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Professor Liang is famous in this field. His research interests center on understanding the microbial control over biogeochemical cycles, especially regarding its role in soil carbon (C) and nitrogen (N) turnover and storage. Recently, he has published many related papers. For example:

1. Liang, C., Amelung, W., Lehmann, J., et al. 2019. Quantitative assessment of microbial necromass contribution to soil organic matter. *Global Change Biology* 25, 3578-3590.
2. Liang, C., Schimel, J.P., Jastrow, J.D., 2017. The importance of anabolism in microbial control over soil carbon storage. *Nature Microbiology* 2, 17105.
3. Shao P.S., Liang, C*, Lynch, L., 2019. Reforestation accelerates soil organic carbon accumulation: Evidence from microbial biomarkers. *Soil Biology and Biochemistry* 131, 182-190.

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Professor Ding research interest is soil carbon cycling and biogeochemistry. She recently published several important papers on the

accumulation of microbial residues in soil and its contribution to SOC.
For example:

1. Ding Xueli, Chen Shengyun, Zhang Bin, Liang Chao, He Hongbo, Horwath William R. 2019. Warming increases microbial residue contribution to soil organic carbon in an alpine meadow. *Soil Biology & Biochemistry*, 135, 13-19.
2. Ding Xueli, Zhang Bin, Lü Xinxin, Wang Jingkuan, William R. Horwath. 2017. Parent material and conifer biome influence microbial residue accumulation in forest soils. *Soil Biology & Biochemistry*, 107, 1-9.
3. Ding Xueli, Liang Chao, Zhang Bin, Yuan Yaru, Han Xiaozeng. 2015. Higher rates of manure application lead to greater accumulation of both fungal and bacterial residues in macroaggregates of a clay soil. *Soil Biology & Biochemistry*, 84, 137-146.
4. Ding Xueli, Zhang Bin, Timothy R. Filley, Tian Chunjie, Zhang Xudong, He Hongbo. 2019. Changes of microbial residues after wetland cultivation and restoration. *Biology and Fertility of Soils*, 55, 405-409
5. Ding Xueli, Qiao Yunfa, Timothy Filley, Wang Haiying, Lü Xinxin, Zhang Bin, Wang Jingkuan. 2017. Long-term changes in land use impact the accumulation of microbial residues in the particle-size fractions of a Mollisol. *Biology and Fertility of Soils*, 53, 281-286.

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Her work is concerned with terrestrial biogeochemical cycling of carbon, nitrogen and phosphorus and the dynamics of terrestrial organic matter at several scales. Her recently important publication: Rumpel, C., 2019.

Soils linked to climate change. *Nature* 572(7770):442-443

Dear editor-in-chief,

We are submitting a manuscript entitled “**Microbial assimilation dynamics differs but total mineralization from added root and straw residues is similar in agricultural Alfisols**” as Original Research Papers to *Soil Biology and Biochemistry*. It was prepared by Yingde Xu, Liangjie Sun, Rattan Lal, Roland Bol, Yang Wang, Xiaodan Gao, Fan Ding, Siwei Liang, Shuangyi Li and Jingkuan Wang.

Microbial transformation of crop residue is the key process of soil organic matter (SOM) formation and degradation. In this paper, we conducted a 500-day *in-situ* field experiment using ^{13}C -labeled maize residue (*Zea mays* L.) as returning substrate to soil to study the role of microbial community composition in the C processing affected by soil fertility and residue type. The technique combined phospholipid fatty acid analysis with stable isotope probing (PLFA-SIP) was used to trace the C turnover by soil microbial communities. We found that soil fertility and residue type did not influence the long-term mineralization of residue C. However, soil fertility (soil internal environment) was more important than the residue type (exogenous OM characteristics) in determining the stability of soil microbial community structure, and they both regulated the dynamics of microbial utilization of crop residue C. Specially, root C was more transformed into soil microbial components (e.g. PLFAs), suggesting that microbial products derived from root residue are more likely to contribute to the stable SOM pool than aboveground plants components. Different temporal dynamic patterns of residue C proportion in PLFAs-C pool were also observed between the two residue-amended treatments, indicating different C sequestration mechanism for residue types. Besides, the microorganisms in the low fertility soil had a greater preference for utilizing residue C in their respiration and microbial biosynthesis, comparing to the microorganisms in the high fertility soil. Above all, this study concluded that soil fertility and residue type could regulate the short-term process of microbial mineralizing and sequestering newly added maize residue, but not for the long-term (500 days) apparent mineralization. These results would provide bases for perfecting the mechanisms of SOC sequestration and SOM formation under different soil and residue conditions. We believe that the findings of this study are relevant to the scope of your journal and will be of interest to its readership.

This manuscript has not been published and is not under consideration for publication elsewhere. All the authors have read and approved the final version of the manuscript. There are no conflicts of interest to declare.

Thank you for your consideration, and we look forward to hearing from you at your earliest convenience.

Sincerely,

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Highlights:

1. Microbial utilization of crop residue C was quantified using PLFA-SIP method.
2. Microorganisms in the low fertility soil prefer to utilize residue C.
3. Root C is more easily immobilized by soil microorganisms than straw C.
4. Residue type does not affect microbial community structure.
5. Soil fertility and residue type do not regulate long-term residue C mineralization.

Microbial assimilation dynamics differs but total mineralization from added root and straw residues is similar in agricultural Alfisols

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Abstract

Microbial transformation of crop residue is the key process of soil organic matter (SOM) formation and mineralization, which determines soil fertility and affects global climate change. However, utilization dynamics of residue-derived carbon (residue C) by various microbial communities is still not well understood, especially under different soil fertility and residue quality conditions over a long-term scale (i.e., >1–2 years). In this study, a 500-day *in-situ* field experiment was conducted using ¹³C-labeled maize (*Zea mays* L.) root and straw (both stem and leaf) to examine the role of microbial community composition on the C processing. Specifically, the mineralization of residue C and incorporation of residue C into microbial biomass in low (LF) and high fertility (HF) soils was investigated. The abundance of ¹³C in soil samples and microbial phospholipid fatty acids (PLFAs) were measured at the days of 60, 90, 150 and 500 since the residues added. The results showed that, for the first 150 days, the mineralization rate of residue C was significantly higher in the LF than that in the HF soil, and the straw-derived C was more susceptible to degradation than root-derived C, but the final mineralization rates (~78%) were not significantly different among treatments on the day 500. Soil fertility significantly affected the relative composition of different microbial groups and distribution of residue C in microbial communities, but residue type did not do so. Furthermore, residue C contributed more to PLFA-C pool in the LF than HF soil, and the proportion of root C in PLFA-C pool was higher than the straw C, indicating easier immobilization of root C by soil microbial anabolism. Accordingly, soil fertility and residue quality could both regulate the kinetics of the microbial immobilization of crop residue C, but overall the available residual quantity of applied (plant-derived) C to enhance or maintain soil C pool did not depend on them in a long term in these agricultural Alfisols.

Key words: soil microbial community; C mineralization; ¹³C-PLFA; fertility; residue quality

1. Introduction

Soil organic carbon (SOC) plays an important role in improving soil fertility, moderating climate change, and strengthening ecosystem sustainability (Liang et al., 2017). Concerns have been raised globally about the decline of SOC and degradation of soil fertility in conventionally cropped fields (Lal, 2004; An et al., 2015a). Thus, it is imperative, under the circumstances, to develop soil and crop management practices

that enhance carbon (C) sequestration (Lal, 2004). Crop residues are the main source of soil organic matter (SOM) in agricultural systems (Schmatz et al., 2016), and they have been applied to cultivated fields for centuries to replenish nutrients and maintain SOC stock (Pan et al., 2016). The process governing the transformation of plant residues into SOM depends ultimately on the microbial growth and activity. Thus, understanding mechanisms of transformation of biomass and sequestration of C in soil necessitate better knowledges of how soil microorganisms utilize residue C and regulate residue decomposition.

