Soil Biology & Biochemistry 65 (2013) 195–203

Contents lists available at SciVerse ScienceDirect

Soil Biology & Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Microbial response to rhizodeposition depending on water regimes in paddy soils

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ARTICLE INFO

Article history:
Received 26 January 2013
Received in revised form
22 May 2013
Accepted 24 May 2013
Available online 12 June 2013

Keywords:
$^{13}CO_2$ pulse labeling
Rhizodeposition
Phospholipid fatty acids (PLFAs)
Microbial community structure
Water conservation techniques
Paddy soil
Root exudation

ABSTRACT

Rhizodeposit-carbon (rhizo-C) serves as a primary energy and C source for microorganisms in the rhizosphere. Despite important progress in understanding the fate of rhizo-C in upland soils, little is known about microbial community dynamics associated with rhizo-C in flooded soils, especially depending on water regimes in rice systems. In this study, rice grown under non-flooded, continuously flooded and alternating water regimes was pulse labeled with $^{13}CO_2$ and the incorporation of rhizo-C into specific microbial groups was determined by ^{13}C in phospholipid fatty acids (PLFAs) at day 2 and 14 after the labeling.

A decreased C released from roots under continuously flooded condition was accompanied with lower total ^{13}C incorporation into microorganisms compared to the non-flooded and alternating water regimes treatments. Continuous flooding caused a relative increase of ^{13}C incorporation in Gram positive bacteria (i14:0, i15:0, a15:0, i16:0, i17:0, a17:0). In contrast, Gram negative bacteria (16:1ω7c, 18:1ω7c, cy17:0, cy 19:0) and fungi (18:2ω6, 9c, 18:1ω9c) showed greater rhizo-C incorporation coupled with a higher turnover under non-flooded and alternating water regimes treatments. These observations suggest that microbial groups processing rhizo-C differed among rice systems with varying water regimes. In contrast to non-flooded and alternating water regimes, there was little to no temporal ^{13}C change in most microbial groups under continuous flooding condition between day 2 and 14 after the labeling, which may demonstrate slower microbial processing turnover. In summary, our findings indicate that belowground C input by rhizodeposition and its biological cycling was significantly influenced by water regimes in rice systems.

1. Introduction

It is widely recognized that rhizodeposit-carbon (rhizo-C) serves as a primary energy and C source for microorganisms in the rhizosphere and that the root–microbial interactions play a key role for soil C cycling as well as for C sequestration (Paterson, 2003; Kuzyakov et al., 2003; Rees et al., 2005). Therefore, better understanding of the mechanisms of rhizo-C utilization by microorganisms and its pathways in soil is necessary. This is particularly the case for paddy soils in rice cultivation.

Rice is the major staple food crop in Asia, and it consumes about 90% of total irrigation water (Bhuiyan, 1992). However, freshwater for rice irrigation is becoming scarce due to increasing competition from industrial and urban demand (Bouman and Tuong, 2001; Fan et al., 2012a). Therefore, water conservation methods for rice production were introduced and developed. These water conservation techniques include non-flooded mulching cultivation (Fan et al., 2005) and alternating wetting and drying irrigation (Yang et al., 2002; Belder et al., 2004). These techniques have been shown to improve rice productivity as well as N and water use efficiency (Yang et al., 2002; Belder et al., 2004; Fan et al., 2005, 2012b).

Soil moisture conditions affect the partitioning and allocation of plant photosynthates in soil (Meharg and Killham, 1990). Henry et al. (2007) found a 26% higher C exudation when growing wheatgrass under drought stress compared to flooding. Rice under alternating wetting and drying or non-flooded conditions has more fine roots and root branching than under flooding (Mishra and

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0038-0717/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.soilbio.2013.05.021

Salokhe, 2011). Alternating wetting and drying practice can improve rice root and shoot morphology (Thakur et al., 2011). This suggests that partitioning of photosynthesized carbon and roots exudation could be affected by water regimes in rice.

Soil moisture also exerts a major effect on microbial activity and community structure (Bossio and Scow, 1998; Drenovsky et al., 2004). Shifts in microbial community structure are expected after conversion from anaerobic to aerobic or alternating aerobic conditions. Previous studies have shown that flooding decreased fungal abundance (Drenovsky et al., 2004; Unger et al., 2009). Higher proportions of branched-chain PLFAs were reported under flooded condition while proportions of straight monounsaturated and straight poly-unsaturated PLFAs were greater under upland condition (Nakamura et al., 2003). This was consistent with earlier study where monounsaturated fatty acids were reduced under flooding (Bossio and Scow, 1998).

A number of studies have documented that rhizosphere microbial communities are strongly influenced by rhizo-C (e.g. Butler et al., 2003; Treonis et al., 2004; Paterson et al., 2007; Denef et al., 2009; Jin and Evans, 2010). Nevertheless, most of these studies have been done in upland systems. Only a few studies have evaluated the rhizo-C utilization by microorganisms in flooded rice systems (Lu et al., 2004; Wu et al., 2009; Ge et al., 2012), especially there is only one study that was related to water status (Yao et al., 2012). Based on a continuous ^{13}C labeling, Yao et al. (2012) showed that utilization of plant derived C by microorganisms in non-flooded is different from that in water-logging condition. However, it still unclear whether water regimes in rice systems affect the input of plant C to soil in this study (Yao et al., 2012). Furthermore, despite advantages of continuous ^{13}C labeling, it was impossible to conclude which rhizosphere microorganisms are the first for utilizing rhizo-C.

Belowground C input by rhizodeposition and its interactions with microorganisms determine to a large extent C dynamics and sequestration in soil. For instance, shifts in microbial growth, such as increases in fungal abundance relative to bacterial populations, have been associated with increased C retention in soil (Six et al., 2006). Rhizodeposition can lead to C accumulation or C consumption due to stimulation of microorganisms (Kuzyakov et al., 2001). For instance, the easily available rhizodeposition may stimulate microorganisms, and then may accelerate (positive priming) or retard (negative priming) SOM decomposition (Kuzyakov et al., 2000; Cheng and Kuzyakov, 2005). Therefore, information on microbial communities associated with rhizo-C dynamics as related to water regimes in rice systems is particularly limited and urgently necessary.

