Organic Matter Decomposition in River Ecosystems: the Role of Microbial Interactions Regulated by Environmental Factors

Yibo Liu
Jilin University
Baiyu Zhang
Memorial University
Yixin Zhang
Soochow University
Yanping Shen
Jilin University
Cheng Cheng
Jilin University
Weilin Yuan
Jilin University
Ping Guo (guoping@jlu.edu.cn)
Jilin University

Research Article

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Abstract

Microbes are the critical contributors to the organic matter decomposition (OMD) in river ecosystems. However, the role of microbial interactions on the OMD in river ecosystems and the regulation of environmental factors to the microbial interactions were not considered previously thus tacked in this study. Cotton strip (CS) as a substitute for organic matter was introduced to Luanhe River Basin in China. The results indicated that CS selectively enriched bacterial and fungal groups related to cellulose decomposition, leading to the cotton strip decomposition (CSD). In these groups, bacterial phyla Proteobacteria, fungal phyla Rozellomycota and Ascomycota were the dominant groups associated with the CSD. Bacteria and fungi on CS cooperatively formed a co-occurrence network to achieve the CSD. In the network, the key modules 2 and 4, mainly composed of phyla Proteobacteria and Ascomycota, directly promoted the CSD. Keystone taxa maintained the stability of microbial network structure and function, and regulated microbial groups associated with CSD in the key modules, rather than directly decomposing the CS. Notably, this study profoundly revealed that water temperature and total nitrogen (TN) regulated the keystone taxa and key modules in microbial interactions and then promoted the OMD. The two key modules 2 and 4 were significantly correlated with water temperature and TN in water, and two keystone taxa (bacterial genera Emticicia and Flavihumibacter) were significantly associated with TN. The research findings help us to understand the microbial mechanism of the OMD in rivers, which provides valuable insights into improving effective management strategies for river ecosystems.

1. Introduction

Organic matter decomposition (OMD), as one of the essential ecosystem functions, is a vital and fundamental process in streams and rivers. It involves the material circulation and energy flow after organic matter enters the ecosystems. It also combines various ecological processes, which are closely related to the nutrient cycle, microbial decomposition, macrobenthic colonization and feeding, etc. [1, 2]. The rate of OMD has been recognized as a good indicator of ecosystem integrity and an alternative measure of river ecosystem health through incorporating with the commonly used structural indicators (e.g., invertebrate community composition) [3]. Microbes are the most important decomposers of organic matter in ecosystems. They can transform the complex organic matter into simple inorganic matter, promote the circulation of life elements and energy flow, and thus maintain the stability of the ecosystem [4, 5]. However, the research on the microbial mechanism of OMD in river ecosystems is so far very limited. Tracking this mechanism is thus promising for effective management of river ecosystems.

Bacteria and fungi in microbes play crucial roles in OMD in river ecosystems [6-10]. They can release extracellular enzymes to directly decompose organic matter [11-12] and provide favorable conditions for macroinvertebrate shredders to proceed with OMD by softening organic matter and improving their nutritional quality [13-14]. In previous studies, more attention was paid to the roles of the biomass, as well as diversity and reproducitvity of microbes on the OMD in river ecosystems [15-17]. It has been recorded that high bacterial and fungal biomass on the organic matter contributed to the rapid OMD [15, 18]. The differences in spore production, species diversity, and reproductive activity of aquatic hyphomycetes on

the organic matter were associated with the OMD in river ecosystems [16, 19]. A few studies also compared the differences in OMD caused by bacterial and aquatic hyphomycete community profiles [20, 21]. As well known, the realization of ecological functions in ecosystems usually depends on various combinations of microbial groups [22]. The OMD in river ecosystems may be controlled by a variety of microbial groups [21]. However, the microbial groups associated with the OMD in river ecosystems have not been accurately identified. This greatly limited our understanding of the microbial mechanism of OMD in river ecosystems.

Undoubtedly, microbial groups usually interact with one another to form a microbial co-occurrence network for achieving ecological functions [23, 24]. Topological properties, interaction patterns, keystone taxa, and modules in microbial networks indicating microbial interactions can offer insight for understanding the microbial mechanism underlying the achievement of specific ecological functions in ecosystems [22, 25, 26]. Topological properties can reveal the structure and characteristics of microbial networks. The positive interactions in the network usually represent the cooperation patterns among microbes, and negative interaction patterns can occur due to competition or interference [27]. Keystone taxa and modules in the network are essential for the in-depth understanding of microbial interactions [22]. Keystone taxa are highly connected taxa in the networks and maintain the stability of microbial community structure and function. Microbial taxa with the same environmental preferences or performing similar functions can converge into modules [22, 28]. Recent studies have also found that the microbial interactions responded to changes in environmental factors, then affected the realization of specific ecological functions [22, 25, 29, 30]. The OMD in river ecosystems affected by multiple environmental factors, such as altitude, land use, channel width, water temperature, nutrient concentration, and pH, etc., has been widely confirmed [8, 10, 31, 32]. However, the dominant environmental factors controlling OMD in river ecosystems were still unclear. Therefore, tracking the microbial interaction pattern in the OMD in river ecosystems, identifying keystone taxa and modules closely associated with OMD, confirming the dominant environmental factors controlling OMD, and then revealing the relationships between keystone taxa and modules and the dominant environmental factors are meaningful to further understand the microbial interaction mechanisms of OMD.

Natural and artificial organic matter can be both used for the examination of OMD in river ecosystems [31, 33]. Leaves, litters, and woods have been widely applied to represent natural organic matter [34-36] since river ecologists observed that aquatic organisms could consume them as energy sources [37, 38]. In recent years, cotton strip (CS), as the substitute for natural organic matter, has a stable structure and quality to facilitate the standardization of OMD testing, and showcases the advantages of simplicity, practicality, economy and sensitivity to environmental changes [8, 10, 31, 39, 40]. Although physical factors (i.e., hydraulic shear force and abrasion) and macroinvertebrates were the additional contributors to the OMD in river ecosystems [41], our pre-experimental results showed few macroinvertebrates colonized on CS in rivers, which implied a fairly low possibility of CS decomposition (CSD) by macroinvertebrates. Moreover, the CS could be placed at the positions where the flow velocity was generally consistent and slow, and was not in contact with the river bottoms, which would reduce or even

eliminate the influence of physical factors on OMD [10]. Therefore, the CSD can be used as a tool to indicate OMD to elucidate the microbial interaction mechanisms of the OMD in river ecosystems.

