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

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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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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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Covering Letter

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 sequestrating 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,

Dr. Liangjie Sun (College of Land and Environment, Shenyang Agricultural University; Tel: +86(24) 15204080038; E-mail: sunlj@syau.edu.cn, sunlj999@126.com)

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.

1 **Microbial assimilation dynamics differs but total mineralization from**

2 **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 ^{13}C-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 ^{13}C 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; ^{13}C-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 (Murase 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

86   contrast, residues of stem and leaf are more easily degraded by soil microorganisms, leading to more

87   release of CO_2 and less sequestration of organic C in soil (Bertrand et al., 2006). However, some studies

88   focusing on the chemical structure of SOC have demonstrated a relatively low stability of lignin-derived

89   residues in soil (Gentile et al., 2011; Rasse et al., 2005; Schmidt et al., 2011), while labile plant constituents

90   would become the main precursors of stable SOC pool due to high microbial use efficiency (Cotrufo et al.,

91   2013). These studies highlighted the greater importance of residue C processing mediated by soil

92   microorganisms in the long term stabilization of SOC (Gentile et al., 2011; Kong et al., 2011; Dungait et al.,

93   2012). Although these contradictory findings increase the difficulty in understanding mechanisms of soil C

94   sequestration, the important role of soil microorganism as control of C processing is widely recognized.

95   Soil microbial activity is an absolute prerequisite for sequestration of crop residue C. Hence, it is necessary

96   to strengthen knowledge about the mechanism by which microbial communities assimilate residues of

97   different quality and their transformation and synthesis into the SOC pool.

98   Soil fertility may be another important factor influencing the utilization of crop residues by soil

99   microbial community through its effects on soil biotic and abiotic environment. Previous research has

100   reported that soil fertility strongly influences the SOC stock, nutrient content, and other soil properties (An

101   et al., 2015a; Fontaine et al., 2011), consequently the population, structure, and function of the microbial

102   community in soil (An et al., 2015a; Cui et al., 2018; Mbuthia et al., 2015; Pei et al., 2015). It has been

103   shown that high fertility soil caused by input of organic manure can decrease the relative abundance of

104   fungi (Wei et al., 2013). However, Marschner et al. (2003) demonstrated that high soil fertility could

105   increase relative proportion of bacteria but had no impact on the eukaryotes. The shift of the microbial

106   community can alter a wide range of soil processes and functions, such as processing of the residue C

107   (Kong et al., 2011). An et al. (2015b) documented that the competition of soil microorganisms for residue C

108   as microbial substrate was influenced by the level of soil fertility. Although the literature is replete with the

109   studies conducted to evaluate the changes of soil microbial community affected by soil fertility and its

110   management (Chen et al., 2018; Farmer et al., 2016; Mbuthia et al., 2015), credible information is scanty

111   regarding shifts in microbial groups and its consequences in assimilating residue C into the soil microbial

112   biomass .

113   Analysis of phospholipid fatty acid, combined with stable isotope probing (PLFA-SIP), is a powerful

114   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 fertilizes.

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 ℃, 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 $^{13}CO_2$ concentration was generated through the reaction between HCl and $Na_2^{13}CO_3$ (99 atom% ¹³C, Sigma-Aldrich). Prior to the generation of $^{13}CO_2$, fans for air cycling and absorption-bottle filled with sodium hydroxide were attached to the chamber to absorb atmospheric CO_2, and to facilitate $^{13}CO_2$ 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 ℃ 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 P_2O_5 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} P_2O_5, and 4 g kg^{-1} K_2O 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)

168 was excavated from PVC container and all visible coarse fragments (e.g., roots and stones) were removed.

169 Residue (root or straw, 120 g per container, equaling to 0.5% of dried soil weight) was mixed with the top

170 soil of each container thoroughly on April 28, 2016. Maize seeds were sown in the container, and only one

171 healthy plant was left to grow until the harvest in autumn. During the second year, no maize residues were

172 applied to soils, and other field management practices were the same as those used during the first year. All

173 treatments were arranged in a randomized design with three replicates.

174 Soil samples were collected on the days of 60 (June 2016), 90 (July 2016), 150 (October 2016), and

175 500 (September 2017) since the day of residue incorporation. From each PVC container (with and without

176 residue addition), three soil cores were taken with a soil corer (diameter 3.5 cm) from the depth of 0–20 cm

177 and then composited into one sample. Fresh soil samples were transported in an ice bag to the laboratory as

178 soon as possible.

179 2.4. Soil physicochemical analysis

180 The SOC, TN and $\delta^{13}C$ values were analyzed using an EAIRMS (Elementar vario PYRO cube

181 coupled to IsoPrime 100 Isotope Ratio Mass Spectrometer, Germany). The soil pH (soil: water, 1:2.5) was

182 measured using a Thunder Magnetic pH Meter (PHS-3B, Shanghai, China). Soil moisture (SM) content

183 was determined by drying at 105 °C constant weight. The ammonium N and nitrate N were extracted by 2

184 M KCl, and then measured using the AA3 continuous flow analytical system (Auto-Analyzer 3, SEAL,

185 Germany).

186 2.5. Soil PLFA analysis

187 The PLFA extraction, quantification, and $\delta^{13}C$ analysis were conducted following the procedure of

188 Bossio and Scow (1995), Denef et al. (2007), and Gomez et al. (2014). Briefly, lipids were extracted from 4

189 g freeze-dried soil in a single-phase chloroform-methanol-citrate buffer solution (1:2:0.8), and transferred

190 into organic phase by mixing with citrate buffer (16 mL) and chloroform (16 mL), and then concentrated

191 under N_2. Phospholipids were separated from neutral lipids and glycolipids in a standard SPE tube (6 mL,

192 500 mg, Supelco Inc., Bellefonte, USA) by eluting with chloroform (8 mL), acetone (16 mL) and methanol

193 (8 mL). Phospholipids were collected in methanol elution and concentrated again under N_2. The collected

