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Soil microbiome-induced changes in the priming effects of ^{13}C-labelled substrates from rice residues

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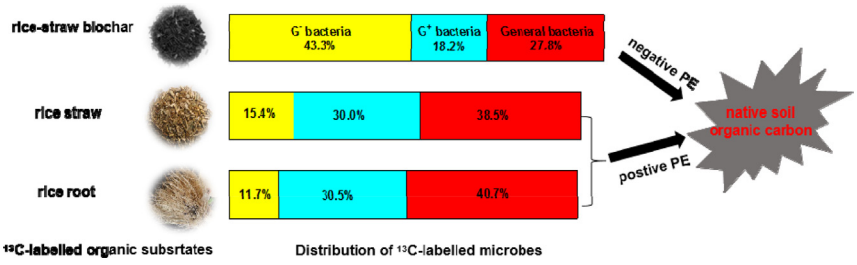
HIGHLIGHTS

- Biochar was preferentially mineralized by G^- bacteria and caused negative PE.
- Root and straw caused positive PE and were metabolized by G^+ and general bacteria.
- Biochar was best in sequestering soil C pool, followed by straw and roots.
- Soil pH, SOC and available nutrients respond to the changes of ^{13}C-labelled PLFA.

GRAPHICAL ABSTRACT



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ABSTRACT

Knowledge gap exists to understand the soil CO_2 emission and microbial group response to substrates of whole plant residues and derived biochar. We used ^{13}C-labelled substrates (rice straw, roots and biochar) to track influences of their decomposition on soil priming effect (PE) and phospholipid fatty acid (PLFA) composition during one-year incubation. Organic substrates at 1% (*w*/w) level increased soil pH, available nitrogen (AN) and available phosphorus (AP), especially during the first 45 days of incubation. After incubation, 44% of the added straw was mineralized to $^{13}CO_2$, followed by roots (~35%) and biochar (~5%). Straw and roots amendment caused positive PE during 4–360 day of the incubation, where a lowest value of 41.9 mg C kg^{-1} was observed. Biochar amendment caused negative PE during 56–150 day of the incubation, where a largest value of −99.0 mg C kg^{-1} was observed. Analysis of ^{13}C-labelled PLFA enabled the differentiation of microbial groups during substrates utilization. Gram positive bacteria (G^+) and general bacteria groups were dominated in co-metabolizing both the native soil organic carbon (SOC) and substrates after straw and roots amendment. Gram negative bacteria (G^-), especially identified by PLFA biomarkers cy17:0 and cy19:0, preferentially utilizes the ^{13}C-labelled biochar but not promoting soil priming effect. Soil pH, SOC, AN and AP all explained changes of total and ^{13}C-labelled PLFA contents (>75%, $p < .05$). Evidences showed that biochar is best in sequestering soil C pool, followed by straw and roots, and soil microbial groups in utilization of organic substances mediated SOC mineralization.

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1. Introduction

As a major representation of terrestrial carbon (C) sink, soil organic carbon (SOC) plays an important role in modulating soil C cycling (Malhi, 2002). The mineralization of SOC, which strongly affected by organic substrates, improves soil fertility and microbial community (Maestrini et al., 2015). Priming effect (PE) is defined as an acceleration or retardation of native SOC mineralization caused by organic substrates (Blagodatsky et al., 2010; Kuzyakov et al., 2009; Maestrini et al., 2015). When the mineralization of SOC increases, it is a positive PE; otherwise, it is a negative PE. Input of easily-decomposable organic substrates such as glucose, sucrose and plant residues speed up the mineralization of SOC, thus causing positive PE (Luo et al., 2017; Nottingham et al., 2009; Zhang et al., 2016). In comparison with these labile C substrates, biochar amendment causes more controversial issues of PE values (Herath et al., 2015; Zimmerman and Ouyang, 2019). On one hand, biochar causes positive PE due to its loaded labile C source, leachable nitrate and phosphate, and porous structures (Zimmerman et al., 2011). All these physico-chemical characteristics stimulate the mineralization of SOC by improving the activity of microbial community. On the other hand, biochar causes negative PE due to its loaded toxic substances (e.g. dioxins, phenols etc.), high alkalinity and specific functional groups (e.g. –COOH, –OH etc.) (Fernandes and Brooks, 2003; Spokas, 2010). These are toxic to microorganisms and reduces the available C sources through soil organic matters adsorption (Zimmerman et al., 2011). As we known, biochar is produced from organic wastes (e.g. crop residue, green manures etc.) through anaerobic pyrolysis (Lehmann and Joseph, 2015). It has been widely used for sequestering soil C, mitigating emission of greenhouse gas and improving soil fertility (Herath et al., 2015; Zimmerman et al., 2011). Biochar types, pyrolysis temperatures, mixtures of biochar and other fresh organic matters (FOM) were all demonstrated to change soil PE (Fang et al., 2015; Zhang et al., 2018). For example, grass derived biochar (250 and 400 °C) had a higher capacity in SOC mineralization (positive PE) than hard woods derived biochar (525 and 650 °C, negative PE) (Zimmerman et al., 2011). Besides the sources, factors such as incubation periods, soil properties are also important in the shift of soil PE. Biochar was reported to cause a positive PE at the early incubation stage, but PE shifted to a negative values as time goes on (Cheng and Lehmann, 2009). Changes in soil microbial groups after biochar amendment contribute to the shift of PE (Zimmerman et al., 2011).

As it have been studied, soil microbial groups and activities regulate soil PE, directly or indirectly affected by the physic-chemical properties of added organic substrates (Qiao et al., 2019; Lenka et al., 2019). ^{13}C stable isotope techniques has been increasingly applied to study the decomposition of organic substrates (e.g. acetate, methane, biochar) and associated microbial composition (McMahon et al., 2005; Wang et al., 2016). The usage of ^{13}C stable isotope labelled phospholipid fatty acid (^{13}C-PLFA) revealed the specific microbial communities (bacteria, fungi, actinomycetes, etc.) responding to the ^{13}C-labelled substrates (Watzinger, 2015; Bore et al., 2017). Wang et al. (2015) added ^{13}C-labelled FOM, leaf of *Zea mays* L., to forest soils and found that the biomass of total microbes and each microbial group all increase with the labile C sources; groups of fungi and actinomycetes adapted to poor nutrient environment such as FOM with higher carbon:nitrogen (C:N) ratio, whereas bacteria needs more labile C and N nutrients for growth; groups of bacteria are responsible for soil positive PE values, because it requires more N contents from both substrates and SOC. According to a "nutrient mining" theory, the aggravated limitation of nutrients for soil microbial growth causes the increased utilization of SOC, and induces positive PE values (Kuzyakov and Bol, 2006; Wang et al., 2015). In comparison with FOM, the proportion of labile components of biochar is lower, and the aromatic structure of biochar makes it more chemically and biologically inert (Downie et al., 2012). Zhang et al. (2018) did a meta-analysis of soil microbes responding to biochar addition. Results showed that properties of soil and biochar influence ratios of soil fungi to bacteria (F/B), also ratios of Gram-positive bacteria to Gram-negative bacteria (G^+/G^-).

Although much work has been done on the differential responses of PE induced by organic substrate such as straw and its derived biochar (Zavalloni et al., 2011; Junna et al., 2014), studies always ignore other tissues such as roots from the same plant. It is necessary to compare the mineralization of SOC (e.g. the direction, size and mechanisms) induced by various tissues (Maestrini et al., 2015), especially during the re-utilization of whole plant residues for a long term period (Bai et al., 2016; Blagodatskaya and Kuzyakov, 2008; Pan et al., 2016). Therefore, we conducted a 360 day of incubation experiment using ^{13}C-labelled organic substrates (rice straw derived biochar, rice straw and roots) from a same plant. We aimed to: (1) examine the PE of organic substrates on SOC during incubation periods; (2) probe the dynamic changes in soil microbial community response to the ^{13}C-labelled organic substrates using ^{13}C-PLFA analysis; (3) explore the role of organic substrate characteristics and microbial communities on soil priming effect.