In this study, CS was introduced to the Luanhe River Basin in China to study the role of microbial interactions on the OMD in river ecosystems and to evaluate the regulation of environmental factors to microbial interactions. The CSD was investigated and the dominant microbial groups and their interaction patterns on CS were identified. The contributions of keystone taxa and modules to CSD were examined. The dominant environmental factors controlling CSD were determined, and their linkages with microbial keystone taxa and modules were disclosed. To the best of our knowledge, this is the first systematic study revealing the microbial interaction mechanisms of the OMD and how this process is regulated by environmental factors in river ecosystems.

2. Materials And Methods

2.1 Study area and sampling points

Luanhe River basin (39°10'-42°30' N, 115°30'-119°15' E) is located in the northern part of Hebei Province of China. Luanhe River has a total length of 888 km and a drainage area of 44750 km^2. Its main tributaries include Xiaoluanhe River, Yixun River, Wulie River, Qinglong River, ect. With the decrease of altitude from northwest to southeast in the basin, the intensity of human activity increase and the land use change obviously. The sampling points are accordingly set up in the rivers of this basin, as shown in Fig. 1. The sampling points SP1-3 were deployed in rivers in the Inner Mongolia grassland. The sampling points SP4-6 were set in the rivers in forest regions with the vegetation of the mixed forest. The sampling points SP7-11 were deployed in the rivers flowing through residential and industrial regions. The sampling points SP12-14 were set in the agricultural rivers with the plants of corn, chestnut, fruit trees, and vegetables. Within these sampling points, SP5, 8, 10 and 13 were located in the main tributaries mentioned above, respectively, and the others were located in the main river. All sampling points were deployed at the outlet of the catchment, because after precipitation, the surface runoff strongly affected by land use from the catchment will eventually collect in rivers located at the outlet of the catchment, and thus leads to an important impact on river ecosystems [42-45]. The background environments around the sampling points are showed in Fig. S1.

2.2 Measurement of environmental parameters

The remote sensing interpretation process was described in the Supplementary Information. After interpretation, the obtained five land use types were prairie, forest, urban, agricultural land, and water area, as shown in Table S1. Using spatial analysis tools in ArcGIS to calculate the area and proportion of land use types in the catchment where each sampling point was located. Land use disturbance in the catchment was calculated as the ratio of the sum of construction and agricultural land area to the total area of the catchment [46]. The altitude and the river channel width of the sampling points were

determined by the altitude measuring instrument (Yili X28, China) and the laser rangefinder (MiLESEEY, X5, China), respectively. Water samples were taken on the first and last day of the CS incubation period. Sampling, storage, preservation, and chemical analysis followed the national standard method for the detection of water and wastewater in China [47] and the environmental quality standards for surface water [48]. Water samples were collected 0.5 m below the water surface and at least 5 m from the riverbank using a 1000 mL organic glass hydrophore. Three duplicate samples were taken out at each sampling point. Water temperature was measured in situ by the water temperature gauge (MR-98501, China). In the laboratory, COD was measured by iodometry. TN was detected using the digestion-UV spectrophotometric method. pH was measured by a multi-parameter analyzer (REX DZS-708, China).

2.3 Preparation, incubation and recovery of CS

The methods of CS preparation, incubation and recovery were adopted from Tiegs et al. [6] and slightly modified. CS with length and width of 8.0 and 2.5 cm, respectively, was prepared by cutting unprimed 100% natural cotton canvas (1.10 mm thick, produced by China cotton canvas factory) accurately. Then CS was gently rubbed with hands to make it wear 3 mm wide around (Fig. S2a). A plastic tie was passed at 7.0 mm on one end (Fig. S2b). The prepared CS was then autoclaved, connected in series with an iron wire (3 mm in diameter), and fixed at one end of the rebar at 40-50 cm. The rebar was hammered into the bottom of the river, but CS did not touch the bottom. Each sampling point was equipped with five rebars, and each rebar was fixed with eight CS. The CS was aligned downstream and paralleled to the current. Each CS was deployed at the position where the flow velocity was basically consistent and slow (≈ 0.30 m/s). On August 30 of 2019, all CS were deployed at the sampling points and recovered after incubation in rivers for four weeks. Right after the recovery, CS on two rebars in each sampling point was placed in a shallow tray with 80% ethanol for approximately 30 s and cleaned gently with a small paintbrush. They were then stored at -4 ℃ and brought back to the laboratory, dried at 40 ℃ to constant weight, and measured tensile strength with a tensiometer (UTM4304X, Shenzhen Sansizongheng technology co. LTD). CS on another two rebars in each sampling point were inserted individually into 50 mL respiration chambers (sterilized 50 mL flat-bottomed plastic centrifuge tubes) after recovery. Each chamber was then submerged in the river with the tube opening pointing upwards so that the tube could be filled with river water, and capped tightly while submerged with no visible air bubbles. Sixteen chambers containing only river water were filled in an identical fashion and the mean of these “control” chambers was used to estimate respiration of the river water from each sampling point. After 2 hours of incubation in the dark at room temperature, the dissolved oxygen (DO) measurement was conducted with a luminescent dissolved oxygen probe (Hach brand, Model #HQ40d, Loveland, CO, USA). Each removed CS was rinsed with ethanol and dried as described above before being weighed to the nearest 0.001 mg. CS on the last rebar in each sampling point were also inserted individually into 50 mL respiration chambers without river water and stored at -20 ℃ for metagenomics sequencing.