194 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 δ^{13}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); 16:1ω7c, 18:1ω7c, cy17:0ω7c, cy19:0ω7c for Gram-negative bacteria (Chen et al., 2018; Pan et al., 2016; Zelles, 1999); 18:2ω6c and 18:1ω9c for saprophytic fungi, and 16:1ω5c for arbuscular mycorrhizal fungi (AMF) (Bach et al., 2010; Olsson, 1999); 10Me 16:0, 10Me 17:0 , 10Me 18:0 and 10Me 17:1ω7c 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 δ^{13}C of each PLFA molecule was corrected using the mass balance equation (Dungait et al., 2011; Pan et al., 2016; Tavi et al., 2013):

$$n_{cd}\delta^{13}C_{cd}=n_c\delta^{13}C_c+n_d\delta^{13}C_d \quad (2)$$

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, $\delta^{13}C_c$ is the abundance of ^{13}C of underivatized compounds, $\delta^{13}C_d$ is the abundance of ^{13}C of derivatizing agents (the δ^{13}C value of methanol was −29.33‰ measured by GC/IRMS), and $\delta^{13}C_{cd}$ 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):

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

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

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

223   2012):

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

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

226   The amount of residue C remaining in the soil ($A_{incorporated}$, g) was calculated with the following

227   equation (Li et al., 2015):

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

229   where, W_{soil} represents the weight of soil (0–20 cm) in each plot which calculated through multiplying

230   the volume by the corresponding bulk density; and C (g kg^{-1}) represents the content of SOC of

231   corresponding soil.

232   The proportion of residue C mineralization ($M_{residue}$) was calculated with the following equation (Xu et

233   al., 2018a):

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

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

2.7. Statistical analysis

237   All results were reported as means of three replicates with standard deviation. The data were

238   individually compared using the ANOVA and Duncan test in different soils, plant residues, and sampling

239   times. Significance was reported at $P<0.05$ level. All data were analyzed with SPSS 19.0 statistical

240   software (IBM Corporation, Chicago, USA) and the graphs were drawn using Origin 8 (Origin Lab

241   Corporation, Northampton, USA). Principal component analysis (PCA) was used to describe the soil

242   microbial community structure in different residue type and soil fertility treatments, based on the relative

243   abundance of the individual PLFA in the total PLFAs and distribution of ^{13}C among PLFAs. PCA was

244   carried out with the standard setting in the program CANOCO 4.5 (Cui et al., 2018).

3. Results

3.1. Mineralization of residue C

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

248 during the later experiment (Figure 1). The LF treatment significantly increased ($P<0.05$) residue

249 mineralization rate as compared to the HF treatment apart from the day 500. Overall, a larger

250 mineralization of residue C was observed in straw-amended treatment compared to the corresponding

251 root-amended treatment, but the difference was only significant ($P<0.05$) on the day 60 and day 150. After

252 500 days of experiment, the total residue C mineralization was approximately 78% relative to initial residue

253 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

255 Maize residue incorporation significantly increased the total PLFAs amount for the entire experiment

256 period ($P<0.05$, Figure 2). The increase of the total PLFAs amount by residue addition in the HF soil was

257 greater than that in the LF soil after 60 days. The total PLFAs amounts of all the residue treatments

258 increased until the day 150 and then decreased. Nevertheless, total PLFAs amount remained essentially

259 unchanged for the controls (without residue addition). Averaged across the sampling events, higher PLFAs

260 amount was measured in the HF (37.5 nmol g^{-1} dry soil) compared with the LF treatments (25.5 nmol g^{-1}

261 dry soil, $P<0.05$). The total PLFAs amounts in straw-amended treatments were slightly higher than that in

262 root-amended treatment before 150 days, while significantly higher total PLFAs amounts were observed in

263 root-amended treatment at the end of the experiment ($P<0.05$).

264 Across the four sampling times, the overall trends in the PLFA contents of different microbial groups

265 were similar with total PLFAs (see Supporting Material; Table S1). The average relative proportions of the

266 different microbial PLFA groups decreased in the following order: Gram-positive (30.0%) > Gram-negative

267 (22.5%) > general (18.5%) > actinomycete (15.1%) > fungi (9.7%) > AMF (4.2%) ($P<0.05$, see Supporting

268 Material; Figure S1). In comparison with the controls, residue addition increased the proportion of fungal

269 PLFA but decreased the proportions of Gram-positive bacterial and actinomycete PLFA, but the relative

270 abundances of microbial groups were not affected by the residue types ($P>0.05$). The Gram-negative

271 bacterial PLFA proportion were higher in the LF treatment than in the HF treatment, but Gram-positive

272 bacterial PLFA had an opposite trend. Meanwhile, the relative abundances of the microbial groups did not

273 changed with experimental time, apart from some fluctuations in actinomycete PLFA.

274 The PCA analysis showed that PC1 and PC2 explained 45.5% and 24.7% of the total variation,

275 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 18:1ω7c, cy17:0ω7c, 18:1ω9c, 18:2ω6c and 16:1ω5c 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 cy19:0ω7c 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).

304   The relative residue C distribution showed that ~50% of PLFA-C derived from the maize residue were

305   incorporated into Gram-positive bacteria and Gram-negative bacteria (26.8% and 23.6%, respectively)

306   across the sampling events (Figure S2), followed by fungi (16.1%), general (16.1%), actinomycete (13.7%)

307   and AMF (3.7%). Residue C distributed more in Gram-negative bacterial PLFA in the LF soil than that in

308   the HF soil, but it distributed more in actinomycete PLFA in the HF soil than that in the LF soil. Although

309   root C contributed more to total PLFA-C than those from straw (Figure 4), relative distribution of residue C

310   had not showed any regular trend under root and straw treatments during the whole experiment period.

