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Long-Term Fertilization Mediates Microbial Keystone Taxa to Regulate Straw-Derived ^{13}C Incorporation in Soil Aggregates

Zhuang Ge [1,2], Roland Bol [3,4], Tianhao Wang [1], Ping Zhu [5], Tingting An [2,*], Shuangyi Li [2,*] and Jingkuan Wang [2]

1 College of Life and Health, Dalian University, Dalian 116622, China; wangtianhao@dlu.edu.cn (T.W.)
2 Northeast Key Laboratory of Conservation and Improvement of Cultivated Land (Shenyang), Ministry of Agriculture, College of Land and Environment, Shenyang Agricultural University, Shenyang 110866, China
3 Institute of Bio- and Geosciences, Agrosphere (IBG-3), Forschungszentrum Jülich GmbH, 52428 Jülich, Germany; r.bol@fz-juelich.de
4 Institute for Biodiversity and Ecosystem Dynamics (IBED), University of Amsterdam, 1090 GE Amsterdam, The Netherlands
5 Jilin Academy of Agricultural Sciences, Gongzhuling 136100, China; zhuping1962@sohu.com
* Correspondence: atting@syau.edu.cn (T.A.); shy_li@syau.edu.cn (S.L.); Tel.: +86-(24)-88487155 (T.A. & S.L.)

Abstract

Soil aggregates are crucial for fertility and organic carbon (C) sequestration, with straw decomposition by soil microbes playing a key role in this process. However, the mechanisms of how fertilization and microbes control straw decomposition and the subsequent formation of straw-derived C in soil aggregates are still unclear. Therefore, topsoil samples (0~20 cm) were collected from three fertilization treatments in a long-term (29-year) Mollisol field experiment: (i) no fertilization control, CK; (ii) inorganic fertilizer, IF; and (iii) inorganic fertilizer plus manure, IFM. Thereafter, an in situ micro-plot incubation experiment was conducted without/with ^{13}C-labeled straw (abbreviated as CKS, IFS, and IFMS, respectively). Soil aggregates were separated into macro- (>0.25 mm) and microaggregates (<0.25 mm). The aggregate-based changes in straw-derived C content, microbial community composition, co-occurrence network, keystone taxa, and functional characteristics were measured on the 1st, 60th, and 150th day after straw addition. The results showed that straw-derived C content increased averagely by 7 (CKS), 13 (IFS), and 20 times (IFMS) from day 1 to day 150 in the macroaggregates. The straw-derived C content in the microaggregates was the highest in the IFS (0.70%) and IFMS (0.67%) treatments on day 60. After straw addition, the relative abundance of Humicola within the soil macroaggregates significantly decreased from 2.9% (CK) to 1.4% (CKS), and that of Penicillium within the soil microaggregates decreased from 7.5% (IF) to 4.0% (IFS) on day 150. Network analysis revealed greater microbial complexity in microaggregates than in macroaggregates, with fungal keystone taxa responding more strongly to straw than bacterial keystone taxa. The SEM model identified bacterial composition and fertilization as key drivers of straw-derived C formation in macro- and microaggregates, respectively. These findings highlight the distinct roles of bacteria and fungi in various sizes of aggregate and the importance of customized soil management for improving soil fertility and C storage.

Keywords: soil aggregate; fertilization; soil microbes; soil organic carbon sequestration

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1. Introduction

The Mollisol region of Northeast China is one of only four main large Mollisol areas in the world [1]. Mollisol is often considered one of the most significant agricultural soil

carbon resources because of its high soil organic C (SOC) and fertility [2]. However, due to intensive agricultural practices and crop cultivation practices, SOC losses have decreased the health and quality of the Mollisols present in Northeast China [3]. Overall, the continued degradation of Mollisol in Northeast China seriously threatens the sustainable development and maintenance of the natural environment. Therefore, it is urgent to better understand the underlying mechanisms of soil organic matter transformation and stabilization to devise practical strategies for improving the fertility and C storage of Mollisol [4].

Soil aggregates are the key component of soil structure, and they play important functions in the soil [5]. Soil aggregates are usually classified as macroaggregates (>0.25 mm) and microaggregates (<0.25 mm) [6]. Different aggregate fractions support various microhabitats for soil microorganisms [7–9]. Differentials in microbial activity and community composition across aggregate size fractions are thought to be linked to SOC turnover, nutrient cycling, and other soil ecological processes that serve multiple purposes [10]. For example, microaggregates are thought to harbor more dynamic and diverse microbial communities; in contrast, macroaggregates are less diverse and more stable [11]. Soil bacteria and fungi are generally more crucial for soil aggregation than soil fauna and plants. Soil bacteria contribute strongly to both macro- and microaggregates, while soil fungi only significantly affect macroaggregates [12]. Therefore, further research is required to explore the microbial community composition and diversity at the aggregate level, which will help us better understand the mechanisms behind the cycling of microbially affected C in soils [7,13].

Straw incorporation is a promising strategy to supply a C source for soil, which would help to improve crop production and affect soil aggregation [14]. Indeed, fresh straw incorporation can help soil microaggregates bind to macroaggregates by promoting the production of soil-binding agents [15]. Moreover, soil microbes do interact with soil aggregate to govern SOC dynamics [8], microbial abundance, and diversity, affecting C cycling [16–18]. Soil aggregates with different particle sizes harbor variable C contents, likely to affect their microbial composition [19]. Macroaggregates have a relatively high fungal abundance and a low bacterial abundance, contain higher nutrient contents, and are favorable for the copiotroph communities (fast-growing microbes favoring nutrient-rich conditions), which are linked to more rapid C turnover [20]. However, microaggregates amass more recalcitrant C, which is more associated with oligotrophic microorganisms and contributes to enhanced C storage [10]. However, still at the aggregate scale, the current understanding of the response of straw-derived C to soil microbes remains rather limited.

Agricultural fertilization is the most well-known strategy to stimulate plant growth and improve crop yield [21]. It can influence soil aggregation. The input of organic fertilizer enhances macroaggregate formation through a higher level of physical protection of organic matter [22]. Moreover, fertilization affects soil microbes within aggregates by altering soil nutrient contents and environmental conditions [23]. For example, long-term organic fertilization has been shown to increase bacterial and fungal biomass within aggregate size fractions [24]. Furthermore, long-term chemical fertilizer additions dramatically alter bacterial and fungal communities across different particle sizes of aggregate fractions [25].

Soil C cycling involves a broad variety of microorganisms, which are arranged in distinct trophic groups and functional niches and may co-exist [26]. Some species are more important in influencing soil function than others, and they are known as keystone taxa, species with a disproportionately large influence on ecosystem functioning despite low abundance [27]. Microbial co-occurrence networks have been used to predict ecosystem functioning and to statistically identify potential keystone taxa by calculating correlations between abundances of species [28,29]. Due to spatial variation, microbial network connections in soil aggregates show generalizable patterns that may be synchronized with

their activities in soil aggregates [30]. However, the information on dynamic responses of microbial network complexity and keystone taxa to straw-derived C turnover under various fertilization conditions, especially in aggregate scales, is still rather restricted.

