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Microplastics shape microbial communities affecting soil organic matter decomposition in paddy soil

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- Bacterial succession over 100-day incubation in MP-amended paddy soils was observed.
- MPs changed C mineralization in a short period after MP addition.
- Bacterial community temporal turnover rates were increased by MP addition.
- MPs promoted organic C mineralization by shifting the bacterial community.

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HIGHLIGHTS GRAPHICAL ABSTRACT

ABSTRACT

Microplastics (MPs) can alter microbial communities and carbon (C) cycling in agricultural soils. However, the mechanism by which MPs affect the decomposition of microbe-driven soil organic matter remains unknown. We investigated the bacterial community succession and temporal turnover during soil organic matter decomposition in MP-amended paddy soils (none, low [0.01% w/w], or high [1% w/w]). We observed that MPs reduced the CO₂ efflux rate on day 3 and subsequently promoted it on day 15 of incubation. This increased CO₂ emission in MP-amended soil may be related to (i) enhanced hydrolase enzyme activities or; (ii) shifts in the Shannon diversity, positive group interactions, and temporal turnover rates (from 0.018 to 0.040). CO₂ efflux was positively correlated (r *>* 0.8, p *<* 0.01) with *Ruminiclostridium_1*, *Mobilitalea*, *Eubacterium xylanophilum*, *Sporomusa*, *Anaerobacteriu*, *Papillibacte*r, *Syntrophomonadaceae*, and *Ruminococcaceae_UCG_013* abundance in soil with high MPs, indicating that these genera play important roles in soil organic C mineralization. These results demonstrate how microorganisms adapt to MPs and thus influence the C cycle in MP-polluted paddy ecosystems.

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1. Introduction

The terrestrial ecosystem has been extensively contaminated with microplastics (MPs), which are defined as plastic particles of diameter less than 5 mm ([Rillig et al., 2017](#page-8-0)). In agroecosystems, MPs may originate from the application of sewage sludge, compost, wastewater irrigation, and plastics in agricultural practices, among which the fragmentation of plastics utilized in agriculture is considered the main source of MPs [\(Hu et al., 2022; Wang et al., 2021a, 2021b; Zhou et al.,](#page-8-0) [2020\)](#page-8-0). It has been reported that farmland soils across China contain 0.1–324.5 kg ha⁻¹ plastic film residues, with an average of 83.6 kg ha⁻¹ ([Huang et al., 2020](#page-8-0)). MPs consist of a highly stable carbon (C) base that resists decomposition and thus exist for hundreds of years in the environment ([Agarwal, 2020](#page-8-0)). However, MPs are classified into non-biodegradable and biodegradable components. Mesophilic bacterial consortium can degrade up to 21.7–22.4% of the biodegradable components of the MPs ([Yuan et al., 2020](#page-9-0)). In addition, small amounts (0.11–0.48%) of the bio-labile dissolved organic C (DOC) released from MPs stimulates microbial activity, thereby participating in the soil organic C (SOC) turnover [\(Romera-Castillo et al., 2018; Zhu et al.,](#page-9-0) [2020\)](#page-9-0). Thus, the microbial degradation of plastic polymers may play an important role in soil C cycling [\(Rillig et al., 2021; Skariyachan et al.,](#page-9-0) [2022\)](#page-9-0). The accumulation of MPs in agricultural soils alters the soil physicochemical properties, such as shaping soil aggregation, reducing bulk density, and increasing water saturation [\(de Souza Machado et al.,](#page-8-0) [2019; Lehmann et al., 2021](#page-8-0)). Consequently, these MP-induced changes alter nutrient- and C-cycling in soils [\(Zhou et al., 2021b](#page-9-0)). For example, previous studies have revealed that MP in soil enhances the photoassimilated-C allocated to the soil and increases the enzyme activity hotspots related to C-, N-, and P-cycling around the MPs [\(Zang](#page-9-0) [et al., 2020; Zhou et al., 2021a\)](#page-9-0). However, MP may decrease dissolved organic matter decomposition and facilitate the accumulation of high-molecular-weight humic-like material ([Liu et al., 2017](#page-8-0)). Although studies have revealed the effects of MPs on biochemical processes in agricultural ecosystems, the underlying biological mechanisms are unclear.

