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Differential long term fertilization changes residue-derived labile organic carbon fractions and microbial community during straw residue decomposition --Manuscript Draft--

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Abstract:	Straw residue amendment is a key global management strategy to achieve more sustainable agriculture. Straw residue returns affect the labile soil organic carbon (SOC) fractions and microbial community in soils. We examined temporal changes in dissolved organic carbon (DOC), microbial biomass carbon (MBC), particulate organic carbon (POC) and microbial community structure in relation to the overall straw-derived residue decomposition. The topsoil (0-20 cm) from three fertilizer management strategies (no fertilization control, CK; inorganic fertilizer, IF; inorganic fertilizer plus manure, IFM) was collected from a unique 29-year long-term field experiment (Mollisols) in Northeast China. An in-situ micro-plot incubation experiment with 13C-labeled maize straw residue (\delta 13C = 246.9\infty\) (i.e., no fertilization control + straw, CKS; inorganic fertilizer + straw, IFS; inorganic fertilizer plus manure + straw, IFMS) and without straw residue was conducted. We used the 13C isotope technique to measure the content of residue-derived labile SOC fractions and used high-throughput sequencing-based amplification of bacterial 16S rRNA and fungal internal transcribed spacer (ITS) rRNA to evaluate the dynamic changes of bacterial and fungal community structure and diversity at different times (the 1st day, 60th day and 150th day) under all six treatments. We found that residue-derived POC was significantly increased, but residue-derived DOC was significantly decreased during straw residue decomposition. The residue-derived MBC content was higher in the fertilized (IFS and IFMS) compared to the unfertilized (CKS) treatment. The Linear discriminant analysis Effect Size (LEfSe) revealed changes after adding straw of soil microbes in CK and IF were significantly higher than in IFM soil. Network analysis showed that straw residue addition decreased the bacterial microbial network complexity in all treatments, but increased fungal network complexity in IFS and IFMS. The random forest model predicted that during straw decom

	POC in labile SOC fractions and was further enhanced in fertilizer management with manure addition. Less pronounced effects on the microbial community following straw addition were found for the combined inorganic and organic fertilizer treatment compared to those without or with only inorganic fertilizer addition. The observed temporal changes in the microbial community suggested that in these Mollisols, independent of agricultural fertilizer management, straw residue-derived POC and DOC promoted fungal C processing, whereas for bacterial this was facilitated only via residue-derived MBC.
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Cover Letter

Dear Prof. Noellemeyer,

We would like to submit our manuscript 'Differential long term fertilization changes residue-derived labile organic carbon fractions and microbial community during straw residue decomposition' prepared by Zhuang Ge, Shuangyi Li, Roland Bol, Ping Zhu, Chang Peng, Tingting An, Na Cheng, Xu Liu, Tingyu Li, Zhiqiang Xu, Jingkuan Wang for evaluation as Research Article in the journal *Soil & Tillage Research*.

The work in this paper can be summarized as follows, the topsoil (0-20 cm) from three fertilizer management strategies (no fertilization control, CK; inorganic fertilizer, IF; inorganic fertilizer plus manure, IFM) was collected from a unique 29-year long-term field experiment (Mollisols) in Northeast China. An in-situ micro-plot incubation experiment with ${}^{13}\text{C}$ -labeled maize straw residue ($\delta^{13}\text{C} = 246.9\%$) (i.e., no fertilization control + straw, CKS; inorganic fertilizer + straw, IFS; inorganic fertilizer plus manure + straw, IFMS) and without straw residue was conducted. We used the ¹³C isotope technique to measure the content of residue-derived labile SOC fractions and used high-throughput sequencing-based amplification of bacterial 16S rRNA and fungal internal transcribed spacer (ITS) rRNA to evaluate the dynamic changes of bacterial and fungal community structure and diversity at different times (the 1st day, 60th day and 150th day) under all six treatments. We found that residue-derived POC was significantly increased, but residue-derived DOC was significantly decreased during straw residue decomposition. The residue-derived MBC content was higher in the fertilized (IFS and IFMS) compared to unfertilized (CKS) treatment. The Linear discriminant analysis Effect Size (LEfSe) revealed changes after adding straw of soil microbes in CK and IF were significantly higher than in IFM soil. Network analysis showed that straw residue addition decreased the bacterial microbial network complexity in all treatments, but increased fungal network complexity in IFS and IFMS. The random forest model predicted that during straw decomposition, Chloroflexi (CKS), Actinobacteria (IFS), Proteobacteria (IFMS) were keystone taxa in the bacterial community, and Ascomycota was the keystone taxon in the fungal community. The straw residue was retained as POC in labile SOC fractions and was further enhanced in fertilizer management with manure addition. Less pronounced effects on the microbial community following straw addition were found for the combined inorganic and organic fertilizer treatment compared to those without or with only inorganic fertilizer addition. The observed temporal changes in the microbial community suggested that in these Mollisols, independent of agricultural fertilizer management, straw residue-derived POC and DOC promoted fungal C processing, whereas for bacterial this was facilitated only via residue-derived MBC.

This manuscript has neither been published and or is under consideration for publication elsewhere. We have no conflicts of interest to disclose. All authors have read and approved the final version of the manuscript. Thank you

in advance the consideration of our manuscript, and we look forward to hearing from you at your earliest convenience.

Yours sincerely,

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- Straw addition decreased the bacterial network complexity in all treatments
- Straw addition increased fungal network complexity in fertilized soils
- Key species in straw decomposition changed under different fertilizer regimes
- Residue-derived POC and DOC increased fungal carbon processing
- Bacterial carbon processing was facilitated only via residue-derived MBC

- 1 Differential long term fertilization changes residue-derived
- 2 labile organic carbon fractions and microbial community
- 3 during straw residue decomposition
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Abstract:

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Straw residue amendment is a key global management strategy to achieve more sustainable agriculture. Straw residue returns affect the labile soil organic carbon (SOC) fractions and microbial community in soils. We examined temporal changes in dissolved organic carbon (DOC), microbial biomass carbon (MBC), particulate organic carbon (POC) and microbial community structure in relation to the overall strawderived residue decomposition. The topsoil (0-20 cm) from three fertilizer management strategies (no fertilization control, CK; inorganic fertilizer, IF; inorganic fertilizer plus manure, IFM) was collected from a unique 29-year long-term field experiment (Mollisols) in Northeast China. An *in-situ* micro-plot incubation experiment with ¹³Clabeled maize straw residue (δ^{13} C = 246.9%) (i.e., no fertilization control + straw, CKS; inorganic fertilizer + straw, IFS; inorganic fertilizer plus manure + straw, IFMS) and without straw residue was conducted. We used the ¹³C isotope technique to measure the content of residue-derived labile SOC fractions and used high-throughput sequencingbased amplification of bacterial 16S rRNA and fungal internal transcribed spacer (ITS) rRNA to evaluate the dynamic changes of bacterial and fungal community structure and diversity at different times (the 1st day, 60th day and 150th day) under all six treatments. We found that residue-derived POC was significantly increased, but residue-derived DOC was significantly decreased during straw residue decomposition. The residuederived MBC content was higher in the fertilized (IFS and IFMS) compared to the unfertilized (CKS) treatment. The Linear discriminant analysis Effect Size (LEfSe) revealed changes after adding straw of soil microbes in CK and IF were significantly higher than in IFM soil. Network analysis showed that straw residue addition decreased the bacterial microbial network complexity in all treatments, but increased fungal network complexity in IFS and IFMS. The random forest model predicted that during straw decomposition, Chloroflexi (CKS), Actinobacteria (IFS), Proteobacteria (IFMS) were keystone taxa in the bacterial community, and Ascomycota was the keystone taxon in the fungal community. The straw residue was retained as POC in labile SOC fractions and was further enhanced in fertilizer management with manure addition. Less pronounced effects on the microbial community following straw addition were found for the combined inorganic and organic fertilizer treatment compared to those without or with only inorganic fertilizer addition. The observed temporal changes in the microbial community suggested that in these Mollisols, independent of agricultural fertilizer management, straw residue-derived POC and DOC promoted fungal C processing, whereas for bacterial this was facilitated only via residue-derived MBC.

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Keywords: Labile soil organic carbon; Soil microbial community; Soil microbial network; Key species; High-throughput sequencing; ¹³C-labelling technique

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1 Introduction

Soil organic carbon (SOC) plays a key role in chemical, physical, and biological properties and processes in soils, thus it is considered to be a crucial factor to affect soil quality (Wiesmeier et al., 2019). Fertilization is an important agricultural practice that aims to improve soil quality and plant nutrition for increasing crop yield (Chen et al., 2014; Xiang et al., 2020). Application of inorganic fertilizer could temporarily slow down food shortage but would lead to soil degradation, soil acidification, and SOC depletion (Stockmann et al., 2013; Zamanian and Kuzyakov, 2019). Organic fertilizer application promoted SOC accumulation, alleviated soil acidification, increased crop yield, therefore would be an alternative option to applicate inorganic fertilizer (Afreh et al., 2018; Han et al., 2016). Moreover, SOC is highly heterogeneous, according to the turnover rates of various SOC fractions, it could be divided into stable and labile fractions (Bol et al., 2009; Six et al., 2002). Labile SOC fractions generally include dissolved organic carbon (DOC), microbial biomass carbon (MBC), and particulate organic carbon (POC) (Franzluebbers et al., 2000; Jardine et al., 1989; Moore et al.,

2000). Labile SOC fractions had relatively short turnover time (weeks to months), they are sensitively responding to agricultural practice (Yan et al., 2007), and are an easily available and important source of energy for soil microbes (Kaye and Hart, 1997; McLauchlan and Hobbie, 2004). Labile SOC pools have been considered as early sensitive indicators of soil quality which affect soil function in special ways due to their different fractions (Blanco-Moure et al., 2016; Rudrappa et al., 2006). The stable isotope technique provided a good way to quantitatively investigate the changes of labile SOC fractions, that results would bring more direct and clear theoretical support for the management of SOC (Amelung et al., 2008). Different fertilizer management strategies also affected SOC pools. For example, fertilization strategies which include organic manure can increase the pool of stable C in the surface soil layer and increase concentrations and proportions of labile C (Li et al., 2018), but apply the different rates of inorganic fertilizer would not affect the concentrations of soil labile fractions (Naylor et al., 2020). Furthermore, previous studies reported that the application of fertilizer affected the diversity and composition of the soil microbial community (Xiang et al., 2020; Zhou et al., 2015). Microbial communities play a key role in soil organic matter (SOM) transformation and nutrient cycling in agricultural soils (Gattinger et al., 2007). Long-term applicated inorganic fertilizer reduced the biodiversity and abundance of

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bacteria, but manure application increased bacterial abundance and diversity (Cui et al., 2018; Zhou et al., 2015). Long-term application of inorganic fertilizer plus pig manure altered fungal community composition by increasing the abundance of *Pezizales*. (Ye et al., 2020). The number of differential populations in bacterial communities in applied manure soils was significantly higher than that in applied inorganic fertilizer soils, whereas those of fungal communities showed the opposite trend, therefore, the responses of different fertilizers to soil bacterial and fungal communities were various (Pan et al., 2020).