Therefore, in the present study, we focused on the dynamic changes in straw-derived C content, microbial community composition, co-occurrence network, keystone taxa, and functional characteristics in a Mollisol over the course of straw decomposition. Considering that straw-derived C dynamics and microbial communities are susceptible to varied fertilizer regimes, we hypothesized that (1) fertilization regulates straw-derived C incorporation into soil aggregates by modifying specific microbial mechanisms, including shifts in microbial diversity and changes in the abundance of functional guilds; (2) the activity of microbial keystone taxa mediate the stability and transformation of straw-derived C, with distinct effects in macroaggregates versus microaggregates; and (3) fertilization-induced changes in microbial community composition and function will differentially influence soil C sequestration in aggregates of different sizes.

2. Materials and Methods

2.1. Study Site Description

This study was conducted at a long-term fertilization experiment site established in 1990 at the Jilin Academy of Agricultural Sciences, Gongzhuling County, Jilin Province, Northeast China (43°30′ N, 124°48′ E, 200 m above sea level). The region experiences a typical continental monsoon climate, with an average annual temperature of 4–5 °C and annual precipitation of 400–600 mm [31]. The soil at the start of the experiment was Mollisol (USDA soil taxonomy), composed of 39% sand, 30% silt, and 31% clay [32]. This study included three application strategies: (1) unfertilized control (CK), (2) balanced inorganic fertilizers (IF), and (3) balanced inorganic fertilizers (IFM). Fully fermented pig manure was applied annually in autumn, after the maize harvest, to the IFM plots [31]. Urea was utilized as nitrogen (N) fertilizer, triple superphosphate was utilized for phosphorus (P) fertilizer, and potassium sulfate was utilized for potassium (K) [33]. The manure contained approximately 112 g kg^{-1} of organic C and 5.0 g kg^{-1} of N and had a $\delta^{13}C$ value of −21.6‰ [34].

2.2. In Situ Field Experiment and Soil Sampling

In the micro-plot experiment, two soil pits (0.9 m × 0.6 m × 0.3 m) were dug in a nearby field. On 5 May 2018, two polyvinyl chloride (PVC) material boxes (0.9 m × 0.6 m × 0.6 m) were vertically placed into the pits [35]. To allow for drainage, the bottoms of the boxes were left open. Each box was divided into nine equal sections, enabling three random replicates of the three treatments (CK, IF, and IFM). Topsoil (0~20 cm) from each fertilization treatment in the long-term field experiment was sieved through a 7 mm mesh to remove crop roots and rocks (see Table S1 for soil properties). The ^{13}C-labeled maize straw was obtained by pulse-labelling mature maize plants with $^{13}CO_2$ [36] and cutting them into 0.5–1.0 cm pieces. In one set of boxes, the soil was mixed with labeled straw (CKS, IFS, IFMS) at 2.3 g straw kg^{-1} soil; in the other set, no straw was added (CK, IF, IFM). During the incubation period, no plants were grown in the boxes. The summary of treatments used in the long-term fertilization and micro-plot straw addition experiment is shown in Table 1. Soil samples were collected at 0~20 cm depth after 1 (6 May 2018), 60 (4 July 2018), and 150 (2 October 2018) days since the day of straw residue incorporation. The samples were placed in sealed bags, kept intact and uncompressed, and stored in a low-temperature incubator upon transfer to the lab.

Table 1. Summary of treatments used in the long-term fertilization and micro-plot straw addition experiment.

Treatment	Fertilization Regime	Fertilizer Rates (kg ha^{-1} yr^{-1})	Organic Amendment	Straw Addition
CK	No fertilization (control)	None	None	No
IF	Inorganic fertilizer	165 N, 82.5 P_2O_5, 82.5 K_2O	None	No
IFM	Inorganic fertilizer + manure	50 N, 82.5 P_2O_5, 82.5 K_2O	115 N from pig manure	No
CKS	No fertilization (control)	None	None	Yes
IFS	Inorganic fertilizer	165 N, 82.5 P_2O_5, 82.5 K_2O	None	Yes
IFMS	Inorganic fertilizer + manure	50 N, 82.5 P_2O_5, 82.5 K_2O	115 N from pig manure	Yes

Note: N, P_2O_5, and K_2O indicate nitrogen, phosphorus, and potassium inputs, respectively. Pig manure was fully fermented before application.

2.3. Soil Aggregate Fractionation

Soil aggregates were isolated by the dry-sieving method in this study. We selected dry sieving because it is less disruptive to soil microhabitats and microbial associations than wet sieving, thereby better preserving the natural spatial distribution of microbes within the aggregates [37]. This preservation is critical for our aim of linking aggregate-specific microbial community structure, keystone taxa, and functional characteristics to straw-derived C dynamics. While wet sieving is often preferred for studying physical stability and organic matter protection, it can alter microbial abundance and community composition by disrupting water-sensitive aggregates and releasing extracellular enzymes. Dry sieving minimizes such alterations, enabling a more accurate assessment of in situ microbial networks [38]. Field-moist clods were cool-dried at 4 °C until reaching a gravimetric water content of ~80 g kg^{-1}, at which point they were able to separate the aggregates [39]. The visible plant residues and stones were removed from the soil, and the large aggregates were gently pressed by hand. The aggregates were separated by placing 100 g of cold-dried (4 °C) soil fragments (<5 mm) on a Retsch AS200 Control (Retsch Technology, Düsseldorf, Germany) [40]. The sieve was mechanically shaken (amplitude 1.5 mm) for 2 min to separate the soil into the following aggregate size fractions: macroaggregates (>0.25 mm) and microaggregates (<0.25 mm) [41].

2.4. Analysis of the Contents of Organic C and Straw-Derived C of Aggregates

The organic C content and its $\delta^{13}C$ value of the aggregates were measured with an elemental analyzer (Elementar Vario PYRO cube, Hanau, Germany) coupled to an isotope ratio mass spectrometer (IsoPrime100, Langenselbold, Germany). The $\delta^{13}C$ value (‰) was expressed relative to the Vienna Pee Dee Belemnite (VPDB) standard. The proportions of straw-derived C of the aggregates (f_{maize}, %) in the soil with maize straw addition were estimated by the following [42]:

$$f_{straw\text{-}C} = (\delta^{13}C_{sample} - \delta^{13}C_{soil}) \times 100/(\delta^{13}C_{straw} - \delta^{13}C_{soil}) \quad (1)$$

where $\delta^{13}C_{sample}$ represents the $\delta^{13}C$ value of aggregate organic C in the treatment with maize straw residue at a certain time; $\delta^{13}C_{soil}$ represents the $\delta^{13}C$ value of aggregate organic C in the initial soil; and $\delta^{13}C_{straw}$ represents the $\delta^{13}C$ value of the initial maize straw.