Microorganisms play important roles in driving biochemical processes ([Banerjee et al., 2016\)](#page-8-0). MPs affect microbial growth and alter the microbial community, inducing changes in the biochemical processes in soil ([Kim and Rillig, 2021; Seeley et al., 2020; Zang et al., 2020; Zhou](#page-8-0) [et al., 2021b](#page-8-0)). For example, MPs serve as "special microbial accumulators" that provide a distinct habitat for microorganisms in farmland soil and establish a special niche ([Xie et al., 2021\)](#page-9-0). MPs in soil may constitute a "rhizosphere-like" niche, which is referred to as the "plasticsphere" ([Guo et al., 2020; Li et al., 2021a, 2021b, 2021c; Yu et al., 2021\)](#page-8-0). Differences in bacterial diversity, richness, community structure, and function may lead to a "neighbor avoidance effect" between microplastic surfaces and the soil, that is the plasticsphere soil (Yu et al., [2021\)](#page-9-0). In soil, MPs may also cause stress to microorganisms by releasing other co-contaminants, such as heavy metals and phthalate esters ([Bandow et al., 2017; Li et al., 2021\)](#page-8-0), which can change the microbial community of the agricultural soil [\(Fei et al., 2020\)](#page-8-0). For example, MPs promote the abundance of members of the dominant bacterial phyla Acidobacteria and Proteobacteria but decrease that of Bacteroidetes, Firmicutes, and Gemmatimonadetes in rice paddy soil ([Liu et al., 2021](#page-8-0)). [Wang et al. \(2020\)](#page-9-0) reported that the microbial community may change intensively over time in polyethylene MP-amended soil. A rapid shift in the microbial population may result in increasingly divergent succession of the microbial community under MP stress [\(Wang et al., 2020\)](#page-9-0). Microbial succession can be estimated by a linear regression of the log-transformed microbial community similarity over time (Liang et al., [2015\)](#page-8-0). However, whether the rapid temporal turnover rate of the bacterial community induced by MPs results in a change in soil organic matter (SOM) decomposition has not been examined.

Alterations in the diversity and structure of the community do not necessarily involve changes in the decomposition rate of SOM because of microbial functional redundancy. This increases the complexity of the effect of MPs on biochemical processes. Several taxa involved in plastic degradation, xenobiotic biodegradation, and amino acid metabolism are abundant in the plasticsphere, which may affect C cycling ([Neis et al.,](#page-8-0) [2015; Zhang et al., 2019\)](#page-8-0). For example, [Zhang et al. \(2021\)](#page-9-0) showed that virgin MPs reduce the abundance of hemicellulose decomposition functional genes (*abfA* and *manB*), whereas aged MPs decrease the abundance of cellulose (*cex*), hemicellulose (*xylA*, *abfA*, and *manB*), and starch (*sga*) decomposition functional genes. This indicates that MPs change C cycling by altering specific functional microbial taxa [\(Sun](#page-9-0) [et al., 2022](#page-9-0)). Keystone microbial taxa are the main contributors to SOM decomposition [\(Banerjee et al., 2016\)](#page-8-0). MPs may influence the distinct soil microbiota, thereby altering microbial functional properties, such as enzyme activities related to C and N cycling (e.g., glucosidase, chitinase, and urease), in soil. This affects SOM decomposition ([Huang et al., 2019;](#page-9-0) [Zhang et al., 2021\)](#page-9-0). Fresh OM decomposition may be initially dominated by opportunistic strategists (e.g., Firmicutes, Bacteroidetes, Actinobacteria, and Proteobacteria) in response to labile components, thus promoting OM decomposition; thereafter, the microbial community is succeeded the species that can decompose degraded OM and reduce the nutrient content ([Fontaine et al., 2003; Wang et al., 2021\)](#page-8-0). Thus, MPs inducing changes in the structure of microbial community and abundance of C/N/P functional genes likely change SOM decomposition in agricultural soil ([Li et al., 2021](#page-8-0)).

In addition to microbial community diversity, composition, and functional traits, the microbial decomposition of SOM may vary with the quality of substrate ([Banerjee et al., 2016; Bao et al., 2020](#page-8-0)). In a previous study, glucose and rice straw were added to soil mixed with polyethylene MPs (none, 0.01% w/w, and 1% w/w) to evaluate the effects of the three different doses of MPs on OM decomposition ([Xiao et al.,](#page-9-0) [2021\)](#page-9-0). The results showed that MPs strongly influenced the priming effect on SOM and rice straw decomposition in rice paddy soil. However, the changes in microbial diversity and taxa in response to MPs during SOM decomposition remain unclear. Therefore, we examined how soil microorganisms regulate SOM decomposition in rice paddy soil in the presence of MPs based on the findings of the previous study [\(Xiao et al.,](#page-9-0) [2021\)](#page-9-0). We hypothesized that (1) the presence of MPs would facilitate the growth of special microbial taxa that accelerate SOM decomposition and (2) MPs could promote SOM decomposition by increasing bacterial community turnover.

2. Materials and methods

2.1. Soil preparation

Typical Stagnic Anthrosol soil was collected from a subtropical rice paddy field (0–20 cm) (113◦20′ 8′′E, 28◦33′ 26′′N, 113 m above sea level) in Hunan, China. The soil was air-dried and sieved through a 2-mm sieve. The soil had a pH of 6.4 (1:2.5 w/v, soil to water ratio), 1.68% organic C, 0.11% total N, and 0.05% total phosphorus;it contained 82% clay, 12% silt, and 6% sand.

2.2. Experimental layout and soil incubation

The soils were thoroughly mixed with MPs and rice straw after preincubation for 2-weeks. The pre-incubated soils (60 g, dry equivalent; 34.3% water content) were then transferred into a 500-mL incubation bottle. The soils were maintained with a 2–3- cm water layer above the soil surface to simulate a flooded condition throughout incubation. Three MP (ultra-high-molecular weight polyethylene; 40–48 µm particle size; Sigma-Aldrich, St. Louis, USA; Fig. S1) treatments were established, namely M0: no MP; M1: 0.1 mg (0.01% w/w; low MPs) MPs g^{-1} dry soil; M2: 10 mg (1% w/w; high MPs) MPs g^{-1} dry soil. The dosages of MPs were based on the actual amounts in the agricultural field [\(Huang et al.,](#page-8-0) [2020; Lv et al., 2019; Sun et al., 2018\)](#page-8-0). Each MP treatment included four sub-treatments: only soil with no C substrate addition; soil $+$ glucose;

 $soil + straw$; $soil + straw + glucose$. Each treatment was replicated three times for a total of 36 experimental units. Rice straw was added at a dose of 2.5 g kg⁻¹ dry soil (1.215 mg C g⁻¹ soil), corresponding to approximately 6 t ha⁻¹ rice straw return rate in the rice paddy field. Glucose solution (4.19 g L $^{-1}$, 0.5 mL per day) was added daily during the first 30 days of incubation using an Eppendorf pipettor. A final rate of 419 μg C g⁻¹ dry soil corresponded to 2.5% SOM in the original soil prepared for this experiment. Glucose was chosen in this experiment to simulate the release of low molecular weight organic matters or DOC from rice litter and green manure ([Gao et al., 2018; Ye and Horwath, 2017](#page-8-0)). An equal amount of distilled water was added to the treatments without glucose addition.

2.3. Sampling

Small plastic vials containing 5 mL of 1 M NaOH were placed inside the bottles to capture any $CO₂$ evolved. The bottles were hermetically sealed and incubated in the dark at 25 ◦C for 100 days. The NaOHcontaining vials were replaced at 1, 3, 5, 7, 10, 15, 20, 25, 30, 35, 42, 52, 65, 83, and 100 days of incubation to analyze the $CO₂$ flux rate. The sampling frequency was set to ensure that all the released $CO₂$ was captured by NaOH. After precipitation using 1 M BaCl₂ solution, the NaOH samples were titrated against 0.1 M HCl using phenolphthalein as an indicator to analyze the total trapped $CO₂$ ([Shahbaz et al., 2018](#page-9-0)). Three control bottles without soil were also evaluated to correct for the total $CO₂$ flux. Other sets of replicates were prepared for soil sampling after 3, 15, and 100 days of incubation, which were based on the intensive, reduced, and stabilized SOM decomposition at the end of incubation. All soil samples (108 in total) were used to analyze the soil properties and microbial communities.

2.4. Chemical analysis, microbial biomass, and enzyme assays

Soil organic C and total N were evaluated using the dry combustion method performed with an elemental analyzer (Vario EL III, Elementar, Langenselbold, Germany). Soil total phosphorus was analyzed based on NaOH fusion, determined at 880 nm using a spectrophotometer (UV2300II, Tech Comp Ltd., Shanghai, China). The soil texture was analyzed with a laser diffraction particle size analyzer (Mastersizer 2000, Malvern Instruments Ltd., Malvern, UK). Soil pH was determined using MetropH320 (Mettler-Toledo Instruments Ltd., Columbus, OH, USA).

Soil microbial biomass was determined using the chloroform fumi-gation-K₂SO₄ extraction method [\(Wu et al., 1990](#page-9-0)). Non-fumigation and fumigation extracts were analyzed using a Multi N/C 2100 TOC/TN analyzer (Analytik Jena, Jena, Germany) to measure soil DOC and dissolved nitrogen (DN). Microbial biomass C was calculated based on the difference in the DOC between the non-fumigation and fumigation extracts and using a *k*ec factor of 0.45 [\(Wu et al., 1990](#page-9-0)). Microbial biomass N was calculated similar to microbial biomass C. The non-fumigation extracts were analyzed spectrophotometrically (PowerWave XS microplate spectrophotometer; BioTek Instruments, Winooski, VT, USA) to determine the ammonium (NH_4^+) concentration.

Activities of five extracellular hydrolytic enzymes, *β*-glucosidase, xylanase (XYL), cellobiohydrolase (CELLO), chitinase (NAG), and leucine aminopeptidase (LAP), were measured according to the fluorimetric protocol described by [Saiya-Cork et al. \(2002\)](#page-9-0) with modifications reported by [DeForest \(2009\)](#page-8-0).

2.5. DNA extraction, amplification, and high-throughput sequencing

Total genomic DNA was extracted from 0.5 g of − 80 ℃ frozen soil using the DNeasy PowerSoil Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. The concentration and quality of the extracted DNA were measured using a Nanodrop spectrophotometer (ND-100; Thermo Fisher Scientific, Waltham, MA, USA). The universal

forward primer 341 F (CCTAYGGGRBGCASCAG) and reverse primer 806 R (GGACTACNNGGGTATCTAAT) were used to amplify the bacterial hypervariable V3–V4 regions of the 16 S rRNA gene ([Wei et al.,](#page-9-0) [2020\)](#page-9-0). Polymerase chain reaction (PCR) was performed under the following conditions: denaturation for 3 min at 95 ◦C; followed by 30 cycles of denaturation at 95 ◦C for 5 s, annealing at 58 ◦C for 30 s, and extension at 72 ◦C for 30 s; and final extension at 72 ◦C for 10 min. The PCR products were further purified using a Qiagen Gel Extraction Kit (Qiagen). The purified amplicons were sequenced on an Illumina MiSeq platform by Novogene Co., Ltd. (Beijing, China). Sequencing libraries were generated using NEBNext® Ultra™ II DNA Library Prep Kit for Illumina® (New England Biolabs, MA, USA), following manufacturer's instructions and index codes were added. The library quality was assessed using the Qubit® 2.0 Fluorometer (Thermo Fisher Scientific, MA, USA). Finally, the library was sequenced on an Illumina platform and 250-bp paired-end reads were generated.

2.6. Bioinformatic analysis

The raw sequences were processed using Quantitative Insights Into Microbial Ecology (QIIME, version 1.7.0) following the online instructions ([Caporaso et al., 2010](#page-8-0)). Briefly, the paired reads were assembled and demultiplexed, and primer sequences and low-quality reads with a quality score of *<* 25 were removed. Sequence analysis was performed using Uparse software with all effective sequences (Uparse v7.0.1001, http://drive5.com/uparse/) ([Edgar, 2013](#page-8-0)). Sequences with \geq 97% similarity were assigned to the same operational taxonomic units (OTUs). Representative sequences for each OTU were screened for further annotation. Bacterial OTUs were assigned to taxonomic groups using the SILVA database (https://www.arb-silva.de/) for species annotation at each taxonomic level using a confidence threshold of 80% ([Quast et al., 2013](#page-8-0)). Because of the variance between sample sequences, all samples from the bacterial OTU tables were randomly rarefied based on the lowest reads, resulting in 8618 bacterial OTUs for downstream analyses.

2.7. Statistics

Statistical analyses were carried out in R version 4.0.5 (The R Project for Statistical Computing, Vienna, Austria). The alpha diversity indices, including Shannon diversity richness (observed species), Chao1 (number of observed OTUs), and ACE were calculated using the "vegan" package. One-way analysis of variance with Tukey's test was used to evaluate the significance of differences between treatments. Microbial beta diversity in different samples was assessed using principal coordinate analysis based on Bray-Curtis dissimilarity in the "vegan" and "ape" packages. The time-decay relationships (TDRs) of soil bacterial communities were evaluated using linear regression between beta-similarity in bacterial communities and temporal distance among samples after logarithmic transformation [\(Liang et al., 2015; Wang et al., 2020\)](#page-8-0). The potential presence of taxonomic groups that may explain the differences between bacterial communities was explored using linear discriminant analysis effect size (LEfSe) with linear discriminant analysis *>* 2 in the Galaxy framework [\(Segata et al., 2011](#page-9-0)). Spearman's correlation index was calculated using the "psych" package [\(Langfelder and Horvath,](#page-8-0) [2012\)](#page-8-0). Taxa with Spearman correlation coefficient *>* 0.8 or *<* − 0.8 and p *<* 0.01 were determined. The main co-occurrence networks were visualized using Gephi 0.9.2 [\(Bastian et al., 2009](#page-8-0)). The $CO₂$ efflux rate correlated with the bacterial genera and their corresponding taxa were considered in the co-occurrence networks. Differences were considered significant at p *<* 0.05 for all statistical tests.

3. Results

3.1. Effects of MPs SSon CO2 flux

MPs reduced the $CO₂$ efflux rate on day 3 of incubation, by 8-27%; however, an increased 1.3-9% CO₂ efflux rate in the later incubation period probably offsets the decreased $CO₂$ emission during the first 3 days (Fig. 1). Thus, MPs showed little effect on accumulative $CO₂$ emission in soil with external C substrates, except for the low MPs that promoted total $CO₂$ emission in soil without amended C sources (Fig. S2).

3.2. Diversity, succession of microbial community and identification of sensitive bacterial species

MP amendment reduced the bacterial Shannon diversity index on day 3 but increased it on day 15 of incubation $(p < 0.05)$ [\(Fig. 2\)](#page-4-0). The dominant bacteria at the beginning of incubation were Clostridia and Bacilli, which belong to the phylum the Firmicutes (Fig. S4). Subsequently, multiple species, including Firmicutes, Proteobacteria (including Alpha-, Beta-, Delta-, and Gamma-proteobacteria), Chloroflexi, and Acidobacteria were prevailent in the soils (Figs. S4 and S5). Soil properties, particularly the DOC, affected the microbial diversity ([Fig. 3\)](#page-4-0).

The LEfSe analysis revealed that 16 microbial biomarkers were sensitive to MP treatments ($p < 0.05$, LDA > 2 ; [Fig. 4](#page-5-0)). On day 3 of incubation, only the family of Syntrophomonadaceae was significantly enriched in the soil without MPs. After 15 days of incubation, *Methanomassiliicoccus* was detected in the soil amended with low MPs, and Acidobacteriales, Clostridia (including *Clostridium_sensu_stricto_8*, Family_XI, *Ruminococcaceae_NK4A214_group*, and *Syntrophomonas*), Pseudomonadaceae, Deinococcaceae, Deinococci and Elusimicrobia were found in high MP-amended soil. On day 100 of incubation, *Reyranella* and *Alcanivorax* genus, and unidentified family of Rhodospirillales were significantly enriched in the soil with low MPs; The genus of *Thermincola* and *Smithella*, and the unidentified family of Thermoplasmatales were detected in the soil amended with high MPs ([Figs. 4 and](#page-5-0) S6).

The high variation of microbial communities between the MP treatments during the incubation induced different microbial succession rates [\(Fig. 5](#page-6-0)). The time-decay relationships (TDRs) of bacterial communities were 0.0175, 0.0399, and 0.0318 in soils without MPs, and with low and high MPs, respectively ([Fig. 5\)](#page-6-0).

3.3. Effect of MPs on soil bacterial community network

The co-occurrence networks were constructed for under each MP amended soil, and they contained both bacterial taxa, soil properties, and $CO₂$ efflux rate [\(Fig. 6](#page-6-0)). The co-occurrence networks comprised 58, 45, and 21 $CO₂$ release-related genera in soils without MPs, or with low and high MPs, respectively ([Fig. 6, Table S1\)](#page-6-0). Therefore, MPs reduced the edges of the co-occurrence networks, which contained 429, 229, 0 negative edges, and 589, 368, 155 positive edges in soils without MPs, or with low and high MPs, respectively (Fig. 6, Table $S1$). The $CO₂$ efflux rate was strongly directly correlated with some bacterial taxa, including *Candidatus_Koribacter*, *Ramlibacter*, unidentified_Acidobacteriaceae_ (Subgroup_1), *Pelosinus*, *Flavisolibacter*, and *Clostridium_sensu_stricto_7* in the soil without MPs; *Clostridium_sensu_stricto_1*, *Ramlibacter*, *Caproiciproducens*, *Nitrosospira*, and *Pelosinus* in the soil with low MP; and *Ruminiclostridium_1*, *Mobilitalea*, Eubacterium_xylanophilum_group, *Sporomusa*, *Anaerobacterium*, *Papillibacter*, *Ruminococcaceae_UCG_013*, and unidentified_Syntrophomonadaceae in the soil with high MPs ([Fig. 6\).](#page-6-0)

4. Discussion

4.1. MP affects bacterial diversity during SOM decomposition

MPs change the $CO₂$ efflux rate in a short period of incubation; however, MPs showed little effect on the accumulative $CO₂$ emission in soil with external C substrates (Fig.1 and S2). This is consistent with the results of a previous study, indicating that low MPs (0.1% w/w) promote accumulative CO₂ emission; whereas, high MPs (1% w/w) showed little effect on $CO₂$ emission in a vegetable farmland soil during 30 d of incubation [\(Gao et al., 2021\)](#page-8-0). Low MPs promote soil microbial growth, and the amended glucose and straw can provide easily available substrates to microorganisms for energy [\(Xiao et al., 2021](#page-9-0)). However, soil without amended C source then decompose SOM for energy and C substrates. MP amendment reduced the bacterial Shannon diversity index on day 3 but increased it on day 15 of incubation ([Fig. 2](#page-4-0)). The lower bacterial diversity indicates that MPs considerably stressed the bacterial population, which is consistent with the findings of a previous study ([Fei et al., 2020](#page-8-0)). However, on day 15, the bacterial diversity was promoted in the presence of MPs after the microorganisms adapted to the new environment. Previous studies have showed that bacterial alpha diversity did not obviously change in polylactic acid MP-amended rice paddy soil and polyethylene MP-amended upland soil ([Chen et al., 2020;](#page-8-0) [Huang et al., 2019\)](#page-8-0). These findings highlight that MPs affect the soil

Fig. 1. CO2 efflux rate from amended C sources in paddy soils with and without microplastics. S, soil without amended C sources; + Glu, soil amended with glucose; $+$ Str, soil with rice straw; $+$ Str & Glu, soil amended with rice straw and glucose combined. M0 = no microplastics; M1 = low (0.01%) microplastics; M2 = high (1%) microplastics. Error bars represent the standard error of the mean $(n = 3)$.

Fig. 2. Shannon diversity (a–c) and principal coordinate analysis of Bray-Curtis distance between bacterial communities (d–g) in the paddy soils with and without microplastics. $M0 =$ no microplastics; $M1 =$ low (0.01%) microplastics; $M2 =$ high (1%) microplastics.

Fig. 3. Relationships between soil properties and alpha diversity of bacterial communities in paddy soils amended with C sources with and without microplastics.

microbial community in diverse manners based on the type of MPs and ecosystems. Polyethylene MPs decreased the stability of the bacterial community structure and created a less complex network pattern by reducing the density of connections between bacterial taxa ([Rong et al.,](#page-9-0)

[2021\)](#page-9-0), providing a foundation for the fluctuating bacterial community and diversity.

Fig. 4. Cladogram of linear discriminant analysis coupled with effect size (LEfSe) results in paddy soils with and without microplastics. M0 = no microplastics; $M1 = low (0.01%) microplastics; M2 = high (1%) microplastics.$

4.2. Keystone taxa and microbial temporal turnover mediated by MPs

MPs promoted the abundance of some specific species, which affected C cycling. Previous studies have revealed that MP surfaces in soil can serve as distinct microbial habitats, which facilitate the colonization of some specific microorganisms [\(Xie et al., 2021; Zhang et al.,](#page-9-0) [2019; Zhu et al., 2021a, 2021b](#page-9-0)). Our results showed that MPs promoted the abundance of some aerobic chemoheterotrophic taxa, such as *Acidobacteriales* and *Pseudomonadaceae*, on day 15 (Fig. 4), thus enhancing microbial respiration. *Pseudomonadaceae* are known as potential MP degrading microorganisms ([Wu et al., 2022](#page-9-0)). This implicates that low MPs may induce the colonization of aerobic chemoheterotrophic species. MPs also promoted the abundance of aerobic chemoheterotrophic and hydrocarbon degradation-related taxa on day 100 (unidentified *Rhodospirillales*, *Reyranella*, and *Alcanivorax*). However, the abundance of xylanolysis and other hydrocarbon degradation-related taxa (*Ralstonia pickettii*, and *Eubacterium xylanophilum group*) also increased significantly in the soil without MPs,

resulting in microbial functional dissimilation but with similar SOM decomposition rates in soils with and without MPs.

The TDR of a bacterial community can be used to estimate the temporal turnover of bacterial species ([Liang et al., 2015](#page-8-0)). The TDR was measured from the slope of linear regression (i.e., TDR value) based on taxonomic diversity using log-transformed community similarity and temporal distance [\(Guo et al., 2018\)](#page-8-0). The TDR value (*w*) is an important indicator of the succession dynamics of bacterial communities ([Wang](#page-9-0) [et al., 2020\)](#page-9-0). The steeper slope observed in the MP-amended soil reflected an increase in the microbial succession rate [\(Fig. 5\)](#page-6-0), which is consistent with the findings of a previous study [\(Wang et al., 2020](#page-9-0)). A higher microbial community temporal turnover rate indicates that MPs accelerate the elimination and replacement of microbial species, which may decrease the stability of bacterial populations in rice paddy soil. This suggests that microbial communities adapt to MP-polluted soil environments by rapidly adjusting their composition. Substrate consumption may increase internal microbial competition because of reduced substrate availability, thus inducing shifts in the soil bacterial

Fig. 5. Time-decay relationships (TDRs) of bacterial communities in amended C sources in paddy soils with and without microplastics. $MO = no$ microplastics; $M1 = low (0.01%) microplastic; M2 = high (1%) microplastic.$

composition ([Goldfarb et al., 2011](#page-8-0)). Increased competition may result in a higher temporal turnover and lower population numbers in soils amended with MPs. This is consistent with the present results, which indicated a low alpha diversity of bacterial communities during the final incubation period (Fig. S3). A drastic turnover of bacterial populations stimulated an increased substrate consumption, thus inducing a higher rate of SOM decomposition in soils containing MPs during the incubation period.

4.3. Microbial mechanisms underlying SOM decomposition in MPenriched soil

Microbial anabolism and catabolism are the two main patterns of SOM metabolism [\(Jeewani et al., 2021; Liang et al., 2017; Luo et al.,](#page-8-0) [2021\)](#page-8-0). MPs may affect microbial metabolism during the utilization of C substrates for energy fueling, microbial reproduction, biomass growth, and metabolite and extracellular enzyme secretion [\(Bhagwat et al.,](#page-8-0) [2021; Oberbeckmann et al., 2015; Xiao et al., 2021; Zang et al., 2020](#page-8-0)). MPs promote the microbial C use efficiency of low molecular weight organic substances, which implicates that MPs can promote microbial growth and reduce respiration over C uptake ([Zang et al., 2020\)](#page-9-0). However, MPs can also induce higher $CO₂$ release without promoting

Fig. 6. Network co-occurrence analysis among soil properties, soil bacterial taxa, and CO₂ efflux rate in response to microplastics in paddy soils. MO = no microplastics; M1 = low (0.01%) microplastics; M2 = high (1%) microplastics. A connection stands for a strong (Spearman's r *>* 0.8) and significant (p *<* 0.01) correlation. Colors of nodes in network represent the bacterial phyla.

-Positive correlation $-$ ---- negative correlation $-$ Reduce $+$ Promote n.s. No significant effect

Fig. 7. Conceptual model showing the shift in microbial community in MP-amended paddy soils, affecting SOM decomposition. ω indicates the microbial community turnover rate evaluated by linear regression between beta-similarity in taxonomic composition and temporal distance among samples after logarithmic transformation. MPs: microplastics.

microbial biomass C [\(Gao et al., 2021](#page-8-0)). Thus, the effect of MPs on SOM decomposition may be controlled by the balance between microbial anabolism and catabolism. An electrochemistry "electron shuttling" hypothesis has been proposed, according to which microbes use MPs as electron sinks or donors during their metabolic processes, which may promote the energy efficiency of microbial metabolism and alter SOC transformation ([Rillig et al., 2021\)](#page-9-0). In addition, polyethylene MPs mainly consist of a C base that includes approximately 90% C, of which 0.11–0.48% may be released during DOC formation, and 22–46% of this released DOC are bio-labile ([Rillig, 2018; Zhu et al., 2020](#page-8-0)). Therefore, soil dissolved organic C could be diluted by complex novel polymers and microbial substrate switching by which easily mineralizable organic C in MPs is preferentially metabolized, thus reducing the soil original SOM decomposition [\(Rillig et al., 2021](#page-9-0)). This is supported by the negative priming effect at the early stage of decomposition in MP-amended paddy soil in a previous study [\(Xiao et al., 2021\)](#page-9-0). However, with the exhaustion of bio-labile C substrate, the energy required for microbial metabolism may be a key driver of SOM decomposition during later microbial succession in the paddy soil ([Gunina and Kuzyakov, 2022; Wei et al., 2020;](#page-8-0) Zhu et al., 2021). The numbers of $CO₂$ release-related genera in soils without and with low and high MPs were 58, 45, and 21, respectively ([Fig. 6, Table S1](#page-6-0)). This result indicates that MPs in paddy soil considerably affected the growth and activity of microbial taxa involved in SOM decomposition. In addition, MPs reduced the negatively correlated edge in the present network ($Fig. 6$), indicating a reduction in the microbial competition toward SOM decomposition. Furthermore, SOM decomposition can be reduced by competition among bacterial pop-ulations [\(Coche et al., 2022\)](#page-8-0), which may have reduced the $CO₂$ efflux rate on day 15 in soils without MP addition ([Fig. 1](#page-3-0)). In the high MP-amended soil, the activities of xylosidase and leucine aminopeptidase were significantly correlated with the $CO₂$ efflux rate-related microbial taxa [\(Fig. 6\)](#page-6-0), suggesting that the addition of high MPs promoted the decomposition of less labile SOM and release of more N for microorganisms. This was consistent with the results showing a significant positive correlation between the activities of these two enzymes and the $CO₂$ efflux rate [\(Fig. 3](#page-4-0)). Soil extracellular enzymes produced by microorganisms regulate SOM decomposition and C sequestration, reflecting the availability of nutrients and resources for the microorganisms ([Yang](#page-9-0) [et al., 2020](#page-9-0)). In the present study, MPs promoted the activities of organic C and N hydrolase enzymes to promote SOM decomposition and provide energy and nutrients for microbial growth.

5. Conclusions

Although the shift in bacterial communities resulting from MPs is well-known, the succession of bacterial communities during SOM decomposition under MP-derived stress remained unclear. In the present study, MPs reduced the $CO₂$ efflux rate on day 3 of SOM decomposition and promoted this effect on day 15, which was subsequently stabilized. SOM decomposition in MP-enriched soil was stimulated by a shift in the microbial community succession and temporal turnover. MPs accelerated the biomass growth of r-strategy bacteria, such as *Ruminiclostridium_1*, *Mobilitalea, Xylanophilum*, *Anaerobacteriu*, *Papillibacter*, *Syntrophomonadaceae*, and *Ruminococcaceae*_UCG_013, which are annotated to the class *Clostridia* and assigned as copiotrophic bacteria. These groups likely induced an increase in $CO₂$ efflux on day 15 of SOM decomposition. MPs reduced the competition among SOM decomposition-related bacterial taxa by reducing the negative correlation between bacterial populations. However, a higher temporal turnover rate indicated that MPs induced a severe succession of bacterial communities. Increased energy and nutrients are required to support bacterial growth and proliferation and accelerate SOM decomposition. The present study revealed the effects of MPs on soil C cycling processes and their microbial mechanisms in rice paddy ecosystems.

CRediT authorship contribution statement

Mouliang Xiao: Methodology, Investigation, Data curation, Writing − original draft, Writing − review & editing. **Ji'na Ding**: Formal analysis, Writing − review & editing. **Yu Luo**: Writing − review & editing. **Haoqing Zhang**: Formal analysis, Writing − review & editing. **Yongxiang Yu**: Writing − review & editing. **Huaiying Yao**: Writing − review & editing. **Zhenke Zhu**: Conceptualization, Writing − review & editing. **David R. Chadwick**: Conceptualization, Writing − review & editing. **Davey Jones**: Conceptualization, Supervision, Resources, Funding acquisition, Writing − review & editing. **Jianping Chen**: Project administration, Resources, Funding acquisition, Writing − review & editing. **Tida Ge**: Conceptualization, Supervision, Project administration, Writing – review $&$ editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.jhazmat.2022.128589.](https://doi.org/10.1016/j.jhazmat.2022.128589)

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