311   The PCA analysis, based on the distribution of residue C among PLFAs showed that PC1 and PC2

312   explained 55.8% and 25.1% of the total variation, respectively (Figure 6). Besides, treatments are roughly

313   separated by sampling time but not by soil fertility and residue type. Overall, highly related microbial

314   community shifted from general and Gram-negative bacteria to Gram-positive bacteria and actinomycete

315   over time.

4. Discussion

4.1 Soil microbial structure dynamics

318   In the present study, residue incorporation significantly increased PLFAs contents (Figure 2, Table S1),

319   and a similar trend was reported by others (Kong et al., 2011; Pan et al., 2016; Tavi et al., 2013). All peak

320   values of PLFAs content appeared on the day 150, but some other studies observed a maximum soil

321   microbial biomass typically less than 7 days after residue addition (Arcand et al., 2016; Bai et al. 2016).

322   Such a few-days quick peak was not observed in the present study because first sampling was done in the

323   second month of experiment. Studies usually attributed the quick peak to the enriched liable components

324   from added residue during the initial stage of decomposition (Pan et al., 2016). However, after that, when

325   recalcitrant components became the main C source for microbial utilization instead of the easily using

326   substrates, microbial community might adjust their strategy to consuming enriched recalcitrant substrates

327   leading microbial PLFAs to reach a peak at day 150.

328   The magnitude of the microbial community responded to crop residue addition varied among soil

329   fertilities and residue types (Figure 2, Table S1). Results obtained in this study indicated that the increase of

330   microbial biomass in the HF soil was greater than that in the LF soil after residue input, suggesting that

331 residue addition has a longer effective impact on microorganism growth in the HF soil due to higher

332 microorganism background values and N content (Table 1). Besides, root and straw residues had different

333 stimulating effects on soil microbial biomass at different decomposition periods. Shahbaz et al. (2017)

334 reported that the leaf and stem were relatively high quality residues with more readily decomposable

335 components, thus, straw could promote microorganism growth within a short period after residue

336 incorporation. Based on the results presented herein, there was a stronger simulation from straw during the

337 period when recalcitrant components gradually became dominate substrate for microorganisms (60–150

338 days), and root had a stronger role in promoting microbial biomass after the peak value appeared on day

339 150. This suggested that although the amount of the labile C reduced over time, it had a legacy effect on

340 microbial growth until the recalcitrant C completely dominated the microbial biomass, but the availability

341 of root C would exceed straw C eventually with the continuation of decomposition.

342 Maize residue incorporation altered soil microbial community structure. The proportion of fungal

343 PLFA increased and the proportion of Gram-positive bacterial and actinomycete PLFA decreased after

344 residue addition (Figure S1), suggesting that fungal community were relatively sensitive to the maize

345 residue decomposition. This phenomenon was attributed to the predominant role of fungi in decomposing

346 cellulose and hemicellulose, the primary components of maize residue (Pan et al., 2016; Six et al., 2006).

347 Besides, we found that relative abundance of Gram-negative bacterial PLFA was higher in the LF treatment

348 whereas Gram-positive bacterial PLFA fraction was higher in the HF treatment. One explanation for this

349 trend was that the soil fertility could lead to the change of microorganisms with different strategies (Cui et

350 al., 2018). It has also been suggested that high fertility soil induced by long-term organic and chemical

351 fertilizer applications was more conductive to increasing *K*-strategy microorganisms (Xu et al., 2018b). In

352 contrast, however, other researchers have suggested that *r*-strategy microorganisms in C limitation soil

353 could strengthen the decay of plant residue to meet their energy demands (Cui et al., 2018), and these may

354 correspond to Gram-positive and Gram-negative bacteria in our study, respectively. PCA analysis results

355 also indicated that Gram-negative bacterial PLFA and Gram-positive bacterial PLFA enriched in the LF and

356 HF soil, respectively (Figure 3).

357 Despite the fact that residue addition changed soil microbial biomass and microbial community

358 structure, residue type did not significantly influence the relative abundance of each PLFA (Figures S1 and

359 3). Possible reason was that root residue had a lower C/N ratio in this study, which could have offset the

360   disadvantage of root having a higher lignin content. Presumably, the heterogeneity between the different

361   residues in the present experiment was much smaller than that of SOM in soil, as a result, the residue

362   quality variation might not be sufficient to cause competition between soil microbial communities which

363   could adapt to the heterogeneous SOM. Besides, some studies demonstrated that the initial residue quality

364   may strongly affect the spatial heterogeneity and composition of some specific species of bacteria or fungi

365   based on DNA stable isotope probing (España et al., 2011; Nicolardot et al., 2007). The data presented

366   herein support a conclusion that residue type might alter the abundance of certain microbial species, but not

367   the microbial community composition form the aspect of whole groups of bacteria and fungi, etc.

4.2 Microbial incorporation of residue C

369   The data showed that residue C contributed to PLFA-C pool to varying degrees and as affected by

370   both residue quality and soil fertility (Figure 4, Table S2). Despite the fact that low microbial biomass and

371   activity limited the microbial incorporation of residue C in the LF soil, the percentage of residue C

372   distributed to PLFA-C pool was significantly higher than that in the HF soil. The possible reason was that

373   microorganisms in the LF soil with limited C source and nutrient had an urgent need and were in a sense

374   always waiting for available substrates, which could be provided by the newly added plant residues

375   (Fontaine et al., 2003). Thus, microorganisms shifted to immobilize the added residue C to satisfy their

376   growth requirements. On the other hand, the LF soil had higher clay content in the study site (An et al.,

377   2015a), and this may increase the contact between substrate and microorganisms. Conversely, the lower

378   proportion of residue C incorporated in PLFAs in the HF soil may be the result of more C source choice

379   from SOM at the beginning. Therefore, the data supported a conclusion that the microorganisms in the LF

380   soil with a limited SOM content had a greater preference for utilizing residue C.