Phospholipid fatty acids (PLFAs) are diverse lipids of cell membranes, and several PLFAs have been used as biomarkers for specific microbial groups (Frostegård et al., 1993; Zelles, 1997, 1999). By combining PLFA analysis with $^{13}CO_2$ labeling and subsequent ^{13}C incorporation in individual PLFA, it is possible to follow C fluxes from the plant into the soil and to identify microorganisms that utilize the rhizo-C (Paterson et al., 2007).

We hypothesized that non-flooded and alternating water regimes would increase the released C from roots into soil because of greater root activity (Mishra and Salokhe, 2011; Thakur et al., 2011). Fungi are sensitive to anaerobic conditions (Schimel et al., 2007) and Gram negative group are usually more abundant at higher substrate availability (Bossio and Scow, 1998; Marschner et al., 2003; Drenovsky et al., 2004). Therefore we also hypothesized that these microbial groups will show higher incorporation of rhizo-C under non-flooded and alternating water regimes treatments than under flooding. To test these hypotheses, rice plants were grown under three water regimes. A $^{13}CO_2$ pulse labeling of the rice shoots was performed and samples were taken at 2 and 14 days after the ^{13}C labeling. We (1) determined the effects of water regimes on rhizo-C and microbial community structure, and (2) assessed the utilization of rhizo-C by microbial groups coupled with temporal changes after the labeling.

2. Materials and methods

2.1. Soil preparation and rice growth condition

The soil characteristics and plant growth conditions have been previously described by Tian et al. (2013). Briefly, soil samples (Anthrosols) were collected from the plough layer (0–20 cm) of a rice field at Dong Kou city, Hunan province, China (110° 62′N and 27° 12′E). The soil was air-dried and sieved (<5 mm), and then 360 g soil were filled into a polycarbonate plant growth pot (SM 16510/11, Sartorius, Göttingen, Germany) and rewetted to 85% of the water holding capacity (WHC). Three healthy germinated rice seedlings were transplanted to each pot (Fig. 1). The soil was amended with urea [CO $(NH_2)_2$] and potassium dihydrophosphate (KH_2PO_4) at the rate of 100 mg N kg^{-1} and 12.5 mg K kg^{-1} soil. 30% N was basal fertilizer, 30% N and 40% N were top dressing on day 15 and 28. All the K was basal fertilizer. Additionally, 3.5 g L^{-1} KH_2PO_4 was directly sprayed to the leaves 11 days after planting.

Three soil water regimes were established: (1) continuously flooded (CF), the pots were always maintained with distilled water to a level of 4–5 cm above soil surface, (2) non-flooded (NF), the pots were maintained at 85–90% of the WHC, (3) alternating wetting and drying (AWD), the pots were flooded as described above for CF, then dried for 3–4 days until the soil water content reached 70–75% of the WHC, then flooded again; this kept the soil under alternating flooded and dried conditions. These three water regimes started after the development of three leaves per plant. The plants were grown at a light intensity of 400 μmol m^{-2} s^{-1} for 14 h per day and a temperature of 27 ± 1 °C and 22 ± 1 °C (day/night). In

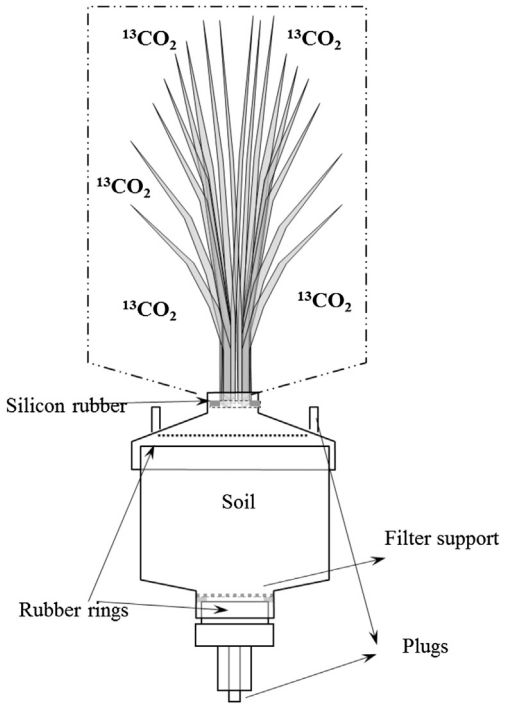

Fig. 1. Experimental set-up for rice growth and labeling of shoots by $^{13}CO_2$ pulse in an airtight chamber (modified after Kuzyakov and Siniakina, 2001).

total, 27 pots were prepared: 18 pots were used for pulse labeling and 9 pots were used as control without ^{13}C labeling.

2.2. $^{13}CO_2$ pulse labeling

The $^{13}CO_2$ pulse labeling took place on day 60 after rice planting. The labeling apparatus consists of a two-compartment Plexiglas chamber that has been previously described by Kuzyakov et al. (2001). The hole around the stem in the lid of the pots was sealed with silicon paste (NG 3170, Thauer & Co., Dresden, Germany) and tested for air leaks the day before labeling. Briefly, 18 pots with 54 plants were placed into one chamber. $^{13}CO_2$ was released into the chamber through the reaction of 1.5 g $Na_2^{13}CO_3$ (99 atom% ^{13}C) and 10 ml 5 M H_2SO_4. The plants were labeled during 5 h in the $^{13}CO_2$ atmosphere. During the pulse labeling, all AWD pots were on stage from flooded to dry, there was obvious water level on surface, but with lower water level above the soil surface compared with continuously flooded pots.