Soil microbial community is the pivotal controller of residue decomposition, nutrients cycling, and the formation of SOM (Moore-Kucera and Dick, 2008; Schimel and Schaeffer, 2012; Andresen et al., 2014). Incorporation of crop residue may alter the composition and abundance of soil microbial community structure (Bai et al., 2016; Pan et al., 2016). Conversely, soil microorganisms, with diverse life cycle and physiology, have different substrate utilization pattern and metabolic capacity to utilize crop residue (Noah et al., 2007; Andresen et al., 2014). Soil microorganisms can utilize substrate differentially in moderating the two critical functions of regulating terrestrial C fluxes: catabolic respiration which causes residue C mineralization, and anabolic process that results in accumulation of stable C (Liang et al., 2017; Schimel and Schaeffer, 2012). Several studies have documented that crop residue C can be rapidly incorporated into soil microbial biomass, which is in accord with the strong assimilation capacity of microorganisms (Murae et al., 2006). To date, a considerable research has focused on microbial process of residue transformation, and a strong progress has been made in understanding the factors affecting the rate of residue decomposition and formation of SOM (Cotrufo et al., 2015; Schimel and Schaeffer, 2012; Schmidt et al., 2011). However, the role of microbial community on the process of residue C sequestration is still a “black box” because of the use of traditional indirect methods and strong heterogeneity of crop residues and soil conditions.

The structural and chemical characteristics of crop residues (residue quality) strongly control their decomposition process and formation of microbial biomass (de Bruijn and Butterbach-Bahl, 2009; Schmatz et al., 2016). The current understanding of the effect of residue quality on decomposition process is still under debate (Schmidt et al., 2011). In general, root residue with high lignin content, which has the characteristic biological recalcitrance, favors the accumulation of SOC through the modification and condensation of the recalcitrant components by microorganism. This transformation is presumably the primary pathway of SOC sequestration and stabilization (Puget and Drinkwater, 2001; Stevenson, 1982). In

contrast, residues of stem and leaf are more easily degraded by soil microorganisms, leading to more release of CO₂ and less sequestration of organic C in soil (Bertrand et al., 2006). However, some studies focusing on the chemical structure of SOC have demonstrated a relatively low stability of lignin-derived residues in soil (Gentile et al., 2011; Rasse et al., 2005; Schmidt et al., 2011), while labile plant constituents would become the main precursors of stable SOC pool due to high microbial use efficiency (Cotrufo et al., 2013). These studies highlighted the greater importance of residue C processing mediated by soil microorganisms in the long term stabilization of SOC (Gentile et al., 2011; Kong et al., 2011; Dungait et al., 2012). Although these contradictory findings increase the difficulty in understanding mechanisms of soil C sequestration, the important role of soil microorganism as control of C processing is widely recognized. Soil microbial activity is an absolute prerequisite for sequestration of crop residue C. Hence, it is necessary to strengthen knowledge about the mechanism by which microbial communities assimilate residues of different quality and their transformation and synthesis into the SOC pool.

Soil fertility may be another important factor influencing the utilization of crop residues by soil microbial community through its effects on soil biotic and abiotic environment. Previous research has reported that soil fertility strongly influences the SOC stock, nutrient content, and other soil properties (An et al., 2015a; Fontaine et al., 2011), consequently the population, structure, and function of the microbial community in soil (An et al., 2015a; Cui et al., 2018; Mbuthia et al., 2015; Pei et al., 2015). It has been shown that high fertility soil caused by input of organic manure can decrease the relative abundance of fungi (Wei et al., 2013). However, Marschner et al. (2003) demonstrated that high soil fertility could increase relative proportion of bacteria but had no impact on the eukaryotes. The shift of the microbial community can alter a wide range of soil processes and functions, such as processing of the residue C (Kong et al., 2011). An et al. (2015b) documented that the competition of soil microorganisms for residue C as microbial substrate was influenced by the level of soil fertility. Although the literature is replete with the studies conducted to evaluate the changes of soil microbial community affected by soil fertility and its management (Chen et al., 2018; Farmer et al., 2016; Mbuthia et al., 2015), credible information is scanty regarding shifts in microbial groups and its consequences in assimilating residue C into the soil microbial biomass.

Analysis of phospholipid fatty acid, combined with stable isotope probing (PLFA-SIP), is a powerful technique to trace the C cycling pathways during the microbial metabolism of particular substrates

(Helfrich et al., 2015; Murase et al., 2006; Yao et al., 2014; Zhang et al., 2019). However, most previous studies using PLFA-SIP technique have been conducted through laboratory incubation over a short period, typically less than half a year (Arcand et al., 2016; Williams et al., 2006). The scientific knowledge is still limited about the interaction effect of soil fertility and residue quality on exogenous residue C incorporation into soil microbial community under field conditions over a relative long-term basis (two years' cropping period). Thus, the present study was designed to understand the dynamics of utilization of maize residue C by the soil microbial community as affected by soil fertility and residue quality. A 500-day *in-situ* field experiment was conducted at the site of the on-going 29-year old fertilizer management. The ^{13}C labeled maize roots or straws were applied into low or high fertility soils to understand the pathways of C transformation. Abundances of ^{13}C were analyzed in SOC and phospholipid fatty acids (PLFAs) to trace C flow from the decomposing residues into soil by different microbial communities. The study was designed to test the following hypotheses: (1) microorganisms in the HF treatment would assimilate more residue C due to greater microbial activity and higher microbial biomass than in the LF treatment; (2) straw C would be mineralized faster, and the microbial assimilation of straw C would be larger, due to higher liable C content in straw than in root; and (3) due to the strong ability of fungi to produce extracellular depolymerizing enzymes, residue C would contribute more to fungal PLFA than other microbial groups, irrespective of residue qualities and soil fertilizers.

2. Materials and Methods

2.1. Site description

This study was conducted at a long-term fertilizer experiment station (41°49'N, 123°34'E), established in 1987 in Shenyang, Northeast China. The experimental site has a temperate, continental, monsoon climate. The mean annual air temperature is 7.6 °C, the mean annual precipitation is 705 mm, and approximately 85% of it occurs during the months from April to September. The soil of this site is classified as a Hapli-Udic Alfisol according to USDA Taxonomy. Maize (*Zea mays* L.) was sown at 5 cm depth in late April and harvested in later September every year, and farmland management followed conventional tillage. The aboveground part of maize was annually removed from the field.

2.2. Preparation of ¹³C-labeled maize residue

At the seedling, booting and tasseling stage in 2014, twenty maize plants were ¹³C pulse-labeled in a plot at a different location. Briefly, an enclosed transparent chamber (2.2 m long × 0.5 m wide × flexible height) was placed to cover the selected plants, and a micro-environment with high ¹³CO₂ concentration was generated through the reaction between HCl and Na₂¹³CO₃ (99 atom% ¹³C, Sigma-Aldrich). Prior to the generation of ¹³CO₂, fans for air cycling and absorption-bottle filled with sodium hydroxide were attached to the chamber to absorb atmospheric CO₂, and to facilitate ¹³CO₂ assimilation by maize plants. The labeling was done on three consecutive bright and sunny days during each growth stage. A detailed description of the chamber system and labeling process was given by An et al. (2015a). In autumn, straws (leaves and stems) and roots were separately harvested and oven dried at 60 °C to a constant weight. The residues were ground and passed through a 40-mesh sieve for the subsequent *in-situ* experiment. The basic properties of maize residues are presented in Table 1.

2.3. *In-situ* experiment and soil sampling

The *in-situ* experiment treatments consisted of the combination of two soil fertility levels with two types of maize residues plus the non-amended controls, resulting in six treatments: (1) and (2) low fertility soil amended with root (LF+R) or straw (LF+S) residues, (3) and (4) high fertility soil amended with root (HF+R) or straw (HF+S) residues, and (5) and (6) non-amended controls in low fertility (LF) and high fertility (HF) treatments.

The LF and HF treatments used for the present study were selected from the long-term fertilizer experiment: (1) no fertilization (defined as low fertility soil, LF); and (2) fertilization with organic manure (270 kg N ha⁻¹ y⁻¹) combined with chemical N (135 kg N ha⁻¹ y⁻¹) and P (67.5 kg P₂O₅ ha⁻¹ y⁻¹) fertilizer (defined as high fertility soil, HF). Organic manure (pig compost) was applied as basal dose before seeding, which contained 150 g kg⁻¹ total organic C, 10 g kg⁻¹ TN, 10 g kg⁻¹ P₂O₅, and 4 g kg⁻¹ K₂O on a dry weight basis. N and P chemical fertilizers were urea and diammonium phosphate, respectively. The basic properties of soils are presented in Table 1.

Polyvinyl chloride (PVC) containers (length × width × height = 0.4 m × 0.3 m × 0.8 m) were randomly inserted into each plot of the two corresponding fertility level treatments. The top soil (0–20 cm)

was excavated from PVC container and all visible coarse fragments (e.g., roots and stones) were removed. Residue (root or straw, 120 g per container, equaling to 0.5% of dried soil weight) was mixed with the top soil of each container thoroughly on April 28, 2016. Maize seeds were sown in the container, and only one healthy plant was left to grow until the harvest in autumn. During the second year, no maize residues were applied to soils, and other field management practices were the same as those used during the first year. All treatments were arranged in a randomized design with three replicates.