381   However, the PLFA-C_{new} content was not significantly affected by soil fertility especially during the

382   early stages of the experiment period (Figure 5, Table S3). The result was mainly due to the higher total

383   PLFAs amount possessed by the HF soil, but lower proportion of residue C to total PLFA-C, which made

384   an offset leading to the situation of the no significant difference of PLFA-C_{new} content. Subsequently,

385   however, the HF soil could be more favorable for soil microbial biosynthesis because of the higher N

386   content than that in the LF soil (Moore-Kucera and Dick, 2008). On day 150 for root-amended treatment,

387   the PLFA-C_{new} content in the HF soil was significantly higher than that in the LF soil, suggesting that the

388 microbial utilization of residue C was regulated simultaneously both by soil fertility and residue quality.

389 These results partially confirmed the first hypothesis, i.e., microorganisms in the HF treatment could

390 assimilate more residue C than those in the LF treatment. It was also pertinent to highlight that the residue

391 C sequestration efficiency of microorganisms in the LF soil was higher, but the total residue C

392 sequestration amount of microorganisms in the HF soil might exceed that of LF soil.

393 The data presented herein also showed that the proportion and content of root C in PLFAs were both

394 higher than straw C especially after 90 days (Figure 4, 5). Hence, the second hypothesis that the

395 assimilation of residue C would be higher in straw treatment was not supported. Similar findings were

396 reported under field conditions by Williams et al. (2006) and Kong et al. (2011), and attributed the result to

397 the microbial preference for root-related substrates during the growing season when the root exudation and

398 the sloughing of root cells thrive in soil, which were all ^{13}C labelled in their experiment. In the present

399 experiment, only labelled root residue, but no labelled root exudation or root cells, were present in the soil.

400 Yet, there was more root C in PLFAs than the straw C. This trend suggested that the growth of soil

401 microbial communities depended more on the root substrate than on the aerial part substrate during the

402 growing season. Further, microbial communities living in soil during the growing season was primarily

403 comprised of those who adapt to consuming the root substrates because of much more prevalence of root

404 substrate than that of leaf or stem substrate during this period.

405 The different temporal dynamic patterns of residue C proportion in PLFAs-C pool between the two

406 residue-amending treatments were observed in the present study (Figure 4). The continuously decreasing

407 proportion of straw C suggested the reduction in substrates available to microorganisms, whereas the

408 occurrence of the peak of root C incorporated into the PLFAs on the day 150 indicated that recalcitrant C

409 became the prominent C source for soil microorganism as the decomposition progressed (Abiven et al.,

410 2005). As mentioned in the section 4.1, the PLFAs contents in both the root and straw treatments reached

411 the peak value on day 150. But the mechanism of this peak seemed to be different based on the proportion

412 of the residue C. In the straw treatment, although its contribution to PLFAs-C pool gradually decreased, the

413 remaining recalcitrant C still significantly stimulated the growth and activity of microorganisms (native

414 SOC was the main C source). In contrast, the effect of root treatment on microbial growth not only came

415 from the stimulating effect of the C substrate, but also contributed more to the microbial biosynthesis

416 (native SOC and root C were both the main C sources).

417   The residue C was not evenly appropriated among diverse microbial PLFA-C pools in this study

418   indicating that soil microorganisms differed in their assimilation and utilization of residue C. The residue C

419   seemingly contributed the most to fungal PLFA (Table S2) (especially 18:2ω6c, data not shown), which

420   supports the third hypothesis, implying that fungi were more efficient user of the residue-derived

421   compounds compared to other microbial groups. The reason might be that fungi could produce different

422   extracellular depolymerizing enzyme and absorb nutrients at the soil-residue interface through their

423   filaments (Acosta-Martínez et al., 2008), which enhance their ability to compete for maize residue.

424   However, in contrast to the most contribution of residue C to fungi, it was bacteria that mobilized the most

425   amount of residue C due to its large biomass, based on the result that 50% of PLFA-immobilized residue C

426   distributed in bacterial PLFA (Figure S2) (mainly in i15:0, data not shown). The results indicated that

427   though fungi had stronger ability to assimilate residue C, bacteria were the primary consumer of residue C

428   due to their higher biomass.

429   In the present study, residue C distributed more in Gram-negative bacterial PLFA in the LF treatment,

430   while it distributed more in actinomycete PLFA in the HF treatment (Figure S2), demonstrating that the

431   utilization process of residue C by different microbial communities was regulated by soil fertility. However,

432   the distribution of residue C in microbial communities did not change significantly between root and straw

433   treatments (Figure S2). Crop residue consisted of a range of recalcitrant biopolymers (Pan et al., 2016),

434   which could be consumed by a range of decomposers in soil. Consequently, the whole soil microbial

435   community with diverse decomposers might have formed strong adaptability to use different kinds of

436   residues (root or straw) (Helfrich et al., 2015). Similarly, although the chemical composition of the residues

437   changed during the decomposition, soil microbial communities could still maintain their own fraction of

438   residue C in a stable state. The above results showed that the synergism among diverse microbial

439   communities moderated the distribution of residue C as it was unaffected by characteristics of exogenous

440   organic matter such as the residue quality.

4.3 Linking C processing with soil microbial community

442   The results showed that the mineralization of residue C was higher in the LF soil than that in the HF

443   soil except on the day 500 (Figure 1). This phenomenon could be explained by the different composition of

444   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 CO_2) 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.

Acknowledgements

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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})	$\delta^{13}C$ 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

650 **Figure 1** The proportion of residue C mineralization relative to the initial added residue C (%) over 500

651 days experiment. Symbols LF and HF represent the soils with low and high fertility, respectively. R and S

652 represent root and straw residues, respectively. Error bars represent the standard deviations from the mean

653 (n=3).

654 **Figure 2** Sum of PLFAs (nmol g^{-1} dry soil) under different treatments over 500 days experiment. Symbols

655 LF and HF represent the soils with low and high fertility, respectively. R and S represent root and straw

656 residues, respectively. Error bars represent the standard deviations from the mean (n=3).