2.4 CSD determination

The tensile strength loss (TSL) and respiration (RES) are the most commonly applied indicators for quantifying the CSD rate [6, 8]. The TSL should be a more comprehensive index reflecting the decomposition status of CS in the culturing process, and is an indicator of the heterotrophic activity of microbes. The RES can indicate the effect of aerobic microbes on CSD, which might include both autotrophic and heterotrophic microbial activities (i.e., autotrophic and heterotrophic respiration) [3]. The TSL and RES are calculated by Eq. (1) and (2) [6].

$$Tensile\ strength\ loss = 1 - [(\frac{Tensile\ Strength_{\ treatment strips}}{Tensile\ Strength_{\ reference strips}} \times 100\ )]/Incubation\ Time \quad (1)$$

where $Tensile\ Strength_{\ treatment\ strips}$ is the maximum tensile strength recorded for each CS incubated in the field. $Tensile\ Strength_{\ reference\ strips}$ is the mean tensile strength of sixteen strips that were not incubated in the field, but were cleaned with ethanol and stored in a desiccator. *Incubation Time* is the duration of incubation (days).

$$Respiration = [(DO_{\ water} - DO_{\ strip\ post}) - (DO_{\ water} - DO_{\ control\ post})] * (V_{H_2O}\ chamber(L))/m_{strip} / t \quad (2)$$

Where $DO_{\ water}$ is the DO concentration in the river water at the start of the 2 h incubation period. $DO_{\ strip\ post}$ is the DO concentration in each CS chamber after the incubation period. $DO_{\ control\ post}$ is the concentration of DO in each control chamber (i.e., that contains river water but no CS) after incubation. V_{H2O} is the volume of water in the respiration chamber, $m_{\ strip}$ is the CS dry mass, and t is time. DO concentration was expressed in mg O_2/L. The strip mass was expressed in grams, and time was recorded in hours.

2.5 Metagenomics Sequencing

Each CS sample stored at -20℃ was sent to Sangon Biotech (Shanghai) Co., Ltd. for bacterial and fungal metagenomic sequencing. The specific steps were described in the supplementary information.

2.6 Statistical analyses

Redundancy analysis (RDA) was performed using CANOCO 5.0 software (Microcomputer Power Company, USA) to examine the relationships between environmental factors and CSD to determine the dominant environmental factors controlling CSD in rivers. A Monte Carlo permutation test (499 permutations) was used to determine the statistical validity of the RDA [49]. The co-occurrence network of the bacterial and fungal genus on CS was created to investigate the interaction patterns among

different bacterial and fungal genus based on CoNet in CYTOSCAPE 3.4.0 [50], with the topological properties and modules of networks obtained by a Network Analyzer [51]. The detected genus were selected, and then a valid co-occurrence was measured by calculating the pairwise associations among the genus with Spearman's correlation coefficient (r)≥0.9 or (r≤-0.9) and the P-value <0.01. The obtained final co-occurrence network was visualized using Gephi [52]. Topological roles of nodes in a network were divided into four types based on the within-module connectivity (*Zi*) and among-module connectivity (*Pi*) of each node: peripheral nodes ($Zi \leq 2.5$, $Pi \leq 0.62$), module hubs ($Zi > 2.5$, $Pi \leq 0.62$), connectors ($Zi \leq 2.5$, $Pi \geq 0.62$) and network hubs ($Zi > 2.5$, $Pi \geq 0.62$). Peripheral nodes have only a few links, and they almost point to nodes in their own modules. Module hubs are highly connected to many nodes in their own modules, while connectors are highly connected to different modules. Network hubs act as both module hubs and connectors. The relationships of keystone taxa and modules with CSD and the dominant environmental factors controlling CSD were analyzed by mantel measurement using Spearman's correlation test.

3. Results

3.1 CSD in rivers

The TSL and RES of CS incubated in rivers are shown in Fig. 2. The TSL and RES ranged from 0.3454 to 0.9984%/d and 0.0067 to 0.0132 mg O_2 g $AFDM^{-1}$ hr^{-1}, with mean values of 0.6972±0.1768 %/d and 0.0097±0.0021 mg O_2 g $AFDM^{-1}$ hr^{-1}, respectively . The TSL at SP1-2, SP5-6, SP8-9 and SP12, as well as the RES at SP1-2, SP5, SP8-9, SP11, and SP13-14, were higher than the average level. However, the TSL at SP3-4, SP7, SP10-11, and SP13-14, as well as the RES at SP3-4, SP6-7, SP10, and SP12, were lower than the average level.

3.2 Bacterial and fungal groups on CS

The metagenomic sequencing for bacteria and fungi on CS in all sampling points was performed. The rarefaction curves tended to be flat, and the sample library coverage rates of each group of bacterial and fungal sequencing were greater than 0.98, indicating that the sequencing depth was enough and the sequencing results could well represent the real situation of the samples. We obtained a higher number of bacterial OTUs than that of fungi on CS in rivers. The number of bacterial and fungal OTU on CS in different sampling points ranged from 2456 to 3791 and from 198 to 735, respectively.

Based on OTU annotation, the obtained bacterial and fungal phyla with a relative abundance of more than 1% could be recognized as dominant microbes, while the rare microbes referred to the phyla with relative abundance less than 1% [53]. In Fig. 3a and b, six bacterial phyla including *Proteobacteria* (53.11%-68.80% of the effective sequences, the same below), *Bacteroidetes* (10.10%-13.23%), *Planctomycetes* (2.93%-9.38%), *Firmicutes* (6.30%-23.57%), *Verrucomicrobia* (2.85%-5.23%), and *Actinobacteria* (1.11%-2.69%), as well as two fungal phyla including

Ascomycota (12.12%-59.37%) and *Rozellomycota* (3.48%-41.80%) were considered to be the shared dominant microbial phyla on CS in all sampling points. The shared dominant bacterial and fungal phyla accounted for 74.52%-85.17% of the total effective sequences of bacteria and fungi.

3.3 Dominant environmental factors controlling CSD

RDA was conducted to reveal the dominant environmental factors controlling CSD in rivers (Fig. 4). The results indicated that the environmental factors significantly impacted CSD variations. It explained 42.75% (P = 0.030) of CSD variations for the first axis and 98.62% (P = 0.020) of CSD variations for all axes. The dominant environmental factors controlling CSD were TN and water temperature with explanatory capacity of 19.9% (P = 0.006) and 15.1% (P = 0.008), respectively. In the RDA ordination biplot, both TN and water temperature were positively correlated with TSL and RES, suggesting that the increase in TN and water temperature could promote the CSD in rivers.

3.4 Bacterial-fungal interactions and their relationships with CSD and the dominant environmental factors

In order to reveal the interactions among bacteria and fungi on CS, and evaluate their relationships with CSD and the dominant environmental factors, we investigated the co-occurrence network of the bacterial and fungal genus using network analysis based on strong and significant correlations (Fig. 5a). The topological characteristics were calculated to describe the complex patterns of biological associations among bacterial and fungal genus on CS (Fig. 5b). The results of topological characteristics showed that the number of nodes was 662 (431 bacteria and 231 fungi). There were 4411 edges in the network, with approximately 42.42% of the edges as bacteria-fungi (B-F), 40.46% as intra-bacteria (B-B), and only 17.12% as intra-fungi (F-F). The numbers of positive and negative correlations in the network were 4248 and 163, respectively. The average clustering coefficient was 0.981. The characteristic path length was 5.888, and the average number of neighbors was 13.326. Network density, diameter, degree centralization, and betweenness centralization were 0.02, 18, 0.059, and 0.027, respectively.

The bacterial-fungal network was divided into modules (i.e., clusters of highly associated nodes) to examine the significant module-trait relationships. The results showed that the calculated modular index was 0.845, which was a typical modular structure, and the bacterial-fungal network could be divided into 61 modules. Ten major modules (>15 nodes, i.e., node number > 2.27% of the total nodes) were shown in Fig. 5a. The edges of the major modules were mainly dominated by B-F (45.27%). Most edges were derived from bacterial phyla *Actinobacteria* and *Proteobacteria*, as well as fungal phyla *Ascomycota* and *Basidiomycota* in the network. The correlation analysis results between ten major modules and CSD showed that the key module 2 had a significant positive correlation with RES, and key module 4 had a significant positive correlation with TSL (P < 0.05, in Fig. 6. Modules significantly associated with CSD were considered as the key modules in this study). Furthermore, interactions within the key module 2 and

4 were predominantly positive. There were direct interactions between the two key modules and other modules (e.g., module 2 and module 1, 9, module 4 and module 1, 10), and their interactions were mainly positive. Notably, the significant positive correlations between the key module 2 and water temperature, and between the key module 4 and TN in water were also observed and stated in Fig. 6 ($P < 0.05$).

Furthermore, a *Zi-Pi* plot was constructed to identify the topological role of each node in the network (Fig. 5c). In this study, peripherals occupied > 96.64% of the total nodes, indicating most of the nodes had only a few links and almost linked to the nodes within their own modules. Module hubs, connectors and network hubs are regarded as the keystone taxa in the network [54]. There were 8 module hubs (1.28% of the total nodes) and 13 connectors (2.08% of the total nodes), but no network hubs were detected in the network. Of the 21 keystone taxa, 14 belonged to bacteria with the relative abundance ranging from 0.01% to 0.24%. Among the 14 keystone taxa, genera *Rhizomicrobium*, *Rhodopseudomonas*, *Burkholderia*, *Phaselicystis*, *Desulfomicrobium*, *Azoarcus*, *Methylomonas*, *Dyella* and *Thermomonas* belonged to phylum *Proteobacteria*. Genera *Emticicia* and *Flavihumibacter* belonged to the phylum *Bacteroidetes*. Genera *Lutispora* and *Youngiibacter* belonged to phylum *Firmicutes*, and genera *Microlunatus* belonged to phylum *Actinobacteria*. Seven fungal keystone taxa were identified, with the relative abundance ranging from 0.001% to 0.040%. Among them, genera *Claviceps*, *Monascus* and *Pseudaleuria* belonged to phylum *Ascomycota*. Genera *Sakaguchia* and *Fomitiporia* belonged to the phylum *Basidiomycota*. Genera *Paramicrosporidium* and *Udeniomyces* belonged to phyla *Rozellomycota* and *Udeniomyces*, respectively. There were 44 edges among these keystone taxa, and all of them were positive edges. There were 9, 6, and 3 keystone taxa located in the module 3, 5 and 1, respectively, and only one keystone taxa in each of the module 12, 15 and 24 (Fig. 7). Keystone taxa *Emticicia* and *Flavihumibacter* in module 1 significantly positively correlated with TN in water ($P < 0.05$, in Fig. 7). However, these 21 keystone taxa and the modules containing them had no significant correlation with TSL and RES (Fig. 7). Furthermore, the keystone taxa displayed significant positive relationships with most of the connected members in their own module and some members of adjacent modules. There were mainly positive direct interactions between module 1 (containing keystone taxa *Emticicia* and *Flavihumibacter*) and the key module 2 and 4.

4. Discussion

4.1 Identification of the dominant microbial groups associated with the CSD

Microbes have been recognized as important and critical contributors to OMD in rivers [9, 16]. Invertebrates can also decompose organic matter, but their contributions are usually influenced by microbial conditions (e.g., providing nutrients and palatability). The relative importance of invertebrates and microbes in OMD may vary depending on the location and conditions of rivers [4]. We did not find macroinvertebrates on CS during sampling in this study, which was consistent with the results of the preliminary experiment. This implied a fairly low possibility of CSD by macroinvertebrates.

Simultaneously, the influences of the hydraulic shear force and abrasion on CSD were systematically reduced or even eliminated by placing CS at positions with consistent and slow flow velocity and not in contact with the sediment. Therefore, the CSD that occurred in rivers was most probably due to the enrichment of bacteria and fungi groups related to cellulose decomposition by the CS.

The CS is mainly composed of cellulose (≈95%), which is a key carbohydrate on earth and a major basal resource in most of Earth's food webs [8]. The hydrolysis of cellulose into smaller molecules that can be assimilated is achieved mainly through cellulases synthesized by cellulose decomposing bacteria and fungi. Cellulases mostly include endoglucanase (EndoG), exoglucanase (ExoG) and β-glucosidase (β-Glu), integrating to achieve the decomposition of cellulose [55]. The six shared dominant bacterial phyla and two shared dominant fungal phyla observed in the present study have been reported as the dominant phyla on cellulose-rich media [56-59]. In order to reveal their role in the CSD, RDA was conducted, respectively. In Fig. S3 and S4, all of the canonical axes significantly explained the variations of CSD, with an explanation rate of 31.38% for bacteria (P = 0.008) and 28.15% for fungi (P = 0.010), respectively. This indicated the shared dominant bacterial and fungal phyla were significantly associated with CSD, respectively. Bacterial phyla *Proteobacteria* was significantly related to CSD with an explanation rate of 12.2% (P = 0.013). *Rozellomycota* and *Ascomycota* were the significant fungal phyla related to CSD with the explanation rate of 16.4% (P = 0.010) and 12.1% (P = 0.012), respectively. These three phyla were positively correlated with TSL, indicating their contributions to TSL. Phyla *Proteobacteria* [60-62], *Rozellomycota* [59, 62] and *Ascomycota* [63-65] have been reported previously to produce cellulase or be detected in cellulose-rich media. Moreover, *Ascomycota* and *Rozellomycota* were the aerobic and anaerobic fungal phyla reported in previous studies [59, 66]. *Ascomycota* and *Rozellomycota* were positively and negatively correlated with RES in this study, indicating they increased and decreased RES, respectively. The aforementioned discussions suggested that CS in rivers could selectively enrich bacteria and fungi groups related to cellulose decomposition, thus resulting in the CSD in rivers.

4.2 Cooperation pattern of bacteria and fungi and contributions of keystone taxa and key modules to CSD

Bacteria and fungi on CS interacted to form a microbial network to achieve the CSD. The network constructed by bacterial and fungal genus had 662 nodes and 4411 edges in this work, indicating that the bacterial and fungal genus on CS were well integrated with each other and formed a co-occurrence network. This was also found in previous studies on microbial co-occurrence patterns in organic-rich soils [27]. One possible explanation was that a great supply of nutrients from CS and river water might release microbes from resource limitation, stimulate microbial growth, and provide more opportunities for interactions among different taxa [67, 68]. The number of F-F edges was the lowest in our network, indicating that fungi linked with more free-living or less connected in the network compared to bacteria [27]. But, their roles on OMD cannot be ignored in aquatic environments, because recalcitrant organic matters like plant litters were mainly decomposed by fungi [69]. The highest number of B-F edges

indicated the close interactions between bacteria and fungi, which are consistent with their ecological associations and common in microbial community. [70]. The number of positive correlations was much higher than that of negative correlations between bacteria and fungi in the network (Fig. 6a), indicating the cooperation of bacterial and fungal genus in CSD. The products such as low molecular weight organic matter released in the decomposition process of recalcitrant organic matters by fungi can be used by bacteria [12, 22]. Meanwhile, nutrients activated by bacteria can facilitate their absorption and utilization by fungi, and bacterial metabolites can also stimulate the growth of fungal hyphae [71]. The synergistic relationship between fungi and bacteria can also be due to the close spatial relationship between them, with bacteria as epiphytes on fungi [72], which deepened the interaction between bacteria and fungi.

The high modularity of microbial network was conducive to the stability of microbial community structure, function and micro-ecosystem, so as to resist the drastic changes of environments [73]. In this study, the network presented obvious modularity and was divided into 61 modules. There were differences in the groups and quantities of bacteria and fungi involved in different modules. This was probably because of the functional specificity of modules, depending on the combinations of bacteria and fungi [22]. The module has also been considered as the niche, and modules identified in the network reflect the environmental preference and habitat heterogeneity of microbes [74]. Although changes in environmental conditions may lead to variations in certain microbes in the module, they can be replaced by microbes with similar ecological characteristics or functional roles on ecosystem stability [75]. Thus, the module is usually relatively stable and preserves its necessary roles in sustaining the ecosystem functions [75]. In our network, most of the interactions within modules were predominantly positive. This suggested that microbes within the same module may form cooperative interactions or share similar niches [74]. Meanwhile, there were some cross-module edges, which were mainly positive, indicating that the modules in this network were not isolated from each other but cooperated with each other. It has been reported that nutrient availability was the important shaping factor in network modules [74]. During the cellulose decomposition process, different and bioavailable nutrients are continuously released, providing various nutrient niches to form different modules. This thus resulted in positive correlations among the modules.

Two key modules were obtained in this study, i.e., modules 2 and 4, which were significantly positively correlated with RES and TSL, respectively. This indicated that members of the two key modules might have their own special functions to contribute to CSD. It was worth mentioning that the bacteria and fungi in the two key modules cooperated to promote RES, because the edges in the key modules were mainly B-F (44.83%) and were predominantly positive. There were 41 bacterial genera and 22 fungal genera involved in the key module 2, such as bacterial genera *Actinotalea*, *Armatimonas/Armatimonadetes_gp1* and *Methylobacter*, as well as fungal genera *Sampaiozyma* and *Cystofilobasidium*. They were typically reported as aerobic or facultative anaerobic [76-79]. Most of them belonged to the shared dominant phyla *Proteobacteria* and *Ascomycota*, which were positively correlated with RES (Fig. S9 and S11). Module 4 included 31 bacterial genera and 13 fungal genera, such as bacterial genera *Flavobacterium* and *Phaeodactylibacter*, and fungal genera *Geotrichum* and *Exophiala*. They have been reported to be related

to cellulose decomposition [79, 80]. Most of these bacteria and fungi belonged to the shared dominant phyla *Proteobacteria* and *Ascomycota*, which were positively correlated with TSL (Fig. S9 and S11), indicating they could synergistically increase TSL. The key modules 2 and 4 positively direct interacted with other modules in the network, indicating that the key modules may connect with other modules through exchanging the nutrients during the cellulose decomposition process, and thus formed a bacterial-fungal cooperative network to achieve the CSD.

Keystone taxa in the network represented the critical regulators driving the microbial interactions [22]. Keystone taxa regulate the community structure and function mainly through acting on the intermediate or effective groups by secreting metabolites, antibiotics and toxins irrespective of their abundance [23, 81]. He et al. emphasized that the keystone taxa might develop strong relationships with other members in the module through antibiotics or secondary metabolites [82]. In this study, 14 bacterial genera and 7 fungal genera were detected as keystone taxa, but their relative abundances were not high. This was consistent with recent microbial network studies in which low abundance taxa might act as the keystone taxa in other different environments [73, 83-86]. These keystone taxa with less abundant may play important roles in determining genetic diversity, functional diversity, and ecosystem stability [86]. Moreover, there were close cooperative relationships among these keystone taxa because all 44 edges were positive. We found that the keystone taxa and the modules containing keystone taxa had no significant correlation with CSD. However, some keystone taxa had direct interactions with the members in key modules 2 and 4. This suggested that the role of the keystone taxa in CSD may be more reflected in their connections with some species contributing to CSD in the key modules, rather than directly decomposing CS. These keystone taxa were very important to understand the CSD caused by the bacterial-fungal network. Removing these keystone taxa may catastrophically disrupt the bacterial-fungal network into many disconnected subnetworks and dramatically decrease the ecological functions at the community level [87]. Taking above together, the bacterial-fungal interactions were dominated with cooperation, reflecting in the keystone taxa, within the key modules, as well as between the key modules and others. The role of keystone taxa was to connect with the microbial groups associated with CSD in key modules, and then the key modules directly promoted the CSD.

4.3 Regulation of the dominant environmental factors to keystone taxa and key modules

Exploring the relationships between the dominant environmental factors and key modules, keystone taxa in the bacteria-fungal network on CS will help to better understand how these microbial interactions are affected by their habitat conditions. Mantel test results showed that key module 2 was significantly positively correlated with water temperature, and key module 4 was significantly positively correlated with TN. Microbes with a similar preference for water temperature and TN tended to gather together to form key modules 2 and 4, respectively, and in turn, these formed modules were susceptible to changes in water temperature and TN. Temperature affects the enzymatic reactions in microbes and is an important control factor for the growth and metabolism of microbes. Nitrogen is a restrictive element of microbes in

environments. It plays an important role in the life and metabolism of microbes by affecting protein synthesis, cell division, etc., processes [88]. It has been widely reported that temperature and TN were the key regulatory factors for the assembly of microbial communities in other environments, and they strongly adjusted microbial interactions [89-91]. Previous studies about soil environments have documented that the modules in microbial networks were significantly positively correlated with environmental temperature and nitrogen concentration [22, 70, 92, 94], which provided support for our results. Two keystone taxa (i.e., *Emticicia* and *Flavihumibacter*) in module 1 were significantly positively correlated with TN in this study. This indicated environmental nitrogen concentration might regulate the stability of microbial network through acting on the keystone taxa preferentially, finally achieving the CSD. Similar results have also been confirmed in other studies in soil environments [86, 91, 95]. A large number of previous studies have reported that water temperature and TN were important factors affecting the OMD in river ecosystems [8, 10, 32]. Our results provided the important mechanisms for those previous studies, i.e., water temperature and TN promoted the OMD in river ecosystems through regulating the keystone taxa and key modules in the bacterial-fungal network.

5. Conclusion

Microbial decomposition is an important mechanism of the OMD in river ecosystems. The occurrence of CSD in rivers was most probably due to the enrichment of bacteria and fungi groups related to cellulose decomposition and the formation of a cooperation-based interaction network on CS. In this network, the key modules and keystone taxa played diverse crucial roles in the CSD. Key modules with the special functions directly contributed to the CSD. Keystone taxa regulated the key modules and maintained the stability of microbial network structure to achieve the OMD function. Notably, the roles of key modules and keystone taxa in CSD in river ecosystems were regulated by the dominant environmental factors, including water temperature and TN. These works will provide important insights for OMD as a powerful tool to improve future river ecosystem management strategies.

Declarations

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Author Contribution

Yibo Liu: Conceptualization, Methodology, Software, Visualization, Formal analysis, Data curation, Investigation, Writing-Original Draft. Yanping Shen, Cheng Cheng, Weilin Yuan: Investigation. Baiyu

Zhang, Yixin Zhang and Ping Guo: Investigation, Resources, Writing-Review & Editing, Supervision, Project administration.

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Figures

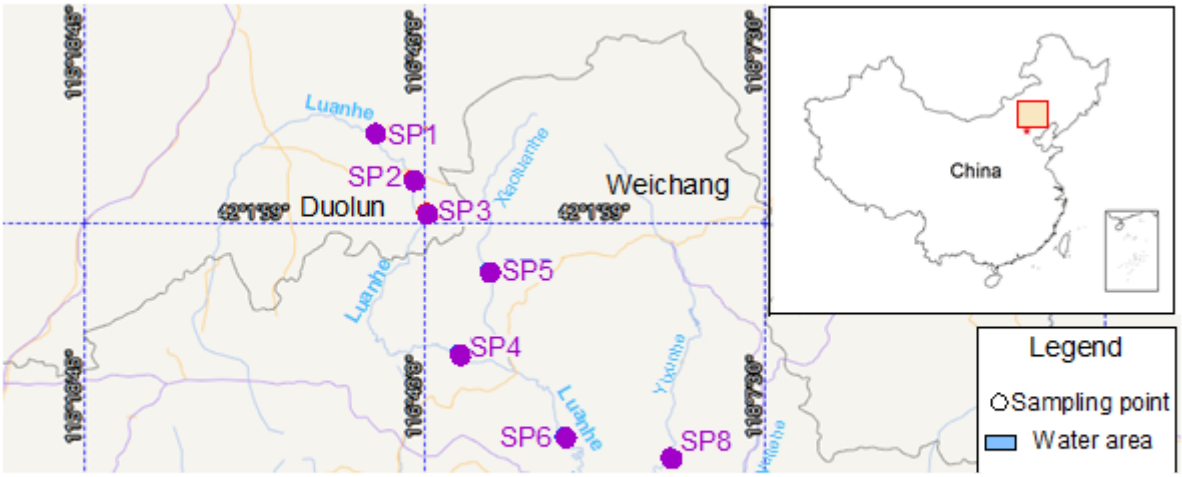

Figure 1

Locations of the sampling points in Luanhe River Basin

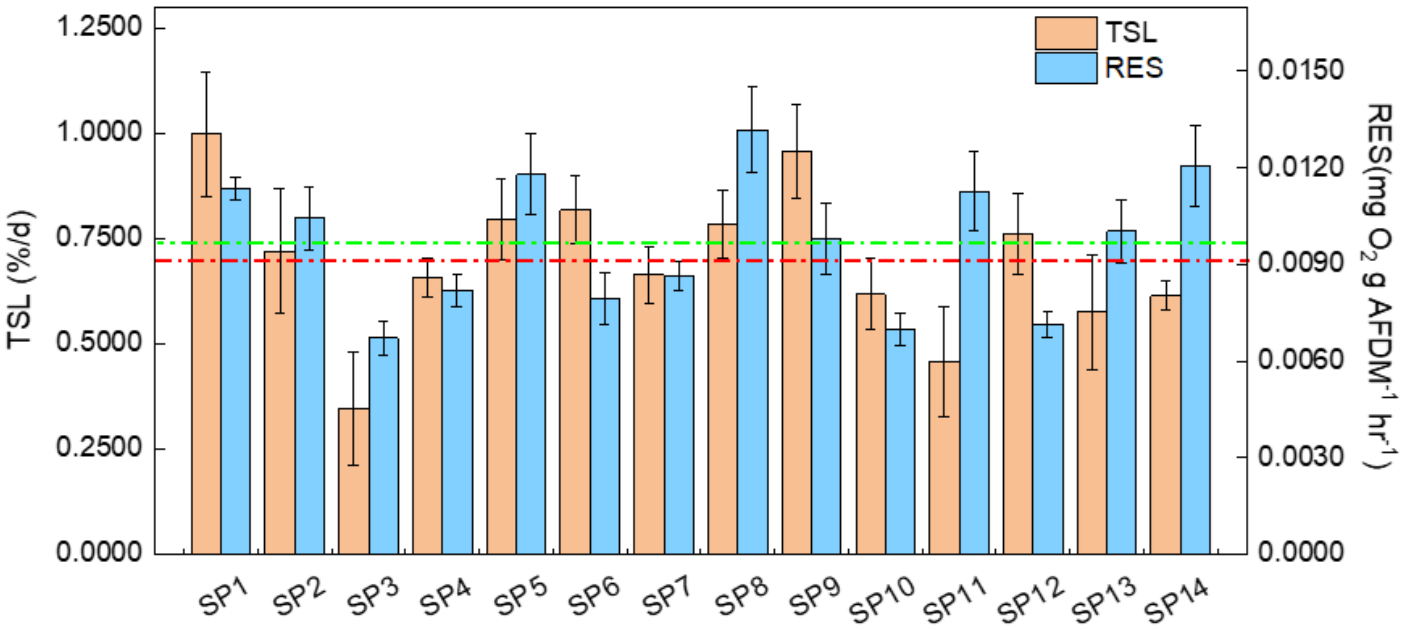

Figure 2

The tensile strength loss (TSL) and respiration (RES) of cotton strip (CS) in rivers. The average values of TSL and RES in all sampling points are indicated by red and green dotted lines, respectively

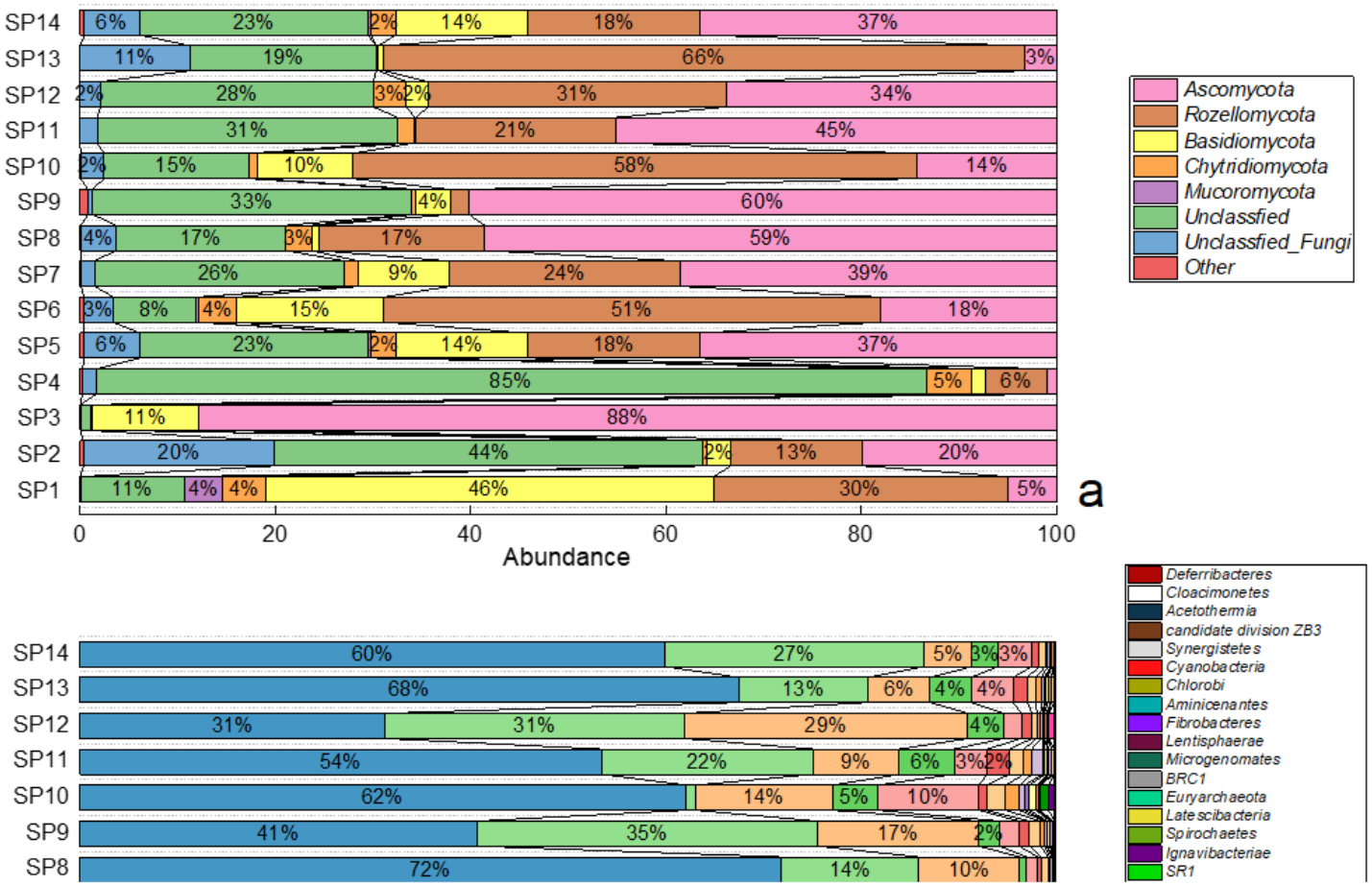

Figure 3

Relative abundance of bacterial (a) and fungal (b) communities at phylum level on cotton strip (CS) in rivers

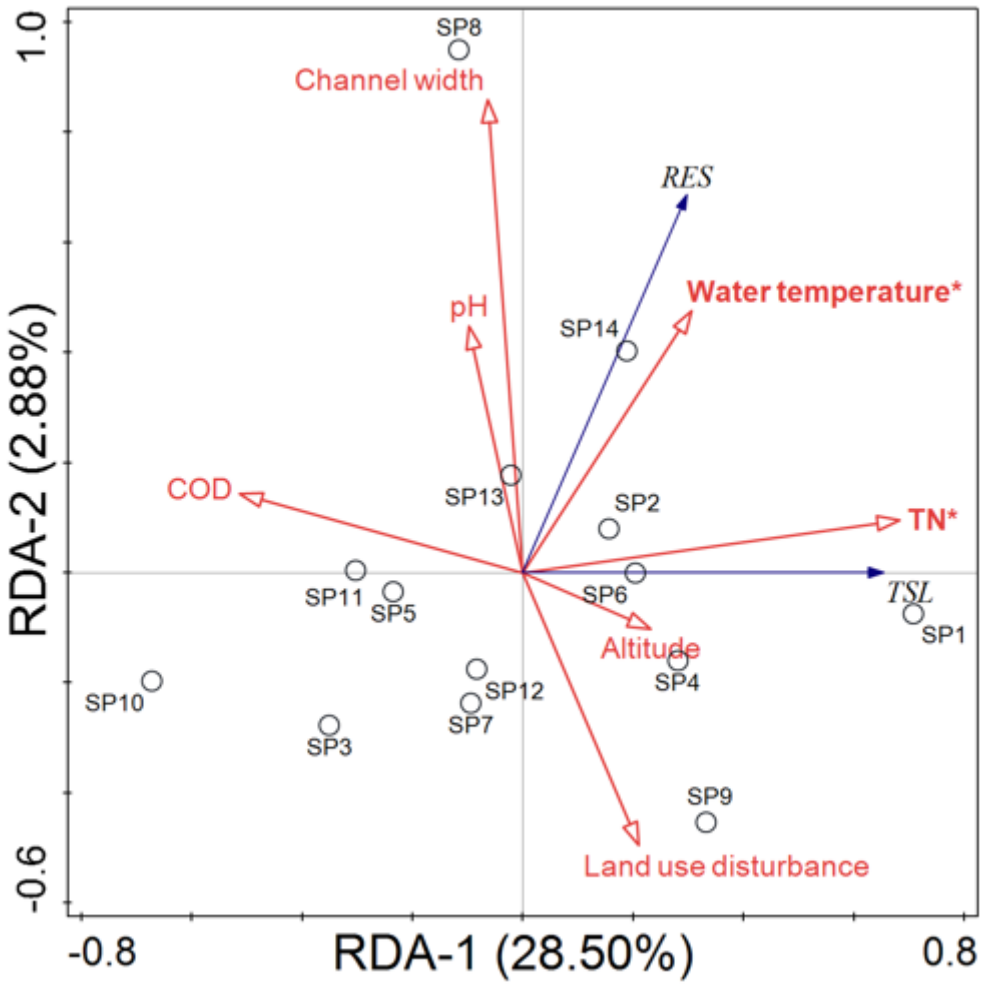

Figure 4

The relationships between cotton strip decomposition (CSD) (represented by blue lines) and the environmental factors (represented by red arrows) using a redundancy analysis (RDA). Note: *Reliability is significant at $P < 0.05$ level

Figure 5

Co-occurrence network of the bacterial and fungal genus on cotton strip (CS) (a). Each circle and square represents a bacterial and fungal genus respectively in the network. Ten major modules are shown in the figure.

Module	Nodes	TSL		RES		TN		Water temperature	
		Correlation coefficients	*p*	Correlation coefficients	*p*	Correlation coefficients	*p*	Correlation coefficients	*p*
Module 1	111	0.04	0.375	0.22	0.128	0.14	0.218	0.04	0.367
Module 2	63	0.08	0.325	**0.30***	0.037	0.24	0.753	**0.50***	0.044
Module 3	53	-0.25	0.896	-0.02	0.648	-0.22	0.834	-0.06	0.497
Module 4	44	**0.57***	0.008	0.20	0.138	**0.59***	0.022	-0.14	0.663
Module 5	39	0.12	0.426	0.30	0.084	0.22	0.725	0.13	0.801
Module 6	30	-0.25	0.776	-0.09	0.513	-0.35	0.67	0.23	0.647
Module 7	23	0.06	0.37	0.11	0.114	0.17	0.837	0.17	0.501
Module 8	18	0.13	0.431	0.20	0.397	0.22	0.641	0.08	0.767
Module 9	17	0.07	0.514	0.19	0.214	0.09	0.604	0.03	0.852
Module 10	16	0.14	0.175	0.23	0.406	0.04	0.716	0.12	0.716

Figure 6

Correlation coefficients and significance between module eigengenes and cotton strip decomposition (CSD), the dominant environmental factors. Only the first ten modules are listed in the table. A Mantel test is conducted to investigate the correlation between the dissimilarity of bacterial and fungal community compositions in module and CSD, the dominant environmental factors. Bold values denote significant relationships by mantel test, * $P < 0.05$

Figure 7

Information description of the keystone taxa and correlation coefficients between keystone taxa and cotton strip decomposition (CSD) as well as the dominant environmental factors controlling CSD. Bold values denote significant relationships by Pearson correlation analysis., * $P < 0.05$.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryMaterial.doc