Thus, the content of maize straw-derived C (C_{straw}) at a certain time in the treatments with maize straw was calculated as follows [43].

$$C_{straw} = C_{sample} \times f_{maize} \quad (2)$$

where C_{sample} represents the organic C content of aggregates in the treatment with maize straw residue at a certain time.

2.5. High-Throughput Sequencing

DNA from soil aggregates of varying sizes was extracted using the MP FastDNA SPIN Kit for Soil (MP Biomedicals, Santa Ana, CA, USA), following the manufacturer's instructions. The DNA concentration and purity were measured with a NanoDrop 2000 UV–vis spectrophotometer (Thermo Scientific, Wilmington, NC, USA), and DNA quality was assessed by 1% agarose gel electrophoresis. The prokaryotic 16S rRNA V3–V4 hypervariable region was amplified via PCR with the primer set 338F/806R, while the fungal ITS1 region was amplified using primers ITS1F/ITS2R. The PCR program was as follows: 95 °C for 3 min; 27 cycles of 95 °C for 30 s, 55 °C for 30 s, 72 °C for 45 s; followed by a final extension at 72 °C for 10 min. Bacterial PCR amplification was carried out in triplicate with a 20 μL reaction containing 4 μL of 5 × FastPfu Buffer, 2 μL of 2.5 mM dNTPs, 0.8 μL of each primer (5 μM), 0.4 μL of FastPfu polymerase, 0.2 μL of BSA, and 10 ng of template DNA. Fungal PCR amplification was similarly performed in triplicate with 2 μL of 10 × Buffer, 2 μL of 2.5 mM dNTPs, 0.8 μL of each primer (5 μM), 0.2 μL of rTaq polymerase, 0.2 μL of BSA, and 10 ng of template DNA. PCR products were extracted from 2% agarose gels, purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA), and quantified with QuantiFluorTM-ST (Promega, Madison, WI, USA) according to the manufacturer's protocol. Equimolar concentrations of purified amplicons were pooled and paired-end sequenced (2 × 300) on an Illumina platform by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China).

2.6. Bioinformatics Analysis

We analyzed the sequences using USEARCH v. 10.0 [44] and VSEARCH v. 2.7.1 [45]. The paired-end Illumina reads were processed with VSEARCH through the following steps: joining paired-end reads, relabeling sequencing names, removing barcodes and primers, filtering low-quality reads, and identifying non-redundant reads. Unique reads were clustered into operational taxonomic units (OTUs) at 97% similarity, with representative sequences selected by UPARSE [46]. Taxonomic identities were assigned using the RDP database [47] for bacteria and the UNITE database [48] for fungi.

Network analysis at the genus level was conducted to examine the interactions within bacterial and fungal taxa. Relative abundances of bacterial and fungal genera were used to construct networks for the respective communities across different treatments during the straw residue decomposition process. Genera with average relative abundances higher than 0.1% were selected for Spearman's correlation analysis. The network analysis was performed using the "igraph" package in R (v4.0) [49]. We assumed that changes in the OTU numbers of keystone taxa between sampling points reflected their microbial activities under different treatments. The highest microbial activity for each keystone taxon was determined based on the sampling point at which its OTU number peaked.

FAPROTAX (v1.1) is a software designed for predicting the ecological functions of bacterial communities based on 16S rRNA sequencing OTU tables, making it especially useful in environmental research [50]. Similarly, FUNGuild (v1.1) provides a comprehensive ecological analysis of fungal communities, identifying trophic types and metabolic functions [51]. In this study, FAPROTAX (v1.1) and FUNGuild (v1.1) were employed to predict the ecological functions of bacterial and fungal communities during straw decomposition. We focused on carbon cycling groups for bacterial functional analysis, while fungal taxa were assigned to functional guilds if their genus matched a single functional group in the FUNGuild database. FUNGuild classifies fungal functions based on taxonomic matches and assigns confidence levels, such as "highly probable" or "probable" [51].

2.7. Structural Equation Model Analysis

Two structural equation models (SEM) were built to gain a mechanistic understanding of how fertilization, microbial community, and keystone taxa affect straw-derived C in different sizes of aggregates. The models were fitted using robust maximum likelihood estimation [52]. Model adjustments were based on nonsignificant probability ($p > 0.05$), high goodness-of-fit index (GFI; GFI > 0.90), minimum discrepancy function by degrees of freedom divided (CMIN/DF; CMIN/DF < 3), and comparative fit index (CFI; CFI > 0.90) to ensure adequate fit [53]. All SEM analyses were performed using Amos 17.0 software (Chicago, IL, USA: Amos Development Corporation).

3. Results

3.1. Organic C and Straw-Derived C Content in Soil Aggregates

After straw addition, organic C contents of macroaggregates and microaggregates in the IFMS treatment were the highest during the whole incubation period (Figure 1). Organic C content in macroaggregates was significantly elevated in the IFMS treatment compared to the other treatments, while microaggregates showed a progressive decline in all treatments over 150 days ($p < 0.05$) (Figure 1a,b).