2. Materials and methods

2.1. Soil characterization

Soil was collected from a paddy field at the Ecological Station of Red Soil, Chinese Academy of Sciences in Yingtan, Jiangxi Province, China (28°15′30″N, 116°55′300″E). It was classified to Anthrosols in China Soil Taxonomy. This region was characterized by a typical subtropical monsoon climate with mean annual precipitation of 1795 mm, mean annual temperature is 17.6 °C (Li et al., 2010). Soil samples were collected from 0 to 20 cm depths, and air-dried. After removing visible root fragments and other plant debris, dry soils were passed through a 2 mm sieve and homogenized.

Soil pH was measured in a soil-water suspension (1:5 *w/v*) with a pH meter (Metler Toledo-Fe20). Soil organic carbon contents were determined by the method of Turin method (Dar and Somaiah, 2015). Total nitrogen (N) content was determined by the method of Kjeldahl (Zhao et al., 2010). Soil available N, P and K were determined by methods of the alkali hydrolysable, the Olsen and the NH_4OAC extraction, respectively (Lu, 1999). Soil pH value was 5.50 and other basic properties were shown in Table 1.

2.2. Preparation and characterization of ^{13}C-labelled organic substrates

Harvested rice plant was selected as the organic material. The growing period of rice was from April to August 2013. To obtain the ^{13}C-labelled organic substrates, we used the multiple pulse labeling technique to mark the rice plant four times at growth periods (tillering stage, jointing stage, heading stage and filling stage) (Malosso et al., 2004; Williams et al., 2006). A special transparent plexiglass chamber (90 cm long × 60 cm wide × 100 cm high) was designed for the preparation of ^{13}C-labelled organic substrates. The schematic diagram was shown in Fig. S1. Each rice plant was accomplished by ^{13}C pulse labeling experiment in four growth periods. Each period was labelled for once, and the labeling time started from 8:30 am to 12:30 am. Details about the ^{13}C pulse labeling experiment were listed as: (1) Rice plant was transferred to the

Table 1
Basic properties of the labelled substrates and soil.

Materials	pH	Total C %	Total N %	$\delta^{13}C$ value ‰	WEOC[a] mg kg^{-1}
Biochar	9.23	56.4	1.73	443	2.18E3
Straw	5.81	39.2	1.06	392	6.47E5
Roots	5.79	35.3	1.05	358	1.3E5
Soil	5.32	2.13	0.26	−25.3	NA

[a] Water extractable organic carbon. NA, data is not available. Values were attained from composite samples, and thus no standard errors of mean were reported.

transparent chamber, where two glass beakers (volume 300 mL for each) included 5.3 g $Ba^{13}CO_3$ (99% ^{13}C-enriched). These two beakers were placed on both sides of the plant. (2) The rice plant started to photosynthesize for 15 min before ^{13}C pulse labeling, improving its enrichment of ^{13}C. (3) At the beginning of labeling, the chamber was covered with a black cloth, then 800 μL/L $^{13}CO_2$ was generated by adding 2 mol/L HCl (200 mL) to the $Ba^{13}CO_3$ beaker through a burette. After the reaction of 5 min, rice plant restarted to photosynthesize without black cloth covering. (4) After 45 min of photosynthesis, we repeated the ^{13}C pulse labeling experiment as shown in step 3. The generated $^{13}CO_2$ from another $Ba^{13}CO_3$ beaker was used to label plant for the second time. (5) After the labeling experiment, the plexiglass chamber was removed and the ^{13}C-labelled rice plant was put back in place. During ^{13}C pulse labeling experiment, a fan hanged on the top of the chamber was used to increase the air flow, and a thermometer was used to monitor the inner temperature at 28–37 °C. Rice straw was harvested on the 10th day after the final labeling. Rice roots were collected by carefully separating them from the soil. A part of the harvested rice straw was washed carefully to remove soil. Then the straw was air-dried, and ground to pass a 1 mm sieve. The ground straw was pyrolyzed by muffle furnace at 400 °C under anaerobic conditions (Yuan et al., 2011). The pyrolysis temperature was raised to 400 °C at a rate of approximately 20 °C min^{-1} and held constant for 5 h. After heating for 5 h, the biochar samples kept cool at the room temperature. Then we obtained the ^{13}C-labelled biochar material. Due to the limited biomass of rice roots, the biochar was produced only from rice shoots. To identify the $^{13}C/^{12}C$ ratio in organic materials, isotope mass spectrometer (flash-2000 Delta V Advantage) was used to analyze the total carbon and $\delta^{13}C$ value (Table 1). ^{13}C contents of rice straw, roots and biochar were enriched and calculated by $\delta^{13}C$ (‰). Biochar had the highest value of $\delta^{13}C$ (443‰), followed by straw (392‰), roots (358‰) and control soil (−25.3‰).

2.3. Mineralization of soil organic carbon

The soil organic carbon mineralization was determined by the alkali absorption method (Goyal et al., 1999). Briefly, each 500 mL glass jar contained 100 g air-dried soil, and deionized water was added to readjust the soil moisture to 60% water holding capacity (WHC). Then these soil samples were pre-cultured for one week in a constant temperature incubator at 25 ± 1 °C. After the pre-incubation, ^{13}C-labelled organic substrates (rice straw, roots, and biochar) were fully mixed with soil samples at a level of 1% (*w*/w). There were four treatments, each in triplicate, listed as: Control treatment (only soil), ^{13}C rice straw treatment (soil + straw), ^{13}C rice root treatment (soil + root), and ^{13}C biochar treatment (soil + biochar). To determine the cumulative emission of CO_2 and ^{13}C-labelled CO_2 ($^{13}CO_2$), we prepared a vial with 20 mL 1 M NaOH inside in the preincubation 500 mL glass jar for each treatment. The vial could be used as a trap for collecting released CO_2. Moreover, three jars with only water and NaOH as above served as reference to consider air CO_2. All the jars were sealed with aseptic membrane to ensure good ventilation conditions. The soil moisture was checked every four days, and deionized water was added to keep the constant condition. The whole incubation period was 360 days under a constant temperature incubator at 25 ± 1 °C.

At the 4, 14, 28, 56, 90, 150, 240 and 360 days of the incubation, the NaOH vials were taken out to determine the evolved CO_2 and $^{13}CO_2$ (‰). Briefly, excess 1.5 M $BaCl_2$ solution and the acid-base indicator were added to the NaOH vials, and then titrated with 0.5 M HCl using a TIM840 autotitrator (Radiometer Analytical, Villeurbanne Cedex, France). After that, the resulting $BaCO_3$ precipitates were filtered and trapped on the glass fibre filters (90 mm, Whatman GF/A, UK), carefully washed and dried at 80 °C, then analyzed for the $^{13}C/^{12}C$ ratio by the MAT-253 isotope mass spectrometer (Thermo Electron, Bremen, Germany), and the $\delta^{13}C$ value was calculated by Eq. (1) (Craig, 1953):

$$\delta^{13}C(‰) = (R_{sample}/R_{PDB} - 1) \times 1000 \quad (1)$$

where R_{sample} is the $^{13}C/^{12}C$ ratio of the sample, and R_{PDB} is the $^{13}C/^{12}C$ ratio of the international Pee Dee belemnite (PDB) standard from cretaceous in South Carolina.

According to Eq. (2), the proportion of ^{13}C from the labelled organic materials (%) in the total released CO_2 was calculated as (Li et al., 2019):

$$^{13}CO_2(\%) = (\delta_{treatment} - \delta_{ck})/(\delta_{feedstock} - \delta_{ck}) \times 100 \quad (2)$$

where $\delta_{treatment}$ is the $\delta^{13}C$(‰) of the soil amended with the ^{13}C-labelled organic substrates, δ_{ck} is the $\delta^{13}C$(‰) of the control soil, and the $\delta_{feedstock}$ is the $\delta^{13}C$ (‰) of the ^{13}C-labelled organic substrate.