Soil samples were collected on the days of 60 (June 2016), 90 (July 2016), 150 (October 2016), and 500 (September 2017) since the day of residue incorporation. From each PVC container (with and without residue addition), three soil cores were taken with a soil corer (diameter 3.5 cm) from the depth of 0–20 cm and then composited into one sample. Fresh soil samples were transported in an ice bag to the laboratory as soon as possible.

2.4. Soil physicochemical analysis

The SOC, TN and $\delta^{13}\text{C}$ values were analyzed using an EAIRMS (Elementar vario PYRO cube coupled to IsoPrime 100 Isotope Ratio Mass Spectrometer, Germany). The soil pH (soil: water, 1:2.5) was measured using a Thunder Magnetic pH Meter (PHS-3B, Shanghai, China). Soil moisture (SM) content was determined by drying at 105 °C constant weight. The ammonium N and nitrate N were extracted by 2 M KCl, and then measured using the AA3 continuous flow analytical system (Auto-Analyzer 3, SEAL, Germany).

2.5. Soil PLFA analysis

The PLFA extraction, quantification, and $\delta^{13}\text{C}$ analysis were conducted following the procedure of Bossio and Scow (1995), Denef et al. (2007), and Gomez et al. (2014). Briefly, lipids were extracted from 4 g freeze-dried soil in a single-phase chloroform-methanol-citrate buffer solution (1:2:0.8), and transferred into organic phase by mixing with citrate buffer (16 mL) and chloroform (16 mL), and then concentrated under N_2 . Phospholipids were separated from neutral lipids and glycolipids in a standard SPE tube (6 mL, 500 mg, Supelco Inc., Bellefonte, USA) by eluting with chloroform (8 mL), acetone (16 mL) and methanol (8 mL). Phospholipids were collected in methanol elution and concentrated again under N_2 . The collected phospholipids were methylated by dissolving them in 1:1 methanol-toluene and 0.2 M KOH-methanol

solution, derivatizing them to their respective fatty acid methyl esters (FAMES). The FAMES were identified and quantified by an Agilent 7890A GC equipped with MIDI peak identification software (Version 4.5; MIDI Inc., Newark, DE, USA). The $\delta^{13}\text{C}$ values of individual PLFAs were determined using a Thermo Scientific Trace GC Ultra attached to a Finnigan MAT 253 IRMS (CuO/Pt Finnigan MAT Mark I combustion interface maintained at 850 °C).

PLFAs 16:0 and 18:0 occur generally in all living cells (Tavi et al., 2013). PLFAs i15:0, a15:0, i16:0, i17:0 and a17:0 were used as biomarkers for Gram-positive bacteria (Bach et al., 2010); 16:1 ω 7c, 18:1 ω 7c, cy17:0 ω 7c, cy19:0 ω 7c for Gram-negative bacteria (Chen et al., 2018; Pan et al., 2016; Zelles, 1999); 18:2 ω 6c and 18:1 ω 9c for saprophytic fungi, and 16:1 ω 5c for arbuscular mycorrhizal fungi (AMF) (Bach et al., 2010; Olsson, 1999); 10Me 16:0, 10Me 17:0, 10Me 18:0 and 10Me 17:1 ω 7c for actinomycetes (Chen et al., 2018; Pan et al., 2016).

2.6. Calculations

As an additional C atom was added to the fatty acid molecule during the methylation step, the $\delta^{13}\text{C}$ of each PLFA molecule was corrected using the mass balance equation (Dungait et al., 2011; Pan et al., 2016; Tavi et al., 2013):

$$n_{cd}\delta^{13}\text{C}_{cd}=n_c\delta^{13}\text{C}_c+n_d\delta^{13}\text{C}_d \quad (2)$$

where, n_c is the number of C atoms of underivatized compounds, n_d is the number of C atoms of derivatizing agents (Methanol, $n_d=1$), n_{cd} is the number of C atoms of corresponding derivatized compounds, $\delta^{13}\text{C}_c$ is the abundance of ^{13}C of underivatized compounds, $\delta^{13}\text{C}_d$ is the abundance of ^{13}C of derivatizing agents (the $\delta^{13}\text{C}$ value of methanol was -29.33‰ measured by GC/IRMS), and $\delta^{13}\text{C}_{cd}$ is the abundance of ^{13}C of corresponding derivatized compounds.

The proportion of residue C in SOC and PLFAs (F_{residue}) was calculated with the following equation (De Troyer et al., 2011):

$$F_{\text{residue}} = \frac{\delta^{13}\text{C}_{\text{sample}} - \delta^{13}\text{C}_{\text{control}}}{\delta^{13}\text{C}_{\text{residue}} - \delta^{13}\text{C}_{\text{control}}} \times 100 \quad (3)$$

where, $\delta^{13}\text{C}_{\text{sample}}$ represents the $\delta^{13}\text{C}$ value of the C pool for the residue-amended treatments; $\delta^{13}\text{C}_{\text{control}}$ represents the $\delta^{13}\text{C}$ value of the C pool for the control soil (without residue addition); and $\delta^{13}\text{C}_{\text{residue}}$ represents the $\delta^{13}\text{C}$ value for the corresponding residue.

The amount of residue C in each PLFA (A) was calculated with the following equation (Blaud et al., 2012):

$$A = C_{\text{PLFA}} \times F_{\text{residue}} / 100 \quad (4)$$

where, C_{PLFA} is the amount of C in each PLFA.

The amount of residue C remaining in the soil ($A_{\text{incorporated}}$, g) was calculated with the following equation (Li et al., 2015):

$$A_{\text{incorporated}} = W_{\text{soil}} \times F_{\text{residue}} \times C \quad (5)$$

where, W_{soil} represents the weight of soil (0–20 cm) in each plot which calculated through multiplying the volume by the corresponding bulk density; and C (g kg^{-1}) represents the content of SOC of corresponding soil.

The proportion of residue C mineralization (M_{residue}) was calculated with the following equation (Xu et al., 2018a):

$$M_{\text{residue}} = 1 - \frac{A_{\text{incorporated}}}{A_{\text{residue}}} \times 100 \quad (6)$$

where, A_{residue} is the amount of initial C (g) of the maize residue.

2.7. Statistical analysis

All results were reported as means of three replicates with standard deviation. The data were individually compared using the ANOVA and Duncan test in different soils, plant residues, and sampling times. Significance was reported at $P < 0.05$ level. All data were analyzed with SPSS 19.0 statistical software (IBM Corporation, Chicago, USA) and the graphs were drawn using Origin 8 (Origin Lab Corporation, Northampton, USA). Principal component analysis (PCA) was used to describe the soil microbial community structure in different residue type and soil fertility treatments, based on the relative abundance of the individual PLFA in the total PLFAs and distribution of ^{13}C among PLFAs. PCA was carried out with the standard setting in the program CANOCO 4.5 (Cui et al., 2018).

3. Results

3.1. Mineralization of residue C

The residue C mineralization sharply increased during the initial 60 days and then slowly increased

during the later experiment (Figure 1). The LF treatment significantly increased ($P<0.05$) residue mineralization rate as compared to the HF treatment apart from the day 500. Overall, a larger mineralization of residue C was observed in straw-amended treatment compared to the corresponding root-amended treatment, but the difference was only significant ($P<0.05$) on the day 60 and day 150. After 500 days of experiment, the total residue C mineralization was approximately 78% relative to initial residue C, which was neither affected by soil fertility nor by residue type ($P>0.05$).

3.2. Kinetics of soil PLFAs and microbial community structure

Maize residue incorporation significantly increased the total PLFAs amount for the entire experiment period ($P<0.05$, Figure 2). The increase of the total PLFAs amount by residue addition in the HF soil was greater than that in the LF soil after 60 days. The total PLFAs amounts of all the residue treatments increased until the day 150 and then decreased. Nevertheless, total PLFAs amount remained essentially unchanged for the controls (without residue addition). Averaged across the sampling events, higher PLFAs amount was measured in the HF (37.5 nmol g⁻¹ dry soil) compared with the LF treatments (25.5 nmol g⁻¹ dry soil, $P<0.05$). The total PLFAs amounts in straw-amended treatments were slightly higher than that in root-amended treatment before 150 days, while significantly higher total PLFAs amounts were observed in root-amended treatment at the end of the experiment ($P<0.05$).