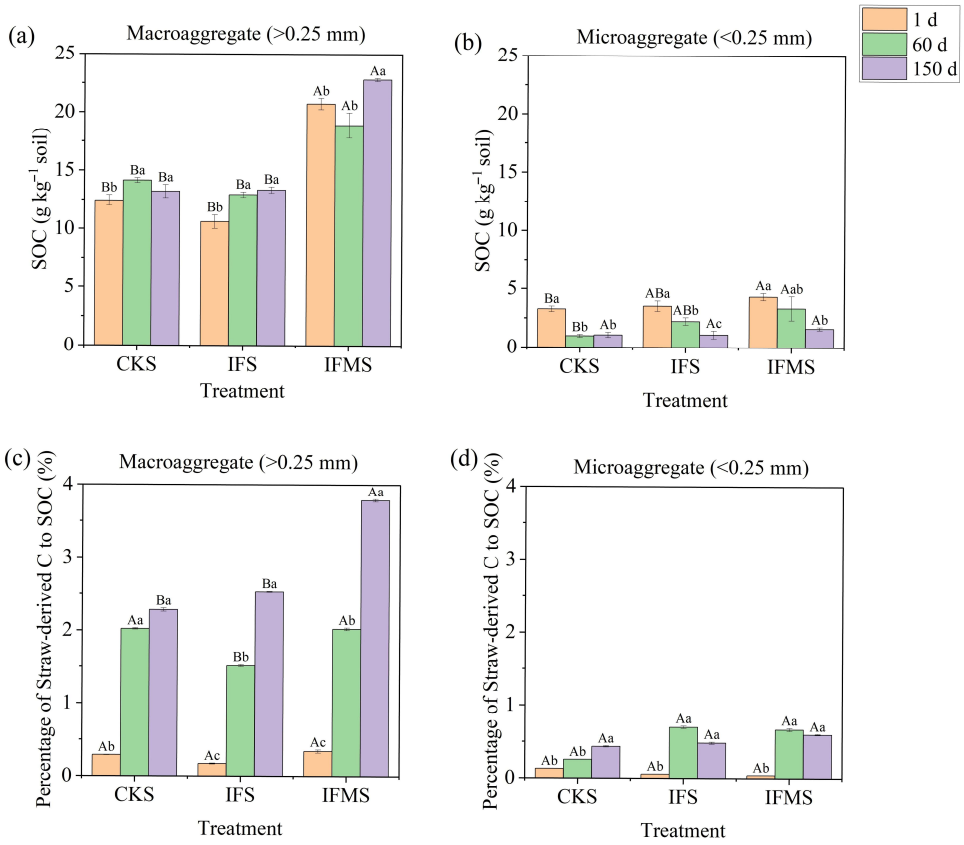

Figure 1. Straw-derived C content in soil aggregates after 1, 60, and 150 day(s) of straw addition to soils with different fertilizer management practices. (**a**) the SOC content in macroaggregate; (**b**) the SOC content in microaggregate; (**c**) the percentage of straw-derived C to SOC in macroaggregate; (**d**) the percentage of straw-derived C to SOC in microaggregate. Treatments include CKS (no fertilization control + straw), IFS (inorganic fertilizer + straw), and IFMS (inorganic fertilizer plus manure + straw). Vertical bars represent the standard error of the mean significant variations (SEM; $n = 3$). Values followed by different letters (uppercase among various fertilizer management practices and lowercase among various incubation periods) mean significant variations ($p < 0.05$) in straw-derived C contents.

Straw-derived C content of macroaggregates was increased by 7 times (CKS), 13 times (IFS), and 20 times (IFMS) from day 1 to day 150. Straw-derived C was highest in macroaggregates with the IFMS treatment. The content of straw-derived C of microaggregates was the highest in the IFS (0.70%) and IFMS (0.67%) treatments on day 60. In the CKS treatment, straw-derived C content continued to increase from day 1 to day 150 and reached the highest value on day 150 (0.43%) (Figure 1d).

3.2. Bacterial and Fungal Community Composition in Soil Aggregates

Within macroaggregates, the relative bacterial abundance of Blastococcus significantly decreased from 5.6% in the IF treatment to 2.9% in the IFS treatment, but that of Arthrobacter was significantly increased from 2.5% in the IF treatment to 4.9% in the IFS treatment on day 60 (Figure 2a). The relative bacterial abundance of Arthrobacter within microaggregates was significantly increased from 2.3% in the IF treatment to 4.5% in the IFS treatment on day 60 (Figure 2b).

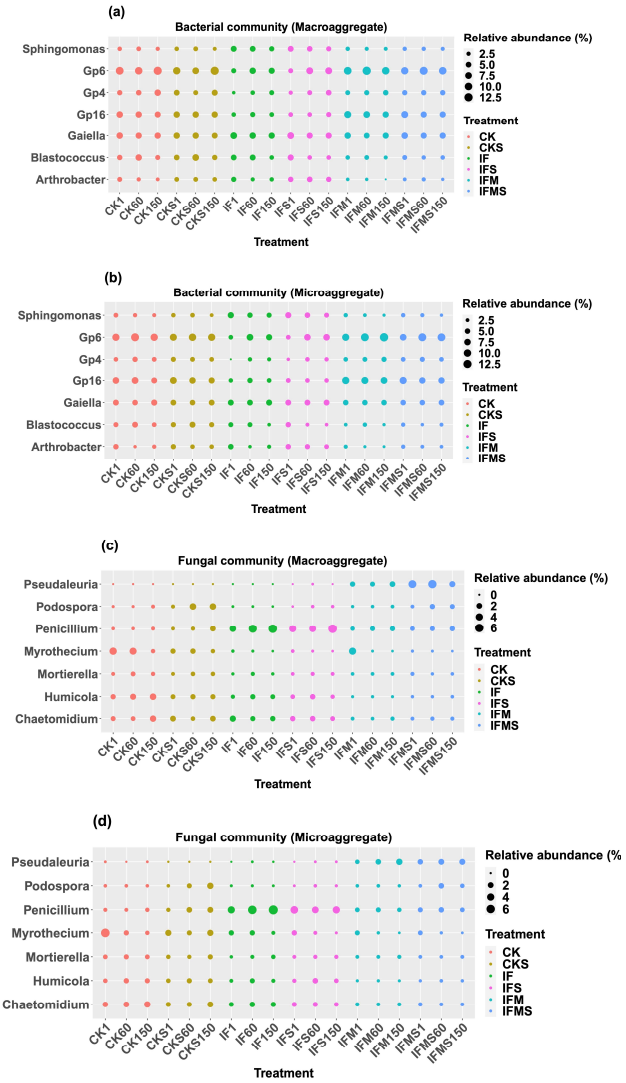

Figure 2. Relative abundance of the taxonomic composition of soil bacterial community in macroaggregates (**a**) and microaggregates (**b**) and that of soil fungal community in macroaggregates (**c**) and microaggregates (**d**) at the genus level after 1, 60, and 150 day(s) of straw addition to soils with different fertilizer management practices. Treatments including CK (control), IF (inorganic fertilizer), IFM (inorganic fertilizer plus manure), CKS (control + straw), IFS (inorganic fertilizer + straw), and IFMS (inorganic fertilizer plus manure + straw). The color of the circle represents different treatments. The size of the circle represents the relative abundance of the genus.

Within macroaggregates, the relative fungal abundance of Myrothecium was 1.25 times higher in the CK versus CKS treatments on day 60, and that of Humicola was almost 2 times higher in the CK versus CKS treatments on day 150. Within macroaggregates, the relative fungal abundance of Myrothecium was significantly decreased from 3.9% in the IFM treatment to 0.2% in the IFMS treatment on day 1, and that of Pseudaleuria significantly increased from 1.3% in the IFM treatment to 5.8% in the IFMS treatment on day 60 (Figure 2c). The relative abundance of Penicillium within microaggregates decreased from 7.5% in the IF treatment to 4.0% in the IFS treatment on day 150 (Figure 2d).

3.3. Bacterial and Fungal Co-Occurrence Network in Soil Aggregates

Long-term fertilizer management practices changed the bacterial and fungal co-occurrence patterns of the soil aggregates (Figures 3 and 4). The topological properties of the bacterial network and fungal network in the soil aggregates are shown in Tables S2 and S3. For bacterial networks, the number of edges in microaggregates was increased by 6%, 34%, and 93% relative to macroaggregates in the CKS, IFS, and IFMS treatments, respectively. This indicated that straw addition resulted in higher complexity in microaggregates than in macroaggregates under all three fertilization conditions. Specifically, straw addition decreased the complexity of bacterial networks within aggregates in the CK treatment but increased it in the IFMS treatment. However, straw addition in the IF treatment decreased the complexity of bacterial networks within the macroaggregate but increased that within the microaggregate (Figure 3).