2.3. Sampling and analyses

Plant and soil samples were taken 2 and 14 days after the pulse labeling. At harvest, shoots were cut at the base and soil was taken out of the pot. The roots were separated from soil by handpicking and washed with 100 ml deionized water to remove the adhering soil. Shoots and roots were dried at 60 °C. Fresh soil samples were stored at −20 °C and 4 °C for PLFAs and microbial biomass C analysis, respectively. A similar sampling procedure as discussed above was performed for the nine control pots before labeling.

Dry samples of shoots, roots and soil were ground in a ball mill prior to analysis. The $\delta^{13}C$ signature and the total C content of shoots, roots and soil were determined by an isotope ratio mass spectrometer (IRMS, Delta Plus, Finnigan MAT, Bremen, Germany) coupled with an elemental analyzer (NA 2500; CE-Instruments, Rodano, Milano, Italy).

Soil microbial biomass C was determined by chloroform fumigation extraction (Wu et al., 1996). Briefly, 10 g fresh soil was extracted with 40 ml of 0.05 M K_2SO_4. Another 10 g fresh soil was fumigated with chloroform for 24 h and extracted in the same way. An aliquot was taken to measure total C concentrations by a TOC/TIC analyzer (Analytik Jena, Jena, Germany). The remaining solution was freeze-dried and weighed for $\delta^{13}C$ analysis by IRMS (Delta Plus; Finnigan MAT, Bremen, Germany).

2.4. PLFA extraction

PLFAs were extracted and purified by a modified method of Frostegård et al. (1991). Fatty acids were extracted from 6 g wet soil samples with a one-phase extraction mixture containing chloroform: methanol: citrate buffer. Phosphatidylcholin-dinonadecanoic acid as internal standard 1 was added before extraction. After purification via activated silica, phospholipids were transformed to fatty acid methyl esters (FAMEs) following the derivatization procedure (Knapp, 1979). Tridecanoic acid methyl ester as internal standard 2 was added to the sample before transferring the samples to autosampler vials for analyses. Then the amounts of FAMEs were analyzed on a GC–MS (GC 5890 with MS 5971A, Agilent, Waldbronn, Germany). The $\delta^{13}C$ of FAMEs were analyzed by GC-C-IRMS via a combustion interface III (Therno Finnigan, Bremen, Germany). Detailed information on the instrumental setup and the used GC-column is presented in Apostel et al. (2013).

Based on previous studies the fatty acids were matched to specific microbial groups (Zelles, 1997, 1999; Bossio and Scow, 1998; Frostegård et al., 1993), we divided 5 microbial groups: Gram positive (i16:0, i17:0, a17:0, i14:0, i15:0, a15:0), Gram negative (18:1ω7c, 16:1ω7c, cy17:0, cy 19:0), Fungi (18:2ω6,9c, 18:1ω9c), AM fungi (16:1ω5c) and Actinomycetes (10Me 16:0, 10Me 18:0).

2.5. Calculations and statistical analysis

2.5.1. ^{13}C in rice-soil system

The obtained $\delta^{13}C$ data were used to calculate $^{13}C_{atom\%}$, and then ^{13}C amount from the pulse labeling incorporated into individual C pool was calculated as follows: (Lu et al., 2002)

$$^{13}C_x = \left[(^{13}C_{atom\%})_{x,L} - (^{13}C_{atom\%})_{x,UL}\right] \Big/ 100 \times C_x \quad (1)$$

where $(^{13}C_{atom\%})_{x,L}$ and $(^{13}C_{atom\%})_{x,UL}$ were $^{13}C_{atom\%}$ in labeled and control samples; C_x is the C content in individual pool in labeled sample.

The ^{13}C incorporation in shoots, roots and soil pools was expressed as the percentage of ^{13}C recovery at each sampling day. The total ^{13}C recovery after sampling in the rice-soil system was the sum of the ^{13}C in shoots, roots and soil.

2.5.2. ^{13}C in PLFA

The $\delta^{13}C$ value of the individual FAME obtained from the GC-C-IRMS was first corrected for the derivatization procedure according to Apostel et al. (2013). Then based on the Eq. (1), the ^{13}C incorporation into individual PLFA (nmol ^{13}C g^{-1} soil) was:

$$^{13}C_{PLFA} = \left[(^{13}C_{atom\%})_{PLFA,L} - (^{13}C_{atom\%})_{PLFA,UL}\right] \Big/ 100 \times PLFA \quad (2)$$

where PLFA was the content of individual PLFA in the labeled samples. Then we calculated the relative ^{13}C distribution in specific microbial group:

$$^{13}C\% = {}^{13}C_{PLFA-groups} / \sum {}^{13}C_{PLFAs} \times 100 \quad (3)$$

2.5.3. ^{13}C in microbial biomass

Based on the Eq. (1), the ^{13}C in microbial biomass was estimated as the difference in ^{13}C in fumigated and unfumigated soil extracts and divided by a factor of 0.45 (Lu et al., 2002).

$$^{13}C - MBC = \left\{\left[(^{13}C_{atom\%})_{f,L} - (^{13}C_{atom\%})_{f,UL}\right] \times C_f - \left[(^{13}C_{atom\%})_{unf,L} - (^{13}C_{atom\%})_{unf,UL}\right] \times C_{unf}\right\} \Big/ 100/0.45 \quad (4)$$

where f means the fumigated soil extract and unf means unfumigated soil extract. C_f and C_{unf} are the total C contents of the fumigated and unfumigated soil extracts.

2.5.4. Statistics

The PLFAs contents were log $(x + 1)$ transformed to focus attention on patterns of the whole community by giving rare fatty acids similar weighting as common fatty acids. Non-metric multidimensional scaling (MDS) was used for plot the PLFAs pattern (Primer-E Ltd, Plymouth Marine Laboratory, Plymouth, UK). In MDS, stress values indicate how well the ordination represents the actual variability in community structure of the samples. Stress values $\leq$0.2 indicate that the ordination was a good reflection of overall community structure (Duong et al., 2009). Significant differences in microbial community structure between treatments

were determined by PERMANOVA (Primer-E Ltd, Plymouth Marine Laboratory, Plymouth, UK).