657 **Figure 3** Principle component analysis (PCA) based on the relative proportion of individual PLFA for

658 different treatments. The numbers next to the symbols represent sampling times. Symbols LF and HF

659 represent the soils with low and high fertility, respectively. R and S represent root and straw residues,

660 respectively.

661 **Figure 4** Contribution of residue C to total PLFA-C pool (%) for different treatments over 500 days

662 experiment. Symbols LF and HF represent the soils with low and high fertility, respectively. R and S

663 represent root and straw residues, respectively. Error bars represent the standard deviations from the mean

664 (n=3).

665 **Figure 5** Sum of residue C content within PLFAs (nmol g^{-1} dry soil) for different treatments over 500

666 days experiment. Symbols LF and HF represent the soils with low and high fertility, respectively. R and S

667 represent root and straw residues, respectively. Error bars represent the standard deviations from the mean

668 (n=3).

669 **Figure 6** PCA analysis based on the distribution of residue C among PLFAs for different treatments. The

670 numbers next to the symbols represent sampling times. Symbols LF and HF represent the soils with low

671 and high fertility, respectively. R and S represent root and straw residues, respectively.

672 **Figure 7** Schematic representation of microbial process of residue C transformation. Symbols LF and HF

673 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
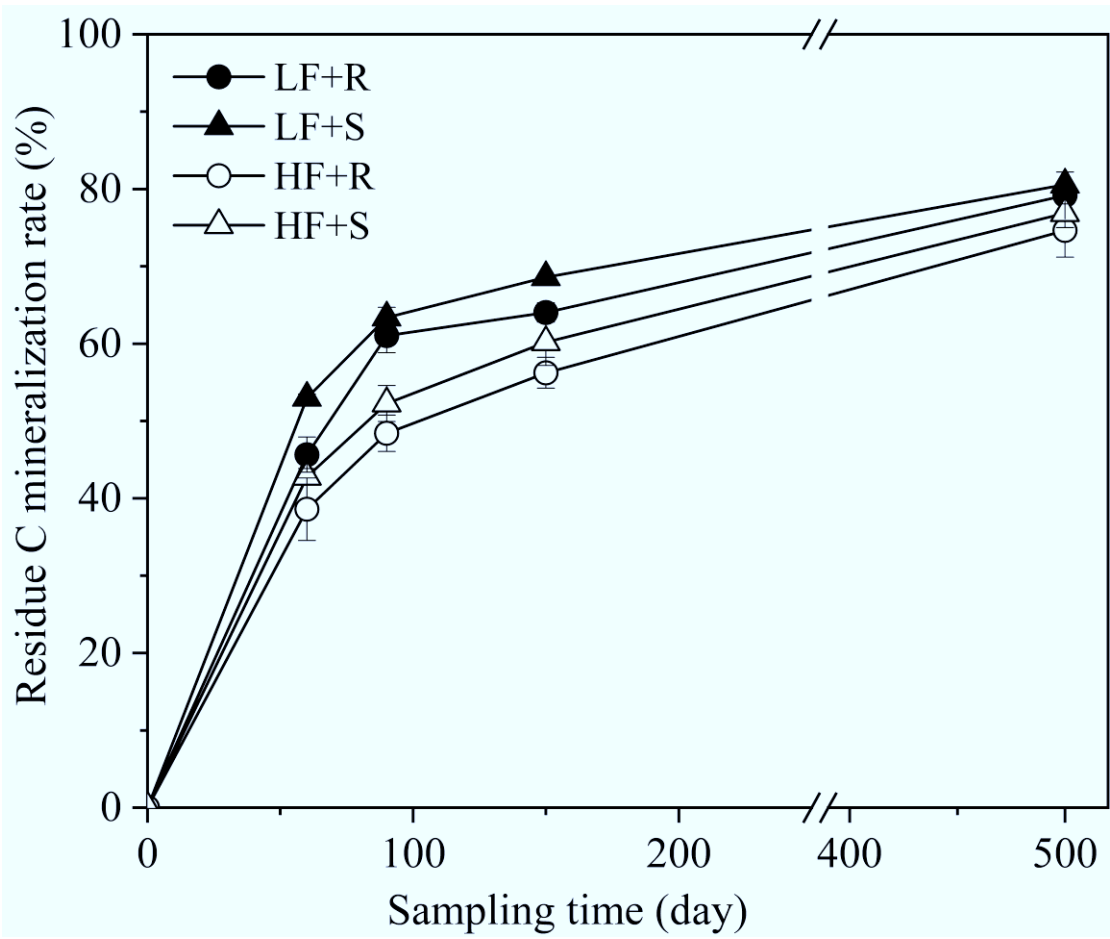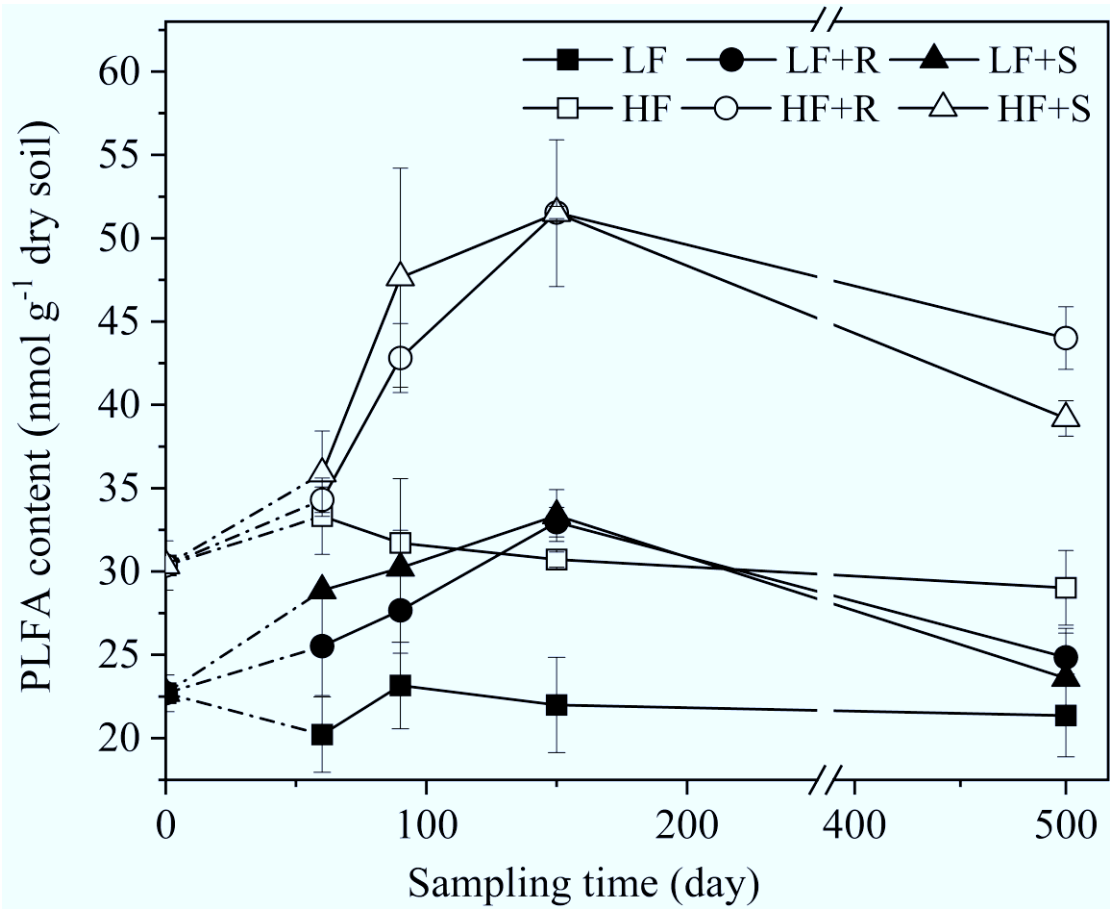
678   responding indicators.