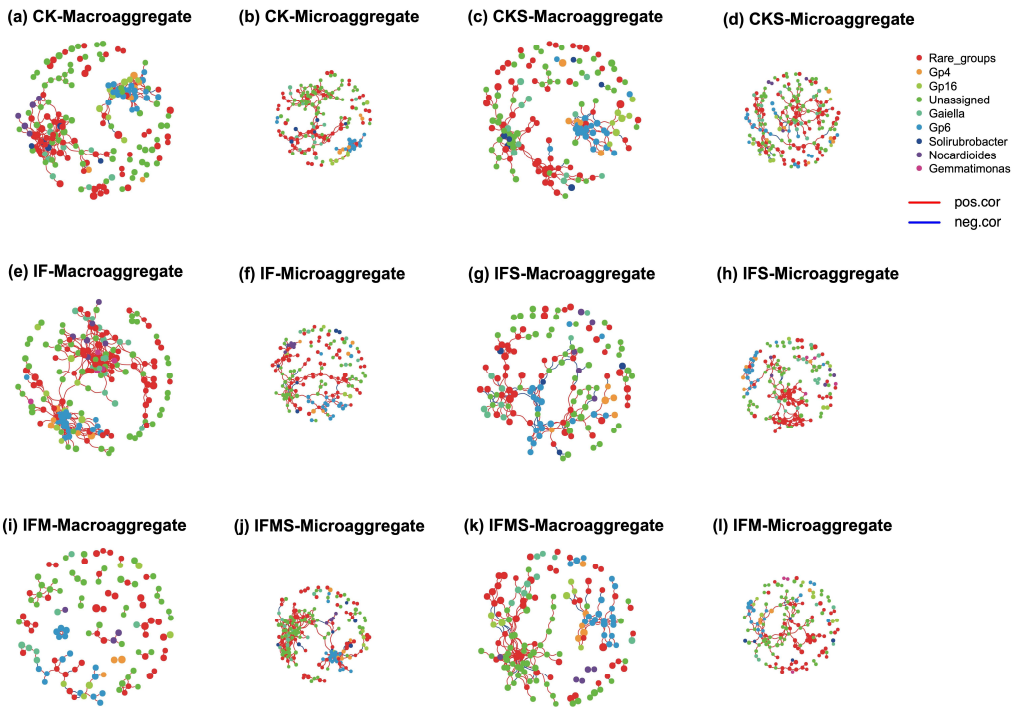

Figure 3. Co-occurrence patterns of bacterial taxa at the genus level in soil aggregates during the whole process of straw residue decomposition. Treatments including CK (control), IF (inorganic fertilizer), IFM (inorganic fertilizer plus manure), CKS (control + straw), IFS (inorganic fertilizer + straw), and IFMS (inorganic fertilizer plus manure + straw) (**f**). Red lines represent significant positive ($p < 0.05$) linear relationships, and blue lines represent negative ($p < 0.05$) linear relationships.

The fungal networks of microaggregates were also more complex than those of macroaggregates after straw addition under all three fertilization conditions (Figure 4). Specifically, in macroaggregates, the number of edges of the fungal network increased by 52% in the IFS compared to the IF treatments. In contrast, it did not alter much in the

CKS and IFMS treatments compared to the corresponding no-straw addition treatments. Straw addition decreased the number of edges in the fungal network of microaggregates by 44% and 8% in the CK and IF treatments but increased the number of edges by 17% in the IFMS treatment.

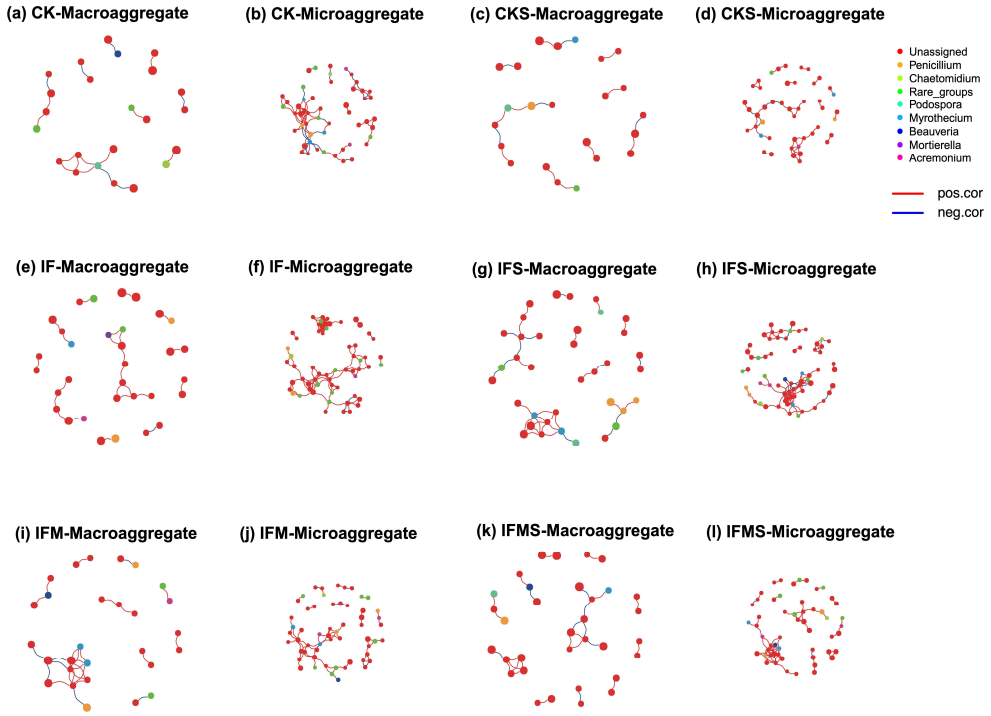

Figure 4. Co-occurrence patterns of fungal taxa at the genus level in soil aggregates during the whole process of straw residue decomposition. Treatments including CK (control), IF (inorganic fertilizer), IFM (inorganic fertilizer plus manure), CKS (control + straw), IFS (inorganic fertilizer + straw), and 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 ($p < 0.05$) linear relationships.

3.4. Bacterial and Fungal Keystone Taxa in Soil Aggregates

Dynamic changes in microbial activities of bacterial keystone taxa and fungal keystone taxa in different sizes of aggregates are shown in Figure S1. Gp6 (OTU_326), Gaiella, and Methyloceanibacter were bacterial keystone taxa, and Podospora (OTU_48), Mrrothecium (OTU_919), and Trichoderma (OTU_173) were fungal keystone taxa in macroaggregates in three straw addition treatments, respectively (Tables S4 and S5). Solirubrobacter Marmoricola and Gp6 (OTU_80) were bacterial keystone taxa, and Acremonium, unassigned genus (OTU_1098), and unassigned genus (OTU_65) were fungal keystone taxa in microaggregates in three treatments, respectively (Tables S4 and S5). The highest microbial activities of bacterial keystone taxa within the aggregates occurred on day 150 in the CKS and IFMS treatments but occurred on day 1 in the IFS treatment. However, the highest microbial activities of fungal keystone taxa within the aggregates occurred on day 150 in the CKS treatment but occurred on day 1 in the IFS and IFMS treatments.

3.5. Bacterial and Fungal Functional Characteristics in Soil Aggregates

The activities of hydrocarbon_degradation (on day 60) and xylanolysis (on day 150) were relatively strong in macroaggregates in the IFMS treatment (Figure 5a). The activities of aerobic_chemoheterotrophy, aromatic_compound_degradation, and methylotrophy on day 1 were relatively strong in microaggregates in the IFS treatment. During the incubation period, the trophic modes differed with the sizes of aggregates under various fertilization

conditions. From day 1 to day 150, in macroaggregates, Saprotroph was the most observed fungi taxa in the CKS treatment, but Pathotroph-Saprotroph was the most observed fungi taxa in the IFS and IFMS treatments. The relative abundance of Saprotroph increased from 44% to 54% (CKS) and 32% to 42% (IFMS). The relative abundance of Pathotroph-Saprotroph increased from 51% to 79% (IFS) but decreased from 36% to 26% (CKS). In microaggregates, the relative abundance of Pathotroph decreased from 16% to 8% (IFS) and 13% to 4% (IFMS). The relative abundance of Pathotroph-Saprotroph decreased from 46% to 28% (CKS) but increased from 71% to 85% (IFS). The relative abundance of Saprotroph increased from 28% to 50% (CKS).

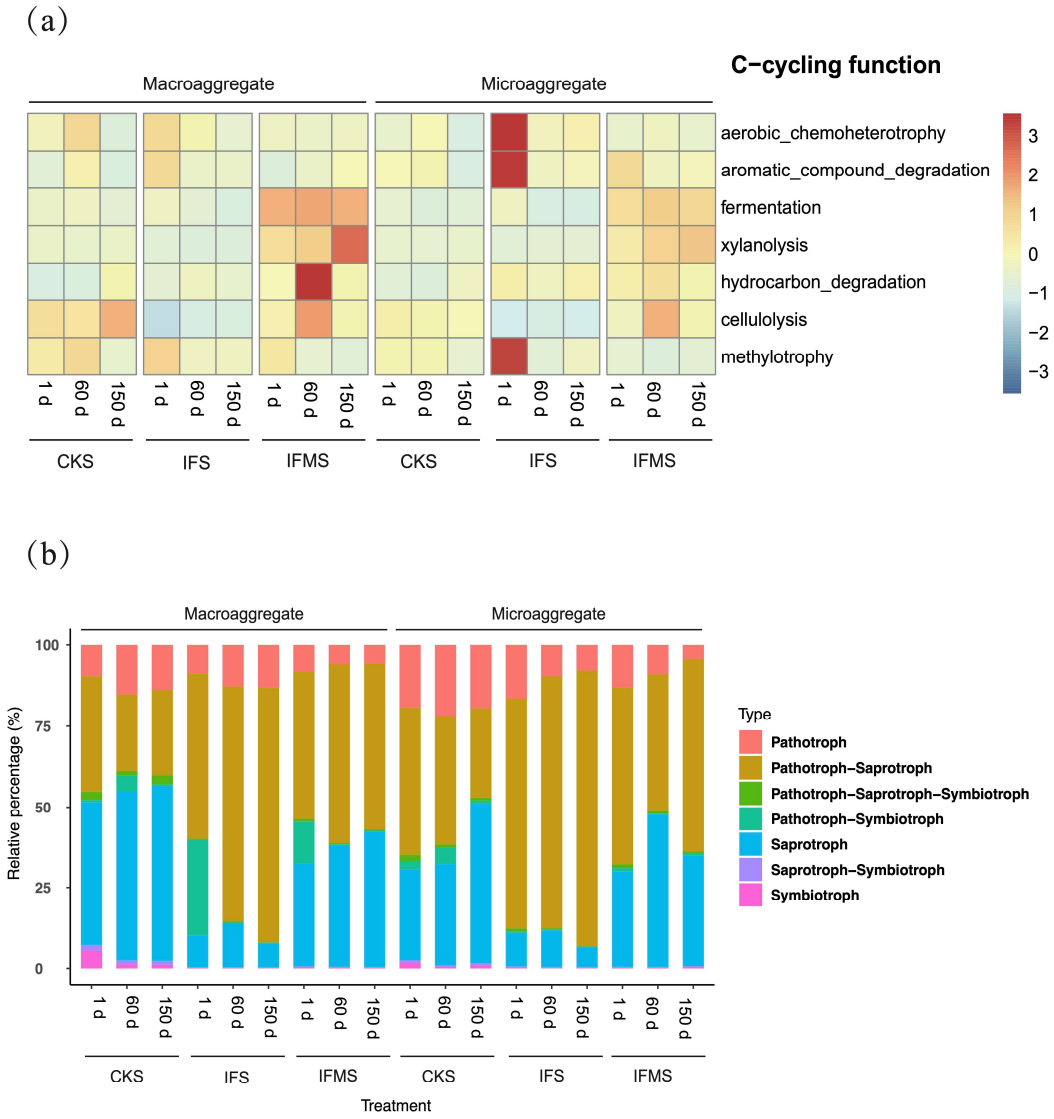

Figure 5. Dynamic changes in bacterial (**a**) and fungal (**b**) functional groups in soil aggregates. Treatments include CKS (control + straw), IFS (inorganic fertilizer + straw), and IFMS (inorganic fertilizer plus manure + straw).

3.6. Fertilization and Microbes Affect Straw-Derived C in Soil Aggregates

The direct and indirect effects of fertilization, microbial community composition, and keystone taxa on straw-derived C were predicted using structural equation modeling (SEM) (Figure 6). Bacterial composition was more important for straw-derived C formation in macroaggregates, while the fungal composition was more critical in microaggregates, and they were also regulated by fertilization. Microbial keystone taxa had a more significant effect on the formation of straw-derived C in macroaggregates than in microaggregates.