Crop residues were assumed to be the dominant carbon (C) source to the arable soil and primary control of the accumulation of SOC (Clemmensen et al., 2013). Therefore crop residue return is believed to be an effective promising approach to improve soil quality, promote agricultural SOC storage, and mitigate climate change (Dikgwatlhe et al., 2014; Lal, 2004; Liu et al., 2014). Straw returns also could affect the SOC pool, especially the labile SOC fractions (Lei et al., 2010; Zhao et al., 2016). Different fertilizer management strategies would influence the decomposition of residue-derived C. Our previous studies showed apply nitrogen fertilization increased the content of residue-derived MBC, apply manure increased the content of residue-derived MBC, apply manure combined with inorganic

fertilizer significantly increased residue-derived POC in Alfisols (Jin et al., 2020; Jin et al., 2018; Wang et al., 2020). Meanwhile, labile SOC is also an essential source in which microorganisms can receive nutrients and energy from it, and it affects the life activities of soil microorganisms. Soil microorganisms simultaneously participate in the processes of labile SOC fractions formation, transformation, and decomposition (Chantigny, 2003). Furthermore, the decomposition of crop residues in the soil is a complex biogeochemical process, and microorganisms are the major drivers of crop residue decomposition and turnover in soils (Marschner et al., 2011). The straw residue is metabolized by soil microbes, transformed into microbial biomass, and becomes stabilized as SOM (Cotrufo et al., 2015). Added crop residue to the soils may greatly influence soil microbial diversity, abundance, and composition thus altering SOC dynamics (Goldstein et al., 2020). Soil bacterial and fungal communities play important roles in the decomposition of SOM and provide available nutrients for plant growth (Allison et al., 2007). Crop residue decomposition usually proceeds through a series of well-characterized stages involving a succession of soil bacterial and fungal communities (Guo et al., 2018). Due to soil bacterial and fungal communities exhibit different dynamic patterns, the microbial succession would lead to the changes of specific microbial taxa during the straw residue decomposition process (Gao et al., 2016;

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Prewitt et al., 2014). Therefore, it is essential to simultaneously detect the dynamic changes of differential populations in soil bacterial and fungal communities in each stage in the process of straw residue decomposition under different fertilizer regimes (Baldrian et al., 2012; Banerjee et al., 2016). In addition, the quantitative relationship between the different residue-derived labile carbon fractions and soil microbes during the process of straw decomposition are still unclear.

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Interactions between microbes are also important aspects in maintaining a diverse microbial community, at the same time, network analyses provide a very useful tool to explore the co-occurrence patterns and reflecting the microbial community in ecosystems (Faust and Raes, 2012; Rottjers and Faust, 2018). Exploring changes in soil microbial community networks can increase our knowledge of the complexity and diversity of microbial communities (Mora-Montes et al., 2010). The application of the organic amendment significantly changed the bacterial and fungal community's network than inorganic fertilizer and therefore fertilization could influence the synergistic interactions between species. (Ling et al., 2016; Xue et al., 2017). However, it remains unclear on exploring the effect of straw residue decomposition to soil bacterial and fungal co-occurrence networks under different fertilizer regimes. Besides, the information on the key role of specific microbial species of soil bacterial and fungal in the process of straw residue decomposition is still limited.

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The North China Plain is one of the most important food production regions in China (Liu et al., 2019). However, in the past several decades, long-term unreasonable field management degraded the soil and deteriorated the soil quality (Liu et al., 2010). Return of straw residue within overall fertilizer management strategies is widely used to mitigate these problems and enhance the storage of SOC in this region (Qiu et al., 2016). Most of the studies only reported the effect of long-term fertilizer or straw incorporation on soil microbial communities (Guo et al., 2020; Pan et al., 2020; Zhao et al., 2019). The microbial community composition and metabolic activity correspond to different fertilizer regimes, and soil fertility affected the residue decomposition process (An et al., 2015b; Zhang et al., 2019). Therefore, deeply revealing the dynamic response mechanisms of the key role in the microbial community during the process of straw residue decomposition in integrated fertility management is crucial for enhancing C sequestration. In this study, we collected arable soils of three fertilizer management strategies from a 29-year fertilization experiment in the North China Plain. We conducted an in-situ micro-plot incubation experiment with and without maize straw residue in these soils to investigate the changes of residue-derived labile SOC fractions content and complex temporal responses of soil microbial communities to straw residue

decomposition, as well as their quantitative relationships under various fertilization regimes. We hypothesized that under the varying fertilizer regimes in Mollisols: (i) Straw residue-derived content of labile SOC fractions would differ between specific management strategies, (ii) Temporal shifts in soil bacterial and fungal population abundances and keystone decomposer species occur during straw decomposition, (iii) Soil bacterial and fungal network complexity would change following straw incorporation, (iv) Different residue-derived labile carbon fractions have varying effects on soil fungal and bacterial population.

2 Materials and methods

2.1 Study site description

A long-term field experiment site used in this study was located at Jilin Academy of Agricultural Sciences at Gongzhuling County, Jilin Province, Northeast China (43° 30′N, 124° 48′E, and 200 m above sea level). The experiment was established in 1990, has a typical continental monsoon climate with mean annual temperatures of 4-5 °C and mean annual precipitation of 400-600 mm (Song et al., 2015). The soil is a Mollisol (classified as a Luvic Phaeozem, FAO) with 39% sand, 30% silt, and 31% clay at the beginning of the experiment (Xie et al., 2014). The three application were selected in this study: (1) unfertilized control (CK), (2) balanced inorganic fertilizers at 165 kg N

ha⁻¹,82.5 kg P₂O₅ ha⁻¹, and 82.5 kg K₂O ha⁻¹ (IF), (3) balanced inorganic fertilizers at 50 kg N ha⁻¹, 82.5 kg P₂O₅ ha⁻¹, and 82.5 kg K₂O ha⁻¹ plus manure at 115 kg N ha⁻¹ (IFM) (Dou et al., 2016). The manure was pig manure and applied in autumn after corn harvesting in the IFM plots each year (Song et al., 2015). The basic soil properties of each treatment are provided in Table S1.

2.2 In-situ field experiment design

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The micro-plot experiment was not undertaken at the main long-term field-site itself, but in a nearby field to avoid any presence of straw influencing future soil. Two soil pits of the following dimensions (length \times width \times height = 1.0 m \times 0.6 m \times 0.3 m) were therefore dug in a nearby field for the micro-plot experiment. Two polyvinyl chloride (PVC) material boxes (length \times width \times height = 1.0 m \times 0.6 m \times 0.6 m) of similar dimensions to the pit were then inserted vertically into field pits on May 5, 2018, i.e., the boxes were 0.3 m above the ground level to avoid any impacts by other soil in the field. The boxes were not closed at the bottom to allow for drainage. Each box consisted of 9 equal sections, allowing 3 random replicates of the 3 treatments under consideration (CK, IF and IFM). The topsoil layer (0-20 cm) was taken from each fertilization treatment of the long-term field experiment and individually passed in the field through a 7 mm sieve to remove crop roots and rocks. More details of soil

properties can be found in Table S1. The ¹³C-labelled maize straw was mature maize plants pulse-labeled using ¹³CO₂ four times over a growing season according to the procedure (An et al., 2015a). The maize straw residue was cut in the size of 0.5-1.0 cm. The method of straw incorporation was based on the concept of full straw incorporation, i.e., where all straw residue after harvest is plowed back into the soil. We added 36 g straw to 15.84 kg of soil per section (equivalent to 2.3 g straw kg⁻¹ soil). No plants were grown in all the boxes during the experimental period. Before completely filling the sections, we first added only soil from three treatments to the bottom 10 cm (20-30 cm depth) in each section box. Subsequently, the upper 20 cm was filled with soil, part of the soils from the three treatments were mixed homogenously with ¹³C-labelled straw. In one box all 9 compartments consisted of the soil mixed with straw (CKS, IFS, IFMS), the other box only contained soil (CK, IF, IFM). Soil samples were collected at the depth of 0-20cm three times: on May 6, 2018 (the 1st day), July 4, 2018 (the 60th day), and October 2, 2018 (the 150th day). Soil samples were sealed in plastic bags, then stored in the 4°C incubator and transported to the laboratory, then the samples were kept at -80 °C for microbial analysis.

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2.3 Measurements of dissolved organic carbon (DOC) and microbial biomass 220 carbon (MBC)

MBC was determined by the chloroform-fumigation extraction method (Vance et

al., 1987). Fresh soil equivalent to 10 g of oven-dried soil was fumigated for 24 h at 25 °C and subsequently extracted with 100 ml of 0.5 mol L⁻¹ K₂SO₄. The same amount of un-fumigated soil was extracted with 100 ml of 0.5 mol L-1 K2SO4. The nonfumigated extract was used to determine DOC. The contents of organic C of soil extracts were determined by the Total Organic Carbon Analyzer (Multi N/C 3100 TOC, Germany). MBC was calculated as the difference in organic C content between fumigated and un-fumigated soil extracts with a correction factor ($k_{\rm EC}$) of 0.45 (Wu et al., 1990). All K₂SO₄ extracts were freeze-dried before further analysis of ¹³C abundance. Soil and K_2SO_4 extracts samples were analyzed for total C and $\delta^{13}C$ values with an elemental analyzer (Elementar Vario PYRO cube, Germany) coupled to an isotope ratio mass spectrometer (IsoPrime 100 Isotope Ratio Mass Spectrometer, Germany).

2.4 Measurements of particle organic carbon (POC)

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POC was isolated from bulk soil using the procedure (Cambardella and Elliott, 1992). A 10 g subsample of bulk soil was passed through a 2 mm sieve, dispersed into 30 ml of 5 g L^{-1} sodium hexametaphosphate, and shaken for 5 h. Next, the suspension was filtered through a 53 μ m sieve. The material remaining on the sieve (POC) was rinsed thoroughly with deionized water, dried at 50 °C for 24 h weighed, and stored

before measurement. Organic C and δ^{13} C in POC were determined using the EA-IRMS.

2.5 Isotopic C analysis and calculations

- The Content of MBC was calculated with the following equation (Vance et al.,
- 243 1987):

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$$C_{MBC} = (C_f - C_{nf}) / k_{EC}$$
 (1)

- where C_f and C_{nf} refer to the amount of dissolved organic C (mg kg⁻¹ soil) from the
- fumigated and the nonfumigated K_2SO_4 extracts, respectively. The k_{EC} value was used
- 247 to convert measured data into biomass C, in this study we used it as 0.45 (Joergensen,
- 248 1996).
- The δ^{13} C of MBC (‰) was calculated with the following equation (Engelking et
- 250 al., 2008):

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$$\delta^{13}C_{MBC} = (\delta^{13}C_{f} \times C_{f} - \delta^{13}C_{nf} \times C_{f}) / (C_{f} - C_{nf})$$
 (2)

- where $C_{\rm f}$ and C_{nf} refer to the amount of dissolved organic C (mg kg⁻¹ soil) from the
- $253 \qquad \text{fumigated and the nonfumigated K_2SO_4 extracts, respectively, and $\delta^{13}C_f$ and $\delta^{13}C_{nf}$ refer}$
- 254 to the $\delta^{13}C$ values (‰) of the fumigated and the nonfumigated K_2SO_4 extracts,
- 255 respectively.
- The proportion of MBC derived from maize straw C in total MBC (f_{MBC}) was

257 calculated according to the following equation (De Troyer et al., 2011):

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$$f_{MBC} = (\delta^{13}C_{MBC} - \delta^{13}C_{MBC-WS}) / (\delta^{13}C_{straw} - \delta^{13}C_{MBC-WS})$$
 (3)

- where $\delta^{13}C_{MBC}$, $\delta^{13}C_{MBC-WS}$, and $\delta^{13}C_{straw}$ are the $\delta^{13}C$ values of MBC from whole soil
- samples with straw, MBC from whole soil samples without straw (WS), and ¹³C-
- labelled maize straw itself, respectively.
- The content of straw-derived MBC (¹³C_{MBC}) was calculated with the following equation
- 263 (Blaud et al., 2012):

$$^{13}C_{MBC} = C_{MBC} \times f_{MBC} \tag{4}$$

- where C_{MBC} denotes the content of MBC, and f_{MBC} denotes the proportion of MBC
- derived from maize straw C in total MBC.
- The proportion of DOC derived from maize straw C in total DOC (fDOC) was
- 268 calculated according to the following equation (De Troyer et al., 2011):

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$$f_{DOC} = (\delta^{13}C_{DOC} - \delta^{13}C_{DOC-WS}) / (\delta^{13}C_{straw} - \delta^{13}C_{DOC-WS})$$
 (4)

- Where $\delta^{13}C_{DOC}$, $\delta^{13}C_{DOC-WS}$, and $\delta^{13}C_{straw}$ are the $\delta^{13}C$ values of DOC from soil samples
- with straw, DOC from soil samples without straw (WS), and ¹³C-labelled maize straw
- itself, respectively.