According to Eq. (3), we can calculate the amount of ^{13}C-labelled organic substrates that decomposed into CO_2 during cultivation ($^{13}CO_2$):

$$^{13}CO_2\left(mg\ kg^{-1}\right) = {}^{13}CO_2(\%) \times CO_2\left(mg\ kg^{-1}\right)/100 \quad (3)$$

where CO_{2total} is the total released CO_2 evolved from the soil amended with ^{13}C-labelled organic substrates.

Then we calculate the priming effect (PE), which is denoted as the promoted or inhibited mineralization of native SOC caused by the organic substrate addition, using the Eq. (4) (Pan et al., 2016).

$$\text{Primed soil } CO_2\left(mg\ kg^{-1}\right) = CO_{2\ treatment} - CO_{2\ control} \quad (4)$$

$CO_{2treatment}$ is the amount of non-labelled CO_2 released in the soil amended with labelled organic substrates, and $CO_{2control}$ is the total amount of CO_2 released in the control treatment.

Moreover, we calculated the degradation residual rate of the organic materials (%) = the amount of CO_2 released by the material/total carbon content of the material ×100% (Eq. (5)).

2.4. Soil prepared for microbial analyses

Soil incubation for microbial analyses were separately prepared by following methods similar to Section 2.3. Briefly, each 500 mL glass jar contained 100 g air-dried soil samples, and ^{13}C-labelled organic substrates were fully mixed with soil samples at 1% (*w*/w) to obtain different treatments. Deionized water was added to readjust the soil moisture to 60% WHC, and the soil moisture was checked every four days. Different treatments were listed as: Control treatment (only soil), ^{13}C rice straw treatment (soil + straw), ^{13}C rice root treatment (soil + root), and ^{13}C biochar treatment (soil + biochar). Three replications for each treatment. These jars were sealed with aseptic membrane and incubated in a constant temperature incubator for 360 days at 25 ± 1 °C. Soil samples were collected at days of 45, 180 and 360, when were used to represent the frequent changes of soil microbes during incubation. These soil samples were divided into three parts. One part was air-dried for soil nutrient content determination; another part was short-term stored at 4 °C and then used for the determination of soil microbial biomass (MBC) and enzyme activity; the last part was stored at −20 °C and then used for soil phospholipid fatty acid (PLFA) analysis.

2.5. Determination of soil microbial biomass carbon and MBC-^{13}C

Soil microbial biomass C (MBC) was determined by chloroform fumigation-extraction method (Vance et al., 1987). Soil samples were extracted with 60 mL 0.05 M K_2SO_4 solution, at a soil-liquid ratio of 1:4. A portion of the extract was used for the MBC-^{13}C determination, and the remaining portion was freeze-dried to detect the $^{13}C/^{12}C$ value by the flash EA-Delta V instrument (Elementar, Germany). Then

we calculated the $\delta^{13}C$ value of MBC as follow:

$$MBC{-}\delta^{13}C\ (‰) = \left(\left(\delta^{13}C_f \times C_f\right) - \left(\delta^{13}C_{uf} \times C_{uf}\right)\right)/(C_f - C_{uf}) \quad (6)$$

where C_f and C_{uf} are the content of soil microbial biomass C in fumigation and non-fumigation soils, respectively. δ^{13} is calculated from Eq. (1) (Potthoff et al., 2003).

Thus, we calculated the proportion of MBC-^{13}C from labelled organic materials in total content of MBC in Eq. (7).

$$MBC{-}^{13}C(\%) = (\delta_t - \delta_c)/(\delta_{feedstock} - \delta_c) \quad (7)$$

where δ_t is the $\delta^{13}C$ (‰) of the soils amended with organic materials, δ_C is the $\delta^{13}C$ (‰) of the control soil sample, and $\delta_{feedstock}$ is the $\delta^{13}C$ (‰) of the labelled organic materials.

2.6. ^{13}C-PLFA analysis

PLFA analysis was adapted to investigate the composition of soil microbial groups. 2.5 g fresh soil sample was analyzed by the modified Bligh–Dyer technique to determine the PLFAs concentration (Bligh and Dyer, 1959). Briefly, soil samples were incubated in a solution of methanol, chloroform, and phosphate buffer in ratio of 2:1:0.8, shaken for 2 h and centrifuged, after which the chloroform phases were collected and stored. Phospholipids were then separated from glycolipids and neutral lipids, saponified and methylated to fatty-acid methyl esters (FAME), and FAME was qualitatively and quantitatively analyzed by GC–MS (Thermo Finnigan Trace GC–MS System). Peaks were identified based on the comparison of retention times with known standards, and the concentration of each PLFA was calculated by comparing the peak area of internal standard. Patterns of PLFA were determined according to Frostegård et al. (1993) and Zelles (1999).

The $^{13}C/^{12}C$ ratio of FAME was determined by gas chromatography-combustion-isotope mass spectrometry Thermo Scientific Trace (GC Ultra-C-IRMS, Finnigan MAT 253), and the $\delta^{13}C$ value was calculated. Since the PLFA esterification process introduces an exogenous carbon atom (CH_3OH), the measured $\delta^{13}C$ value needs to be corrected during data processing. The abundance value of the measured FAME is converted to the abundance value of the corresponding PLFA using the following mass balance formula (Zhang et al., 2013)

$$n_{cd}\delta^{13}C_{cd} = n_c\delta^{13}C_c + n_d\delta^{13}C_d \quad (8)$$

where *n* represents the number of carbon atoms, and *c, dss* and *cd* represent the $\delta^{13}C$ values of the PLFA before derivatization, the derivatized medium (methanol) and the derivatized FAME, respectively. Among them, $\delta^{13}C$ of methanol was −42.5‰.

PLFA biomarkers were detected and grouped as follows: i13:0, i14:0, a15:0, i15:0, i16:0, a17:0 and i17:0 are fatty acids derived from Gram-positive bacteria (G^+); cy17:0, cy19:0, 16:1ω7c and 16:1ω9c are Gram-negative bacteria (G^-) derived fatty acids; 18:1ω9c, 18:1ω9t and 18:2ω9,12c are fungal derived fatty acids; 10Me16:0,10Me17: 0 and 10Me18:0 are actinomycete derived fatty acids. 12:0, 13:0, 14:0, 15:0, 16:0, 17:0, 18:0, and 20:0 are also used as general bacterial source fatty acids, but are not classified as G^+ or G^- (Li et al., 2019; Orwin et al., 2018; Zelles, 1999). The relative abundance (mol %) of PLFA was used to characterize the composition of soil microbial groups.

According to the requirements for isotope analysis, only 20 types of the PLFA biomarkers can be detected. The remaining 5 types of PLFA biomarkers (12:0, 13:0, i13:0, i14:0 and 18:2ω9, 12c) were not be included in the calculation below. The proportion of C (P_i) from the labelled organic material (F) in each PLFA is calculated by using Eq. (9) (Lemanski and Scheu, 2014):

$$P_i = \left(\delta^{13}C_t - \delta^{13}C_0\right)/\left(\delta^{13}C_F - \delta^{13}C_0\right) \quad (9)$$

where $\delta^{13}C_t$ and $\delta^{13}C_0$ represent the $\delta^{13}C$ value (‰) of a single PLFA in soils with and without organic materials addition, respectively. $\delta^{13}C_F$ represents the $\delta^{13}C$ value (‰) of the labelled organic materials.

2.7. Statistical analysis

Data analysis was conducted using SPSS 18.0. One-way ANOVA and Tukey HSD comparison to analyze the significance of differences in soil properties among treatments. The mean deviation (MD) analysis was used to describe the abundances in the $\delta^{13}C$-PLFA values of the soil amended with ^{13}C-labelled organic substrates relative to the control soil (Luo et al., 2010), as shown in Fig. S2. Redundancy analysis (RDA) was used to study the relationships between soil chemical properties and variations in soil microbial groups. Multivariate analysis was carried out using the Vegan package in R software (3.1.0) and graphs were obtained by using sigmaplot 12.5.