Figure1

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Figure2

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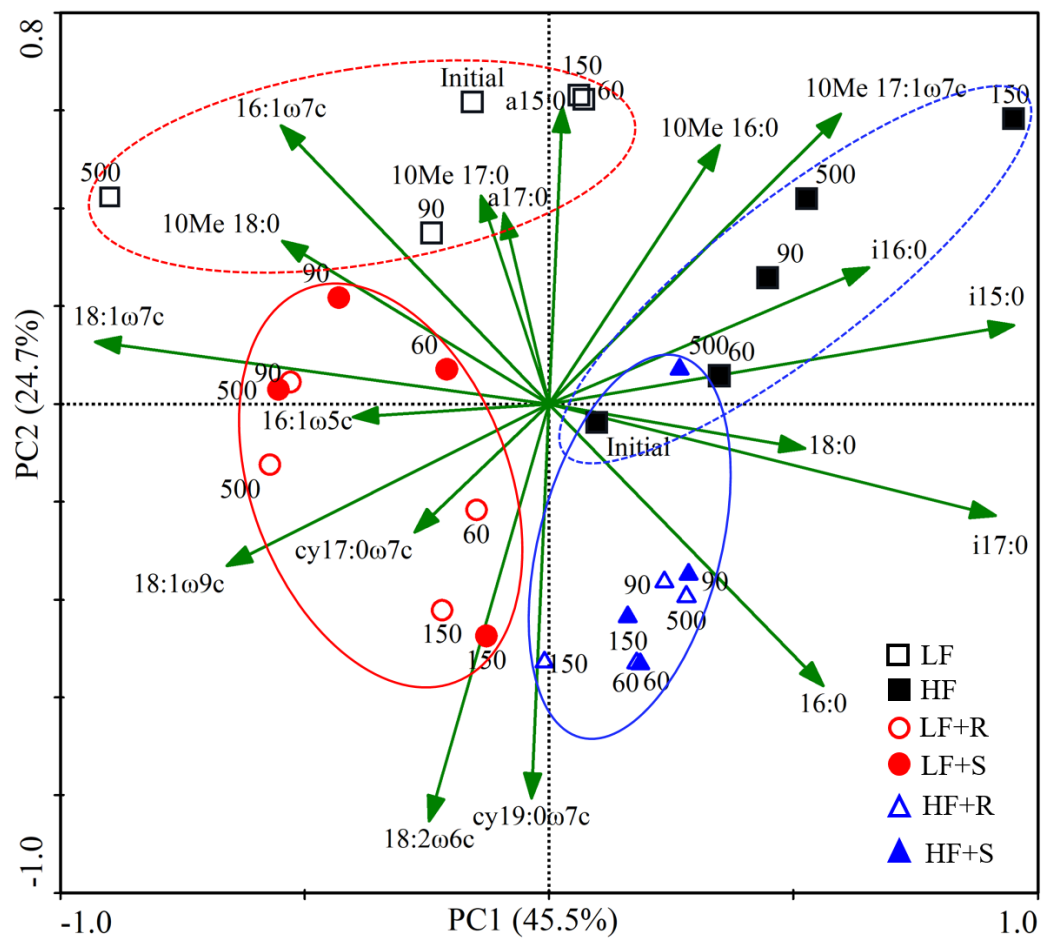
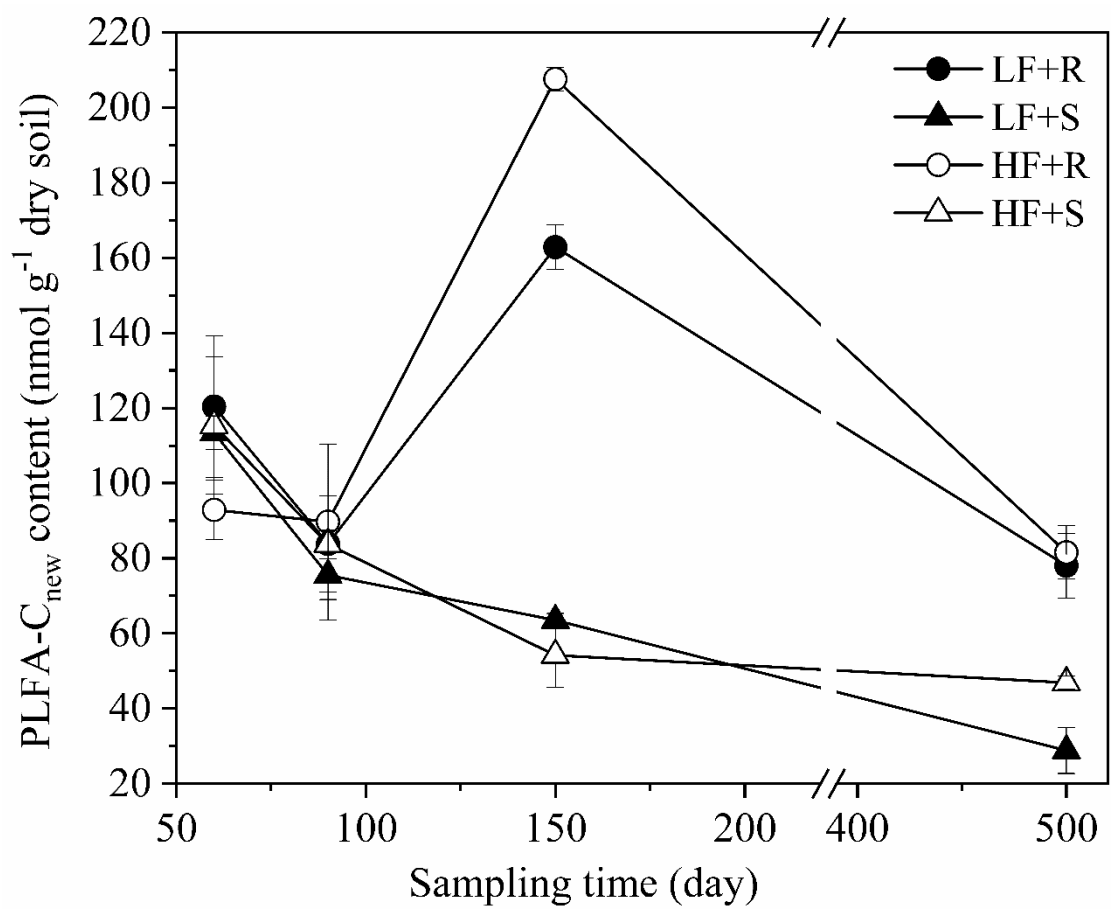**Figure4**