The content of straw-derived DOC (C_{DOC}) was calculated using the following equation (Blaud et al., 2012):

$$^{13}C_{DOC} = C_{DOC} \times f_{DOC}$$
 (5)

- where C_{DOC} denotes the content of DOC, and f_{DOC} denotes the proportion of DOC
- derived from maize straw C in total DOC.
- The proportion of POC derived from maize straw C in total POC (fpoc) was
- 279 calculated according to the following equation (De Troyer et al., 2011):

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$$f_{POC} = (\delta^{13}C_{POC} - \delta^{13}C_{POC-WS}) / (\delta^{13}C_{straw} - \delta^{13}C_{POC-WS})$$
 (6)

- Where $\delta^{13}C_{POC},\,\delta^{13}C_{POC\text{-ws}},$ and $\delta^{13}C_{straw}$ are the $\delta^{13}C$ values of POC from soil samples
- with straw, POC from soil samples without straw (WS), and ¹³C-labelled maize straw
- 283 itself, respectively.
- The content of straw-derived POC (CPOC) was calculated using the following
- 285 equation (Blaud et al., 2012)

$$^{13}C_{POC} = C_{POC} \times f_{POC} \tag{7}$$

- 287 where C_{POC} denotes the content of POC, and f_{POC} denotes the proportion of POC
- derived from maize straw C in total POC.

All results are shown as the mean of the three plot replicates with standard error. Significant differences between treatments (P < 0.05) were calculated by one-way analysis of variance (ANOVA) in combination with Duncan's test using SPSS 19.0 and OriginPro 2019.

2.6 High-throughput sequencing

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Soil total DNA was extracted from 0.5 g of soil using the MP FastDNA SPIN Kit for Soil (MP Biomedicals) according to the manufacturer's instructions. The final DNA concentration and purification were quantified by NanoDrop 2000 UV-vis spectrophotometer (Thermo Scientific, Wilmington, USA), and DNA quality was checked by 1% agarose gel electrophoresis. We performed Polymerase chain reaction (PCR) amplification of the prokaryotic 16S rRNA V3-V4 hypervariable region with primers set 338F/806R (338F, 5'-ACTCCTACGGGAGGCAGCAG-3' and 806R, 5'-GGACTACHVGGGTWTCTAAT-3') (Lee et al., 2012) and fungal ITS1 region with primers set ITS1F/ITS2R (ITS1F, 5'-CTTGGTCATTTAGAGGAAGTAA-3', ITS2R, 5'-GCTGCGTTCTTCATCGATGC-3') (Adams et al., 2013) primer pairs. The PCR reactions were conducted using the following program: 3 min at 95°C; 27 cycles of 30 s at 95°C, 30 s at 55°C, and 45 s at 72°C; and 72°C for 10 min. The bacterial PCR amplification was performed in triplicate 20-μL mixtures containing 4 μL of 5 × FastPfu

Buffer, 2 µL of 2.5 mM dNTPs, 0.8 µL of each primer (5µM), 0.4 µL of FastPfu polymerase, 0.2 µL of BSA, and 10 ng of template DNA. The fungal PCR amplification was performed in triplicate 20- μ L mixtures containing 2 μ L of 10 × Buffer, 2 μ L of 2.5 mM dNTPs, 0.8 μL of each primer (5μM), 0.2 μL of rTaq Polymerase, 0.2 μL of BSA, and 10 ng of template DNA. The products were extracted from 2% agarose gels, purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA), and quantified using QuantiFluorTM-ST (Promega, Madison, WI, USA) according to the manufacturer's protocol. Purified amplicons were pooled in equimolar concentration and paired-end sequenced (2 × 300) on an Illumina platform (Illumina, San Diego, CA, USA) according to the standard protocols by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China). Sequences were deposited in the Sequence Read Archive (SRA) of the National Center for Biotechnology Information (NCBI; Bethesda, MD, USA) under studies PRJNA644514 and PRJNA644592 for the 16S rRNA and ITS rRNA genes, respectively.

2.7 Bioinformatics and statistics analysis

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The sequences were processed using USEARCH v.10.0 (Edgar, 2010) and VSEARCH v.2.7.1 (Rognes et al., 2016). The paired-end Illumina reads processed in the following steps by VSEARCH: joining of paired-end reads and relabeling of

sequencing names; removal of barcodes and primers; filtering of low-quality reads; finding non-redundancy reads. Unique reads were clustered into operational taxonomic units (OTUs) with 97% similarity. The representative sequences were picked by UPARSE (Edgar, 2013). The OTU table was generated by USEARCH. We used the RDP database (Wang et al., 2007) for the taxonomic identity of each bacteria and the UNITE database (Nilsson et al., 2018) for the taxonomic identity of fungi by USEARCH. A total of 2,516,669 16S rRNA and 3,211,166 ITS paired-end high-quality sequences were obtained from all of the 54 soil samples, respectively. The α -diversity index of Shannon and Chao 1 was calculated by USEARCH software. Each sample had a different number of reads, to even the varying read numbers, all the samples were randomly re-sampled to the lowest read number. Therefore, 17229 and 33746 randomly selected 16S rRNA and ITS sequences were used to calculate bacterial and fungal αdiversity.

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Statistical tests and graphical representations were carried out in the R environment (version 3.6.1; https://www.r-project.org/). Differences in the microbial α -diversity were compared using analysis of variance (ANOVA) followed by Tukey's HSD test. P<0.05 was considered to reflect a statistically significant difference. The linear discriminant analysis effect size (LEfSe) method was conducted to identify

different abundance values (at all taxonomic levels) that reflected the different fertilization treatments in each incubation time. Only taxa meeting an LDA significance threshold of 2 for bacterial and fungal communities are shown.

Co-occurrence network analysis (based on class level) was performed to examine the connections within bacterial and fungal taxa. Relative abundances of bacterial and fungal genera were used to construct networks for bacterial and fungal classes in different treatments during the whole process of straw residue decomposition. Average relative abundances of the class level higher than 0.1% were selected for Spearman's correlation analysis. Network analysis was then performed using the "igraph" package in R v. 3.6.1 (Rottjers and Faust, 2018).

To obtain the best discriminant performance of the taxa across the whole incubation time of straw residue decomposition in different fertilizer managements, we regressed the relative abundances of the bacterial and fungal taxa at the order level against the incubation time of straw residue decomposition in the field using the randomForest package (version 4.6-14) in R using default parameters (ntree = 1000, mtry is p/3, where p is the number of taxa in the class) (Liaw and Wiener, 2002). Lists of taxa ranked by Random Forests in order of feature importance were decided more than 100 iterations. The 10-fold cross-validation was performed by using the rfcv ()

function in the randomForest package for selecting appropriate features with five repeats.

The structural equation model (SEM) was obtained using Amos 17.0 software (Chicago, IL: Amos Development Corporation) to quantify the significant effects of residue-derived labile SOC fractions on the changes of soil bacterial and fungal community structure in response to different fertilizer management strategies and incubation time. Three fertilizer treatments (CKS, IFS, IFMS) were categorical variables with three levels: 0 (CKS), 1 (IFS), and 2 (IFMS). We used the robust maximum likelihood estimation to fit the covariance matrix to the model (Wang et al., 2016). The theoretical model was adjusted according to the principle of the low Chisquare $(\gamma^2; 0 \le \gamma^2 \le 2)$, nonsignificant probability (P; P > 0.05), high goodness-of-fitindex (GFI; GFI > 0.90) and root mean square error of approximation (RMSEA; RMSEA < 0.05) to ensure that the final model was adequately fitted (Grace and Keeley, 2006).

3 Results

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3.1 Incorporation of residue-derived labile SOC fractions under different fertilizer

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The content of straw derived active SOC in three treatments occurred various trends (Fig. 1). The content of straw-derived DOC was significantly decreased by 43% (CKS), 50% (IFS) and 77% (IFMS) from the 1st day to the 60th day, respectively (Fig. 1a). On the 150th day, that content in IFMS treatment was 0.49 mg kg⁻¹, significantly lower than in CKS (2.82 mg kg⁻¹) and IFS (4.30 mg kg⁻¹) treatment. The changes in the content of straw-derived MBC over time were different in three treatments (Fig. 1b). The contents of straw-derived MBC in IFS treatment are 59 mg kg⁻¹ (the 1st day), 60 mg kg⁻¹ (the 60th day) and 41 mg kg⁻¹ (the 150th day), which were the highest in three treatments during the whole incubation time. At the end of incubation time, the order of the content of straw-derived MBC in three treatments is IFS > IFMS > CKS. The content of strawderived POC was significantly increased during the incubation time in three treatments (Fig. 1c). On the 150th day, straw-derived POC in IFMS treatment was 198 mg kg⁻¹, which was twice more than in CKS and 42% more than in IFS treatment.

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3.2 Soil bacterial and fungal community diversity and structure

Different fertilizer management significantly changed the α -diversity of the bacterial community. During straw residue decomposition, α -diversity indices (Chao1 and Shannon) in IFM was the highest, the α -diversity in IF was the lowest in the bacterial community (Fig. S1). However, there was no significant difference in the α -

diversity indices (Chao1 and Shannon) of the fungal community among three fertilizer management during straw residue decomposition (Fig. S2).

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The most abundant bacterial and fungal phyla in all soil samples were shown in Fig. S3a and Fig. 2a. During the incubation time, Actinobacteria, Acidobacteria, and Proteobacteria were the dominant phyla in all soil samples. There was no significant difference in the relative abundance of bacterial phylum level in each treatment after adding straw residue (Fig. S3a). With regards to the fungal communities, the relative abundance of Ascomycota was significantly decreased from 45.0% (CK) to 25.4% (CKS), but *Basidiomycota* was significantly increased from 3.4% (CK) to 9.4% (CKS) on the 60th day of the incubation. The relative abundance of Basidiomycota was significantly increased from 1.9% (IF) to 5.7% (IFS) on the 1st day, Zygomycota was significantly decreased from 7.0% (IF) to 3.7% (IFS) on the 150th day of the incubation. The relative abundance of *Basidiomycota* was significantly increased from 0.7% (IFM) to 8.3% (IFMS) on the 150^{th} day (Fig. 2a) of the incubation.