3. Results

3.1. Soil properties

Due to the high pH values of organic substrates especially biochar, soil pH were all increased after organic substrates amendment at the first 45 day of incubation ($p < 0.05$, Table 2). Biochar amended soil had the highest pH of 4.51. Soil pH decreased with the incubation time from day 45 to 360. Biochar amended soil had the highest pH reduction of 6.43%, followed by straw, roots and control treatments. Contents of SOC also decreased with incubation time from day 45 to 360 ($p < 0.05$). After incubation of 45 days, biochar, straw and roots amendments increased the SOC contents by 22.6%, 12.4% and 26.1% when compared with control treatment, respectively. Until the end of incubation (360 day), biochar amended soil had a lowest SOC reduction of 9.03%, followed by control (10.6%), straw (14.6%) and roots (21.9%) treatments.

Be similar with soil pH and SOC, soil total nitrogen (TN) contents decreased with incubation time ($p < 0.05$). Biochar amended soil had the highest TN contents with the incubation periods, due to biochar loaded N contents. However, biochar amended soil had the highest TN reduction of 9.66%, followed by roots, straw and control treatments. In comparison with straw, both biochar and roots amendment had lower contents of soil AN at the first 45 day of incubation ($p < 0.05$, Table 2). Differences in AN contents among organic substrates amendment diminished with incubation time, except for a reduction in control soil. As shown in Table 2, biochar amendment always had the highest contents of soil AP and available potassium (AK). Except for straw amendment, there were no significant difference in contents of soil AP between the incubation of 45 and 360 days. However, contents of soil AK increased with incubation time. Biochar amended soil had the highest content of soil AK, followed by straw, roots and control soils.

3.2. Dynamics of soil CO_2 emission and PE.

The cumulative amount of CO_2 released from the straw and roots amended soils were higher than the biochar amended and control soils (Fig. 1a). Straw amendment significantly increased the total CO_2 emission than other treatments, while there was no significant difference between biochar amendment and control soil. At the end of incubation, straw and roots amendment increased the cumulative CO_2 emission by 76.3% and 72.1% relative to control, respectively (Fig. 1a). The cumulative PE values after organic amendments were shown in Fig. 1b. During 0–4 day of the incubation, there was no significant effect of straw and roots amendments on the mineralization of SOC, as the observed PE values around zero ($p < .05$). After that, PE values for straw and roots amendments shifted to positive throughout incubation. The significant upward peaks of PE values were observed around 150 day of the incubation. Differences in PE values between straw and roots

Table 2
Effects of organic substrates on soil pH and nutrient contents.

Treatments	pH		SOC g kg^{-1}		TN g kg^{-1}		AN mg kg^{-1}		AP mg kg^{-1}		AK mg kg^{-1}	
	45d	360d	45d	360d	45d	360d	45d	360d	45d	360d	45d	360d
Control	4.32^{Ad}	4.20^{Bab}	22.6^{Ac}	20.2^{Bb}	2.18^{Ab}	2.10^{Bb}	239^{Aa}	207^{Ba}	44^{Ab}	45^{Ab}	50^{Bd}	59Ad
Biochar	4.51^{Aa}	4.22^{Ba}	27.7^{Aa}	25.2^{Ba}	2.38^{Aa}	2.15^{Ba}	207^{Ab}	218^{Aa}	54^{Aa}	58^{Aa}	438^{Ba}	521^{Aa}
Straw	4.46^{Ab}	4.22^{Ba}	25.4^{Ab}	21.7^{Bb}	2.18^{Ab}	2.05^{Bc}	234^{Aa}	226^{Aa}	43^{Bb}	50^{Ab}	175^{Bb}	229^{Ab}
Roots	4.37^{Ac}	4.17^{Bb}	28.5^{Aa}	21.4^{Bb}	2.28^{Aab}	2.09^{Bb}	217^{Ab}	220^{Aa}	42^{Ab}	41^{Ab}	69^{Bc}	83^{Ac}

Means followed by the same lowercase letter within a column and the same uppercase within a row are not significant at the 0.05 level. TN: total nitrogen; AN: available nitrogen; AP: available phosphorus; AK: available potassium.

amendments were non-significant during incubation, except for the incubation of 360 day. At the end of incubation, the PE values of straw amendment (161 ± 160 mg kg^{-1}) were significantly lower than roots (715 ± 117 mg kg^{-1}). Taking the error bars of each PE value after biochar amendment into consideration, the cumulative PE values were positive but non-significant during 0–56 day of the incubation. During 56–150 day of the incubation, PE values shifted to be negative ($p < .05$). However, the negative PE values were transient and shifted to be positive (non-significant) during 150–360 day of the incubation. A significant downward peak of PE values after biochar amendment was observed around 150 day of the incubation.

Analysis of ^{13}C isotope showed that contributions of organic substrates to soil CO_2 emission sharply decreased at the first 150 day of the incubation, and then decreased to a relative stable phase during 150–360 day of the incubation. Straw amendment had a highest contribution of 67.3%, followed by roots (45.2%) and biochar (8.1%) amendment (Fig. 1c). However, straw amendment had the lowest residue of 58.2%, followed by roots (66.4%) and biochar (98.1%) (Fig. 1d).

3.3. Dynamic soil microbial biomass carbon and groups

At the early stage (day 45) of incubation, organic substrates amendment increased soil MBC, relative to the control. (Fig. 2a). No significant changes in contents of MBC were found during 45–180 day of the incubation for each treatment. Contents of MBC were sharply decreased during 180–360 day of the incubation. At the end stage (day 360) of incubation, straw and roots amended soils had higher contents of MBC than biochar amended and control soils. Analysis of ^{13}C incorporated into contents of MBC showed that straw had the highest contributions of 17.5% to 20.6%, followed by roots (11.0% to 13.2%) and biochar (<1%) (Fig. 2b).

At the early stage of incubation, roots and straw amendments had higher contents of total PLFAs and each microbial taxonomic group associated PLFAs than other two treatments (Fig. 3). There were no significant changes in contents of total PLFAs during 45–180 day of the incubation for each treatment (Fig. 3a). For both straw and roots treatments, contents of total PLFAs were sharply decreased during