Across the four sampling times, the overall trends in the PLFA contents of different microbial groups were similar with total PLFAs (see Supporting Material; Table S1). The average relative proportions of the different microbial PLFA groups decreased in the following order: Gram-positive (30.0%) > Gram-negative (22.5%) > general (18.5%) > actinomycete (15.1%) > fungi (9.7%) > AMF (4.2%) ($P<0.05$, see Supporting Material; Figure S1). In comparison with the controls, residue addition increased the proportion of fungal PLFA but decreased the proportions of Gram-positive bacterial and actinomycete PLFA, but the relative abundances of microbial groups were not affected by the residue types ($P>0.05$). The Gram-negative bacterial PLFA proportion were higher in the LF treatment than in the HF treatment, but Gram-positive bacterial PLFA had an opposite trend. Meanwhile, the relative abundances of the microbial groups did not changed with experimental time, apart from some fluctuations in actinomycete PLFA.

The PCA analysis showed that PC1 and PC2 explained 45.5% and 24.7% of the total variation, respectively (Figure 3). Residue-amended treatments had shifted the microbial community structure to a

distinct position from their controls (dotted ellipse and solid ellipse), but root and straw treatments had similar microbial community structure. The LF and HF treatments also clearly separated from each other (red ellipse and blue ellipse). In residue-amended treatments, factor loadings of PLFAs suggested that the PLFAs 18:1 ω 7c, cy17:0 ω 7c, 18:1 ω 9c, 18:2 ω 6c and 16:1 ω 5c could explain the main microbial changes in the LF treatments, which are considered as indicators of Gram-negative bacteria, fungi and AMF (arrows laid on the left side of the plot). The PLFAs i15:0, i16:0 and cy19:0 ω 7c could explain the main microbial changes in the HF treatments, which are considered as indicators of Gram-positive bacteria and Gram-negative bacteria (arrows laid on the right side of the plot).

3.3 Incorporation of residue C into PLFAs

Soil fertility and residue type significantly affected the contribution of residue C to total PLFA-C pool (Figure 4). During the 500-day experiment, the proportion of residue C in total PLFA-C in the LF soil was 41.3% greater than that in the HF soil ($P<0.05$). The root amendment resulted in a higher residue-C percentage in PLFA-C than straw amendment in most sampling events. Moreover, different temporal dynamic patterns were observed between the two residue-amended treatments, i.e., fraction of residue C in straw-amended treatment sharply decreased before 150 days and remained essentially unchanged afterward, but in root-amended treatment, residue C percent sharply decreased before 90 days, and increased to the highest level on the day 150 and then showed a decrease at the end of the experiment. Averaged across the sampling times, the contribution of residue C to fungal PLFA (23.4%) was the highest under all treatments (Table S2).

The temporal dynamics of total residue-derived PLFA-C content (PLFA-C_{new}) was consistent with its proportion in total PLFA-C (Figure 5). The differences among treatments were not significant ($P>0.05$) before 90 days except for the significant lower value under HF+R on the day 60 ($P<0.05$). After 90 days, the total PLFA-C_{new} content was significantly greater in root amendment than that in straw amendment ($P<0.05$). PLFA-C_{new} content of LF+R and HF+R treatments reached their peak on day 150, with values of 162.8 nmol g⁻¹ dry soil and 207.6 nmol g⁻¹ dry soil, respectively. At the end of experiment, Gram-positive bacterial PLFA signatures contributed by residue C (on average of 25.0 nmol g⁻¹ dry soil) were the most abundant in all treatments, and the least was AMF PLFAs (3.5 nmol g⁻¹ dry soil) under all treatments (Table S3).

The relative residue C distribution showed that ~50% of PLFA-C derived from the maize residue were incorporated into Gram-positive bacteria and Gram-negative bacteria (26.8% and 23.6%, respectively) across the sampling events (Figure S2), followed by fungi (16.1%), general (16.1%), actinomycete (13.7%) and AMF (3.7%). Residue C distributed more in Gram-negative bacterial PLFA in the LF soil than that in the HF soil, but it distributed more in actinomycete PLFA in the HF soil than that in the LF soil. Although root C contributed more to total PLFA-C than those from straw (Figure 4), relative distribution of residue C had not showed any regular trend under root and straw treatments during the whole experiment period.

The PCA analysis, based on the distribution of residue C among PLFAs showed that PC1 and PC2 explained 55.8% and 25.1% of the total variation, respectively (Figure 6). Besides, treatments are roughly separated by sampling time but not by soil fertility and residue type. Overall, highly related microbial community shifted from general and Gram-negative bacteria to Gram-positive bacteria and actinomycete over time.

4. Discussion

4.1 Soil microbial structure dynamics

In the present study, residue incorporation significantly increased PLFAs contents (Figure 2, Table S1), and a similar trend was reported by others (Kong et al., 2011; Pan et al., 2016; Tavi et al., 2013). All peak values of PLFAs content appeared on the day 150, but some other studies observed a maximum soil microbial biomass typically less than 7 days after residue addition (Arcand et al., 2016; Bai et al. 2016). Such a few-days quick peak was not observed in the present study because first sampling was done in the second month of experiment. Studies usually attributed the quick peak to the enriched labile components from added residue during the initial stage of decomposition (Pan et al., 2016). However, after that, when recalcitrant components became the main C source for microbial utilization instead of the easily using substrates, microbial community might adjust their strategy to consuming enriched recalcitrant substrates leading microbial PLFAs to reach a peak at day 150.

The magnitude of the microbial community responded to crop residue addition varied among soil fertilities and residue types (Figure 2, Table S1). Results obtained in this study indicated that the increase of microbial biomass in the HF soil was greater than that in the LF soil after residue input, suggesting that

residue addition has a longer effective impact on microorganism growth in the HF soil due to higher microorganism background values and N content (Table 1). Besides, root and straw residues had different stimulating effects on soil microbial biomass at different decomposition periods. Shahbaz et al. (2017) reported that the leaf and stem were relatively high quality residues with more readily decomposable components, thus, straw could promote microorganism growth within a short period after residue incorporation. Based on the results presented herein, there was a stronger stimulation from straw during the period when recalcitrant components gradually became dominant substrate for microorganisms (60–150 days), and root had a stronger role in promoting microbial biomass after the peak value appeared on day 150. This suggested that although the amount of the labile C reduced over time, it had a legacy effect on microbial growth until the recalcitrant C completely dominated the microbial biomass, but the availability of root C would exceed straw C eventually with the continuation of decomposition.

Maize residue incorporation altered soil microbial community structure. The proportion of fungal PLFA increased and the proportion of Gram-positive bacterial and actinomycete PLFA decreased after residue addition (Figure S1), suggesting that fungal community were relatively sensitive to the maize residue decomposition. This phenomenon was attributed to the predominant role of fungi in decomposing cellulose and hemicellulose, the primary components of maize residue (Pan et al., 2016; Six et al., 2006). Besides, we found that relative abundance of Gram-negative bacterial PLFA was higher in the LF treatment whereas Gram-positive bacterial PLFA fraction was higher in the HF treatment. One explanation for this trend was that the soil fertility could lead to the change of microorganisms with different strategies (Cui et al., 2018). It has also been suggested that high fertility soil induced by long-term organic and chemical fertilizer applications was more conducive to increasing *K*-strategy microorganisms (Xu et al., 2018b). In contrast, however, other researchers have suggested that *r*-strategy microorganisms in C limitation soil could strengthen the decay of plant residue to meet their energy demands (Cui et al., 2018), and these may correspond to Gram-positive and Gram-negative bacteria in our study, respectively. PCA analysis results also indicated that Gram-negative bacterial PLFA and Gram-positive bacterial PLFA enriched in the LF and HF soil, respectively (Figure 3).

Despite the fact that residue addition changed soil microbial biomass and microbial community structure, residue type did not significantly influence the relative abundance of each PLFA (Figures S1 and 3). Possible reason was that root residue had a lower C/N ratio in this study, which could have offset the

disadvantage of root having a higher lignin content. Presumably, the heterogeneity between the different residues in the present experiment was much smaller than that of SOM in soil, as a result, the residue quality variation might not be sufficient to cause competition between soil microbial communities which could adapt to the heterogeneous SOM. Besides, some studies demonstrated that the initial residue quality may strongly affect the spatial heterogeneity and composition of some specific species of bacteria or fungi based on DNA stable isotope probing (España et al., 2011; Nicolardot et al., 2007). The data presented herein support a conclusion that residue type might alter the abundance of certain microbial species, but not the microbial community composition from the aspect of whole groups of bacteria and fungi, etc.