The top bacterial and fungal classes were detailed in Fig. S3b and Fig. 2b. The relative abundances of *Acidobacteria_Gp6* and *Acidobacteria_Gp4* were significantly increased from 6.2% (IF) and 4.1% (IF) to 10.5% (IFS) and 6.4% (IFS) on the 60th day of the incubation in the bacterial community, respectively (Fig. S3b). Regarding the

fungal classes, compared to CK treatment, the relative abundance of *Sordariomycetes* was significantly decreased on the 1st day in CKS treatment. The relative abundance of *Agaricomycetes* was significantly increased during the incubation time, and *Pezizomycetes* was significantly increased on the 1st day in IFS than in IF treatment. The relative abundance of *Agaricomycetes* was increased more than double in IFMS than in IFM treatment on the 1st day (Fig. 2b). Furthermore, the relative abundance of *Dothideomycetes* in IF, IFS, IFM, IFM treatments was higher than in CK and CKS treatments, the relative abundance of *Sordariomycetes* had the opposite trend.

3.3 Taxonomic biomarkers of soil microbial communities

The LEfSe analysis from phylum to genus levels was performed to identify high-dimensional biomarker taxa with different abundances among three fertilization regimes after adding straw residues in each incubation time (Fig. 3, 4, and 5). From an overall perspective, after adding straw residue, more significantly different taxa occurred between CK and CKS treatment, IF and IFS treatment than IFM and IFMS treatment during the incubation time (Fig. 3, 4, and 5).

For the bacterial communities, in CKS treatment, the *Nitrospirae* was the most abundant biomarkers on the 1st day (Fig. 3a); the *Actinomycetales* and *Cytophagaceae* were especially enriched on the 60th day (Fig. 3b); the *Gammaproteobacteria* was

enriched on the 150th day (Fig. 3c). In IFS treatment, the *Actinobacteria* was enriched on the 1st day (Fig. 4a); the *Nitrospirae* and *Deltaproteobacteria* were significantly enriched on the 60th day (Fig. 4b); the *Actinobacteria* and *Burkholderiaceae* were the significantly different abundance on the 150th day (Fig. 4c). In IFMS treatment, the *Chloroflexi* was the most differentially abundant phyla on the 60th day (Fig. 5b).

Regarding the fungal communities, in CKS treatment, the *Eurotiomycetes*, *Dothideomycetes*, *Chytridiomycota* were significantly changed on the 1st day (Fig. 3d); the *Eurotiales* was significantly different on the 60th day (Fig. 3e); the *Eurotiales*, *Wallemiomycetes*, and *Lasiosphaeriaceae* were enriched on the 150th day (Fig. 3f). In IFS treatment, the *Hypocreales fam_Incertae_sedis* showed the highest abundance on the 1st day (Fig. 4d); the *Sordariomycetes* and *Basidiomycota* were significantly enriched on the 60th day (Fig. 4e); the *Sordarlales* was the most abundant biomarker on the 150th day (Fig. 4f). In IFMS treatment, the *Basidiomycota* was the most differentially abundant phylum on the 1st day (Fig. 5d).

3.4 Co-occurrence network in soil bacterial and fungal community

To determine the effects of fertilizer treatments on the soil microbial community after adding straw residue, networks were constructed for three fertilization treatments with and without added straw residue (Fig. 6 and 7). Long-term different fertilizer

management changed bacterial and fungal co-occurrence patterns, the application of IF and IFM fertilizer increased the complexity of the bacterial community but decreased the complexity of the fungal community (Fig. 6a, b, and c; Fig. 7a, b, and c). After adding straw residue, the num edges and average degree of bacterial communities were decreased in all treatments, the most decreased in IFMS treatment particularly. In fungal communities, the num edges and average degree in CKS treatment were also decreased than in CK treatment but in IFS and IFMS treatments were increased than in IF and IFM treatments (Table 1 and 2). These results indicated that during the process of straw residue decomposition the complexity of microbial network in bacterial communities was decreased, the IFMS treatment was more affected than CKS and IFS treatment. In fungal communities, the complexity of microbial networks in IFS and IFMS treatment were increased than in IF and IFM treatment, but in CKS treatment was decreased than in CK treatment (Fig. 6 and 7).

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3.5 Keystone taxa of straw microbiota during straw residue decomposition

To examine the bacterial and fungal keystone taxa of the whole process of straw residue decomposition in various fertilizer regimes, we regressed the relative abundances of bacteria and fungi at the order level using a random forest regression.

We performed 10-fold cross-validation with five repeats to determine the importance

of bacterial and fungal classes. We showed the 11 most important bacterial and fungal orders as keystone taxa. We defined these taxa as keystone taxa in the model in order of discriminatory importance as shown in Fig. 8. We found bacterial and fungal keystone taxa during the straw residue decomposition in different fertilizer regimes were various. During the whole incubation time, in the bacterial community, the Anaerolineales order of the Chloroflexi phylum was the most important taxa in CKS treatment; Actinomycetales and Solirubrobacterales orders of Actinobacteria phylum were the taxa in IFS treatment; Myxococcales, Rhodocycales, and Rhodobacterales orders of Proteobacteria phylum were the taxa in IFMS treatment. In the fungal community, the Saccharomycetales order of Ascomycota phylum was the vital taxon in CKS and IFS treatment; the Hypocreales order was the essential taxon in IFMS treatment during the whole straw decomposition process.

3.6 SEM analysis on the significant effects of residue-derived labile SOC fractions

on soil microbes

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To assess the significant effects of residue-derived labile SOC fractions on the changes of soil bacterial and fungal community structure in response to different fertilizer management strategies and incubation time, a SEM model was conducted (Fig. 9). Fertilization had positive direct impacts on both the bacterial (+0.49) and the fungal

community (+ 0.46), but incubation time only had a positive direct impact on the bacterial community (+ 0.98). All the content of residue-derived labile SOC fractions had positive impacts on the fungal community, the content of residue-derived POC contributed the greatest impact (+ 0.76). The bacterial community was only affected by the content of residue-derived MBC (+ 0.54).

4 Discussion

- 4.1 Straw residue-derived labile SOC fractions as affected by fertilizer
- 493 management strategies and incubation time
 - DOC was produced from the decomposition of SOM which is primarily driven by soil microorganisms (Marschner and Bredow, 2002). The content of residue-derived DOC decreased quickly, this result is consistent with our previous study in Alfisol (Jin et al., 2020), which indicated residue-derived DOC would be primarily utilized by microorganisms after added straw residue (Kalbitz et al., 2000). Application of organic fertilizers would maintain a steady flow of nutrients into the soil, but they released nutrients more slowly (Baghdadi et al., 2018), which may be the reason that the content of residue-derived DOC in IFMS treatment is lower than CKS and IFS treatment.
 - MBC is the living microbial component of SOC and is considered a sensitive

indicator of microbial activity (Broos et al., 2007; Paul, 1984). In this study, the content of residue-derived MBC in IFS and CKS treatment was higher than the IFMS treatment at the beginning of the incubation time. That because microbial biomass from CKS and IFS treatment responded rapidly (i.e., within 1 day) to the residue returning by accumulating residue-derived C, this result indicated compared with IFM treatment, CK and IF treatment lacked sufficient nutrients. After adding straw, microbes would quickly participate in the decomposition of residue-derived C in a starved state (Bastida et al., 2013). At the end of the incubation time, the content of residue-derived MBC in IFS and IFMS treatment were higher than CKS treatment, which indicated with the organic fertilizer released nutrient, the treatment included fertilizer would promote the fixation of residue-derived MBC by soil microorganisms (Luan et al., 2020). In this study, there was an inverse trend between the content of residue-derived DOC and residue-derived POC in each treatment during the incubation time. That because POC is composed of decomposing plant and microbial residues, and it is served as essential sources of plant nutrients and decomposed by microbes (Feller and Beare,

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1997; Plaza et al., 2018; Xiao et al., 2017). Thus, with the process of straw residue

decomposition, the content of residue-derived POC increased. At the end of incubation

is that application of manure improves the soil structure, promotes the formation of soil aggregates, and allows part of the free SOC to be protected by the soil aggregates, thereby promoting the increase in the content of straw residue-derived POC (Mi et al., 2016; Verma and Sharma, 2007).

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4.2 Microbial community structure responses to straw residue decomposition

Straw residue returned to the soil affected the soil fungal communities more strongly than the soil bacterial communities on phylum and class levels (Fig. 2), which was consistent with the previous study (Maarastawi et al., 2018). Straw is beneficial to enhance microbial processing in CK and IF treatment and has no negative effect on IFM treatment (Fig. 3, 4, and 5). It is known that saprotrophic fungi contribute to the decomposition of residue-derived C and thus boost C mineralization in soil (Dini-Andreote et al., 2016). Dothideomycetes have been implicated in assimilating C derived from plants (Freedman et al., 2015). In CKS treatment, the Dothideomycetes quickly responded on the 1st day. After adding straw residue, the relative abundance of Basidiomycota significantly changed but these varieties initiated at different incubation stages under various fertilizer regimes (Fig. 2a). Basidiomycota played particularly important roles in degrading plant litter with high lignin contents in soils (Entwistle et al., 2018). The response of Basidiomycota to add straw residue in IFS and IFMS

treatment was much quicker than in CKS treatment. The IF and IFM treatment had more nutrients than in CK treatment, which could be the driver for this, and is in line with previous studies that Basidiomycota is better adapted to high nutrient levels (Hannula et al., 2012). From the 60th day to the 150th day during the incubation time, the relative abundance of Sordariomycetes significantly increased in IFS and IFMS treatments (Fig. 4e, 4f, 5e, and 5f). A similar result was found in another study (Ma et al., 2018), probably due to IF and IFM treatment had abundant nutrients and can promote the growth of microbial groups due to their diverse metabolic capacity so that Sordariomycetes can play the role of straw degradation effectively (Ding et al., 2017; Koranda et al., 2014). Sordariomycetes class is one of the largest classes in the phylum Ascomycota and is also known to play a role in the degradation of crop residue (Qin et al., 2014; Tardy et al., 2015). Fungi link the allocation of C and sequestration of nutrients from organic substrates, and it is important in decomposing plant-derived substrates (Hobara et al., 2014; Quirk et al., 2012). Different fertilization regimes affect soil fungal community composition in various ways. The organic application directly increased the amount of SOC and intensively affected soil fungal community composition (Sun et al., 2016). Inorganic fertilization regulated the quality and quantity of plant-derived C inputs to indirectly affect the soil fungal community (Weber et al.,

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4.3 Straw residue decomposition and fertilization changed the co-occurrence

patterns of microbial community

In the network for the whole process of straw residue decomposition, we found the bacterial and fungal communities exhibit different co-occurrence patterns (Fig. 6 and 7). The further addition of straw residues results in a beneficial increase of the overall microbial abundance together with an enhancement of the complexity of the fungi community in applying fertilizer field, but as observed in our study for all fertilizer strategies lead to a reduction in the bacterial complexity. These results indicated that straw addition is always detrimental to bacterial complexity independent of fertilizer management. It showed strong competitive interactions between bacterial species for composing straw residue impeded species coexistence and decreased bacterial communities' stability (Ratzke et al., 2020). Although bacterial complexity was decreased, that did not affect the role of soil microbes on straw residue decomposition, because of redundancy of functions within the microbial community, i.e., the other microbes would also do the function of residue decomposition (Wagg et al., 2019). Regarding fungal communities, the changes in the complexity of the network in IFS and IFMS treatment were different to those found within CKS treatment during the

whole process of residue decomposition (Fig. 7). That results suggested added organic materials to the soil of a high fertility level would provide a good habitat for the growth of the fungi, lead to better stability and provide stronger resistance to the disturbance of the fungal community (Scheffer et al., 2012). Therefore, straw addition is beneficial to increase fungal complexity in the applied fertilizer field, independent if inorganic fertilizer or mixed inorganic fertilizer and manure. Fungal species however would have competition for experienced the resource due to lack of nutrients in CKS treatment so that here we observed a decreased stability of the network (Fuhrman, 2009). Moreover, added straw residue decreased the percentage of positive links in the bacterial community among three treatments, which suggested that straw residue positively affected bacterial species competition and niche separation (Deng et al., 2016; Yu et al., 2018). In the fungal community, the percentage of positive links also decreased in CKS and IF treatment (Fig. 7). This result indicated IFMS treatment had more diverse organic compounds and increased fungal community cooperation and niche overlap during straw residue decomposition (Kong et al., 2020).