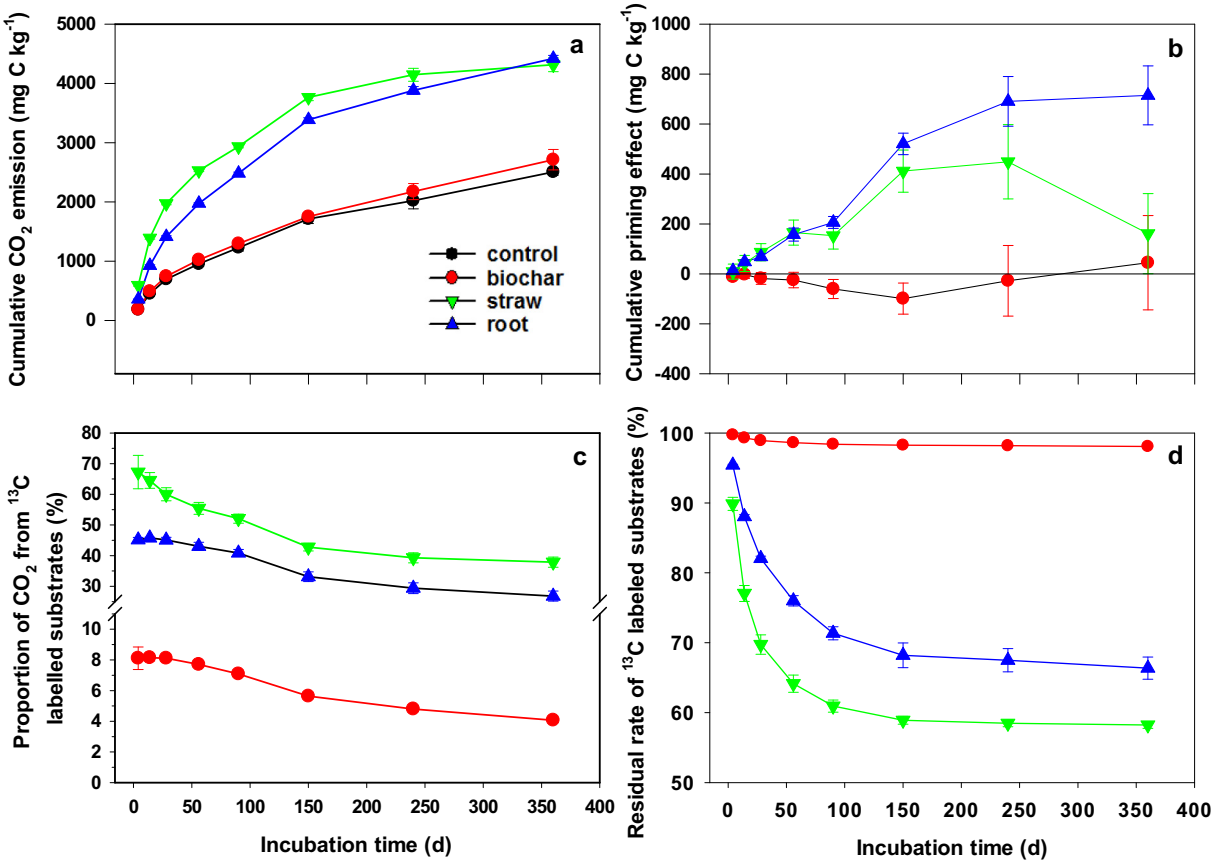

Fig. 1. Effects of organic substrates on soil organic carbon mineralization (a: cumulative CO_2 emission; b: cumulative priming effect induced by ^{13}C-labelled substrates on soil organic carbon; c: ^{13}C-labelled substrates derived CO_2 evolution; d: residue rate of ^{13}C-labelled substrates).

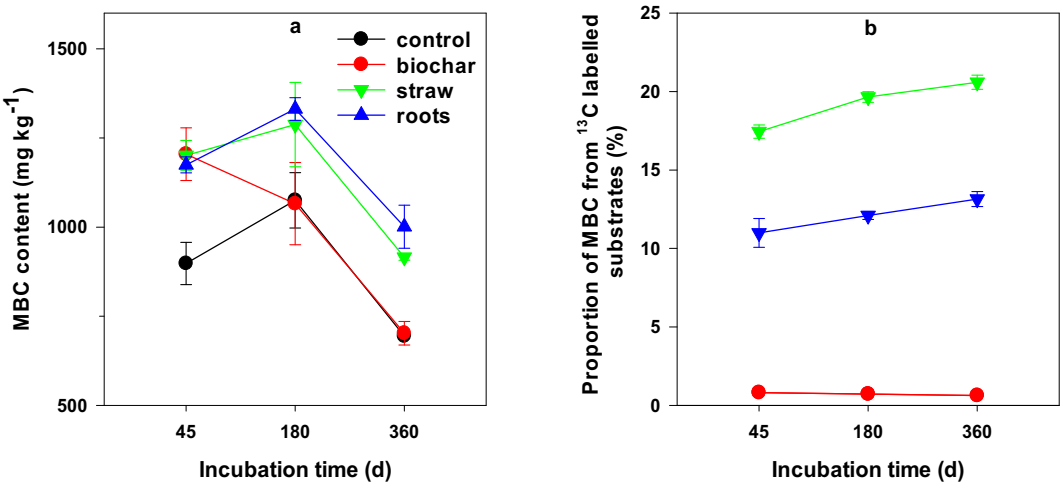

Fig. 2. Microbial biomass carbon (MBC) content in soils with or without organic substrates amendment (a), and the proportion of ^{13}C-labelled substrates incorporated into MBC (b).

180–360 day of the incubation. Differences in contents of total PLFAs among treatments were disappeared at the end stage of incubation. Similar phenomena were observed in the contents of PLFAs associated with microbial taxonomic groups of general bacterial, Gram-positive (G^+) bacterial and actinomycetes (Fig. 3b-d). Groups of general bacterial and G^+ bacterial were the dominant parts in total PLFAs, followed by fungi, Gram-negative (G^-) bacterial and actinomycetes. According to Fig. 3e-f, contents of fungi and G^- bacterial PFLAs straightly decreased with incubation time, and differences in contents of PLFAs for each group were disappeared at the end stage of incubation. G^+ to G^- bacterial ratios (G^+/G^-) were not significantly affected by amendments of organic substrates, while these ratios increased with incubation time (Fig. 3g). Fungi to bacteria ratios (F/B) were also not significantly affected by amendments of organic substrates, but significantly decreased with incubation time (Fig. 3h).

According to the RDA analysis, soil chemical properties were used as the explanatory variables and the PLFAs of soil microbial groups were the response variables (Fig. 4). The first two axes of RDA together explained 44.3% of the total variation in the microbial community groups. Results confirmed that soil pH ($F = 16.1$, $p = .01$), AP ($F = 6.92$, $p = .01$) and AN ($F = 2.53$, $p = .03$) were significantly correlated with contents of soil PLFAs, and explained 49.2, 21.1 and 7.7% of the soil PLFAs variation, respectively.

3.4. ^{13}C-labelled soil microbial groups

Soil microbial community responds to the utilization of ^{13}C-labelled organic substrates was showed in Fig. 5a-c. According to the percentages of ^{13}C-labelled organic substrates to total C in PLFAs of microbial groups, G^- and the general bacterial were the dominant microbial groups in biochar amended soil, while G^+ and the general bacterial were the dominant microbial groups in both roots and straw amended soils. Regardless of the dominant groups, ^{13}C distribution proportion within G^+ and actinomycetes groups generally increased with incubation time, whereas that of fungi group decreased sensitively in biochar amended soil (Fig. 5a). For both straw and roots amended soils, ^{13}C distribution proportion within fungi and actinomycetes groups generally increased with incubation time, whereas that of G^- group decreased sensitively (Fig. 5b-c). During 180 day of incubation, a significant percentage peak of bacteria groups (G^+ and general bacteria) were observed in three organic substrates. Distributions of biomarkers ^{13}C-PLFA showed that there is an order of magnitude higher in straw and roots amended soils than biochar (Fig. S2). For biochar amended soil, G^- bacteria biomarkers of cy17:0 and cy19:0 were dominant in the abundance of ^{13}C-PLFAs. While the straw and roots amended soils were dominated in the ^{13}C-PLFA of G^- bacteria biomarkers (cy17:0 and cy19:0) and fungal biomarker (18:1ω9t) at the early 45 days of incubation. However, the abundance of fungal biomarker (18:1ω9t) ^{13}C-PLFAs decreased with incubation time, and shifted to the actinomycetes biomarker (10Me16:0) until the end of incubation (Fig. S2). In correspondence, percentages of soil microbial community responds to the utilization of SOC were shown in Fig. 6d-e. G^+ and the general bacterial were the dominant microbial groups (>50%) for three organic substrates amended soils. Other groups of soil microbes followed as fungi > G^- ≈ actinomycetes. During incubation, the percentage of G^+ group increased while fungi group decreased. Slight changes in other microbes were observed.

RDA analysis of ^{13}C-PLFA data showed that there are significant differences in soil microbial communities between biochar and raw organic substrates amendment (Fig. 6). The first and second axes of RDA together explained 88.1% of the total variation in the microbial groups, when responding to the variations of the soil chemical properties. In total, soil pH, SOC, TN, AN, AP and AK were all significantly correlated with contents of soil ^{13}C-PLFAs, and could explain 97.6% of the soil ^{13}C-PLFAs variation. The importance of variables on microorganism were followed as AP > SOC > AN > pH > TN.