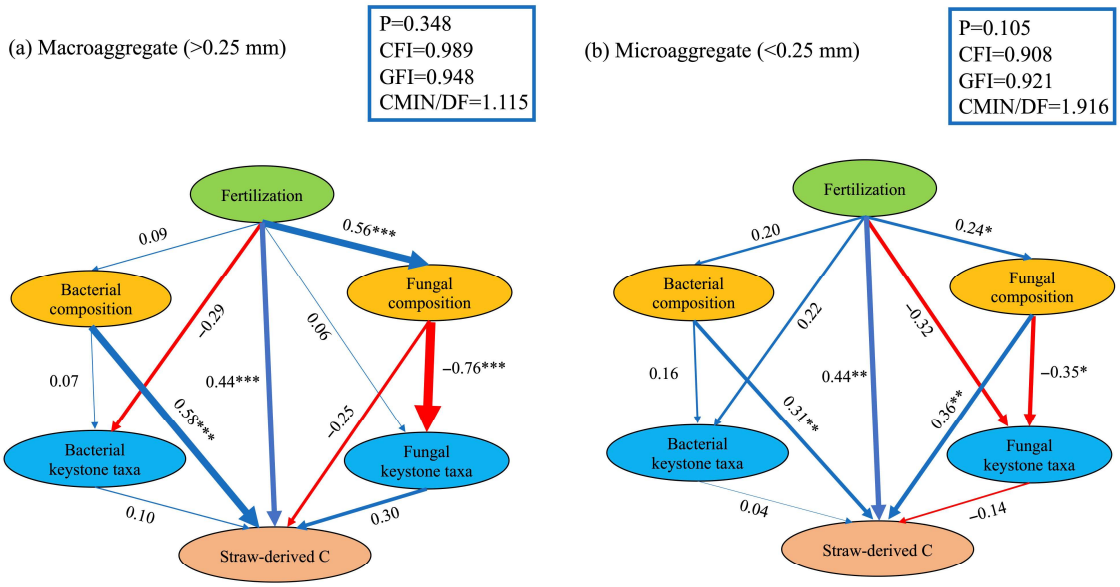

Figure 6. Effects of fertilization, microbial community composition, and microbial keystone taxa on straw-derived C in macroaggregates (**a**) and microaggregates (**b**) estimated using the structural equation model. The width of the arrows indicates the strength of significant standardized path coefficients ($p < 0.05$). Asterisks (*, **, ***) denote statistical significance levels for path coefficients, corresponding to p-values of <0.05, <0.01, and <0.001, respectively. Blue lines indicate significant positive relationships ($p < 0.05$). Red lines indicate significant negative relationships ($p < 0.05$). The bacterial and fungal communities are represented by their first dominant eigengenes.

4. Discussion

4.1. Fertilization Effects

The application of inorganic fertilizer plus manure (IFMS) improved soil fertility and increased soil aggregate organic C [54]. The organic C content of aggregates was the highest in the IFMS treatment among all straw addition treatments during the incubation period. In contrast, straw addition results in positive priming effects on organic C of microaggregates [55]. The organic C content of microaggregates decreased during the incubation period.

Straw-derived C increased and was mainly stored in the macroaggregate size fraction at day 150. Previous work also found that, in the short-term (224 days) experiment, exogenous fresh straw C generally persisted in macroaggregates in the short term [56]. This could be explained by the higher nutrient content in macroaggregates, which favor copiotroph communities, known to enhance C turnover [23]. Therefore, macroaggregates represent the point where exogenous fresh straw-derived C enters the system of aggregates [57]. In microaggregates, IFS and IFMS treatments harbor higher nutrient and microbial activities, straw addition could rapidly activate soil microbes, and easily decomposable parts of straw quickly decomposed and form as straw-derived C [58]. However, CKS treatment harbors low nutrient and microbial activities, microbes respond slowly to straw input, and straw-derived C keeps increasing [59].

4.2. Microbial Composition Changes in Bacterial and Fungal Communities

Fertilization and straw-derived C induced significant changes in bacterial and fungal community composition of soil aggregates. Nutrient availability is generally considered to play an important role in mediating microbial community composition in soils [60]. It is well established that Blastococcus can break down organic material by production proteolysis and is involved in the metabolization of intermediate straw decomposition

products [61,62]. The decreases in relative abundances of Blastococcus in the IFS treatment indicated straw-derived C was mainly assimilated in macroaggregates. Arthrobacter could be characterized as consumers of easily available C and helps to accumulate SOC [63]. The increase in straw-derived C content led to the highest activities of Arthrobacter in the IFS treatment. Fungi have a broader profile of nutrient utilization than bacteria [64]. We found that Humicola in the macroaggregate decreased in the CKS treatment. Humicola is known to produce cutinases, which can accelerate straw degradation [65]. Pseudaleuria content was enriched in the IFMS treatment. Similar findings also occurred in Alfisol, Inceptisol, and Ultisol [66]. Pseudaleuria is the most abundant genus of Pyronemataceae and is found to be dominant in healthy soils [67]. Pyronemataceae is a fungal symbiont with roots and obtains carbohydrates from plants and, in turn, is beneficial for the uptake of nutrients by plants [68]. Macroaggregates had more nutrients; therefore, Pseudaleuria increased in our study. We also observed that Myrothecium decreased in the IFMS (day 1) and CKS (day 60) treatment because Myrothecium is the fastest-growing genus in Ascomycota, which contains dominant responders to labile C utilization [35]. Overall, we noted more changes in the fungal microbial composition as compared to the bacterial communities in aggregates under different fertilizer regimes.

4.3. Straw Decomposition and Aggregate Network Complexities

Different aggregate fractions had variable microbial network structures due to the spatial heterogeneity of soil aggregates, which also may harbor distinct microbial communities [69]. Larger-sized aggregates contain more inorganic nutrients and have more extensive pore spaces and a more heterogeneous structure than smaller soil aggregates, which can provide a variety of microhabitats and allow for more varied bacterial interactions and niches [70,71]. The combination of inorganic fertilizer and manure ensures a continuous supply of both organic and inorganic nutrients, promoting bacterial growth and interactions within the confined spaces of microaggregates [72]. Manure addition provided a rich source of organic matter and nutrients to the soil, and these nutrients are more evenly distributed within microaggregates, which can support a diverse and active bacterial community [73].

Straw is a rich source of organic matter and cellulose, which provides a substrate for bacterial and fungal decomposition [74]. Microaggregates, with their larger surface area relative to volume, offer more sites for organic matter to adhere, facilitating microbial colonization and activity [75]. The decomposition process of straw within microaggregates creates numerous microhabitats and nutrient hotspots, encouraging diverse microbial interactions and network formation [76]. The breakdown of straw releases a range of nutrients and organic compounds into the soil [77]. In microaggregates, these nutrients are more evenly distributed and readily available to microbial communities [10]. The fine structure of microaggregates provides various niches where bacteria and fungi can thrive, leading to increased microbial diversity and network complexity [78]. Microaggregates promote close interactions between different microbial species [7]. These interactions include competition for resources and cooperation in the breakdown of straw [79]. Such close interactions can lead to more intricate microbial networks, as bacteria and fungi establish cooperative and competitive relationships to maximize resource utilization [80].

4.4. Microbial Keystone Taxa Exhibit Different Patterns Within Aggregates

In our study, Gaiella and Marmoricola were identified as the bacterial keystone taxa in the IFS treatment. These genera are known for their roles in the degradation of recalcitrant organic matter and nutrient cycling, suggesting they may act as central regulators of C flow within the soil microbial community [81,82]. Ecologically, their loss could disrupt

network integrity and C turnover pathways [83]. From a practical standpoint, these findings highlight the potential for targeted management strategies, such as optimizing straw incorporation timing or co-application of amendments that favor these keystone taxa, to enhance microbial network stability and promote efficient C sequestration [84]. Integrating microbial network metrics into soil management decisions could maximize the persistence of straw-derived C, supporting sustainable soil fertility and climate mitigation goals [85]. Also, both Gaiella and Marmoricola are classified as Actinobacteria, which are usually defined as r-strategists [86]. However, the application of inorganic fertilizer is said to reduce soil bacterial diversities, although straw-derived C increases it [87]. The effects on microbial activities of bacterial keystone taxa were mainly regulated by inorganic fertilizer in the IFS treatment. Gp6, Solirubrobacter, and Methyloceanibacter exhibited copiotrophic lifestyle; therefore, their activities could be higher with an increase in straw-derived C in the CKS and IFMS treatments [88].

The microbial activities of fungal keystone taxa increased gradually in the non-fertilized soil (CKS) but decreased gradually in the fertilized soil (IFS and IFMS) after straw addition. Podospora and Myrothecium are known as decomposer fungi [89]. They have evolved mechanisms to break down complex organic materials, such as cellulose and lignin found in straw. In nutrient-poor soils, these fungi can efficiently utilize C sources from the straw, giving them a competitive advantage [90]. Therefore, their microbial activities increased during the straw decomposition in the CKS treatment due to poor nutrient conditions. Trichoderma and Acremonium are generally saprotrophic and reported to have the ability to degrade more recalcitrant soil organic matter rather than solely labile C [91]. Therefore, their microbial activities would be decreased in IFS and IFMS treatments due to abundant nutrient conditions.

4.5. Microbial Function Shifted Within Aggregates During Straw Decomposition

FAPROTAX was adopted to annotate and then evaluate the key ecological function of bacterial communities that contributed to C cycling to further explore bacterial activities for straw decomposition. In macroaggregates, hydrocarbon_degradation significantly increased on day 60 in inorganic fertilizer plus manure soil, suggesting that straw addition could accelerate the C mineralization processes in this treatment [92]. In microaggregates, aerobic_chemoheterotrophy is regarded as the primary pathway of C flow in microbial communities [93]; thus, it had a rapid response at the beginning of straw addition in the IFS treatment. The activities of Methylotrophy decreased in the IFS treatment because it can be reduced by organic material amendments [94]. The degradation of aromatic compounds was also inhibited by the straw addition. It is suggested that the amendment of plant residue in combination with inorganic fertilizer would be able to accelerate the decomposition of the fresh straw residue. However, it would not impact the mineralization of native soil organic matter [95].

FUNGuild analysis exhibited a prediction for the fungal community and revealed that straw addition altered function characteristics in aggregates. Saprotrophs have been identified as the most important decomposers in soil [96]. Straw addition increased Saprotroph in the CKS treatment, indicating that Saprotroph flourishes in response to increases in soil organic C and available nutrients [97]. Pathortroph-Saprotroph was the dominant type in the IFS treatment and increased during the straw decomposition process. Phaeosphaeriaceae belonged to the Pathortroph-Saprotroph mode, and the application of inorganic fertilizer could stimulate the growth of Phaeosphaeriaceae [98]. Macroaggregates have a much stronger capacity to degrade recalcitrant substances than microaggregates, which can help Saprotroph to assimilate maize straw [99].

4.6. Straw-Derived C Within Aggregates Was Regulated by Fertilization and Microbial Keystone Taxa

From the SEM model, we confirmed that bacterial community composition was the dominant control on the formation of straw-derived C in macroaggregates (Figure 6). Because up to 90% of soil bacteria are associated with macroaggregates, these bacteria are predominantly copiotrophs, which are generally more abundant in nutrient-rich conditions [100,101]. The abundance of these bacteria is more likely to increase when straw is added [102]. Therefore, with a greater abundance of bacteria, the formation of straw-derived C increased by straw addition [103]. Moreover, fungal community composition showed a significant impact on the formation of straw-derived C in microaggregates. This is most likely due to the fact that, as aggregate size decreased, physical protection and recalcitrant C derived by microbes increased, and oxygen availability and labile C content declined [10]. Stronger collaboration between fungal communities in microaggregates is needed to break down recalcitrant C and resist adverse soil conditions [104]. Moreover, with fewer species that are closely connected, the keystone interactions may not be as significant as the microbial community composition [105]. Furthermore, in microaggregates, keystone taxa had a less significant effect on the formation of straw-derived C than they had in macroaggregates. This result is due to fewer microbial species in microaggregates having a direct correlation with C cycling than in macroaggregates [99].

4.7. Limitations and Future Directions

This study has several limitations that should be acknowledged. First, our sampling was conducted at a single long-term experimental site in the Mollisol region of Northeast China, which may limit the generalizability of the findings to other soil types, climates, or management systems. Mollisols are known for their high organic C content and fertility, so microbial community responses and straw-derived C dynamics may differ in more degraded or nutrient-poor soils [106]. Second, although we replicated treatments in the field, spatial variability within and between plots could still influence microbial composition and functional predictions, especially at the microaggregate scale where heterogeneity is high [107]. Third, our functional analyses relied on FAPROTAX and FUNGuild predictions based on taxonomic assignments from amplicon sequencing. While these tools are widely used, they do not directly measure functional genes or their expression [108]. As such, predicted functional shifts should be interpreted with caution and ideally validated using shotgun metagenomics or metatranscriptomics [109,110]. Fourth, the structural equation models (SEM) used to explore causal relationships between fertilization, microbial communities, keystone taxa, and straw-derived C depend on the quality of the input data and the correctness of model assumptions (e.g., linearity, directionality of effects). Alternative model structures or additional environmental variables might yield different outcomes [111]. Future research could address these limitations by including multiple sites across diverse soil types, integrating multi-omics approaches for functional validation, and applying complementary statistical modeling frameworks to confirm causal pathways.

5. Conclusions

Distinct pathways exist for straw-derived C formation in macroaggregates versus microaggregates. Macroaggregates accumulated straw-derived C over the 150-day period under all practices, enhanced by bacterial-driven processes. In contrast, microaggregates showed a straw-derived C decline under inorganic fertilization (IFS, IFMS); here, fertilizer practices critically influenced C formation. Microbial networks were more complex in microaggregates. Fertilizer management and aggregate size strongly shaped keystone microbial taxa and functional dynamics during decomposition. Therefore, optimize C

sequestration by aggregate size tailored management by, (i) in macroaggregate-dominated Mollisols, prioritizing manure addition to boost bacterial-mediated C cycling, and, (ii) in soils with high microaggregate content, reducing inorganic fertilization and emphasizing fungal-centric practices to minimize C loss.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy15092116/s1, Figure S1. Dynamic changes of microbial activities of bacterial keystone taxa in macroaggregate (**a**–**c**) and in microaggregates (**d**–**f**), and fungal keystone taxa in macroaggregate (**g**–**i**) and in microaggregates (**j**–**l**). Table S1. Soil basic properties of different fertilizer management strategies in 2018. Table S2. Topological properties of bacterial network in soil aggregates. Table S3. Topological properties of fungal network in soil aggregates. Table S4. The keystone taxa in the bacterial networks in soil aggregates. Table S5. The keystone taxa in the fungal networks in soil aggregates.

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