day 0 [24]) or re-cycling of $^{13}$C among the populations. By summarizing six labeling events, $^{13}$C distribution showed that 16–32% of $^{13}$C was distributed to Br PLFAs whereas about 27–53% of $^{13}$C was distributed to Mono PLFAs (Table 1). During the later stages of plant growth, approximately 40% to half of the total $^{13}$C was retained in Mono PLFAs (Table 1). Thus, the pattern of $^{13}$C distribution differed greatly from that of total PLFA profile (Fig. 1(b)). Apparently, the populations with the characteristics of straight chain fatty acids, especially those of PLFAs 16:0, 16:1ω7c, 18:1ω7&9, were faster growing, and more active and competitive for the available substrates released from rice plants than the populations with the characteristics of Br PLFAs. It, however, should be noted that the effect of PLFAs derived from living rice roots could not be completely excluded.

Although PLFA composition of rice roots was unavailable, the measurement of sea grass roots showed that plant roots contained mainly PLFAs 16:0, 18:2ω6c, 18:3ω3 and 18:1ω9 (over 90% of total PLFAs in the case of Spartina alterniflora) [33]. Assuming rice roots contained similar PLFA composition, total and $^{13}$C PLFAs, especially those of 16:0 and 18:1ω9 might be overestimated due to possible incomplete separation of living roots from soil. However, significant artifacts did not seem to occur in our experiment. Under the chromatography conditions used for measurement of total PLFAs, 18:1ω7 and 18:1ω9 were clearly separated. 18:1ω7, a mainly bacterial specific compound, significantly increased, whereas 18:1ω9, a compound both bacteria and plants, decreased with plant growth. This trend would not be expected if plant roots had a significant contribution to the measured PLFAs. Furthermore, 18:2ω6c was detected in total PLFA but not found in $^{13}$C PLFA, and 18:2ω3 was undetectable in both total and $^{13}$C PLFA measurements. Both of these PLFAs were major compounds of plant PLFAs [33]. Therefore, we assumed that the two-step separation procedure used in our experiment was efficient for removing most of the fine living roots to avoid this significant artifact for the measurement of microbial PLFAs.

Total PLFAs increased towards the end of the growing season as shown in Fig. 1(a). The carbon source for new biomass synthesis came from plant carbon and/or native soil organic carbon. Fig. 3 showed total PLFAs at the end of growing season, which were divided into three fractions: (i) ‘original’ PLFAs (presumably identical to those at the start of experiment); (ii) new PLFAs formed from plant carbon; and (iii) new PLFAs formed from soil carbon. The plant carbon contribution was estimated based on six pulse-chase labeling events. The soil carbon contribution was calculated from mass balance: total PLFAs at the end of the growing season minus ‘original’ PLFAs and minus

Table 1
The $^{13}$C incorporation into phospholipid fatty acids (PLFAs) and the distribution among groups of PLFAs

<table>
<thead>
<tr>
<th>Labeling time</th>
<th>July 4</th>
<th>July 19</th>
<th>August 4</th>
<th>August 21</th>
<th>September 4</th>
<th>September 20</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{13}$C-PLFA at Day 0 (μg kg$^{-1}$ soil)</td>
<td>2.30 (0.37)$^a$</td>
<td>6.93 (0.78)</td>
<td>3.21 (0.67)</td>
<td>2.84 (0.62)</td>
<td>0.92 (0.14)</td>
<td>1.14 (0.30)</td>
</tr>
<tr>
<td>Br PLFA (%)</td>
<td>25.2 (4.9)</td>
<td>27.2 (0.9)</td>
<td>28.1 (1.9)</td>
<td>23.3 (3.5)</td>
<td>16.0 (1.2)</td>
<td>26.9 (4.2)</td>
</tr>
<tr>
<td>Mono PLFA (%)</td>
<td>28.9 (1.9)</td>
<td>28.8 (1.1)</td>
<td>26.8 (0.3)</td>
<td>33.7 (4.3)</td>
<td>36.7 (1.5)</td>
<td>30.6 (3.2)</td>
</tr>
<tr>
<td>Pu PLFA (%)</td>
<td>0.2 (0.1)</td>
<td>0.1 (0.1)</td>
<td>0.4 (0.1)</td>
<td>0.2 (0.2)</td>
<td>3.1 (2.0)</td>
<td>0.5 (0.2)</td>
</tr>
<tr>
<td>Sa PLFA (%)</td>
<td>45.7 (2.9)</td>
<td>43.9 (2.1)</td>
<td>44.8 (1.6)</td>
<td>42.8 (4.1)</td>
<td>44.2 (3.4)</td>
<td>42.1 (1.9)</td>
</tr>
<tr>
<td>$^{13}$C-PLFA at Harvest (μg kg$^{-1}$ soil)</td>
<td>7.62 (0.61)</td>
<td>23.9 (2.1)</td>
<td>10.70 (1.3)</td>
<td>5.90 (0.76)</td>
<td>6.83 (0.43)</td>
<td>6.27 (0.73)</td>
</tr>
<tr>
<td>Br PLFA (%)</td>
<td>31.8 (3.1)</td>
<td>28.9 (0.6)</td>
<td>28.0 (2.1)</td>
<td>22.3 (1.3)</td>
<td>18.2 (1.7)</td>
<td>16.3 (1.4)</td>
</tr>
<tr>
<td>Mono PLFA (%)</td>
<td>32.6 (1.2)</td>
<td>34.3 (1.0)</td>
<td>43.9 (3.4)</td>
<td>41.9 (3.1)</td>
<td>51.2 (2.3)</td>
<td>52.7 (3.3)</td>
</tr>
<tr>
<td>Pu PLFA (%)</td>
<td>1.2 (0.2)</td>
<td>0.7 (0.3)</td>
<td>0.8 (0.3)</td>
<td>1.2 (0.1)</td>
<td>1.0 (0.2)</td>
<td>1.4 (0.3)</td>
</tr>
<tr>
<td>Sa PLFA (%)</td>
<td>34.4 (2.3)</td>
<td>36.1 (1.7)</td>
<td>27.4 (1.1)</td>
<td>34.7 (3.6)</td>
<td>29.6 (1.8)</td>
<td>29.6 (2.2)</td>
</tr>
</tbody>
</table>