4. Discussion

4.1. Impact of organic substrates on PE

Soil CO_2 emission is strongly affected by the type of organic substrates and incubation time. Prior reports employed sucrose, maize leaf and biochar to test soil CO_2 emission (Aye et al., 2018; Luo et al., 2017; Nottingham et al., 2009). Those C additives increase soil CO_2 emission in a short-term incubation (especially for labile C sources); however, effects of C additives are attenuated over time (Chaker et al., 2018; Wang et al., 2015). In our study, similar observation was made over longer incubation period (1 year; Fig. 1). Labile carbon of rice straw caused the most cumulative $^{13}CO_2$ emission, followed by roots which contain higher lignin. The proportion of $^{13}CO_2$ derived from ^{13}C-labelled organic substrates decreased with incubation time, indicating the existence of native SOC mineralization. Priming effect (PE) is used to evaluate the capacity of organic substrates on native SOC mineralization (Kuzyakov et al., 2009; Zimmerman et al., 2011).

Several mechanisms are proposed to explain the differences in PE values induced by various organic substrates (Maestrini et al., 2015). Among them, the added substrate containing labile carbon favors the growth of specific soil microbes which co-metabolize the native SOC and are responsible for the soil primed CO_2 emission (Maestrini et al.,

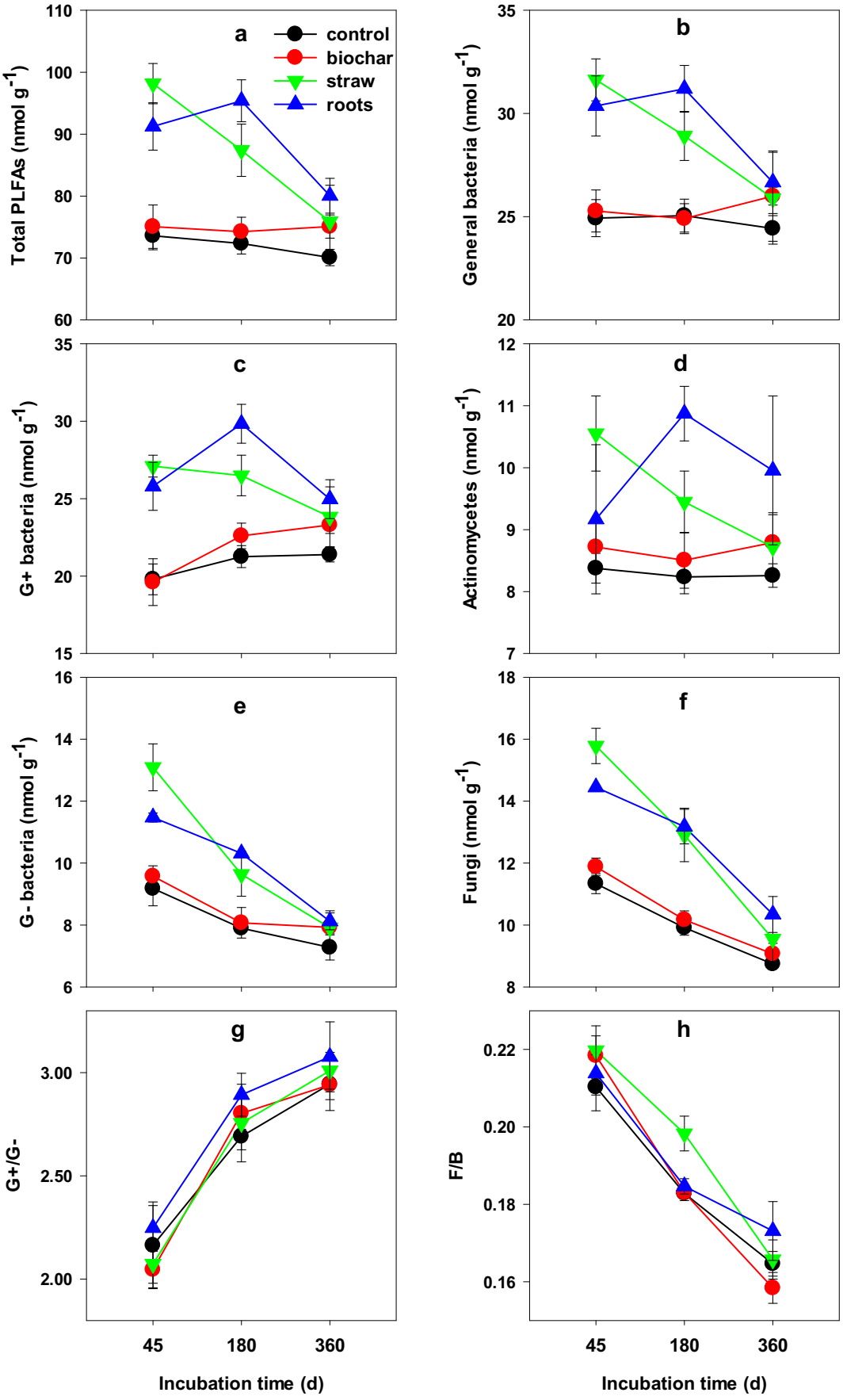

Fig. 3. Soil microbial PLFA contents (a) and community compositions (b-h) affected by three organic substrates.

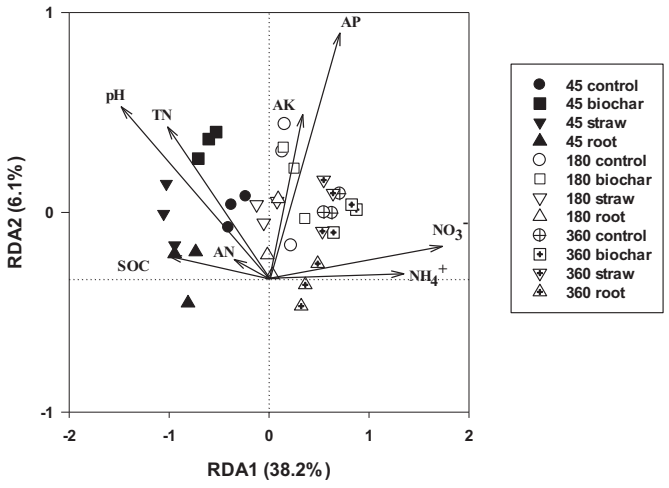

Fig. 4. RDA analysis for soil microbial groups and chemical properties with or without organic substrates amendment, sampling for the 45, 180 and 360 day of incubation.

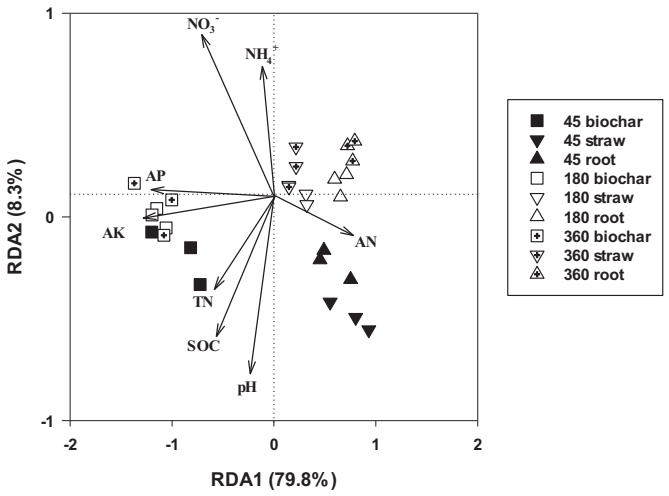

Fig. 6. RDA analysis for soil microbial group response to the ^{13}C-labelled organic substrates utilization and chemical properties, sampling for the 45, 180 and 360 day of incubation.