4.2 Microbial incorporation of residue C

The data showed that residue C contributed to PLFA-C pool to varying degrees and as affected by both residue quality and soil fertility (Figure 4, Table S2). Despite the fact that low microbial biomass and activity limited the microbial incorporation of residue C in the LF soil, the percentage of residue C distributed to PLFA-C pool was significantly higher than that in the HF soil. The possible reason was that microorganisms in the LF soil with limited C source and nutrient had an urgent need and were in a sense always waiting for available substrates, which could be provided by the newly added plant residues (Fontaine et al., 2003). Thus, microorganisms shifted to immobilize the added residue C to satisfy their growth requirements. On the other hand, the LF soil had higher clay content in the study site (An et al., 2015a), and this may increase the contact between substrate and microorganisms. Conversely, the lower proportion of residue C incorporated in PLFAs in the HF soil may be the result of more C source choice from SOM at the beginning. Therefore, the data supported a conclusion that the microorganisms in the LF soil with a limited SOM content had a greater preference for utilizing residue C.

However, the PLFA-C_{new} content was not significantly affected by soil fertility especially during the early stages of the experiment period (Figure 5, Table S3). The result was mainly due to the higher total PLFAs amount possessed by the HF soil, but lower proportion of residue C to total PLFA-C, which made an offset leading to the situation of the no significant difference of PLFA-C_{new} content. Subsequently, however, the HF soil could be more favorable for soil microbial biosynthesis because of the higher N content than that in the LF soil (Moore-Kucera and Dick, 2008). On day 150 for root-amended treatment, the PLFA-C_{new} content in the HF soil was significantly higher than that in the LF soil, suggesting that the

microbial utilization of residue C was regulated simultaneously both by soil fertility and residue quality. These results partially confirmed the first hypothesis, i.e., microorganisms in the HF treatment could assimilate more residue C than those in the LF treatment. It was also pertinent to highlight that the residue C sequestration efficiency of microorganisms in the LF soil was higher, but the total residue C sequestration amount of microorganisms in the HF soil might exceed that of LF soil.

The data presented herein also showed that the proportion and content of root C in PLFAs were both higher than straw C especially after 90 days (Figure 4, 5). Hence, the second hypothesis that the assimilation of residue C would be higher in straw treatment was not supported. Similar findings were reported under field conditions by Williams et al. (2006) and Kong et al. (2011), and attributed the result to the microbial preference for root-related substrates during the growing season when the root exudation and the sloughing of root cells thrive in soil, which were all ^{13}C labelled in their experiment. In the present experiment, only labelled root residue, but no labelled root exudation or root cells, were present in the soil. Yet, there was more root C in PLFAs than the straw C. This trend suggested that the growth of soil microbial communities depended more on the root substrate than on the aerial part substrate during the growing season. Further, microbial communities living in soil during the growing season was primarily comprised of those who adapt to consuming the root substrates because of much more prevalence of root substrate than that of leaf or stem substrate during this period.

The different temporal dynamic patterns of residue C proportion in PLFAs-C pool between the two residue-amending treatments were observed in the present study (Figure 4). The continuously decreasing proportion of straw C suggested the reduction in substrates available to microorganisms, whereas the occurrence of the peak of root C incorporated into the PLFAs on the day 150 indicated that recalcitrant C became the prominent C source for soil microorganism as the decomposition progressed (Abiven et al., 2005). As mentioned in the section 4.1, the PLFAs contents in both the root and straw treatments reached the peak value on day 150. But the mechanism of this peak seemed to be different based on the proportion of the residue C. In the straw treatment, although its contribution to PLFAs-C pool gradually decreased, the remaining recalcitrant C still significantly stimulated the growth and activity of microorganisms (native SOC was the main C source). In contrast, the effect of root treatment on microbial growth not only came from the stimulating effect of the C substrate, but also contributed more to the microbial biosynthesis (native SOC and root C were both the main C sources).

The residue C was not evenly appropriated among diverse microbial PLFA-C pools in this study indicating that soil microorganisms differed in their assimilation and utilization of residue C. The residue C seemingly contributed the most to fungal PLFA (Table S2) (especially 18:2 ω 6c, data not shown), which supports the third hypothesis, implying that fungi were more efficient user of the residue-derived compounds compared to other microbial groups. The reason might be that fungi could produce different extracellular depolymerizing enzyme and absorb nutrients at the soil-residue interface through their filaments (Acosta-Martínez et al., 2008), which enhance their ability to compete for maize residue. However, in contrast to the most contribution of residue C to fungi, it was bacteria that mobilized the most amount of residue C due to its large biomass, based on the result that 50% of PLFA-immobilized residue C distributed in bacterial PLFA (Figure S2) (mainly in i15:0, data not shown). The results indicated that though fungi had stronger ability to assimilate residue C, bacteria were the primary consumer of residue C due to their higher biomass.

In the present study, residue C distributed more in Gram-negative bacterial PLFA in the LF treatment, while it distributed more in actinomycete PLFA in the HF treatment (Figure S2), demonstrating that the utilization process of residue C by different microbial communities was regulated by soil fertility. However, the distribution of residue C in microbial communities did not change significantly between root and straw treatments (Figure S2). Crop residue consisted of a range of recalcitrant biopolymers (Pan et al., 2016), which could be consumed by a range of decomposers in soil. Consequently, the whole soil microbial community with diverse decomposers might have formed strong adaptability to use different kinds of residues (root or straw) (Helfrich et al., 2015). Similarly, although the chemical composition of the residues changed during the decomposition, soil microbial communities could still maintain their own fraction of residue C in a stable state. The above results showed that the synergism among diverse microbial communities moderated the distribution of residue C as it was unaffected by characteristics of exogenous organic matter such as the residue quality.

4.3 Linking C processing with soil microbial community

The results showed that the mineralization of residue C was higher in the LF soil than that in the HF soil except on the day 500 (Figure 1). This phenomenon could be explained by the different composition of substrates in the LF and HF soils. The LF soil was deficient in organic matter for energy production and N

nutrient (Table 1). In this case, soil microorganisms would preferentially choose the easily digestible substrate, newly added maize residues, to meet their growth requirements. Similarly, reduced C mineralization in the HF soil might be the result of abundant-C-sources environment even before the residue addition. Compared with HF treatment, more residue C was mineralized by microorganisms to produce energy (and lost as CO₂) in the LF soil, and contributed to microbial growth as PLFA-C, combining the result of incorporation of residue C in PLFAs (Figure 4, 7). This trend indicated that the microorganisms in the LF soil were more eager for the extra C substrate from residue, both catabolically and anabolically (Figure 7). Besides, plant root C was mineralized slower than was the straw C generally because of differences in their chemical composition (Abiven et al., 2005). These trends were confirmed in the present study from the result before 500 days, but root C was more susceptible to become microbial component as PLFAs was also observed (Figure 4), suggesting that root residue may be more likely to contribute to SOM in the form of microbial products (Figure 7).

However, the data also showed that soil fertility and residue type did not affect the total residue C mineralization amount by the end of the experiment (Figure 1). A potential reason for this phenomenon might be that the available components in the straws were reduced more rapidly, which could also be implied by the results of continuously decreasing of PLFA-C_{new} content (Figure 5). In contrast, root residue with complex structure would stimulate microbial activity continuously during the later decomposition period though the liable components were depleted, thereby, its mineralization amount was gradually equal to that in the straw treatment. For soils of different fertility, the LF soil with lower microbial biomass and activity probably reduced residue C mineralization more than in the HF soil when the available components were exhausted. Taking all together, soil fertility and residue type might not be determinants of long-term apparent mineralization and sequestration of residue C (Figure 7). Recent observations highlighted physical protection and sorption mechanisms rather than intrinsic biochemical recalcitrance of C played more important roles in controlling long term SOC stabilization, which was also in accord with the results presented herein (Lehmann and Kleber, 2015). Therefore, soil fertility and residue quality could regulate most of the residue degradation process, but the threshold for the final sequestration of residue C in soil might be determined only by the protective capacity of soil particles (Wang et al., 2017).

At the end of experiment, approximately 78% of residue C was lost by soil microbial respiration, and residue C found in any individual PLFA accounted for only a small fraction. Accordingly, although a large

part of the residues could be used by soil microorganisms, only a small fraction was eventually allocated to the biomass of microorganisms, and most was consumed as energy substrates. Besides, it should be noted that lower straw C was detected in PLFAs but total mineralization rates of root- and straw C were similar, which indicated that more straw C had been directly deposited in the soil without or after microbial utilization (Figure 7). We emphasized that although the residue C mineralization (or sequestration) rates among all residue-amended treatments were similar, the transformation routes and sequestration mechanisms of different residues in soils were different (Figure 7).