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4.4 Temporal responses of keystone taxa dynamics during straw residue decomposition

A dominant species often affects ecosystem functioning or a specific process exclusively by its sheer abundance, but keystone taxa can also, more subtly, utilize their

influence on microbial system function irrespective of abundance (Banerjee et al., 2018; Fierer, 2017). We further identified 11 bacterial and fungal orders as keystone taxa of microbial community dynamics during the process of straw residue decomposition by random forest model (Fig. 8). These results may be useful for identifying the most closely related bacterial and fungal taxa during the process. We found the keystone taxa in the phylum level in the bacterial community were different in various fertilizer regimes, but the keystone taxon in the fungal community was the same, i.e., Ascomycota. Chloroflexi was considered as the keystone taxon in CKS treatment (Fig. 8a). Chloroflexi was the oligotrophic bacteria phylum and it can grow under low substrate concentrations (Pepe-Ranney et al., 2016). In this study, the relative abundance of *Chloroflexi* was significantly changed at the early stage of straw residue decomposition in CKS treatment (Fig. 3a), which indicated that CKS treatment had a lower nutrient and limited growth of copiotrophic decomposers, when added straw residue to CK treatment, Chloroflexi would actively participate in assimilating straw residue (Tardy et al., 2015). Actinobacteria was considered as keystone taxon in IFS treatment (Fig. 8b). Actinobacteria was the coptotrophic phylum and more sensitive to carbon sources in the soil and maybe abundant after adding labile SOC (Goldfarb et al., 2011). They are vital saprophytes capable of degrading complex plant debris (Barka et

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al., 2015). Proteobacteria acted as keystone taxon in IFMS treatment (Fig. 8c). 612 613 Proteobacteria was a copiotrophic phylum and showed saprophytic lifestyles, the nutrient supply in IFMS treatment was sufficient, thus Proteobacteria fit in this 614 situation and grow fast during the process of straw residue decomposition (Zhan et al., 615 616 2018). Ascomycota was the keystone taxon in the fungal community among the three 617 fertilizer regimes regardless of whether the nutrients are sufficient (Fig. 8d, e, and, f). That because *Ascomycota* is known as the largest and most diverse fungal phylum as 618 619 well as the key decomposers in the decomposition of the organic materials, it harbored a wide scale of substrate utilization and is essential in breaking down the recalcitrant 620 621 organic compounds (Schoch et al., 2009; Wang et al., 2018; Wang et al., 2021). 4.5 Relationship of residue-derived labile SOC fractions and soil microbes in 622 623 response to fertilizer management and straw residue returned 624 The SEM model showed different residue-derived labile SOC fractions had various 625 impacts on the bacterial and fungal communities. The residue-derived DOC, POC, and MBC significantly affected the fungal community while the bacterial community was 626 only significantly affected by residue-derived MBC, that indicated the fungal 627 community was more sensitive bacterial community to the residue-derived labile SOC, 628

consistent with other studies (Kong et al., 2020; Zhong et al., 2020). The residuederived MBC significantly affected both bacterial and fungal communities, which indicated MBC is an important indicator of soil microbes and would affect microbial community construction. The residue-derived POC contributed the greatest impact on the fungal community, the reason might be more residue-derived C accumulated and formed POC during the whole process of straw residue decomposition and then significantly affect the fungal community (Goldstein et al., 2020). The influence level of the fungal community from residue-derived DOC was similar to POC, the difference between these two fractions is that residue-derived DOC brought impacts on the initial stage, whereas residue-derived DOC worked at the end of the straw residue decomposition process. This result also verified the changes in the content of residuederived DOC and POC in our present study.

Conclusions

Straw residue addition decreased the bacterial microbial network complexity in all treatments, but increased fungal network complexity in IFS and IFMS. *Chloroflexi* (CKS), *Actinobacteria* (IFS), *Proteobacteria* (IFMS) were keystone taxa in the bacterial community, and *Ascomycota* was the keystone taxon in the fungal community. The straw residue was retained as POC in labile SOC fractions and was further

enhanced in fertilizer management with manure addition. Residue-derived MBC was higher under fertilized conditions. Less pronounced effects on the microbial community following straw addition were found for the combined inorganic and organic fertilizer treatment compared to those without or with only inorganic fertilizer addition. The observed temporal changes in the microbial community suggested that in these Mollisols, independent of agricultural fertilizer management, straw residue-derived POC and DOC promoted fungal C processing, whereas for bacterial this was facilitated only via residue-derived MBC.

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Conflict of Interest:

The authors declare that they have no conflict of interest.

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1058 Tables

Table 1 Topological properties of the co-occurrence network in the bacterial community under various fertilizer regimes during the process of straw residue decomposition

	CK	CKS	IF	IFS	IFM	IFMS
Num.edges	55	46	211	99	139	38
pos.edges (percentage)	55 (100)	43 (93)	209 (99)	91 (92)	134 (96)	36 (94)
neg.edges (percentage)	0 (0)	3 (7)	2(1)	8 (8)	5(4)	2 (6)
num.vertices	67	67	116	87	117	55
connectance	0.02	0.02	0.03	0.03	0.02	0.03
average.degree	1.64	1.37	3.64	2.28	2.38	1.38
average.path.length	2.43	1.51	4.36	3.47	3.24	1.65
diameter	7	4	11	7	8	4
clustering.coefficient	0.53	0.29	0.52	0.41	0.50	0.24
no.clusters	22	24	11	20	24	19
centralization.degree	0.05	0.02	0.06	0.05	0.05	0.03
centralization.betweenness	0.01	0.00	0.11	0.08	0.02	0.01

Note: CK, IF, IFM, CKS, IFS, and IFMS denote control, inorganic fertilizer, inorganic fertilizer plus manure, control + straw, inorganic fertilizer + straw, and inorganic fertilizer plus manure + straw treatment, respectively.

Table 2 Topological properties of the co-occurrence network in the fungal community under various fertilizer regimes during the process of straw residue decomposition

	CK	CKS	IF	IFS	IFM	IFMS
Num.edges	63	27	16	42	8	33
pos.edges (percentage)	62 (98)	19 (70)	15 (93)	38 (90)	6 (75)	30 (90)
neg.edges (percentage)	1 (2)	8 (30)	1 (7)	4 (10)	2 (25)	3 (10)
num.vertices	45	38	22	43	13	39
connectance	0.06	0.04	0.07	0.05	0.10	0.04
average.degree	2.80	1.42	1.45	1.95	1.23	1.69
average.path.length	2.91	1.44	1.35	1.34	1.00	1.96
diameter	6	4	3	3	1	5
clustering.coefficient	0.45	0.60	0.67	0.82	1.00	0.33
no.clusters	8	14	9	14	6	11
centralization.degree	0.10	0.04	0.12	0.07	0.06	0.06
centralization.betweenness	0.13	0.01	0.02	0.01	0.00	0.02

Note: CK, IF, IFM, CKS, IFS, and IFMS denote control, inorganic fertilizer, inorganic fertilizer plus manure, control + straw, inorganic fertilizer + straw, and inorganic fertilizer plus manure + straw treatment, respectively.

1069 Figures Captions

- 1070 Figure 1 Content of residue-derived dissolved organic carbon (DOC) (a), residue-
- derived microbial biomass carbon (MBC) (b), and residue-derived particle organic
- 1072 carbon (POC) (c). Different capital letters indicated significant differences (P < 0.05)
- among different incubation time under the same fertilization treatment. Different
- lowercase letters indicated significant differences (P < 0.05). The values presented in
- the figures are given as mean \pm standard errors. CKS denotes control + straw, IFS
- denotes inorganic fertilizer + straw, and IFMS denotes inorganic fertilizer plus manure
- + straw, respectively.
- 1078 **Figure 2** Relative abundance of the taxonomic composition of soil fungal community
- at the phylum level (a) and class level (b), respectively. Treatments including CK
- 1080 (control), CKS (control + straw), IF (inorganic fertilizer), IFS (inorganic fertilizer +
- straw), IFM (inorganic fertilizer plus manure), IFMS (inorganic fertilizer plus manure
- + straw). Soil samplings were conducted in 1 day, 60 days, and 150 days after added
- straw residue. Phylum and class names were color-coded on the right listed above.
- 1084 Figure 3 Cladogram plotted from LEfSe analysis revealed the bacterial taxonomic
- levels with different abundance values in 1 day (a), 60 days (b), and 150 days (c), and
- fungal taxonomic levels with different abundance values in 1 day (d), 60 days (e), and
- 1087 150 days (f) between CK (control) and CKS (control + straw) treatment. The circular
- 1088 ring from inside to outside represents phylum, class, order, family, and genus,
- 1089 respectively.
- 1090 **Figure 4** Cladogram plotted from LEfSe analysis revealed the bacterial taxonomic
- levels with different abundance values in 1 day (a), 60 days (b), and 150 days (c), and
- fungal taxonomic levels with different abundance values in 1 day (d), 60 days (e), and
- 1093 150 days (f) between IF (inorganic fertilizer) and IFS (inorganic fertilizer + straw)
- treatment. The circular ring from inside to outside represents phylum, class, order,
- family, and genus, respectively.
- 1096 **Figure 5** Cladogram plotted from LEfSe analysis revealed the bacterial taxonomic
- levels with different abundance values in 1 day (a), 60 days (b), and 150 days (c), and
- fungal taxonomic levels with different abundance values in 1 day (d), 60 days (e), and
- 1099 150 days (f) in IFM (inorganic fertilizer plus manure) and IFMS (inorganic fertilizer