The $^{13}$C-PLFAs were measured twice after the 6-h pulse labeling: immediately after labeling (Day 0); and at the end of the growing season (Harvest).

$^a$ Values are means of three replications with standard errors in parentheses.

$^b$ Br PLFA, branched phospholipid fatty acids; Mono PLFA, monounsaturated phospholipid fatty acids; Pu PLFA, polyunsaturated phospholipid fatty acids; Sa PLFA, saturated phospholipid fatty acids.
plant carbon contributions. Since the turnover of PLFAs within the growing season was not taken into account, the plant and soil carbon contributions were possibly underestimated. The seasonal increases (new PLFAs formed from plant and soil carbon) were most significant for PLFAs 18:1o7&9 and 16:0, followed by PLFAs 16:1o7c, i15:0 and ai15:0 (Fig. 3). The plant carbon contribution accounted for approximately 70–100% of total seasonal increases. In correspondence to 13C distribution among PLFAs (Fig. 2), the plant carbon contributions to newly formed PLFAs were not proportional to the ‘original’ PLFAs (Fig. 3). The plant C contribution caused almost a doubling of PLFAs 18:1o7&9,16:1o7c and 16:0 over the plant growing season, whereas the contribution to most Br PLFAs corresponded roughly to less than half of their ‘original’ values, or approximately 30% of the total at the end of the season. The soil carbon contribution within the plant growing season had only a minor contribution to PLFAs 18:1o7&9, 16:1o7c, 20:4o6c and ai15:0 (Fig. 3). The mineralization of labile soil organic matter possibly contributed partly to the new biomass formation. Thus, the analysis of 13C-PLFAs revealed that the increase of microbial biomass resulted predominantly from the plant carbon contribution (Fig. 3). This result corroborated our previous observation using the chloroform-fumigation-extraction approach, which showed that the plant carbon contribution accounted for 100% of its seasonal increase or approximately 28% of total microbial biomass at the end of the growing season [9].

Although the analysis of 13C-PLFAs suggested the existence of the populations that differed in their ability to assimilate plant-derived carbon, it was not easy to assign taxonomic identities based on PLFA analysis. This was not only because of the lower identification power of the PLFA approach compared to DNA-based analysis in resolving the microbial community structure [29,34], but also because that the plant-derived materials, with their components ranging from simple sugars to the complex mucilage polymers, were widely available for the micro-organisms in the soil. Some speculations, however, are proposed. The studies using either substrate-enriched cultivation or phylogenetic analysis of DNA sequences retrieved directly from the environmental samples revealed that the bacterial populations in the anaerobic rice soil were dominated by Clostridium, Bacillus, Bacteroidetes, Verrucomicrobiales, and Actinobacteria [35,36]. The Clostridium spp., in particular, appeared to be predominant in the anoxic zone of rice soil [37] and when organic matters such as rice straw were incorporated [38]. The Clostridium spp., which are usually anaerobic and Gram-positive, contain mainly the straight chain fatty acids 14:0, 16:0, 16:1, 18:1 [39]. The Bacillus spp. (aerobic and Gram-positive) and Bacteroidetes (anaerobic and Gram-negative), on the other hand, contain mainly the branched chain fatty acids i15:0, i17:0, ai15:0 and ai17:0 [39,40]. Probably, the straight chain PLFAs (16:0, 16:1o7c, 18:1o7&9) detected in this study represented mainly Clostridium spp. and the Br PLFAs indicated Bacillus spp. and Bacteroidetes. Indeed, an increase in Clostridium with rice growth has been previously reported in a rice field soil [41].

In conclusion, by using phospholipid fatty acid-based 13C-pulse chase labeling, the present study demonstrated that the rice plants not only promoted microbial activity and growth by providing the readily-available substrates in the form of photosynthate rhizodeposition, but also caused a change in microbial community structure due to differential distribution of the photosynthates among the microbial populations. Plant-microorganism interaction plays the central role in carbon and nutrient cycling in plant-soil systems. The seasonal change in microbial community structure may lead to change in seasonal patterns of carbon and nutrient cycling in the soil.

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