The significant differences of ^{13}C incorporation into shoots, roots, soil and microbial biomass and microbial groups between treatments and sampling days were determined by two-way ANOVA with SPSS (Version 11.0, SPSS Inc., USA). Differences were considered significant at $p < 0.05$ with the least significant difference (LSD) test.

3. Results

3.1. Shoots and roots biomass

The shoots biomass were higher under continuously flooding condition than those of non-flooded and alternating wetting and drying treatments ($p < 0.05$; Table 1). There were differences in roots biomass between the continuously flooded and non-flooded treatments ($p < 0.05$; Table 1).

3.2. ^{13}C within rice-soil system

The total ^{13}C incorporation within rice-soil systems (the sum of ^{13}C in shoots, roots and soils) were 5.23 and 4.72 mg ^{13}C pot^{-1} for non-flooded, 5.73 and 5.56 mg ^{13}C pot^{-1} for continuously flooded and 5.43 and 5.35 mg ^{13}C pot^{-1} for alternating water regimes treatment at day 2 and 14, respectively (Table 1). The continuously flooded treatment resulted in a higher ^{13}C recovery in the shoots compared with non-flooded and alternating water regimes treatments but in a lower ^{13}C recovery of the soil ($p < 0.05$; Fig. 2A and C). The ^{13}C recovery of the roots did not differ significantly between the treatments (Fig. 2B). Moreover, we could not detect an effect of the sampling time, with the exception of the alternating water regime treatment for which the ^{13}C recovery of the soil decreased from day 2 to day 14 (Fig. 2C).

3.3. Microbial biomass C, total PLFAs and soil microbial community

Continuous flooding condition decreased microbial biomass C and total PLFAs contents in comparison with the non-flooded soil ($p < 0.05$; Table 2). Microbial biomass C and total PLFA contents did not change between day 2 and day 14 for any of the treatments (Table 2). The pattern of PLFA composition was significantly influenced by water regimes ($p < 0.01$; Fig. 3). The stress value of the MDS plot was 0.08, indicating that the ordination was a good reflection of overall microbial community structure. The abundance of microbial groups varied under the different water regimes while showed little to no temporal effect (Tables 2 and 3). Continuous flooding decreased the abundance of Gram negative, fungi and AM fungi groups ($p < 0.05$; Table 2), but the Gram positive and the actinomycetes groups were not significantly affected. The fungi/bacteria ratio of the continuously flooded treatment was 1.4 and 1.2 times lower than the non-flooded and the alternating water regime treatments, respectively ($p < 0.05$; Table 2).

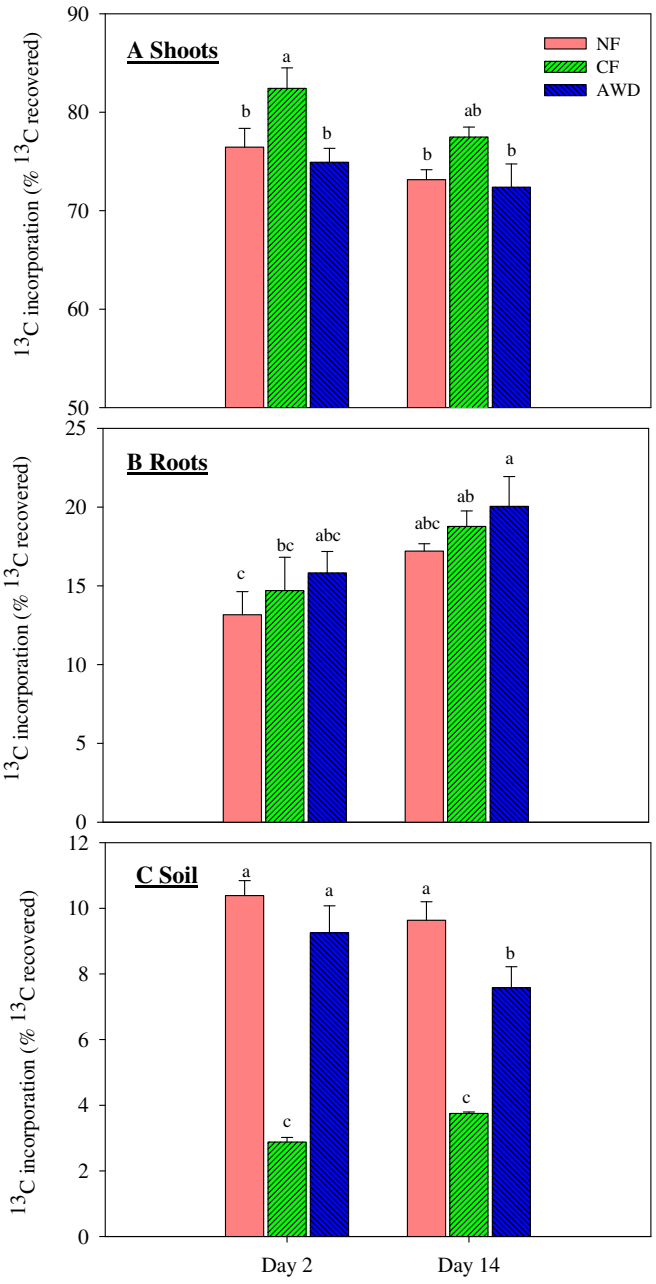

Fig. 2. Effect of water regimes on the amount of ^{13}C incorporated into the shoots (top), roots (middle) and soil (bottom) 2 and 14 days after $^{13}CO_2$ pulse labeling. Different low-case letters indicate significant differences. Error bars represent standard errors of the means ($n = 3$). NF: non-flooded; CF: continuously flooded; AWD: alternating wetting and drying.