- plus manure + straw) treatment. The circular ring from inside to outside represents
- phylum, class, order, family, and genus, respectively.
- 1102 **Figure 6** The network analysis showed the co-occurrence patterns of bacterial taxa at
- class level in different treatments including CK (control) (a), IF (inorganic fertilizer)
- 1104 (b), IFM (inorganic fertilizer plus manure) (c), CKS (control + straw) (d), IFS
- (inorganic fertilizer + straw) (e), IFMS (inorganic fertilizer plus manure + straw) (f)
- during the whole process of straw residue decomposition. Red lines represent
- significant positive (P<0.05) linear relationships and blue lines represent negative
- 1108 (P<0.05) linear relationships.
- 1109 **Figure 7** The network analysis showed the co-occurrence patterns of fungal taxa at
- class level in different treatments including CK (control) (a), IF (inorganic fertilizer)
- 1111 (b), IFM (inorganic fertilizer plus manure) (c), CKS (control + straw) (d), IFS
- 1112 (inorganic fertilizer + straw) (e), IFMS (inorganic fertilizer plus manure + straw) (f)
- during the whole process of straw residue decomposition. Red lines represent
- significant positive (P<0.05) linear relationships and blue lines represent negative
- 1115 (P<0.05) linear relationships.
- 1116 **Figure 8** Random-forest model detects bacterial and fungal taxa that predict bacterial
- keystone taxa in CKS (control + straw) (a), IFS (inorganic fertilizer + straw) (b), IFMS
- 1118 (inorganic fertilizer plus manure + straw) (c) and fungal keystone taxa in CKS (control
- + straw) (d), IFS (inorganic fertilizer + straw) (e) and IFMS (inorganic fertilizer plus
- manure + straw) (f) during the whole process of straw residue decomposition. The top
- 1121 11 bacterial and fungal orders were identified by applying a random forest regression
- of their relative abundances in the straw residue decomposition against the incubation
- time of different fertilizer managements. Keystone taxa are ranked in descending order
- of importance to the accuracy of the model.
- 1125 Figure 9 A structural equation model (SEM) assesses the significant effects of residue-
- derived labile SOC fractions on the changes of soil bacterial and fungal community
- structure in response to different fertilizer management strategies and incubation time.
- Numbers adjacent to arrows represent path coefficients. The width of arrows indicates
- the strength of the standardized path coefficient. The blue lines indicate positive path
- 1130 coefficients, red lines indicate negative path coefficients, and grey lines indicate non-
- 1131 coefficients, respectively. Significance levels are denoted with *P < 0.05, **P < 0.01,

- ***P < 0.001. Chi-square ($\chi^2 = 0.320$), probability level (P = 0.811), goodness-of-fit
- index (GFI = 0.990), and root-mean-square errors of approximation (RMSEA = 0.000)
- indicate that our data matches the hypothetical model.

Supplementary Materials

Table S1Soil basic properties of different fertilizer management strategies in 2018.

Treatment	SOC (g kg ⁻¹)	δ^{13} C (‰)	TN (g kg ⁻¹)	C/N ratio	AP (mg kg ⁻¹)	AK(mg kg ⁻¹)
CK	15.0±0.1 b	-18.8±0.0 a	1.4±0.1 c	10.6±0.4 a	15.32±0.75 c	65.04±3.18 c
IF	15.0±0.1 b	-19.4±0.0 c	1.5±0.0 b	9.7±0.0 b	20.90±0.70 b	81.42±1.40 b
IFM	25.2±0.2 a	-19.1±0.1 b	2.6±0.0 a	9.7±0.0 b	80.66±2.56 a	137.37±3.87 a

Note: The CK denotes no fertilization control treatment, IF inorganic fertilizer treatment, IFM inorganic fertilizer plus manure treatment. SOC denotes soil organic carbon, TN denotes total nitrogen, AP denotes available phosphorous, AK denotes available potassium. Different lowercase letters mean significant differences (P<0.05) in various fertilizer management strategies.

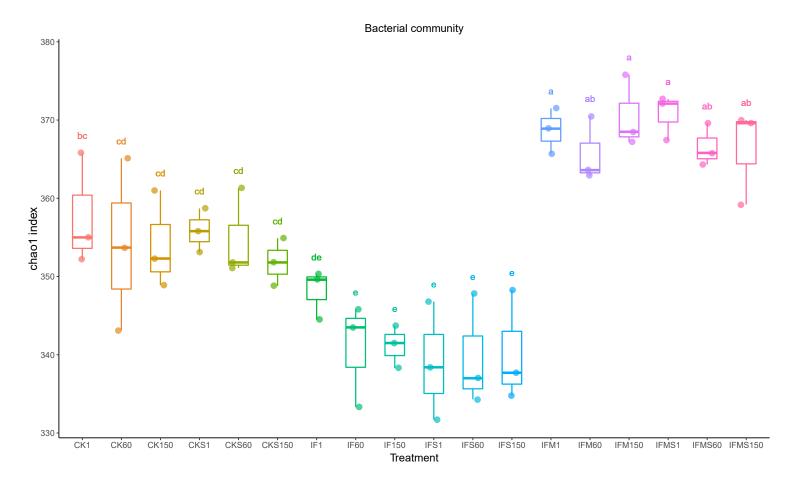
Table S2

Content of soil dissolved organic carbon (DOC), microbial biomass carbon (MBC), and particle organic carbon (POC) with and without straw residue under different fertilizer management strategies during the incubation time.

Treatment	Incubation time (day)	DOC (mg kg ⁻¹)	MBC (mg kg ⁻¹)	POC (g kg ⁻¹)
	1	828 ± 26	843 ± 33	3.79 ± 0.64
CK	60	371 ± 32	106 ± 38	2.94 ± 0.18
	150	329 ± 52	157 ± 1.7	3.39 ± 0.18
	1	930 ± 1.8	904 ± 25	3.61 ± 0.44
IF	60	332 ± 32	89 ± 6.5	2.85 ± 0.02
	150	308 ± 2.3	111 ± 47	3.32 ± 0.05
	1	925 ± 11	904 ± 4.8	9.68 ± 0.35
IFM	60	412 ± 8.3	247 ± 31	8.44 ± 0.36
	150	268 ± 43	420 ± 12	9.07 ± 0.46
	1	946 ± 59	857 ± 16	3.46 ± 0.1
CKS	60	408 ± 28	145 ± 24	3.83 ± 0.26
	150	369 ± 32	251 ± 81	4.04 ± 0.25
	1	930 ± 31	846 ± 64	3.61 ± 0.08
IFS	60	334 ± 8.6	232 ± 28	3.25 ± 0.29
	150	312 ± 8.1	267 ± 5.3	3.37 ± 0.37
	1	933 ± 16	881 ± 5.2	10.37 ± 1.02
IFMS	60	424 ± 15	236 ± 32	9.04 ± 0.98
	150	326 ± 30	435 ± 24	9.33 ± 0.55

Note: CK denotes control, CKS denotes control + straw, IF denotes inorganic fertilizer, IFS denotes inorganic fertilizer + straw, IFM denotes inorganic fertilizer plus manure, IFMS denotes inorganic fertilizer plus manure + straw.







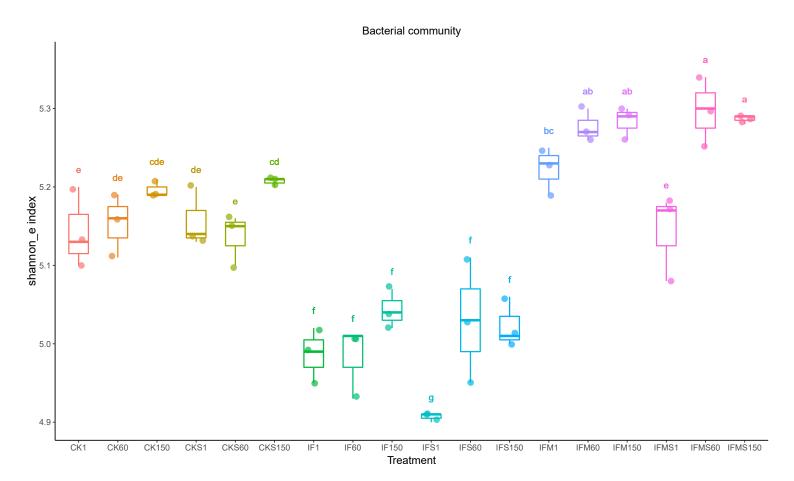
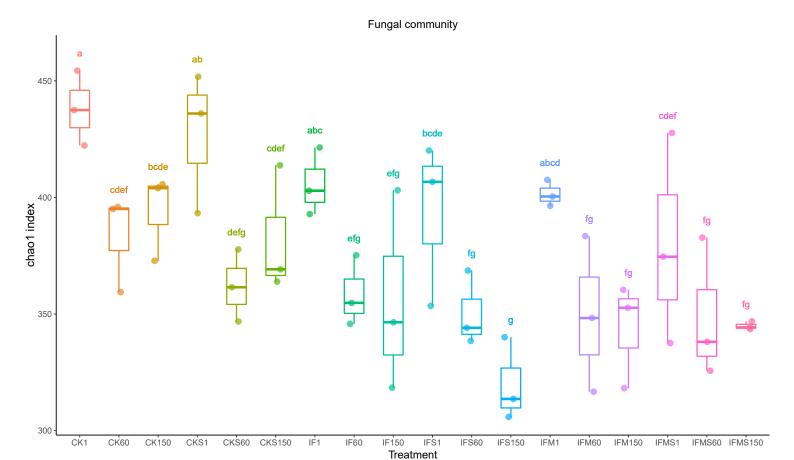


Figure S1 Box plots of Chao 1 (a) and Shannon (b) diversity indices of soil bacterial community in CK (control), IF (inorganic fertilizer), IFM (inorganic fertilizer plus manure), CKS (control + straw), IFS (inorganic fertilizer + straw), and IFMS (inorganic fertilizer plus manure + straw) after adding straw residue in 1 day, 60 days and 150 days.





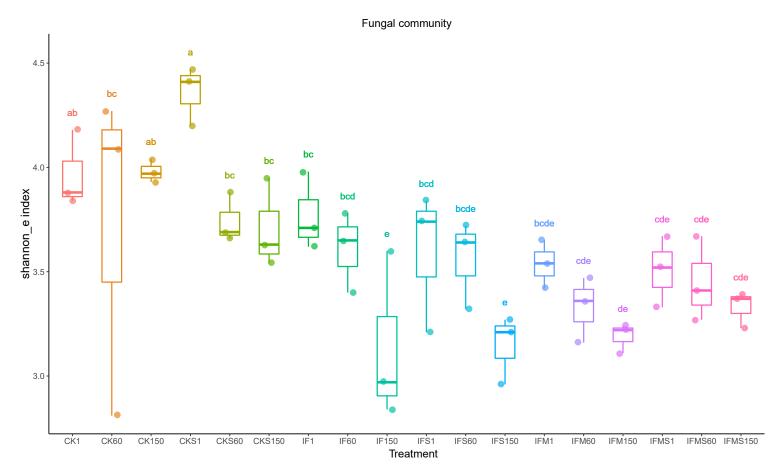
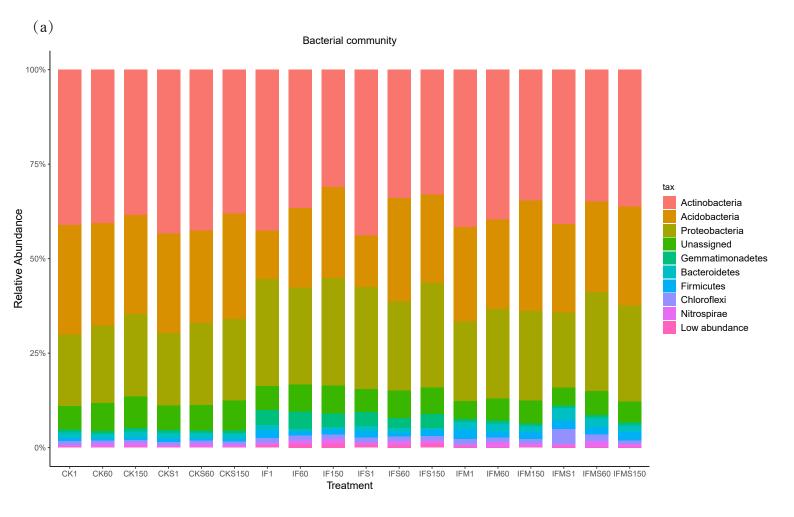


Figure S2 Box plots of Chao 1 (a) and Shannon (b) diversity indices of soil fungal community in CK (control), IF (inorganic fertilizer), IFM (inorganic fertilizer plus manure), CKS (control + straw), IFS (inorganic fertilizer + straw), and IFMS (inorganic fertilizer plus manure + straw) after adding straw residue in 1 day, 60 days and 150 days.