5. Conclusions

Soil fertility and residue type did not influence the long-term apparent mineralization and sequestration of residue C. However, soil fertility (soil internal environment) was more important than the residue type (exogenous OM characteristics) in determining the stability of soil microbial community structure, and they both regulated the dynamics of microbial utilization of crop residue C. Microorganisms in the LF soil had a greater preference for utilizing residue C than that in the HF soil. The straw C was more susceptible to microbial mineralization, whereas the root C contributed more to soil microbial components (e.g. PLFAs), and more likely contributed to the stable SOM pool.

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646 **Tables**

647 **Table 1** Basic properties of soil samples and ^{13}C labeled maize residues.

	Total organic C (g kg ⁻¹)	TN (g kg ⁻¹)	$\delta^{13}\text{C}$ values (‰)	ammonium N (mg kg ⁻¹)	nitrate N (mg kg ⁻¹)	C/N	total PLFAs (nmol g ⁻¹)	pH (H ₂ O)	Lignin (g kg ⁻¹)
LF soil	11.2	1.1	-	12.5	33.0	10.2	22.7	6.0	-
HF soil	17.6	2.2	-	13.5	198.3	8.0	30.4	5.7	-
Root	435.6	15.1	392.0	-	-	28.8	-	-	135.3
Straw	443.9	14.0	699.6	-	-	31.7	-	-	71.4

648 Symbols LF and HF represent the soils with low and high fertility, respectively.

Figures captions

Figure 1 The proportion of residue C mineralization relative to the initial added residue C (%) over 500 days experiment. Symbols LF and HF represent the soils with low and high fertility, respectively. R and S represent root and straw residues, respectively. Error bars represent the standard deviations from the mean (n=3).

Figure 2 Sum of PLFAs (nmol g⁻¹ dry soil) under different treatments over 500 days experiment. Symbols LF and HF represent the soils with low and high fertility, respectively. R and S represent root and straw residues, respectively. Error bars represent the standard deviations from the mean (n=3).

Figure 3 Principle component analysis (PCA) based on the relative proportion of individual PLFA for different treatments. The numbers next to the symbols represent sampling times. Symbols LF and HF represent the soils with low and high fertility, respectively. R and S represent root and straw residues, respectively.

Figure 4 Contribution of residue C to total PLFA-C pool (%) for different treatments over 500 days experiment. Symbols LF and HF represent the soils with low and high fertility, respectively. R and S represent root and straw residues, respectively. Error bars represent the standard deviations from the mean (n=3).

Figure 5 Sum of residue C content within PLFAs (nmol g⁻¹ dry soil) for different treatments over 500 days experiment. Symbols LF and HF represent the soils with low and high fertility, respectively. R and S represent root and straw residues, respectively. Error bars represent the standard deviations from the mean (n=3).

Figure 6 PCA analysis based on the distribution of residue C among PLFAs for different treatments. The numbers next to the symbols represent sampling times. Symbols LF and HF represent the soils with low and high fertility, respectively. R and S represent root and straw residues, respectively.

Figure 7 Schematic representation of microbial process of residue C transformation. Symbols LF and HF represent the soils with low and high fertility, respectively. The blue triangles represent accumulative

674 mineralization rates of residue C at a certain stage of residue decomposition. The blue circles represent
675 amounts of PLFA-C_{new} derived from maize residues. The green rectangles represent the total microbial
676 biomasses. The green and yellow ellipses represent the bacterial and fungal biomasses, respectively. The
677 number or size of the geometric figures (e.g., triangle, circle, and rectangle) represent the amounts of
678 responding indicators.