Table 1
Effect of water regimes on the dry weight for shoots and roots, and ^{13}C incorporation in rice-soil 2 and 14 days after $^{13}CO_2$ pulse labeling.

	Day 2			Day 14		
	NF	CF	AWD	NF	CF	AWD
Shoots biomass (g pot^{-1})	3.71 (0.11)^{b}	4.38 (0.14)^{a}	3.93 (0.08)^{b}	4.33 (0.08)^{b}	5.42 (0.16)^{a}	4.59 (0.10)^{b}
Roots biomass (g pot^{-1})	1.78 (0.19)^{b}	2.86 (0.11)^{a}	2.21 (0.27)^{ab}	1.82 (0.09)^{b}	3.03 (0.23)^{a}	2.39 (0.24)^{ab}
^{13}C in rice-soil system (mg pot^{-1})	5.23 (0.60)^{a}	5.73 (0.53)^{a}	5.43 (0.46)^{a}	4.72 (0.26)^{a}	5.56 (0.58)^{a}	5.35 (0.57)^{a}

Different low-case letters indicate significant differences between three treatments. Numbers in bracket represent standard error of the means ($n = 3$).
NF: non-flooded; CF: continuously flooded; AWD: alternating wetting and drying.

Table 2
Abundance of microbial groups under three water regimes 2 and 14 days after $^{13}CO_2$ pulse labeling.

	Day 2			Day 14		
	NF	CF	AWD	NF	CF	AWD
Total PLFA (nmol g^{-1})	158 (31)^{a}	135 (21)^{b}	145 (23)^{ab}	140 (35)^{a}	134 (24)^{a}	145 (21)^{a}
Microbial biomass C (mg C kg^{-1})	410 (18)^{a}	324 (32)^{b}	396 (5.7)^{ab}	410 (16)^{a}	307 (14)^{b}	363 (17)^{a}
PLFA groups (nmol g^{-1})						
Gram positive bacteria	42.2 (1.2)	42.5 (2.7)	40.2 (1.6)	38.3 (2.0)	43.9 (1.1)	41.4 (1.9)
Gram negative bacteria	38.6 (0.79)^{a}	28.4 (1.6)^{b}	30.8 (0.52)^{b}	30.7 (1.8)^{a}	24.4 (0.84)^{b}	32.5 (0.34)^{a}
Fungi	18.1 (0.64)^{a}	11.1 (0.62)^{b}	13.5 (0.92)^{b}	13.4 (1.0)^{a}	10.7 (1.2)^{b}	12.4 (0.60)^{a}
AM fungi	4.00 (0.12)^{a}	2.20 (0.17)^{c}	3.07 (0.16)^{b}	3.11 (0.42)^{a}	1.93 (0.12)^{b}	3.64 (0.07)^{a}
Actinomycetes	12.8 (0.25)	10.6 (0.76)	11.8 (0.46)	11.6 (0.79)	10.8 (0.42)	12.9 (0.54)
Fungi/bacteria	0.27 (0.007)^{a}	0.18 (0.003)^{c}	0.23 (0.006)^{b}	0.24 (0.01)^{a}	0.18 (0.01)^{b}	0.22 (0.005)^{a}

Different low-case letters indicate significant differences between three treatments within one sampling day. Error bars represent standard error of the means ($n = 3$).
NF: non-flooded; CF: continuously flooded; AWD: alternating wetting and drying.

3.4. Incorporation of rhizo-C into microbial biomass and in total PLFAs

The total ^{13}C recovery in both microbial biomass and total PLFAs was lower under continuously flooded as compared with non-flooded and alternating water regimes ($p < 0.05$; Fig. 4). The ^{13}C recovery in microbial biomass decreased more than ^{13}C in total PLFAs between day 2 and 14 irrespective of water regimes reflecting faster turnover of C within whole microorganisms as compared to cell walls. The ^{13}C recovery in microbial biomass under the non-flooded and alternating water regimes at day 2 were 1.7 and 2.4 times higher than those of day 14 ($p < 0.05$; Fig. 4A). There was a significant decline of the ^{13}C in total PLFAs between day 2 and 14 only under non-flooded treatment ($p < 0.05$; Fig. 4B).

3.5. Incorporation of rhizo-C into soil microbial groups

The ^{13}C originating from rhizodeposits was not evenly distributed among microbial groups indicates that they differed in uptake and utilization of rhizo-C. Across all water regimes and both samplings, the relative ^{13}C distribution showed that 23% of ^{13}C in the total PLFAs was incorporated into Gram negative group (16:1ω7, 18:1ω7c, cy17:0, cy18:0) (Fig. 5). The following group with high ^{13}C incorporation was fungi (18:2ω6, 9c, 18:1ω9c), containing about 8–14% of ^{13}C in total PLFAs.

Compared with non-flooded and alternating water regime, continuously flooded treatment resulted in lower relative ^{13}C in all Gram negative, fungi, AM fungi and actinomycetes, but higher incorporation in Gram positive group only at day 14 ($p < 0.05$; Fig. 5). There were significant temporal effects on the ^{13}C distribution between microbial groups with varying water regimes (Table 3). For the non-flooded and alternating water regime treatments, a decrease of the incorporated ^{13}C between day 2 and day 14 was measured for fungi and Gram negative groups (Fig. 6). This decrease accounts on average 49% and 14% of the ^{13}C incorporation at day 2 for fungi and Gram negative groups, respectively. ^{13}C of most microbial groups showed little to no temporal variation under continuously flooded condition (Fig. 6).