Figure 4
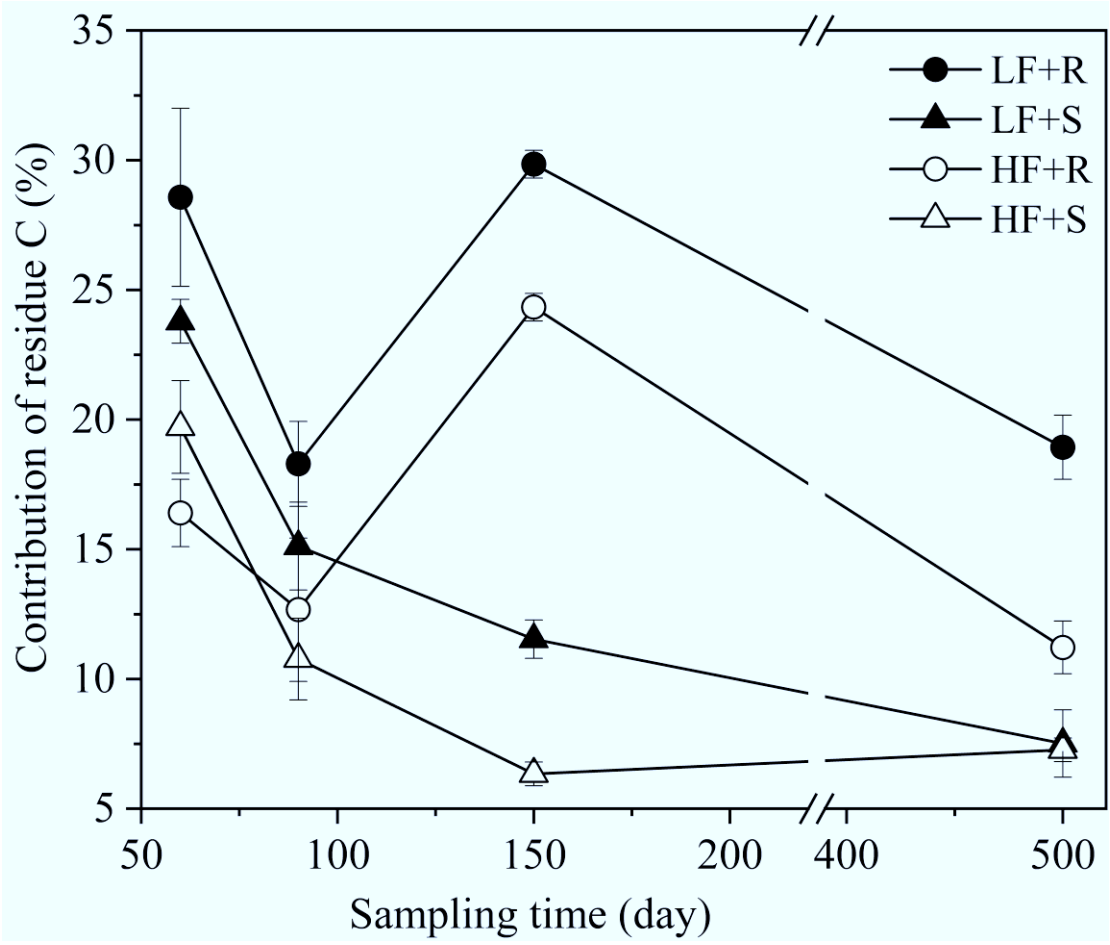
Figure5

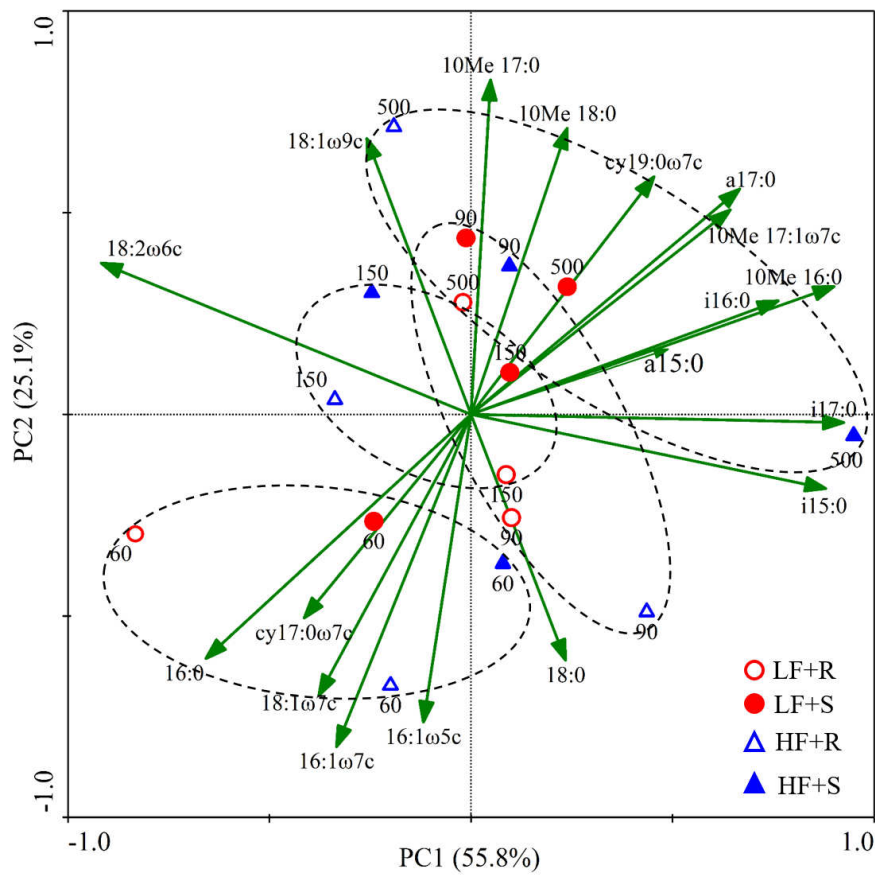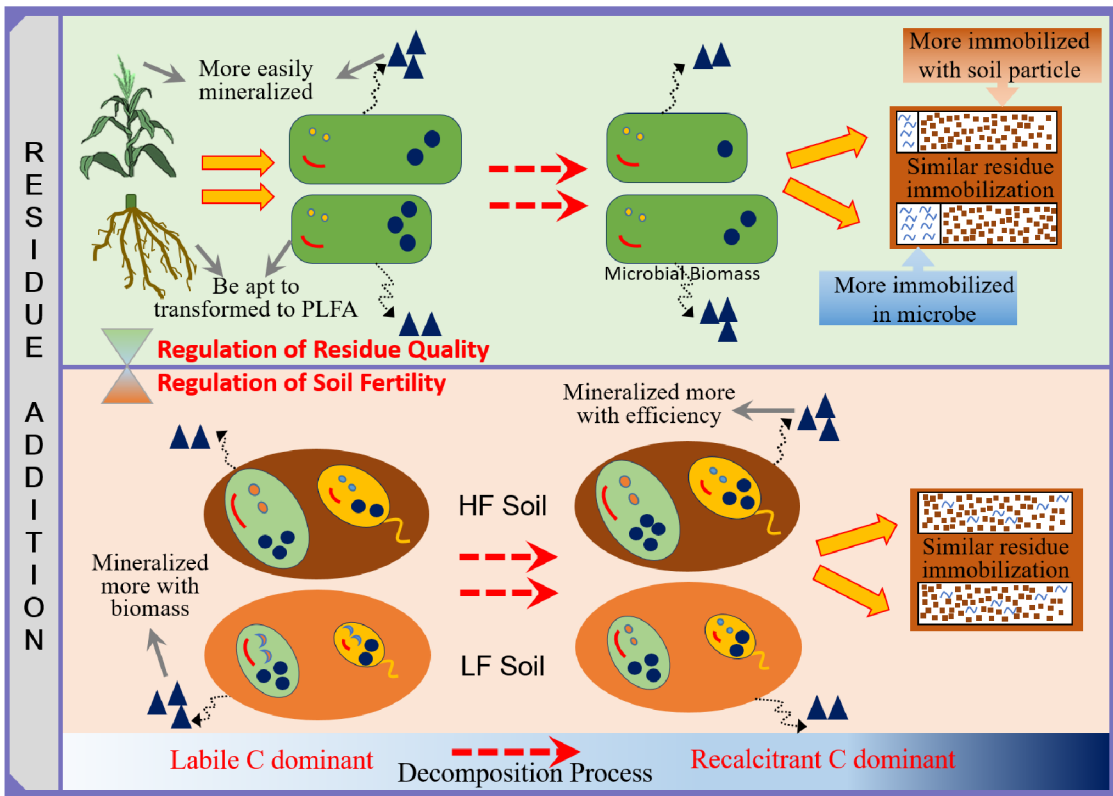
Figure 5

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*Declaration of Interest Statement

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