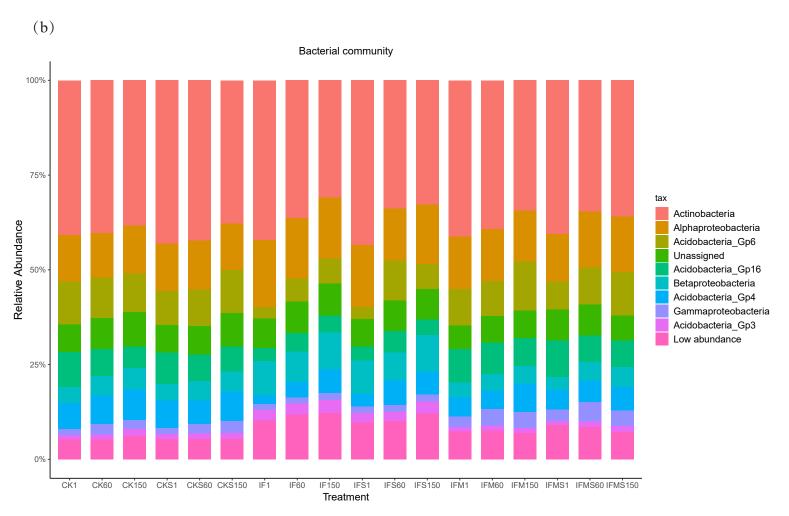


Figure S3 Relative abundance of taxonomic composition of soil bacterial community at phylum level (a) and class level (b), respectively. Treatments including CK (control), CKS (control + straw), IF (inorganic fertilizer), IFS (inorganic fertilizer + straw), IFM (inorganic fertilizer plus manure), IFMS (inorganic fertilizer plus manure + straw). Soil samplings were conducted in 1 day, 60 days and 150 days after added straw residue. Phylum and class names were color-code on the right listed aboved.

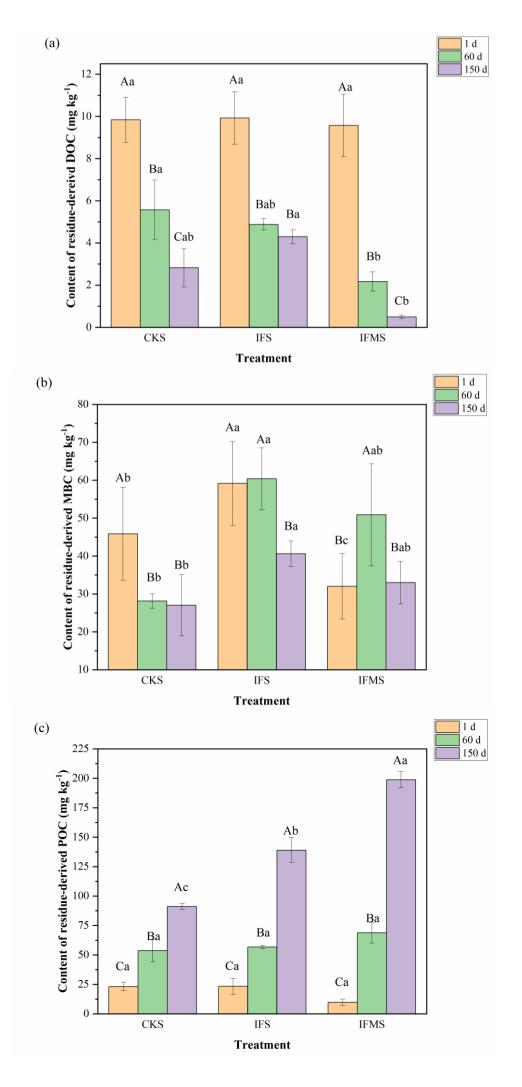
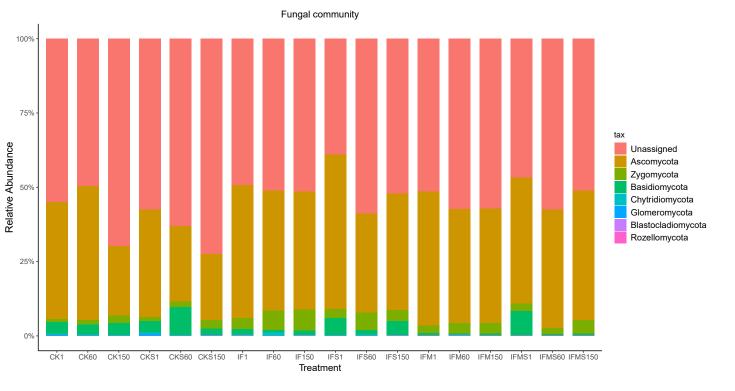