4. Discussion

4.1. ^{13}C allocation in rice-soil system under three water regimes

Soil water status affects the partitioning and allocation of plant photosynthates (Meharg and Killham, 1990). A 26% or even 50% lower C exudation was observed when growing plants under anaerobic condition compared to aerobic condition (Meharg and Killham, 1990; Henry et al., 2007). In our study, continuous flooding resulted in a lower ^{13}C recovery in soil compared to non-flooded or alternating water regime treatments (Fig. 2C). The 3.75% recovery in soil in continuous flooding condition in the present study is in good agreement to Watanabe et al. (2004) recorded that 3.4% of the recovered C was transferred to continuously flooded soil during the booting stage on day 14. Moreover, in an earlier study we could

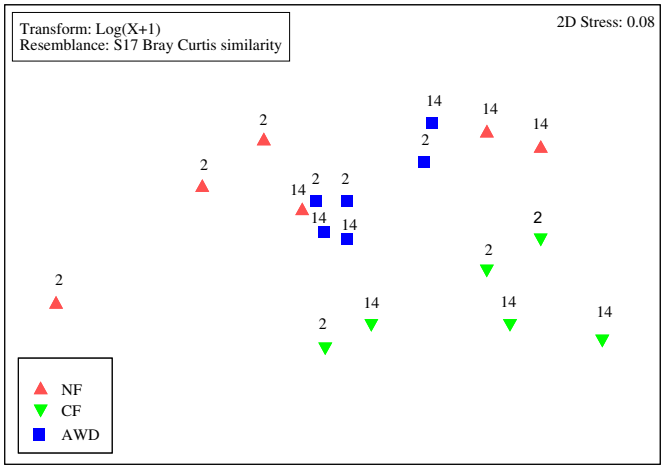

Fig. 3. MDS plots of total PLFA patterns based on Bray–Curtis similarities under three water regimes and sampling days. NF: non-flooded; CF: continuously flooded; AWD: alternating wetting and drying. The number (2, 14) in the symbol means sampling day.

Table 3
ANOVA analyses across water regimes and sampling days for abundance and relative ^{13}C incorporation of microbial groups.

Groups	Effect	df	PLFA abundance (nmol g^{-1})		Relative ^{13}C incorporation (%)	
			F	*p* Value	*F*	*p* Value
Gram positive bacteria	Water regimes	2	1.01	0.39	4.37	**0.03**
	Day	1	0.06	0.81	5.73	**0.03**
	Interaction	2	0.94	0.42	0.94	0.42
Gram negative bacteria	Water regimes	2	12.4	**0.0012**	56.2	<**0.0001**
	Day	1	6.07	**0.02**	32.1	**0.0001**
	Interaction	2	4.12	**0.04**	4.66	**0.03**
Fungi	Water regimes	2	6.36	**0.01**	2.22	0.15
	Day	1	3.38	0.09	42.5	<**0.0001**
	Interaction	2	1.43	0.27	2.34	0.14
AM fungi	Water regimes	2	30.5	<**0.0001**	24.6	<**0.0001**
	Day	1	1.38	0.26	26.6	**0.0002**
	Interaction	2	6.19	0.01	6.51	**0.0122**
Actinomycetes	Water regimes	2	3.86	**0.05**	205	<**0.0001**
	Day	1	0.01	0.92	394	<**0.0001**
	Interaction	2	1.5	0.26	25.1	<**0.0001**

Values in bold indicate significant different.

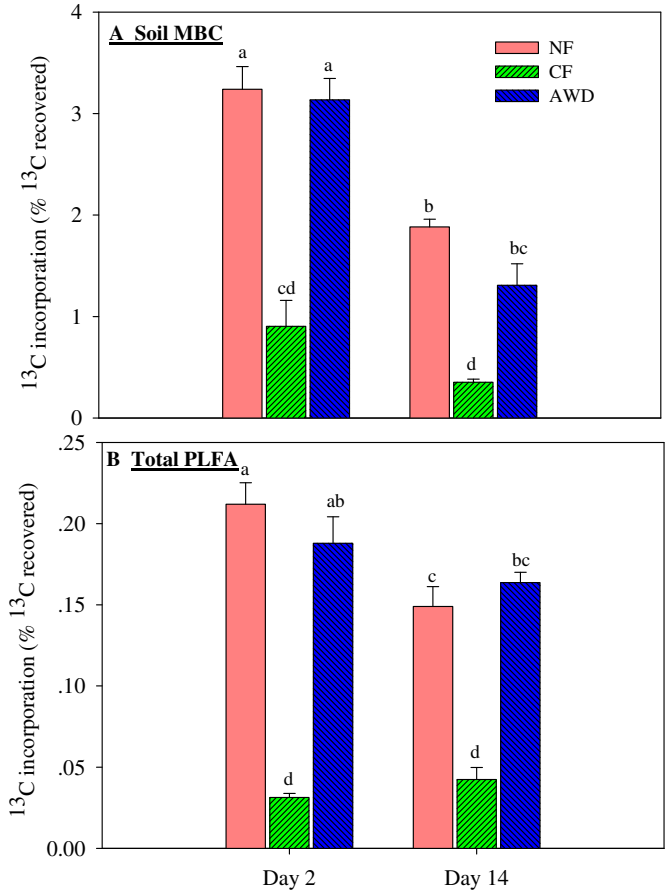

Fig. 4. Effect of water regimes on ^{13}C incorporation into microbial biomass and total PLFAs 2 and 14 days after $^{13}CO_2$ pulse labeling. Different low-case letters indicate significant differences. Error bars represent standard error of the means ($n = 3$). NF: non-flooded; CF: continuously flooded; AWD: alternating wetting and drying.

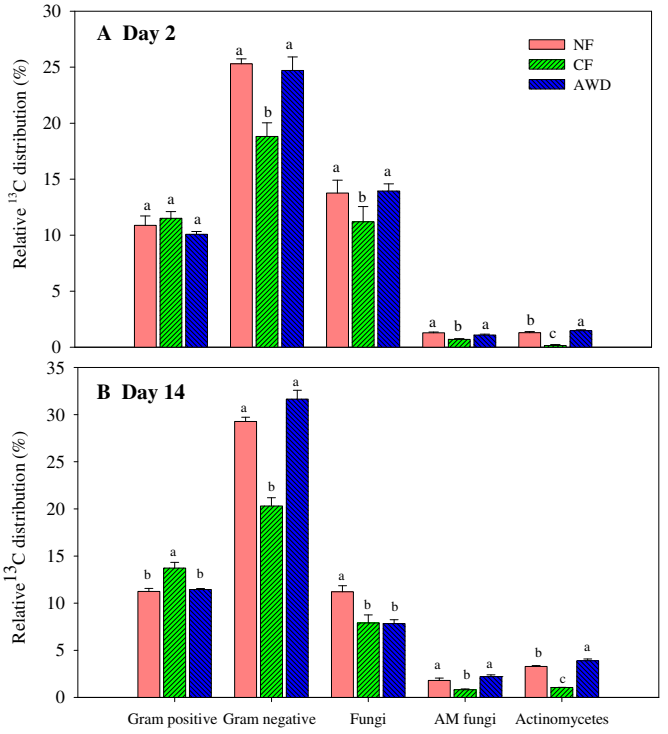

Fig. 5. Relative ^{13}C incorporation in microbial groups under water regimes 2 and 14 days after $^{13}CO_2$ pulse labeling. Different low-case letters indicate significant differences for all treatment within one signature group. Error bars represent standard error of the mean ($n = 3$). NF: non-flooded; CF: continuously flooded; AWD: alternating wetting and drying.

show an allocation of 5.3% of the recovered C to soil under flooded conditions when rice plants were labeled at a younger stage (35 day) (Tian et al., 2013). These differences reflect the plant development stage, which influences C incorporation into roots and soil (Swinnen et al., 1994).

Roots biomass followed the order of continuously flooded > alternating water regime > non-flooded, with significant differences observed between continuously flooded and non-flooded at both day 2 and 14 (Table 1). This suggested that the roots biomass were not cause of higher rhizodeposition for non-flooded and alternating conditions. Rice grown under alternating wetting and drying and non-flooded conditions can enhance roots activity due to the higher oxygen availability and hence, may develop more fine roots and branching (Mishra and Salokhe, 2011). Further, new assimilates are allocated primarily to root tips and increased exudation was correlated with increasing number of root tips (Thornton et al., 2004; Pausch and Kuzyakov, 2011; Wichern et al., 2011). Consequently, higher roots activity under non-flooded and alternating wetting and drying than continuously flooded conditions may lead to higher rhizodeposition as we observed in the present study. Conversely, roots under anaerobic conditions may produce substances such as ethanol and lactate, which are toxic to plants and microorganisms, thus decrease roots respiration and decomposition of rhizo-C (rhizomicrobial respiration). This was proved in our recent study: a higher root-derived CO_2 (sum of roots and rhizomicrobial respiration) was released when rice was grown under non-flooded and alternating water regimes as compared with continuous flooding (Tian et al., 2013). Therefore, more assimilates may remain in rice in continuous flooding condition as the reduced roots exudation or roots-derived respiration. This line of reasoning is also supported by the significantly higher ^{13}C incorporation in shoots of rice under continuous flooding compared to the other treatments (day 2; Fig. 2A). We also observed that most roots were dark brown and with less branching under flooding condition, indirectly indicated lower roots activity (Thakur et al., 2011) and thus may reduce exudation.

4.2. Effect of water regimes on soil microbial community composition

The observed significant decrease of Gram negative bacteria in continuous flooding condition (Table 2), are in agreement with

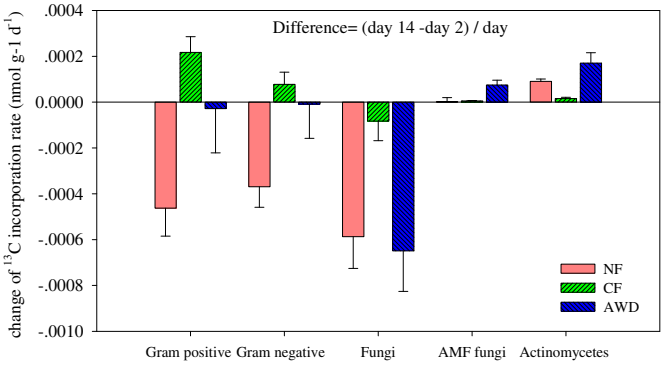

Fig. 6. Changes of ^{13}C incorporation rate under water regimes between day 2 and day 14 after $^{13}CO_2$ pulse labeling. Error bars represent standard error of the mean ($n = 3$). NF: non-flooded; CF: continuously flooded; AWD: alternating wetting and drying.

previous studies that monounsaturated fatty acids are usually associated with aerobic growth (Guckert et al., 1985; Bossio and Scow, 1998; Bossio et al., 2006). The decrease of fungi and AM fungi under continuous flooding in our study (Table 2) and previous studies (Bossio and Scow, 1998; Drenovsky et al., 2004) shows that fungi are sensitive to flooding. Contrary to previous studies (Schimel et al., 2007; Gordon et al., 2008), alternating water regime did not decrease the abundance of fungi. This may be ascribed to relative short duration or intensity of drying–rewetting cycles (Borken and Matzner, 2009). Water regimes significantly influenced the microbial profiles (Table 2 and Fig. 3). This is in agreement with earlier studies showing that water status was an important factor for soil microbial community composition (Bossio and Scow, 1998; Bossio et al., 2006; Mentzer et al., 2006; Unger et al., 2009). Additionally, most studies documented that nutrient availability, the quality of SOC and roots exudates composition are also key factors affecting soil microbial communities (Cookson et al., 2005; Steenwerth et al., 2006; Paterson et al., 2007; Tian et al., 2012). We observed the allocation of C to roots respond very fast to water regimes after pulse labeling (Fig. 2), therefore, the changes of amounts and composition of exudates between treatments also can not be ruled out as a potential cause for the difference of soil microbial community.

4.3. Incorporation of rhizo-C into microbial groups under three water regimes

The lower ^{13}C incorporation in microbial biomass and total PLFAs in flooding conditions coincides with smaller ^{13}C recovery in soil (Figs. 2C and 4), demonstrating the strong influence of rhizo-C on soil microorganisms with varying soil water status. Similarly, Yao et al. (2012) reported that the ^{13}C incorporation into total PLFAs was significantly lower under continuous water-logging compared with the non-flooded conditions after a continuous labeling. The ^{13}C in microbial biomass decreased faster between day 2 and 14 than the total PLFAs regardless of the water regimes (based on the absolute ^{13}C data in MBC and PLFAs during two sampling times). This can be explained by the faster turnover of the intracellular pool of microbial biomass (extracted by chloroform fumigation extraction) compared to the PLFAs turnover as they are allocated in cell membranes.

The ^{13}C incorporation was not evenly distributed among microbial groups. The higher relative ^{13}C in Gram negative bacteria and fungi in non-flooded and alternating water regime vs. continuous flooding condition (Fig. 5) confirmed our second hypothesis. These results suggest that Gram negative and fungi actively utilize more newly rhizo-C, and those microorganisms are important initial rhizodeposition sinks under non-flooded and alternating water regime conditions in paddy soils as did in upland systems (Treonis et al., 2004; Leake et al., 2006; Denef et al., 2007; Jin and Evans, 2010). Gram negative bacteria strongly increased with higher substrate availability with organic fertilizers amendments (Bossio and Scow, 1998; Marschner et al., 2003; Drenovsky et al., 2004). Preferential utilization of plant exudates by Gram negative bacteria than Gram positive bacteria was observed after labeling young beech trees (Esperschütz et al., 2009). Additionally, Gram negative bacteria groups are usually associated with aerobic growth (Guckert et al., 1985; Bossio and Scow, 1998; Bossio et al., 2006). Presumably, the improved soil oxygen condition and the higher amounts of exudations under non-flooded and alternating water regime benefit to ^{13}C incorporation into Gram negative bacteria. The ^{13}C in Gram negative and fungi declined significantly between day 2 and 14 in the non-flooded and alternating water regimes conditions, indicating fast turnover for uptake and utilization of rhizo-C (Fig. 6).

Contrasting with the non-flooded and alternating water regimes treatments, the significantly higher ^{13}C in Gram positive bacteria at day 14 (Fig. 5) indicates that these microorganisms were more active in processing rhizo-C and showed higher contributions to the utilization in continuous flooding paddy soils. However, the slower incorporation (higher ^{13}C on day 14 than day 2) suggests that Gram positive bacteria were less active in processing rhizo-C. Further, most microbial groups even showed little to no temporal ^{13}C incorporation change under flooding condition (Fig. 6). Presumably, this may be caused by lower microbial processing turnover under flooding condition. Lower rhizo-C input to soil and anaerobic environment under continuously flooded system (Fig. 2C) may also be partly responsible to microbial activity. Additionally, anaerobic environment may strongly affect the composition of root exudates, and then affect the rhizo-C utilization by microorganism. E.g. higher concentrations of fumaric and succinic acid when growing wheatgrass under drought condition than flooding were observed (Henry et al., 2007). Aulakh et al. (2001) characterized the roots exudates of rice, and found that among organic acids, malic acid showed the highest concentration followed by tartaric, succinic, citric and lactic acids. Thus, how the composition of rhizodeposits influence the microorganisms should be investigated in future.

The ^{13}C incorporation in soil and microorganism under water regimes provided us important implications for C cycling and sequestration in rice systems. Most C additions to soil originates from plants, and it has been widely reported that around 50% of the photosynthesized-fixed C was transferred belowground and about 5–10% of the net fixed C can be recovered in soil (Nguyen, 2003; Rees et al., 2005). Previous studies also reported the preferential retention and greater contribution of rhizo-C to SOM-C as compared to aboveground litter (Kong and Six, 2012; Rasse et al., 2005). Flooding condition decreased belowground C input by rhizodeposition, then accompanied with lower microbial processing turnover. Therefore, future investigations on mechanisms which are of importance for microbial utilization of rhizodeposits and effects on C cycling and sequestration in rice systems with focus on water conversation techniques are necessary.

5. Conclusions

Continuous flooding resulted in lower ^{13}C incorporation in soil and microorganisms as compared with non-flooded and alternating water regimes. The higher abundance of Gram negative bacteria and fungi together with ^{13}C incorporation under non-flooded and alternating water regimes, suggests and those microorganisms are important initial rhizodeposition sinks under non-flooded and alternating water regime conditions in paddy soils. In contrast, Gram positive bacteria showed higher contribution for uptake of rhizo-C in continuous flooding as compared with the other two treatments. The decrease of ^{13}C in Gram negative bacteria and fungi groups between day 2 and 14 showed higher process of rhizo-C under non-flooded and alternating water regime, while ^{13}C in most microbial groups under continuous flooding showed lower microbial turnover.

Summarizing, the study showed that water regimes used for rice production had fundamental effects on fate of rhizodeposition and its biological pathways. Future studies are required to investigate these findings under field conditions and also how the composition of rhizodeposits influence the microorganism as related to water regimes.

Acknowledgments

The authors thank China scholarship council for providing fund to Jing Tian to pursue her study in Germany and center for stable

isotope research and analysis (KOSI) at university of Göttingen for ^{13}C analyses. We would also like to thank professor Petra Marschner for her great help during data analyzing, and her useful suggestions on the earlier version of the manuscript. This work was supported by the National Basic Research Program of China (2011CB100505), the Innovative Group Grant of the National Science Foundation of China (31121062), and National Natural Science Foundation of China (41171195).

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