Figure1

Figure 1

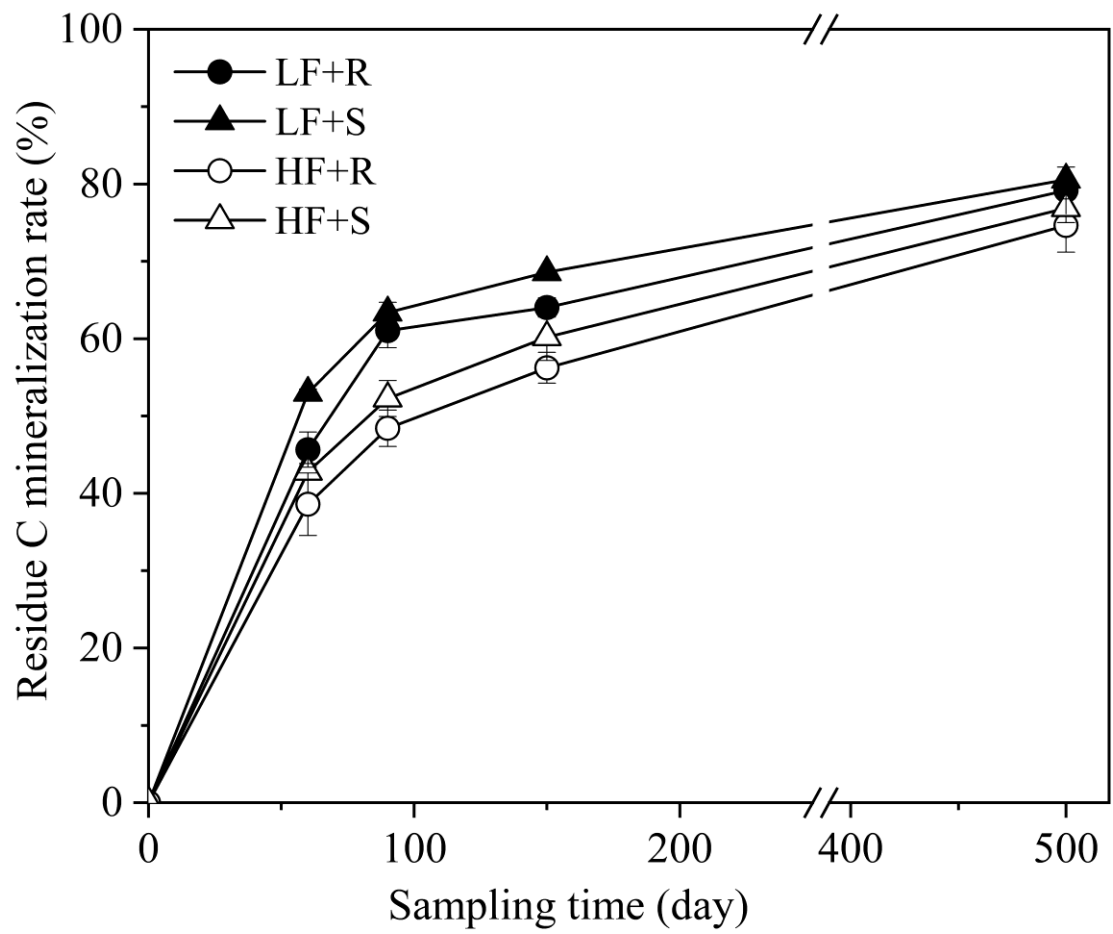


Figure2

Figure 2

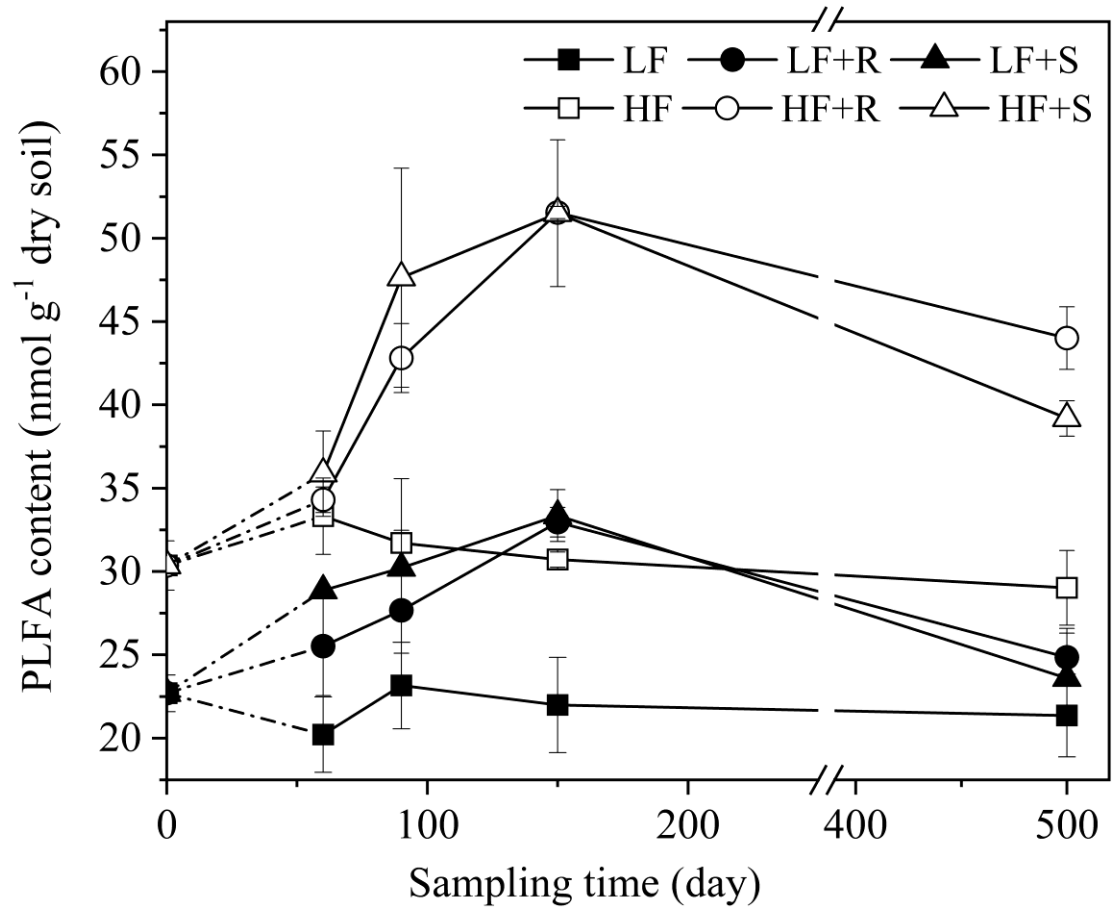


Figure3

Figure 3

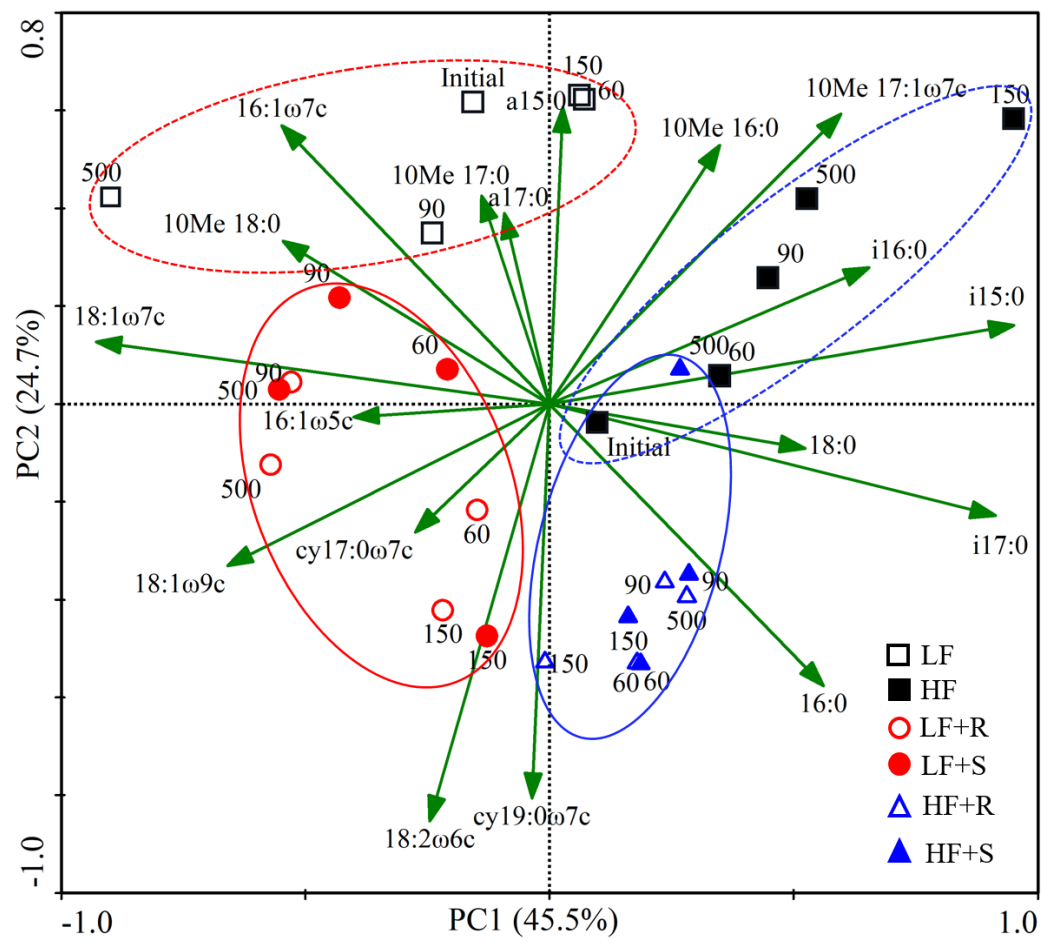


Figure4

Figure 4

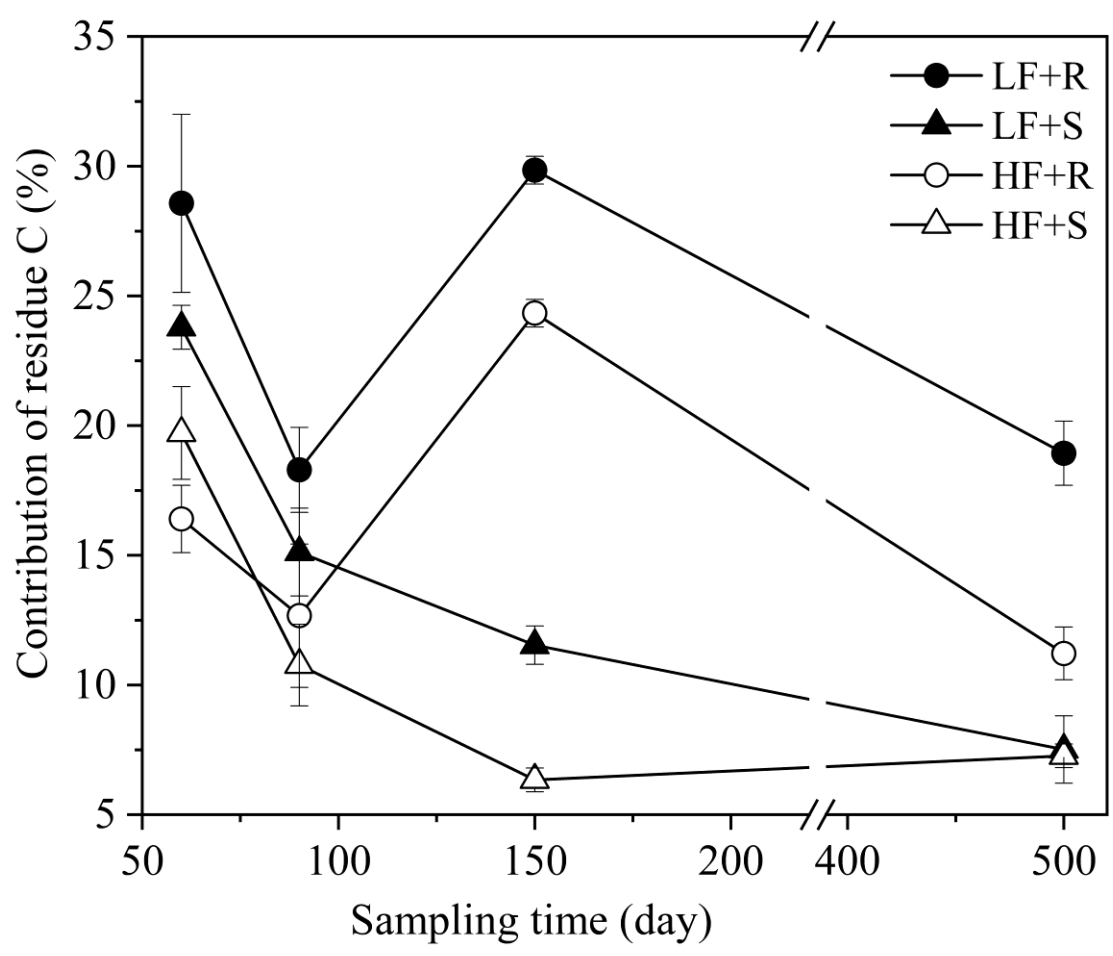


Figure5

Figure 5

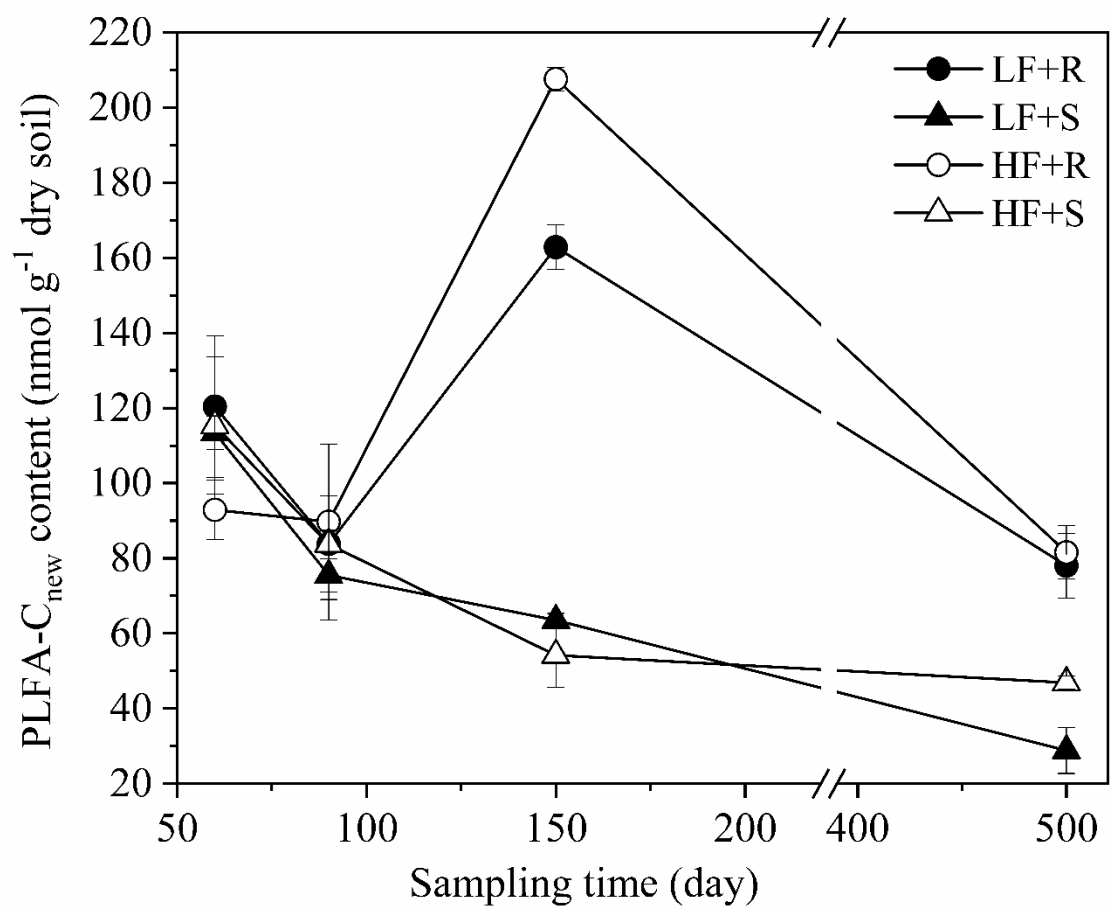


Figure6

Figure 6

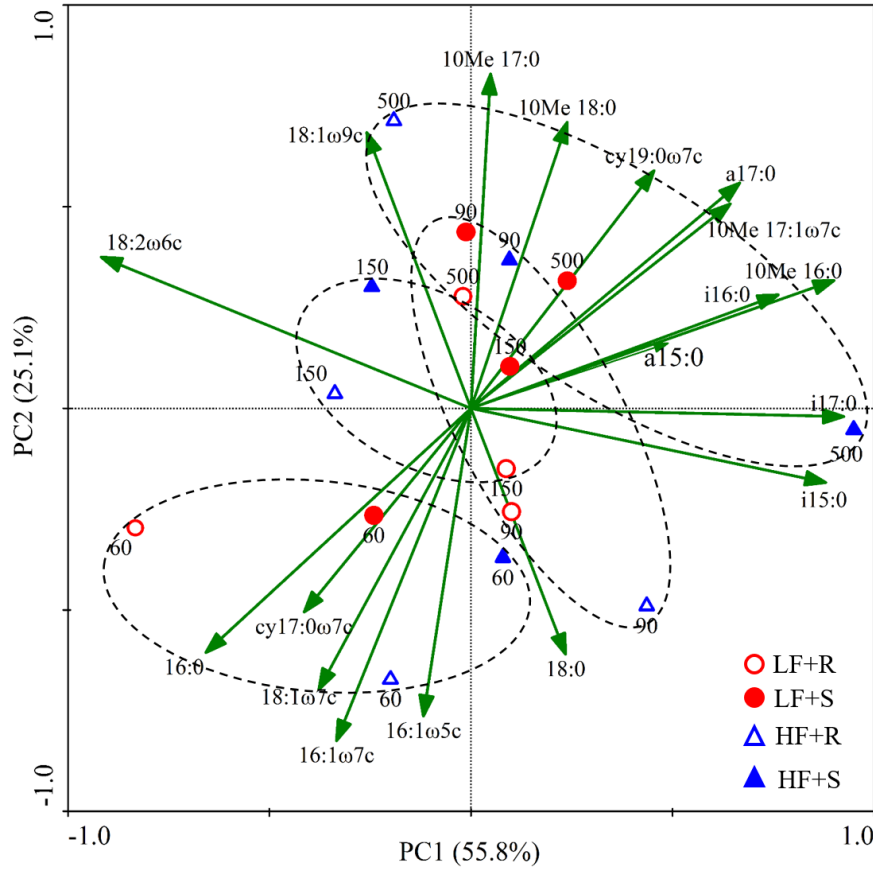
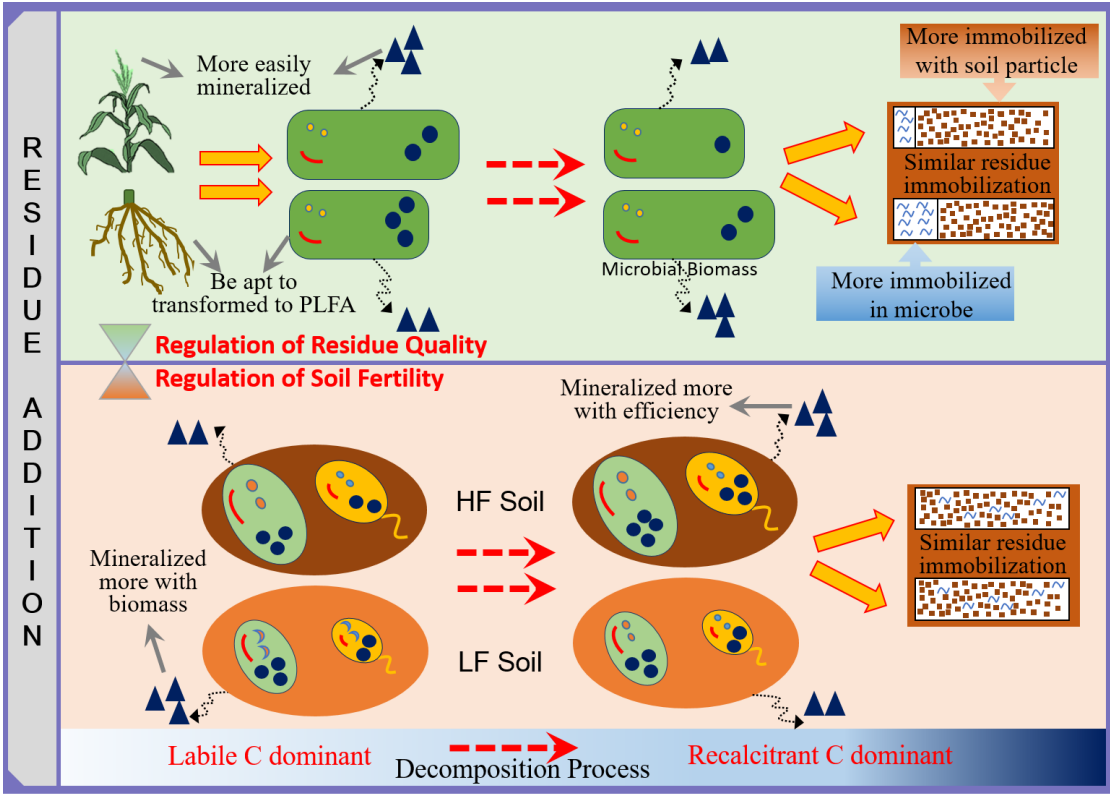


Figure7

Figure 7



Declaration of interests

☒ 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.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

None