2015; Shahbaz et al., 2018). Compared with raw substrates, biochar contains fewer labile carbon fractions and had the highest residual rate after incubation. Though some studies proposed that substrate associated labile carbon leads to a positive priming effect on the short-term incubation (Cross and Sohi, 2011; Zimmerman et al., 2011), no significant positive PE of biochar was observed in our study. During 360 days of the incubation, we observed the non-significant positive PE (0–56 day) of biochar amendment shifts to negative PE

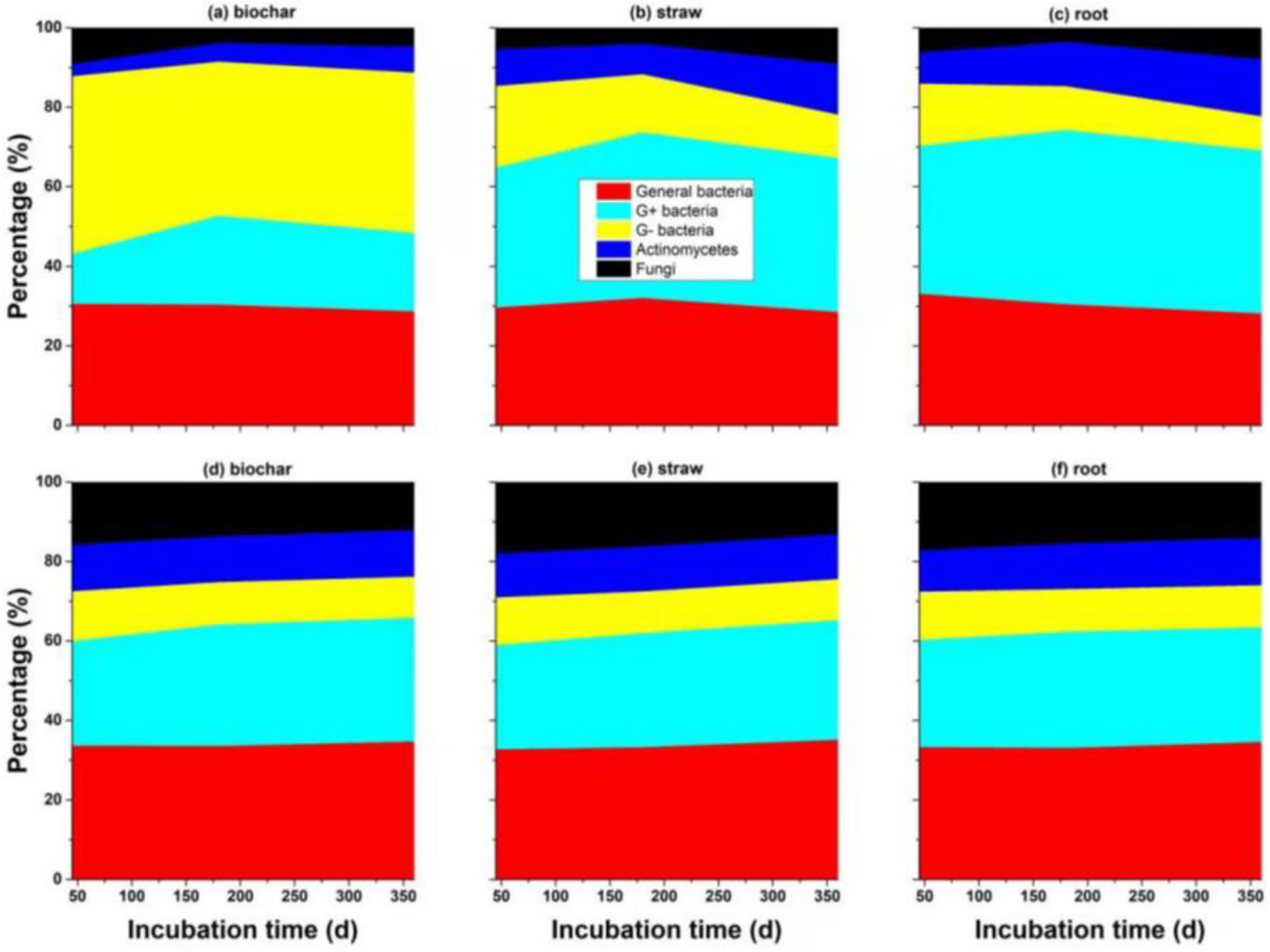

Fig. 5. Distribution percentages of ^{13}C-labelled PLFA content of each soil microbial group to total contents of ^{13}C-labelled PLFA, and that of PLFA content of each soil microbe group to total contents of PLFA with three organic substrates amendment (a, d: biochar, b, e: straw, c, f: root).

(56–150 day) and then fades away until the end of incubation (150–360 day). More complex mechanisms existed in the mineralization of SOC by biochar amendment. As reported, the abiotic behavior such as the sorption of soil dissolved organic matters on biochar surface caused negative PE, which may offset the possible existed positive PE caused by the few easily decomposable C fractions of biochar (Downie et al., 2012; Maestrini et al., 2015). Moreover, biochar amendment changed soil properties as shown in Table 2. The equilibrium between CO_2 and soil carbonates (HCO_3^- and CO_3^{2-}) could be altered by pH values in soil solution (Maestrini et al., 2015). Therefore, the higher soil pH caused by biochar amendment at the early incubation period would be responsible for the emerged negative PE. As time went, the effect of biochar amendment on soil pH decreased and the negative PE depleted. Shifts in soil microbial activity and community caused by biochar amendment also contributed to the changes in PE (discussed further below) (Sheng and Zhu, 2018; Steinbeiss et al., 2009). In accordance with previous study (Wang et al., 2015), the fresh organic substrate such as rice straw and roots amendment caused positive PE during incubation period. However, during the early 0–4 day of incubation, there was no significant positive PE observed after amendment. Two reasons are speculated from this study as: (1) the increase of soil pH after organic substrates amendment at this stage (Table 2); (2) the major contribution of ^{13}C-labelled organic substrates to soil CO_2 emission at this stage, nearly accounting for 67.3% for straw and 45.2% for roots (Fig. 1c). According to these soil PE values, biochar has the largest capacity in sequestering soil C pool, followed by straw and then roots from the same plant.

4.2. Impact of fresh organic substrates on the composition of soil microbial groups

Organic substrates are known to alter the soil microbial community composition (Ding et al., 2018; Kuzyakov et al., 2009; Sheng and Zhu, 2018). As shown by results of MBC and PLFA contents, we proved that both soil microbial biomass and groups are changed by the amendment of three organic substrates. Fresh organic substrates provided C sources, nutrient contents (AP, AN and AK) for the growth of soil microbes, which were consistent with previous studies (Ding et al., 2018; Sheng and Zhu, 2018). The highest contents of soil MBC after straw and roots amendment were found at day 180 of incubation, when was in accordance with the upward peaks of PE values in Fig. 1b. Only 19.7% and 12.1% of the MBC content came from the ^{13}C-labelled straw and roots substrates, respectively (Fig. 2b). Therefore, most of the soil microbes utilized native SOC, and resulting in the positive PE. Rice straw was more easily utilized by soil microbes, but contributed less to soil C mineralization than roots. With the rapid depletion of labile C from straw substrate, the decreased contents of MBC were responsible for the changed soil PE values at the end of incubation (day 180 to 360), but not totally. Soil microbial communities play a vital role in explaining the dynamic changes of soil PE. As reported, there are mainly two mechanisms involved in modulating the mineralization of SOC by soil microbes (Zimmerman et al., 2011). Firstly, the 'r-strategist' points out that there are soil microbes responding to the added organic substrates quickly, then microbes co-metabolize the native SOC and improve the soil nutrients. Most of the r-strategist response appeared as an immediately increasement of positive soil PE (Zimmerman et al., 2011). Secondly, the 'k-strategists' points out that there are soil microbes continuously utilize the native SOC, and they co-metabolize the added organic substrates through their release of extra-cellular enzymes. Most of the k-strategist response was found in the condition of complex substrates such as straw. Actually, a balance between r- and k-strategists are responsible for the soil priming effects, not likely a single mechanism (Zimmerman et al., 2011).

Through ^{13}C-PLFA analysis, both G^+ and general bacteria groups were dominated in the mineralization of native SOC and substrates after straw and roots amendment. Dynamic changes in percentages of microbial species throughout the incubation time indicated that: (1) Both G^+ and general bacteria groups cause positive PE based on the co-metabolism mechanism. (2) G^+ bacteria firstly responds to the labile C sources of added organic substrates and then co-metabolize the native SOC ('r-strategist'), while general bacteria utilizes the native SOC continuously, and then co-metabolizes the organic substrates ('k-strategist'). The mineralization mechanisms of these two groups are interactive and dynamic. An upward peak of percentages of G^+ and general bacteria groups was observed around 180 day of the incubation, which is in accordance with the dynamic changes in soil PE values. We speculated that 'r-strategist' is dominant in soil priming effect during 0–180 day of the incubation, while it changes to 'k-strategist' during 180–360 day of the incubation. Reasons were possibly due to the depletion of labile C source, available nutrients (AP and AN) from substrates at the early incubation time, and then bacteria groups (G^+ and general bacteria) acquired more sources from the native soil environment (Wang et al., 2015). As shown by the RDA analysis, soil available nutrients and pH properties all contributed to the changes in the contents of PLFA and ^{13}C-labled PLFA. Moreover, we analyzed the abundance of microbial biomarkers ^{13}C-PLFAs after straw amendment throughout the incubation. Results indicated that G^- bacteria (biomarkers: cy17:0 and cy19:0) and fungi (biomarker: 18:1ω9t) were dominated in utilizing the ^{13}C-labelled substrates at the early stage of incubation. These dominant biomarkers shifted to the G^- bacteria biomarker (cy17:0) and actinomycetes biomarker (10Me16:0) at the end of incubation (Fig. S2). As reported by Wang et al., (2015), who pointed out that fungi and actinomycetes are better adapted to nutrient poor environment than bacteria, and maybe contribute less to the enhancement of soil positive PE values. Here, at the 360 day of the incubation, percentages of total actinomycetes and fungi groups were higher in roots amended soil (26.1% for non-labelled PLFA, and 22.3% for ^{13}C-labelled PLFA) than in straw amended soil (24.4% for non-labelled PLFA, and 21.8% for ^{13}C-labelled PLFA). Though distributions of actinomycetes and fungi were lower (<30%) among treatments, the pearson correlation analysis showed that soil PE sensitively respond to the changes in ^{13}C-labelled PLFA of actinomycetes and fungi groups ($r > 0.75$) (Table S1). Thus, we proposed that groups of actinomycetes and fungi contribute to the differences in soil PE values at the end of incubation between straw and roots amendment, possibly based on the 'r-strategist' mechanism.

4.3. Impact of biochar substrates on soil microbial groups

In comparison with the fresh organic substrates, soil microbial community and associated priming effects induced by biochar amendment could be more complex. Soil priming effect depended on many factors, such as the properties of biochar, incubation time and soil properties (Maestrini et al., 2015; Sheng and Zhu, 2018). Due to the pyrolysis process of fresh organic matters in anaerobic environment, the produced biochar material has special physico-chemical properties. Biochar has the porous structure, limited liable C and enhanced available nutrients (AP, AK etc.) contents, and a variety of surface functional groups (Lehmann and Joseph, 2015). All these properties affected both the soil microbial biomass and groups, finally causing differences in soil priming effect (Wang et al., 2016). Though the contribution of ^{13}C-labelled biochar to soil MBC contents was lower (<5%), the special "charsphere" provided habitat, C sources and nutrients for the growth of soil microbes (Maestrini et al., 2015; Sheng and Zhu, 2018; Steinbeiss et al., 2009; Wang et al., 2016). These microbes utilize the limited C sources of biochar and cause negative or positive soil PE values. An 'r-strategist' mechanism can induce the co-metabolizing of the native SOC and liable C sources, thus causing a positive PE. However, Wang et al. (2015) proposed that at the short period a theory of 'preferential substrate utilization' would be responsible for metabolizing the liable C sources, thus causing a negative PE. Here, non-significant negative or positive PE value was observed at the early stage of

incubation (<56 day), we proposed that 'r-strategist' mechanism together with the theory of 'preferential substrate utilization' induce soil priming effects. Negative PE phenomenon is always found after biochar amendment. Reasons are mainly ascribed to: (1) biochar shows a higher capacity in sorbing soil organic matters, thus reducing the available C sources, nutrients for microbes (Zimmerman et al., 2011). (2) biochar releases toxic substances such as VOCs, heavy metals, which is toxicity to microbes (Fernandes and Brooks, 2003; Spokas, 2010). (3) biochar changes the soil physico-chemical properties such as the distributions of soil aggregate, soil pH, which affect the activity of soil microbes (Zheng et al., 2018). (4) changes of biochar surface with incubation time, such as the surface oxidation, directly or indirectly affect the activity of soil microbes (Cheng and Lehmann, 2009; Zimmerman et al., 2011).

Through the analysis of ^{13}C-PLFA, results indicated that G- bacteria (cy17:0 and cy19:0) preferentially utilizes the ^{13}C-labelled biochar but not promoting soil priming effect. Though percentages of G^+ bacteria utilizing the ^{13}C-labelled biochar were lowest among three substrates, its important role in mediating soil priming effect had been reported in this study. Study of Zhang et al. (2018) proposed that ratios of G^+/G^- sensitively respond to biochar properties (temperature, pore structure, and porosity etc.). Here, we observed that changes of G^+/G^- ratios (0.28–0.57 - 0.48) are in accordance with that of soil PE values (non-significant - negative - non-significant). We speculated that G^+ and G^- bacteria preferentially utilize the C sources of biochar (a modified 'preferential substrate utilization' theory), thus causing a peak value of negative PE around the 150 day of incubation. During this stage, we did not deny the co-existence of the mineralization of native SOC. We even proposed that both G^+ and general bacteria cannot adapt to the poor nutrient environment of biochar during 150–360 day of the incubation. Thus, emission of CO_2 from utilization of SOC by G^+ and general bacteria increases and negative PE gradually vanish. In accordance with the straw and roots, contributions of fungi and actinomycetes groups to soil PE decreased with incubation time after biochar amendment. However, its lower percentages in ^{13}C-PLFA indicated that their co-metabolism mechanism gives way to the theory of modified 'preferential substrate utilization'.

5. Conclusion

Significant differences between biochar and its feedstock substrates in soil priming effects and microbial communities were found, after 360 days of the incubation. These differences were not constant but dynamic with incubation period. Straw and roots amendment caused positive soil PE except the first 4 days of the incubation, while significant negative PE caused by biochar amendment is found during 56–150 days of the incubation. Through the RDA and ^{13}C-PLFA analysis, we found that both soil properties (pH, AP, AN and SOC etc.) and the composition of microbial groups affect PE values. Groups of G^+ and general bacteria were dominated in co-metabolizing both the substrate C and SOC after straw and roots amendments, causing positive PE. The differentiation of actinomycetes and fungi groups were responsible for the decreased PE values after straw amendment. G^- bacteria was preferable in utilizing biochar C, causing negative PE; while groups of G^+ and general bacteria were dominated in co-metabolizing both the biochar C and SOC. Their dynamic balance throughout the whole incubation caused the changes in soil PE after biochar amendment. Biochar was best in sequestering soil C pool, followed by straw and then roots from the same plant. However, plant species and the complexity of pyrolysised biochar properties such as temperature, aging time on affecting soil PE still need further study.

CRediT authorship contribution statement

Yi-min Wang: Writing - original draft, Methodology, Writing - review & editing. **Ming Li:** Writing - original draft, Methodology. **Chun-yu Jiang:** Methodology, Data curation. **Ming Liu:** Investigation. **Meng Wu:** Formal analysis. **Ping Liu:** Methodology. **Zhong-pei Li:** Formal analysis, Supervision, Writing - review & editing. **Minori Uchimiya:** Writing - review & editing. **Xu-yin Yuan:** Conceptualization, Methodology.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2020.138562.

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