Figure 1







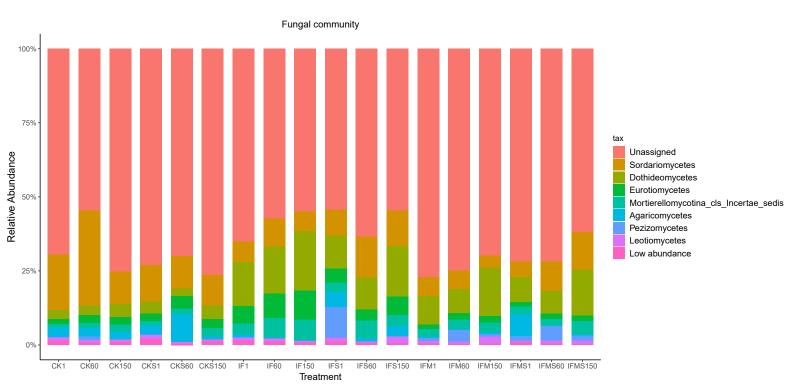


Figure 2

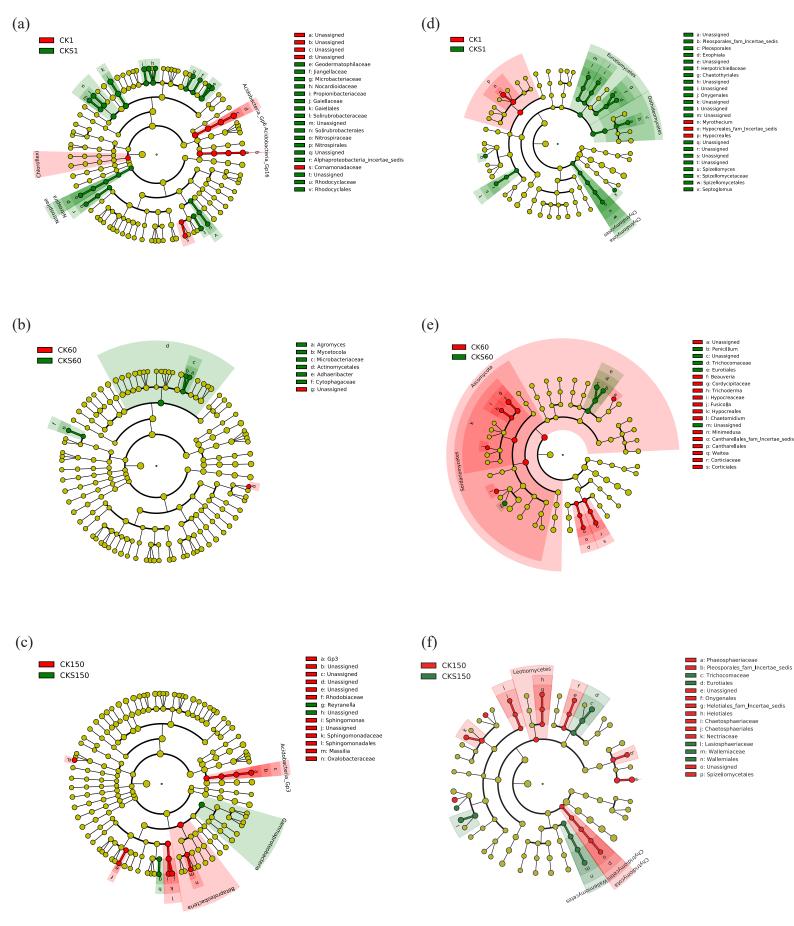


Figure 3

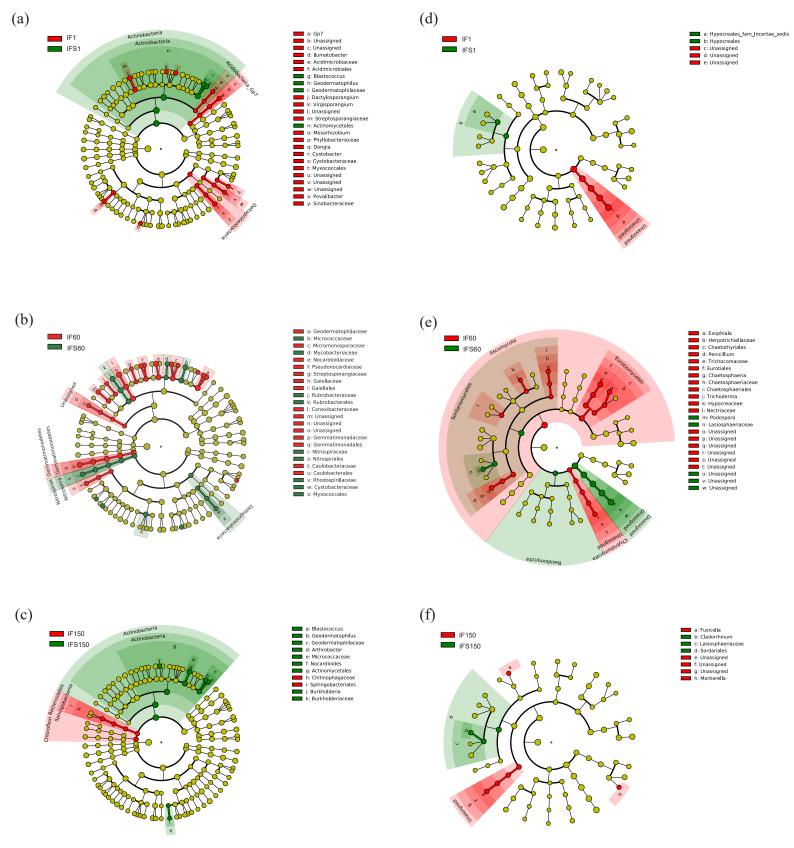


Figure 4

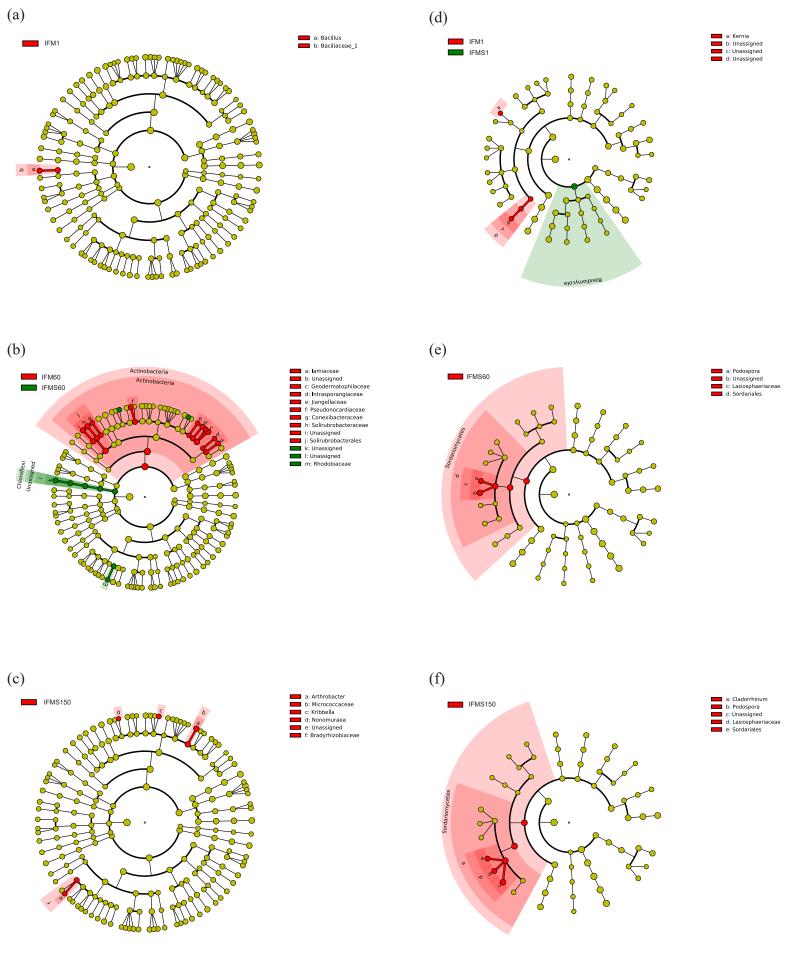


Figure 5

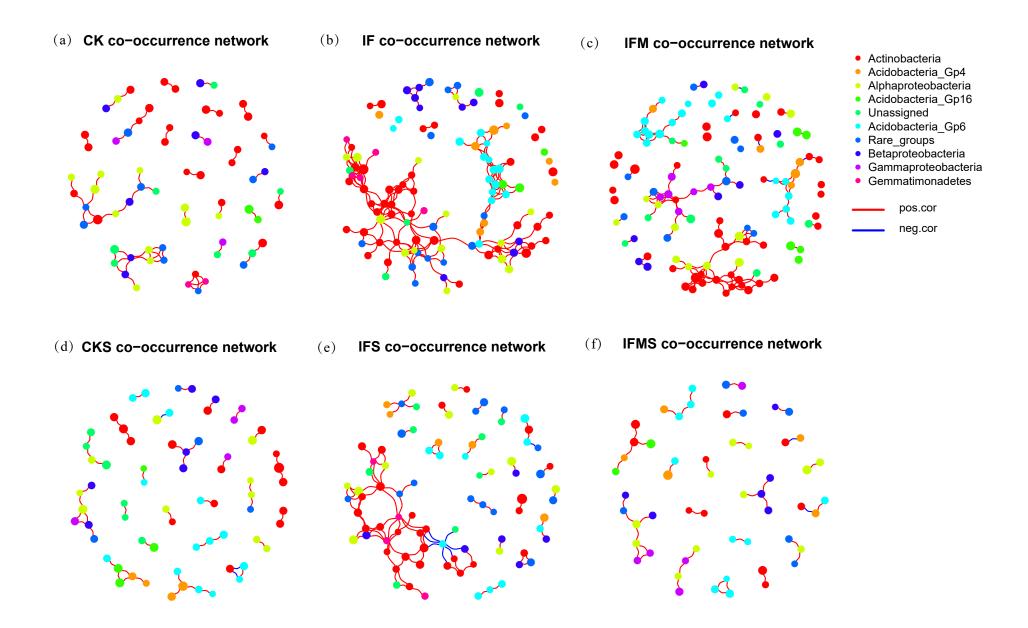


Figure 6

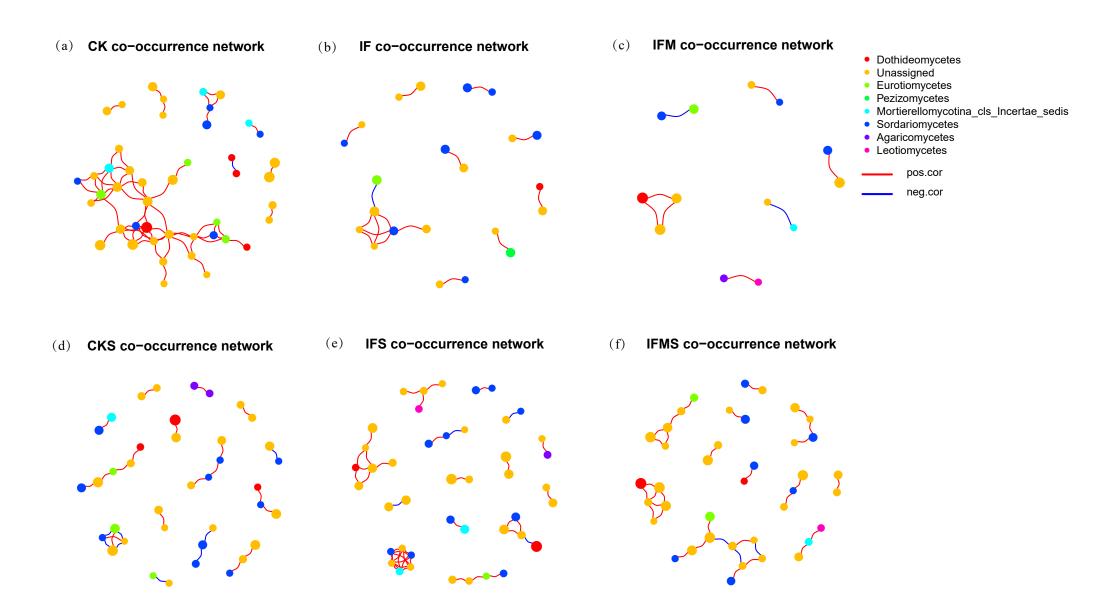


Figure 7

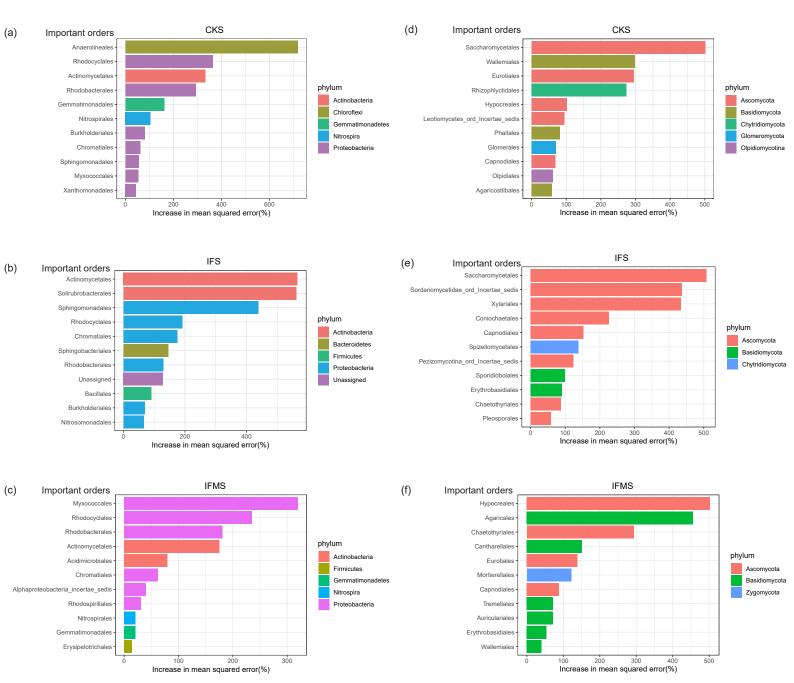


Figure 8

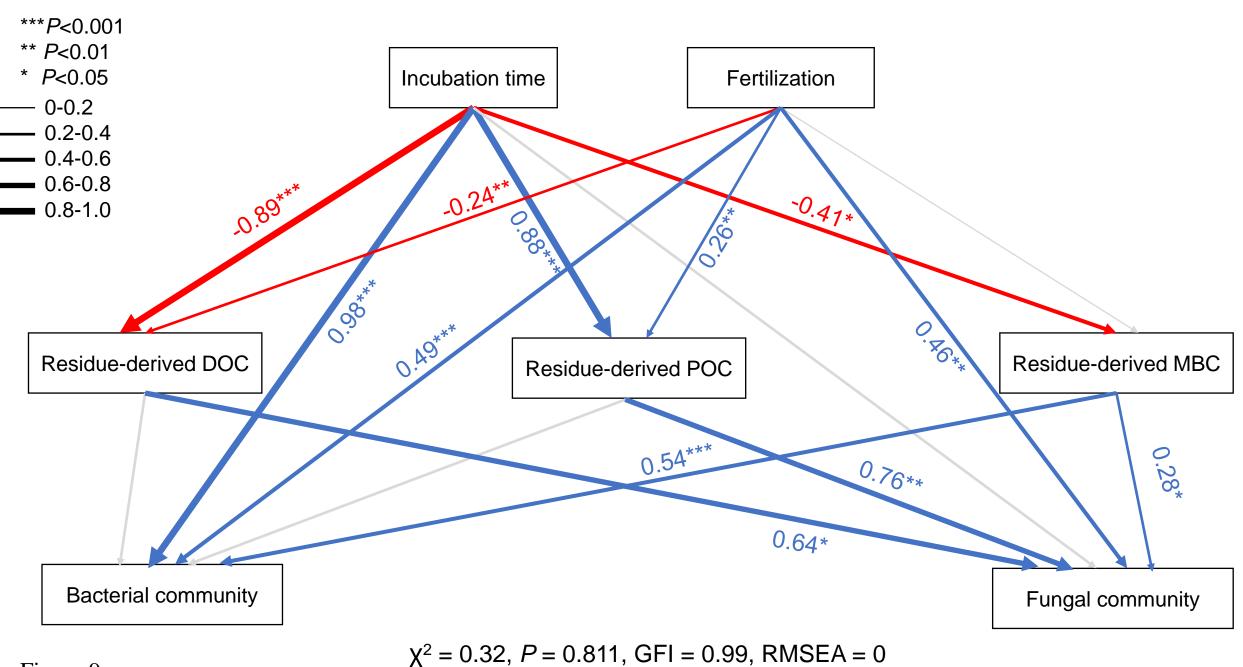


Figure 9

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: