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Deciphering microbial mechanisms underlying soil organic carbon storage in a wheat-maize rotation system

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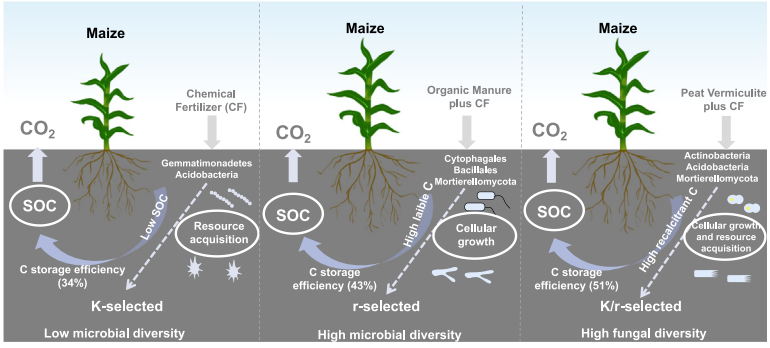
HIGHLIGHTS

- Organic material amendments significantly increased soil carbon storage efficiency.
- Organic manure addition enriched for genes indicative of cellular growth strategies.
- Chemical fertilizer enriched for genes involved in resource acquisition and competition.
- Soil amended with peat-vermiculite was dominated by a mix of *r*- and *K*-selected microbes.
- *Gemmatimonadetes* showed a negative correlation to soil carbon storage efficiency.

GRAPHICAL ABSTRACT



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ABSTRACT

A link between microbial life history strategies and soil organic carbon storage in agroecosystems is presumed, but largely unexplored at the gene level. We aimed to elucidate whether and how differential organic material amendments (manure versus peat-vermiculite) affect, relative to sole chemical fertilizer application, the link between microbial life history strategies and soil organic carbon storage in a wheat-maize rotation field experiment. To achieve this goal, we combined bacterial 16S rRNA gene and fungal ITS amplicon sequencing, metagenomics and the assembly of genomes. Fertilizer treatments had a significantly greater effect on microbial community composition than aggregate size, with soil available phosphorus and potassium being the most important community-shaping factors. Limitation in labile carbon was linked to a *K*-selected oligotrophic life history strategy (*Gemmatimonadetes*, *Acidobacteria*) under sole chemical fertilizer application; defined by a significant enrichment of genes involved in resource acquisition, polymer hydrolysis, and competition. By contrast, excess of labile carbon promoted an *r*-selected copiotrophic life history strategy (*Cytophagales*, *Bacillales*, *Mortierellomycota*) under manure treatment; defined by a significant enrichment of genes involved in cellular growth. A distinct life history strategy was not observed under peat-vermiculite treatment, but rather a mix of both *K*-selected (*Acidobacteria*) and *r*-selected (*Actinobacteria*, *Mortierellomycota*) microorganisms. Compared to sole chemical

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fertilizer application, soil organic carbon storage efficiency was significantly increased by 26.5% and 50.0% under manure and peat-vermiculite treatments, respectively. Taken together, our results highlight the importance of organic material amendments, but in particular a one-time peat-vermiculite application, to promote soil organic carbon storage as a potential management strategy for sustainable agriculture.

1. Introduction

The accumulation of soil organic carbon in agroecosystems is a global priority for increasing crop productivity, while concurrently compensating for the adverse effects of greenhouse gas emissions (Lal, 2004; Wiesmeier et al., 2019). Recycling organic materials to fields is critical for mitigating land degradation and improving soil fertility (Liu et al., 2020). A global meta-analysis revealed that plant residue incorporation can, on average, increase soil organic carbon (SOC) level by 14.9% (Xia et al., 2018). However, soil organic carbon storage efficiency (CSE) varies depending on soil type, geographical location, and organic material (Yan et al., 2013; Dodor et al., 2018; Wan et al., 2021). Soil CSE has been shown to be positively or negatively affected by organic material amendments (Zhou et al., 2016; Dodor et al., 2018). Despite the fact that organic material amendments can build up SOC due to mineral binding, the subsequent organic carbon decomposition by priming effects can potentially lead to SOC losses and offset the efficiency of SOC sequestration (Luo et al., 2017; Feng and Zhu, 2021). For instance, glucose addition can simultaneously induce both CO_2 release and organic carbon decomposition through microbial biomass turnover (Bastida et al., 2019; Luo et al., 2017). This uncertain fate of newly formed SOC is mainly determined by the activity of soil microbes, which mediate 80–90% of soil carbon cycling (Liang et al., 2017). Therefore, deciphering the microbial mechanisms driving organic material amendments towards SOC storage is crucial for sustainable carbon management in agroecosystems.

Microbial traits involved in SOC storage can be differentiated into two distinct microbial life history strategies: oligotrophy versus copiotrophy (slow-growing vs. fast-growing microorganisms) (Fierer et al., 2007; Fierer et al., 2012; Ho et al., 2017). *K*-selected oligotrophs with long life expectancy and less investment into reproduction are well adapted to a wide range of niches defined by environmental stress (e.g., nutrient limitation, high salinity, or low pH) (Malik et al., 2019; Malik et al., 2020). Contrary to stress-tolerant oligotrophs, *r*-selected copiotrophs thrive in niches with high nutrient availability and low stress exposure, but have low life expectancy (Pianka, 1970; Ramirez et al., 2012). Copiotrophs have been shown to invest more energy than oligotrophs into cellular growth and cell reproduction with high biomass yield (Lipson, 2015). Trade-offs in functional traits and ecological strategies determine the microbial decomposition of SOC, primarily due to the investment into enzyme production including carbohydrate-active enzymes (Wilhelm et al., 2019; Shao et al., 2021). In consequence, *K*-selected microbes are expected to make a major investment into the decomposition of complex organic matter by release of extracellular enzymes (Ho et al., 2017; Malik et al., 2020). By contrast, the activity of *r*-selected microbes results in transiently high microbial biomass, thereby contributing to long-term SOC storage by recalcitrant necromass accumulation (Kallenbach et al., 2016; Liang et al., 2017). Current advances in meta-omics approaches allow us to characterize the functional traits that are linked to distinct microbial life history strategies (Leff et al., 2015; Chen et al., 2020; Malik et al., 2020). A better understanding of the factors governing soil organic carbon decomposition versus SOC storage demands a deeper insight into the diversity, structure, and genetic potential of microbial communities involved in soil carbon cycling.

Soil aggregate formation is known to be an important factor that controls soil carbon sequestration (Six et al., 2006; Garcia-Franco et al., 2015). Microbes can be spatially and functionally interconnected and closely associated with soil aggregates (Dang et al., 2021; Wilpiszeski et al., 2019). Different aggregate size classes harbor diverse microbiota. For instance, macroaggregates (0.25–2 mm), composed of more labile substrates, are abundantly colonized by *Actinobacteria*, *Planctomycetes*, and *Alphaproteobacteria* (Trivedi et al., 2015), while *Acidobacteria* and *Bacteroidetes* were found to be more prominent on microaggregates that are characterized by a high proportion of recalcitrant organic matter (Lin et al., 2019; Trivedi et al., 2017). Therefore, microbial utilization of organic carbon and, in consequence, microbial life history strategies should differ among soil aggregate size classes. However, environmental genomics, or metagenomics, has not yet been widely applied to disentangle the genetic potential associated with particular aggregate size classes under distinct fertilizer treatments.

Recycling organic materials to fields is a traditional organic farming practice in agroecosystem. Organic manure is more widely applied and studied than peat-vermiculite (Zhong et al., 2010; Ding et al., 2017). However, peat-vermiculite amendments can greatly improve soil structure, thereby increasing both SOC and crop yield (Pan et al., 2020; Wu et al., 2021). Therefore, we conducted a field experiment with a single-time application of peat-vermiculite plus continuous chemical fertilizer treatments (PV) versus seasonal treatments with cattle manure plus chemical fertilizer (OM). The study site is located in Northern China Plain with a typical wheat-maize rotation system. The overall goals of this study are to address: (i) What are the direct and indirect effects of organic material amendments on microbiome composition and its genetic potential; and (ii) how strongly is soil CSE affected by biotic versus abiotic factors. Sole application of chemical fertilizer (CF) each season was conducted as a control treatment. The three fertilizer practices strongly differ in SOC quantity and quality. Organic manure is defined by labile compounds that are easily accessible by microbes, while peat is composed of recalcitrant polymers that are difficult to be degraded by microbes (Kirchman et al., 2004). Therefore, we hypothesize the following: (i) The microbiomes enriched by the OM and PV treatments will show distinct life history strategies that are closely linked to CSE. (ii) The CF treatment will show a high abundance of genes encoding carbohydrate-active enzymes (CAZymes) given that the CF microbiome is limited in labile carbon, but not in other nutrients. In order to test our hypothesis, Soil CSE was calculated based on the changes in SOC stock divided by total carbon inputs during the experimental period. Genes involved in the decomposition of complex carbon sources, such as cellulose, hemicellulose and xylan, were identified using a genome-resolved metagenomics approach. Altogether, we determined multiple biotic and abiotic soil parameters, bacterial and fungal diversity, and the metagenomic distribution of genes indicative of distinct life history strategies, including carbon decomposition potential (e.g., CAZymes), across the three fertilizer treatments and their aggregate size classes.

2. Materials and methods

2.1. Study site

The study site is a wheat-maize rotation system located in Quzhou, Hebei Province (115.0°E, 36.9°N). The soil is silty clay and classified as calcareous fluvo-aquic soil (IUSS Working Group WRB, 2015). This area has a monsoonal, continental and sub-humid climate with average annual temperature and precipitation of 13.2 °C and 490 mm, respectively. Initiated in October 2014 (maize season), the field experiment included three different treatments. The chemical fertilizer (CF)

contained 150 kg N ha^{-1}, 45 kg P ha^{-1}, and 45 kg K ha^{-1}. CF was applied as a basic fertilizer in each wheat and maize season as a control treatment. Organic manure plus chemical fertilizer (OM) was applied with the same chemical fertilizer application as CF plus 12 Mg cattle manure ha^{-1} (OM applied before wheat sowing each year). The peat-vermiculite plus chemical fertilizer (PV), with peat-vermiculite being applied with 129 Mg ha^{-1} for a single time in October 2014 and the chemical fertilizer being continuously applied in each wheat and maize season. The chemical properties of the two organic material amendments are shown in Table S1. All three experimental treatments (CF, OM, PV) were managed with the integrated soil-crop system that had been proposed to yield more grain with lower environmental costs (Chen et al., 2011). Winter wheat and summer maize were planted in October and July after the previous crop harvest, respectively. As a basic carbon stock, all the crop straw residues were returned to the field after harvest. Irrigation was applied in wheat season according to crop demand and precipitation. The experimental field was plowed at a depth of 30 cm before wheat sowing each year.

2.2. Soil sampling

Using an aluminum corer, three undisturbed soil cores (5 (height) × 10 cm (diameter)) were taken from the tillage soil after maize harvest from each plot in 2019. The area of each plot was 46 m^2. A total of 27 soil undisturbed soil cores were collected randomly (3 treatments × 3 replicates × 3 cores) and immediately transported on ice to the laboratory. Soil cores were carefully broken apart along the natural break points. Rocks, crop residues, and roots were removed. The three cores taken from the same plot were combined to produce a composite sample for each plot. Aliquots of each sample were stored at 4 °C for analysis of soil chemical properties.

2.3. Soil aggregate fractionation

To minimize disruption of the microbial community during the aggregate size fractionation, we applied the microbially-focused method as described by Bach and Hofmockel (2014). In brief, plant roots and leaves were removed after the soil had been spread onto a sterile vessel and air-dried to a consistent moisture content (~10%). Soil aggregates were separated by placing soil on a series of sieves. The stack was shaken for 4 min at a rate of 30 times per minute. The soil was sieved through 2 mm and 0.25 mm mesh to obtain large macroaggregates (>2 mm, LM), small macroaggregates (0.25–2 mm, SM), and microaggregates (<0.25 mm, M). A total of 27 soil aggregate samples were produced (3 treatments × 3 plots × 3 different aggregate size classes).

2.4. Soil properties characterization

SOC and total N contents were determined using the Vario Max CN elemental analyzer after removal of soil carbonate by 1 mol L^{-1} HCl (Elementar, Langenselbold, Germany). Briefly, 5 g air-dried soil was treated with 50 mL 1 mol L^{-1} HCl for 24 h. Then, the soil was water-washed to remove Cl^{-1} for CN analysis. Soil mineral nitrogen, including NO_3^--N and NH_4^+-N, was extracted by 0.01 mol L^{-1} $CaCl_2$ solution and measured in continuous flow analysis (Pan et al., 2020; Wu et al., 2021). Soil available K and available P were measured according to Hanway and Heidel (1952) and Olsen et al. (1954), respectively. Soil pH was determined using a 1:2.5 ratio of soil to deionized water. Measurements were done with a pH meter (FE20-FiveEasy™ pH, Mettler Toledo). SOC stock was calculated using the following equation: SOC stock (Mg ha^{-1}) = SOC (g kg^{-1}) × BD (Mg m^{-3}) × D (m)/10 (Novara et al., 2015). The BD and D in the equation represented soil bulk density and depth. Crop residue and root carbon inputs were included in the total carbon inputs according to wheat and maize above-ground biomass in the five years of field experiment (2015 to 2019). Specifically, root carbon inputs were calculated as the 30% of total above-ground biomass carbon according to Kundu et al. (2007). SOC storage ratio (%) = (SOC stock treatment − SOC initial)/total organic carbon inputs. SOC stock treatment and SOC initial were the SOC storage before the experiment and SOC storage in the sampling time (maize harvest in October 2019) (Yan et al., 2013).

2.5. DNA extraction and amplicon sequencing

Genomic DNA was extracted from 2 g soil of each of the 27 aggregate-size class samples, using the FastDNA Spin Kit for Soil (MP Biochemicals, LLC, America) according to the manufacturer's instructions. DNA quality and quantity were determined by NanoDrop spectrophotometry (Thermo Fisher Scientific). The V4-V5 region of bacterial 16S rRNA genes and fungal ITS regions were PCR-amplified for sequencing using the primer pairs 515F/909R (Tamaki et al., 2011) and ITS3-2024F/ITS4-2409R (Bellemain et al., 2010), respectively. These primers contained an 8-nucleotide barcode sequence unique to each sample. PCR reaction conditions and amplification steps are listed in Table S2. PCR products were purified using the Qiagen Gel Extraction Kit (Qiagen, Germany) following the manufacturer's instructions. The indexed and purified PCR products were pooled in equal amounts and sequenced using the Illumina MiSeq platform in paired-end mode (2 × 250 bp) (Novogene, Tianjin, China). Paired-end reads were quality filtered and trimmed using Trimmomatic with default parameters (Bolger et al., 2014). The quality-trimmed paired-end reads were merged using FLASH v.1.2.9 (Magoc and Salzberg, 2011) with default settings. These high-quality reads were subjected to chimera detection using UCHIME (Edgar, 2011). Sequence similarities of 97% and 95% were applied to cluster the paired-end reads into operational taxonomy units (OTUs) using the UPARSE series of scripts for bacteria and fungi, respectively (Edgar, 2013). Singletons and chimeric reads were excluded from further OTU analysis. Representative sequences of each OTU of the 16S rRNA gene and ITS data sets were taxonomically assigned employing the RDP classifier (Wang et al., 2007) against the SILVA SSU rRNA database 138 (Quast et al., 2013) and UNITE database (version 7.2) (Nilsson et al., 2019), respectively. OTUs that could not be assigned to the bacterial domain or fungal kingdom were removed from the OTU tables. On average, 60,257 and 80,161 reads per sample were obtained for each bacterial 16S rRNA gene and fungal ITS sequence dataset, respectively. Less than 10-fold difference among the library sizes was observed in our study (Weiss et al., 2017). Thus, the sequence datasets were rarefied to 36,804 and 64,981 reads per sample for the 16S rRNA gene and ITS region, respectively.

2.6. Metagenomic sequencing and CAZyme annotation

The 27 DNA extracts subjected to amplicon sequencing were also used for metagenomic analysis. Sequencing was done using Illumina Hiseq 2000 platform in 2 × 150 bp paired-end mode, resulting in a total dataset of 412.84 Gb. Low-quality reads were filtered by Trimmomatic (Bolger et al., 2014). Then, the high-quality reads were assembled with MEGAHIT (mink = 27, maxk = 141, and step = 20) (Li et al., 2015). CAZyme-encoding genes involved in the decomposition of cellulose, xylan, pectin, hemicellulose and chitin were annotated as described previously (Peng et al., 2018). Briefly, genes on metagenome contigs were queried against the CAZy database (2019) maintained by the dbCAN consortium (Zhang et al., 2018) using diamond software (*E*-value threshold of 10^{-5}). FragGeneScan 1.30 (Rho et al., 2010) was used to predict genes on all contigs and convert their nucleotide sequences into amino acid sequences. Individual CAZyme modules (e.g., cellulose, hemicellulose, etc.) were defined by grouping related enzymatic functions based on their enzyme commission numbers. A mapping file linking dbCAN sequences to defined CAZyme modules was generated using a custom python script (Peng et al., 2018). The resulting annotations are based on matching dbCAN top hits against the mapping file (Yin et al., 2012). All datasets were normalized.

2.7. Reconstruction of metagenome-assembled genomes (MAGs)

Assembled contigs with a length >1000 bp were further binned using metaBAT2 with default settings (Kang et al., 2015). Completeness and contamination of the binned genomes were checked by CheckM (v1.1.2; Parks et al., 2015). MAGs estimated to have >70% completeness and <10% contamination were selected for taxonomic assignment and functional annotation. Taxonomic classification and phylogenetic treeing of assembled MAGs were performed using GTDB-Tk (v1.3.0, Chaumeil et al., 2019). High-quality MAGs were subjected to gene prediction using Prokka (v1.14.6). Predicted genes were blasted against NCBI non-redundant protein database and Kyoto Encyclopedia of Genes and Genomes (KEGG) database with Diamond using an E-value threshold of 10^{-5} (Kanehisa and Goto, 2000).

2.8. Statistical analysis

All statistical analyses were performed using R (version 3.6.1). Samples from fertilizer treatments (CF, OM and PV) and, respectively, their aggregate sizes (LM, SM and M) were pooled for Duncan multiple comparisons test. *P* values were False Discovery Rate (FDR) corrected by multiple testing using the Benjamini-Hochberg method (Ferreira, 2007). To assess the impact of the different fertilizer treatments on microbial functional traits and life history strategies, genetic traits of the soil microbiomes were classified into the categories "resource acquisition" versus "cellular growth" based on literature (Chen et al., 2020; Malik et al., 2020; Shao et al., 2021). Briefly, genes involved in polymer hydrolysis (e.g., encoding extracellular enzymes) and organic carbon decomposition, but also those involved in defense and antagonism, were assigned to "resource acquisition", while genes involved in biosynthesis of cellular components (e.g., amino acids, fatty acids, and nucleic acids) were assigned to "cellular growth" (Malik et al., 2020). Alpha diversity, represented by Shannon index, was visualized using *ggplot2* package (Gómez-Rubio, 2017). Unconstrained principal coordinates analysis (PCoA) based on a Bray-Curtis distance matrix was performed to quantify the major variance components of beta diversity. Permutational multivariate analysis of variance (PERMANOVA) was further applied to quantify the effects of fertilization treatments and soil aggregate sizes on the microbial community based on Bray-Curtis distances using the *vegan* package (Oksanen et al., 2007). Correlations between microbial community/functional traits and soil physico-chemical parameters were determined by Spearman's correlations analysis. The abundance of dominant phyla was *Z*-scored transformed and visualized in the format of a heatmap. Random forest analysis was applied to assess the impact of particular soil properties on microbial diversity using the R package *randomForest*. The impact of each predictor is indicated by the mean squared error (MSE). Regression analysis was used to test the significance of each predictor.

2.9. Data deposition

A total of 54 amplicon sequence datasets (27 for bacterial 16S rRNA genes and 27 for fungal ITS regions) and, in addition, 27 metagenomic sequence datasets were deposited with NCBI Sequence Read Archive (SRA) under the accession numbers PRJNA610098 and PRJNA640885.

3. Results

3.1. Soil properties and SOC storage

SOC content was significantly increased in the OM (20.16 g kg^{-1}) and PV treatments (22.41 g kg^{-1}) relative to the CF treatment (12.51 g kg^{-1}) ($P_{FDR} < 0.05$) (Fig. S1). Total N content concomitantly increased 1.5-fold (OM) and 1.4-fold (PV) relative to the CF treatment, but nitrogen was also non-limiting for the microbial community associated with the CF treatment (Fig. S1). No significant difference in SOC and total N contents was observed between the different soil aggregate size classes of each treatment (Fig. S1). The OM treatment showed the greatest available P and available K contents. These were significantly different from those of the CF and PV treatments ($P_{FDR} < 0.001$). Soil NO_3^--N significantly increased in the OM and PV treatments relative to the CF treatment ($P_{FDR} < 0.05$), while all three treatments had no differential effect on the soil NH_4^+-N content and pH. Compared to CF treatment, the OM and PV treatments significantly increased soil CSE by 26.5% and 50.0%, respectively ($P_{FDR} < 0.05$; Table 1).

3.2. Diversity and composition of the soil microbial community

Bacterial α-diversity, as expressed by Shannon diversity index, was the greatest in the manure treatment (OM). Fungal α-diversity showed a significantly positive correlation with SOC content ($P_{FDR} < 0.01$) (Fig. 1). However, soil aggregate size had no significant effect on bacterial and fungal α-diversity. The fertilization treatments had a much greater effect on the bacterial and fungal community composition (27.0% vs. 56.7%; $P_{FDR} < 0.001$) than aggregate size (8.9% vs. 9.4%; $P_{FDR} < 0.01$) (PERMANOVA, Fig. S2). Bacterial and fungal communities formed distinct, treatment-specific clusters (PCoA of β-diversity, Fig. 1e, f). Soil C:N, available K, available P, and SOC were the dominant predictors for CSE (random forest analysis, Fig. S3). Correlation analysis revealed that available K and available P were positively linked to microbial diversity, while the C:P and N:P ratios were negatively correlated with microbial diversity ($P_{FDR} < 0.05$; Fig. S4).

Organic material amendments had significant effects on microbial community composition (Figs. 2 & S5). The phylum *Gemmatimonadetes* was highly abundant in CF treatment and negatively correlated with SOC content ($P_{FDR} < 0.01$). *Actinobacteria* were particularly enriched in samples of the PV treatment, while *Cytophagales* (*Bacteroidetes*) and *Bacillales* (*Firmicutes*) showed a greater relative abundance in the OM treatment than in the other two treatments ($P_{FDR} < 0.05$; Figs. 2; S5). The *Mortierellomycota*, with the fungal order *Mortierellales* being highly abundant (Fig. S5), was identified to be the most important predictor for soil CSE, followed by *Gemmatimonadetes*, *Actinobacteria*, and *Rokubacteria* (Fig. 2). The relative abundances of both *Mortierellomycota* and *Actinobacteria* were positively and significantly correlated with soil CSE, while *Gemmatimonadetes* and *Rokubacteria* were negatively and significantly correlated with soil CSE ($P_{FDR} < 0.05$; Fig. 2).

3.3. Treatment-specific effects on genetic life history potential

On KEGG level 3, genes involved in resource acquisition (e.g., *starch and sucrose metabolism*, *secretion system*, *sulfur metabolism*, *fructose and*

Table 1
SOC storage efficiency.

Treatments	SOC stock in 2015	SOC stock in 2019	Changes in in SOC stock	Total organic carbon input (Mg ha^{-1})		SOC storage efficiency from total organic carbon input
	(Mg ha^{-1})	(Mg ha^{-1})	(Mg ha^{-1})	Straw	Amendments	(%)
CF	34.29	48.07	13.78	40.55	0.00	0.34 a[1]
OM	46.57	78.31	31.74	49.44	24.48	0.43 b
PV	34.29	79.51	45.22	47.51	40.50	0.51 b

[1] Different letters (a, b) indicate significant differences in SOC storage efficiency between the fertilizer treatments ($P_{FDR} < 0.05$).

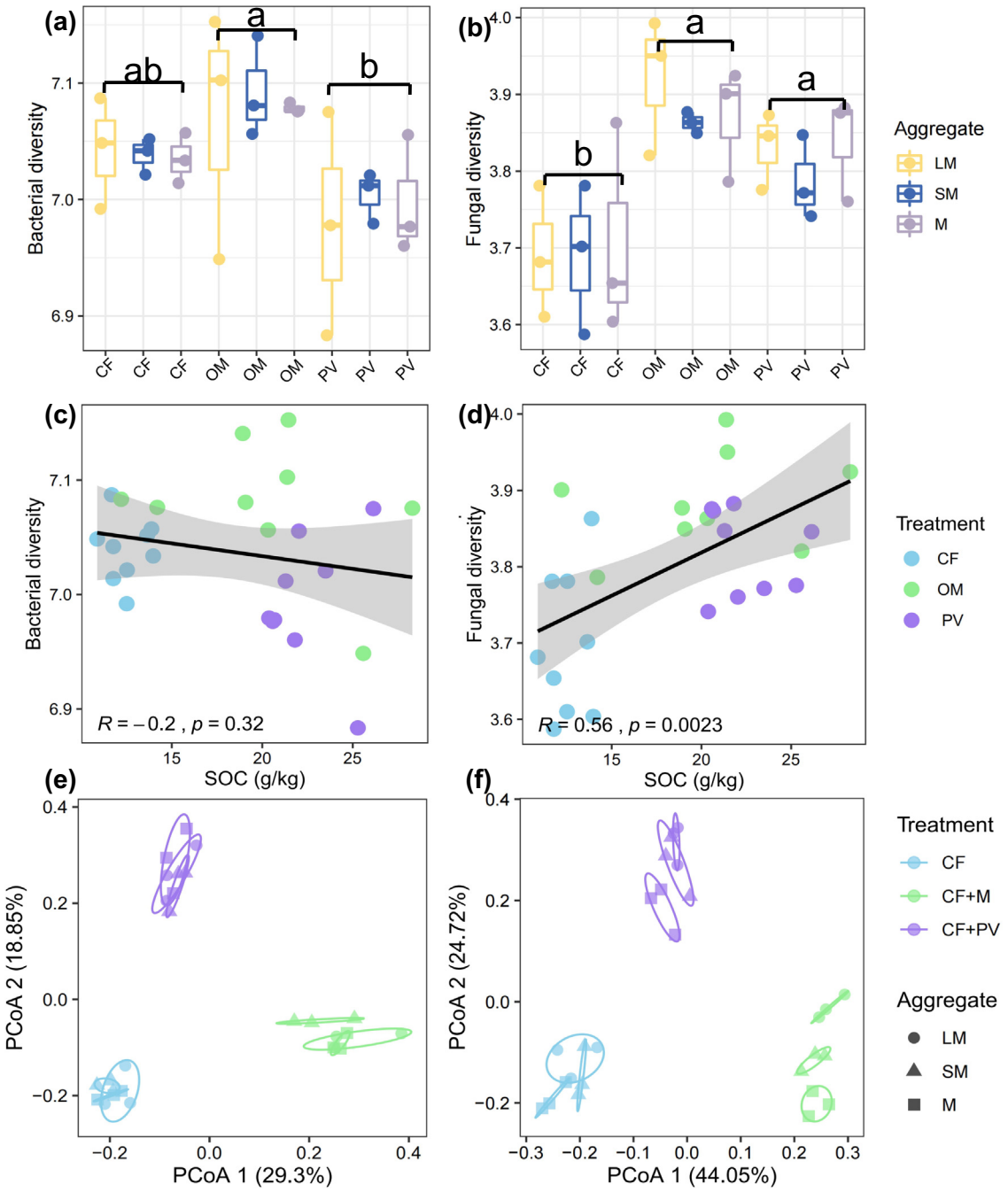

Fig. 1. Boxplots showing the bacterial and fungal alpha (Shannon) diversity across the aggregate size classes of each fertilizer treatment (a, b); correlation of bacterial and fungal alpha diversity with soil SOC content (c, d); and Principal Component Analysis (PCoA) of bacterial and fungal communities based on Bray-Curtis dissimilarities (e, f). The analyses are based on amplicon sequencing of bacterial 16S rRNA genes and fungal ITS region. CF: chemical fertilizer; OM: organic manure plus chemical fertilizer; PV: peat-vermiculite plus chemical fertilizer. LM: large macroaggregates; SM: small macroaggregates; M: microaggregates. Different letters (a, b, c) indicate significant differences between fertilizer treatments ($P_{FDR} < 0.05$).

mannose metabolism, and *bacterial secretion system*) and competition (e.g., *antimicrobial resistance genes* and *enediyne antibiotics*) were significantly enriched in the microbiome associated with the CF treatment ($P_{FDR} < 0.05$). By contrast, genes involved in cellular growth, such as *ribosome biogenesis*, *transfer RNA biogenesis*, *transcription factors*, *arginine* and *lysine biosynthesis*, and *lipid metabolism* were, relative to the CF treatment, significantly enriched in the microbiome associated with the OM treatment ($P_{FDR} < 0.05$). In the microbiome associated with the PV treatment, the abundance of genes affiliated with either resource acquisition or cellular growth did not significantly differ to those in both CF and OM treatments (Fig. 3).

3.4. CAZyme genes involved in C decomposition

Compared to CF treatment, the microbiomes associated with the OM and PV treatments showed a lower abundance of polymeric carbon decomposition-related genes (Fig. 4). In particular, the abundance of CAZyme genes involved in cellulose, hemicellulose, and pectin utilization was significantly lower in the microbial communities associated with the OM treatment ($P_{FDR} < 0.05$). A similar but statistically insignificant trend was observed for the microbiome associated with the PV treatment ($P_{FDR} > 0.05$). The microbial communities residing on large macroaggregates (>2 mm) and macroaggregates

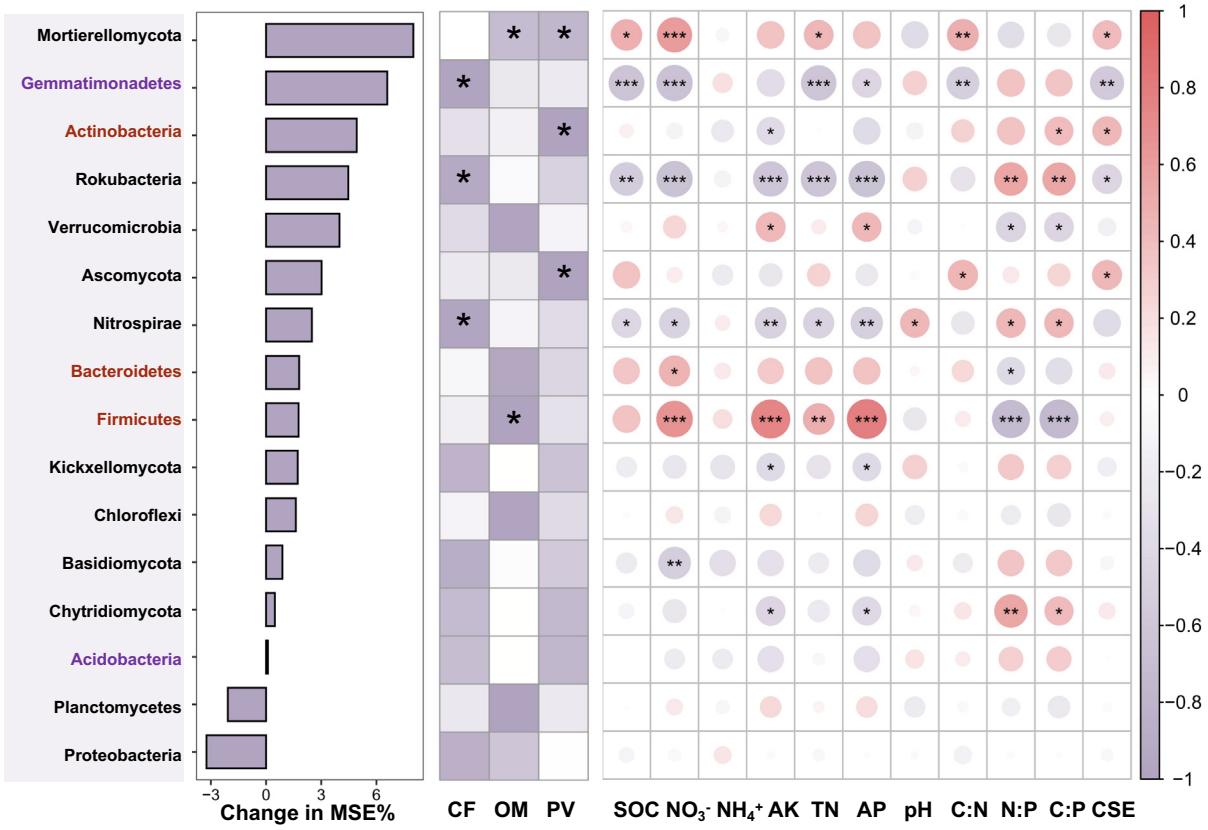

Fig. 2. Impact of dominant bacterial phyla and fungal kingdoms on SOC storage as predicted for the fertilizer treatments by random forest analysis; and abundance correlation between particular microbial taxa and soil chemical properties. The phyla *Bacteroidetes* and *Firmicutes* refer to the orders *Cytophagales* and *Bacillales*. The analyses are based on PCR sequencing of bacterial 16S rRNA genes and fungal ITS regind classified as calcareouson. MSE% indicates the importance of each taxonomic group (=predictor) for soil CSE. The abundance of each taxon was z-scored transformed and is shown in the format of a heatmap, involving the combined analysis of all three aggregate size classes for each treatment (CF, OM, and PV). The key shows the z-scores of the relative abundances. Each asterisk indicates that the given taxon was significantly enriched in a particular fertilizer treatment relative to the other treatment(s) ($P_{FDR} < 0.05$). Asterisks, but also dot size and color (see scale), indicate a significant association between microbial taxa and particular soil chemical properties: "*" $P_{FDR} < 0.05$, "**" $P_{FDR} < 0.01$, and "***" $P_{FDR} < 0.001$.

(0.25–2 mm) harbored, relative to those associated with microaggregates (<0.25 mm), a significantly greater number of CAZyme genes involved in cellulose and xylan utilization ($P_{FDR} < 0.05$). The microbial populations associated with macroaggregates (0.25–2 mm) showed a significantly greater number of CAZyme genes involved in chitin and hemicellulose utilization than those associated with large macroaggregates (>2 mm) and microaggregates (<0.25 mm) ($P_{FDR} < 0.05$) (Fig. S6).

3.5. MAGs driving enhanced soil CSE

Twenty high-quality MAGs (>70% completeness and <10% contamination) were reconstructed and classified as belonging to the *Acidobacteria* (3), *Actinobacteria* (5), *Chloroflexi* (2), *Gemmatimonadetes* (1), *Krumholzibacteriota* (1), and *Proteobacteria* (8) (Fig. 5, Table S4). Four MAGs assigned to *Acidobacteria* (bin_OL2.19, bin_OL2.50 and bin_OM3.11) and *Gemmatimonadetes* (bin_OL2.37) were highly abundant in the CF treatment across all soil aggregate size classes, while five MAGs classified as *Actinobacteria* (bin_GS2.13, bin_GS2.5, bin_OS3.17, bin_GS3.19 and bin_OL2.27) were more abundant in the OM and PV treatments. The *Gemmatimonadetes* MAG (bin_OL2.37) was detected with relatively high metagenomic abundance of up to 0.36% on large macroaggregates (>2 mm) of the CF treatment. By contrast, two MAGs assigned to *Actinobacteria* (bin_GS3.19) and *Chloroflexi* (bin_GS2.21) showed a high metagenomic abundance of up to 0.34% and 0.24% on microaggregates (<0.25 mm) of the OM and PV treatments, respectively (Fig. S7). CAZyme genes were detected with high but varying genomic abundance across all 20 MAGs. In fact, CAZyme genes involved in the hydrolysis of cellulose, cellobiose, xylan, pectin, starch/glycogen, peptidoglycan, and lignin ranged from 364 to 953 genes per MAG. The relative abundance of five high-quality MAGs affiliated to the *Krumholzibacteria*, *Steroidobacterales* (*Gammaproteobacteria*), *Chloroflexi*, *Solirubrobacterales*, and *Jiangellales* were positively associated with soil CSE, while two MAGs (*Thermoanaerobaculia* and *Gemmatimonadetes*) were negatively correlated with soil CSE (Figs. 5 and S7). The orders *Solirubrobacterales* and *Jiangellales* belong to the *Actinobacteria*, while the *Thermoanaerobaculia* represent a class in the *Acidobacteria* (Dedysh and Lawson, 2020).

4. Discussion

Previous metagenomic research confirmed trade-offs between different microbial life history strategies, but in particular between the cellular growth and resource acquisition strategies (Chen et al., 2020; Malik et al., 2020). These trade-offs depend on microbial resource availability and microbial community composition (Ramirez et al., 2010; Melillo et al., 2017). In our study, both PCR-sequencing and functional metagenomics provided evidence that the three fertilizer regimes (CF, OM, PV) promoted distinct life history trade-offs affecting SOC storage, due to the major differences in microbial resource availability. Moreover, PERMANOVA showed that fertilizer type, rather than differing soil aggregate size classes, determined microbial community composition and life history strategy. In our further discussion, we therefore mainly focus on the fertilization effects.

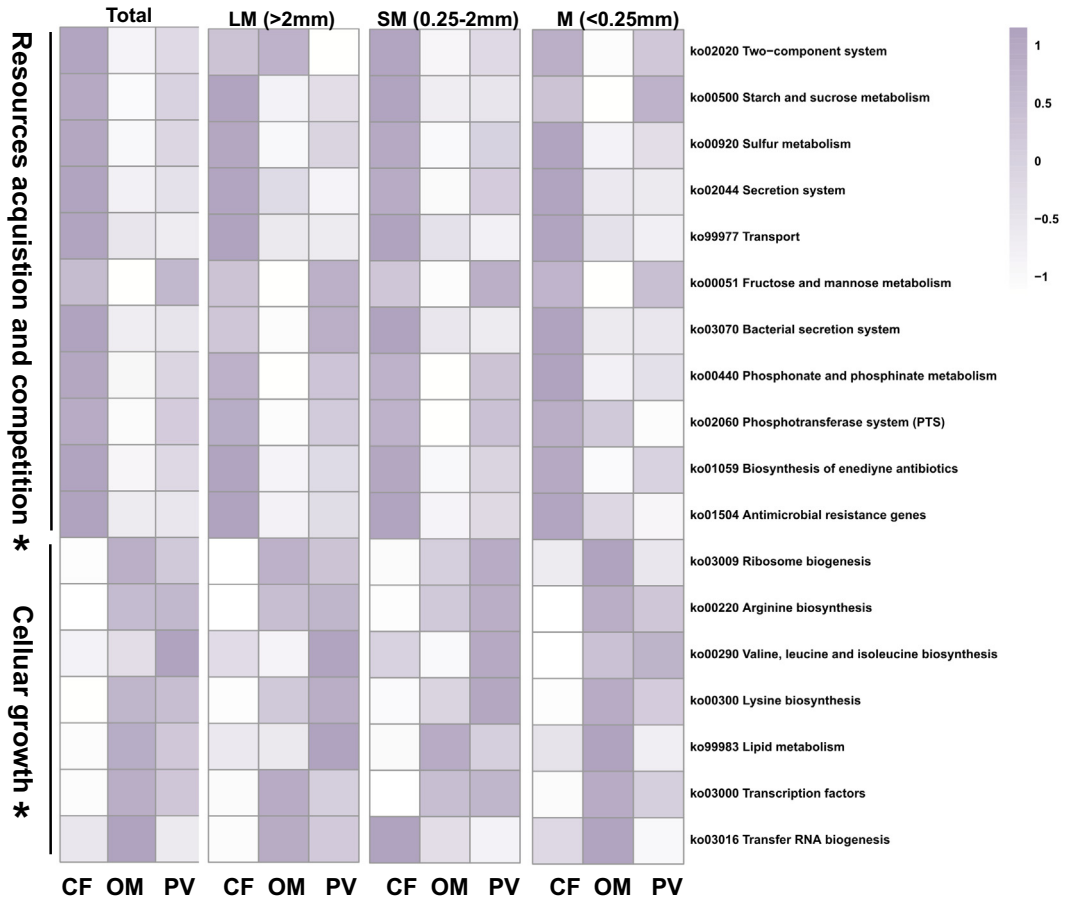

Fig. 3. The genetic life history potential of the microbiomes associated with particular fertilizer treatments and aggregate size classes. The gene abundance of each KEGG level 3 category was z-scored transformed and is visualized in the format of a heatmap. The functional KEGG categories were grouped according to Malik et al. (2020), being indicative of two distinct life history strategies: "resource acquisition and competition" (e.g., transport, nutrient metabolism) and "cellular growth" (biosynthesis). The asterisks aside the life history strategies indicate that these genetic traits were significantly enriched in either CF treatment ("resource acquisition and competition") or OM treatment ("cellular growth") ($P_{FDR} < 0.05$). The abundance of genes affiliated with either "resource acquisition" or "cellular growth" did not significantly differ under PV treatment to those in the CF and OM treatments. The key shows the z-scores of the relative abundances. CF: chemical fertilizer; OM: organic manure plus chemical fertilizer; PV: peat-vermiculite plus chemical fertilizer.

4.1. The impact of the fertilizer regimes on microbial life history strategies

The CF treatment was poor in labile carbon, but received a seasonal input of plant residues. Previous research has shown that glycosyl hydrolase-encoding genes are abundant and diverse among microbial communities that inhabit soils with limited nutrient supply of labile carbon (Zhalnina et al., 2018; Malik et al., 2020). We therefore assumed that the CF microbiome strongly depends on the production of extracellular enzymes to hydrolyze plant polymers. In fact, the CF metagenome showed a significantly greater abundance of glycosyl hydrolase genes involved in decomposing cellulose, hemicellulose and pectin than the OM and PV metagenomes (Fig. 4). We concluded that the microbiome associated with CF treatment had a *K*-selected life history strategy, i.e., a resource-acquisition strategy defined by slow growth rates, efficient nutrient uptake systems and high substrate affinity (Shao et al., 2021). Indeed, the abundance of metabolic pathway genes involved in nutrient acquisition (e.g., ABC transporters, secretion system, sulfur metabolism, fructose and mannose metabolism) was greatest in the CF metagenome and differed significantly from those in the OM and PV metagenomes. With high production of extracellular enzymes for nutrient acquisition, microbial investment into cell growth decreases. The resource-acquisition life history strategy is frequently linked to an increased competition between community members for nutrient resources (Malik et al., 2020). Thus, the particular enrichment of genes involved in antimicrobial resistance and biosynthesis of enediyne antibiotics in the CF metagenome is another line of evidence for this microbial life history strategy under nutrient-limited conditions (Fig. 3). Collectively, these findings may explain why the CF treatment was previously observed to harbor the lowest microbial biomass carbon and nitrogen (Pan et al., 2020).

Contrary to the CF soil, the OM soil was rich in labile carbon and other nutrients that were applied in each season, in addition to plant residues. We therefore assumed that the OM microbiome had an *r*-selected life history strategy, i.e., a strategy defined by cellular reproduction and high growth yield (Malik et al., 2020). Indeed, the abundance of genes involved in cellular reproduction was greatest in the OM metagenome and differed significantly from those in the CF and PV metagenomes (Fig. 3). This indicates that high growth yield under optimal resource conditions is a major functional trait of the soil microbes associated with the OM treatment (Malik et al., 2020). Concurrently, the OM metagenome contained a significantly lower abundance of glycosyl hydrolase genes involved in polysaccharide decomposition than the CF and PV metagenomes (Fig. 4), suggesting that compared to the CF and PV microbiomes, the ability to produce extracellular enzymes is ecologically less important for the OM microbiome (Wei et al., 2020). Thus, our results collectively corroborate that under nutrient-rich conditions, soil microbiomes invest in cell reproduction rather than resource acquisition. A characteristic of such nutrient-rich conditions is reduced environmental pressure to compete for resources, which agrees well with the fact that greatest bacterial and fungal alpha diversity was observed in the OM treatment (Fig. 1).

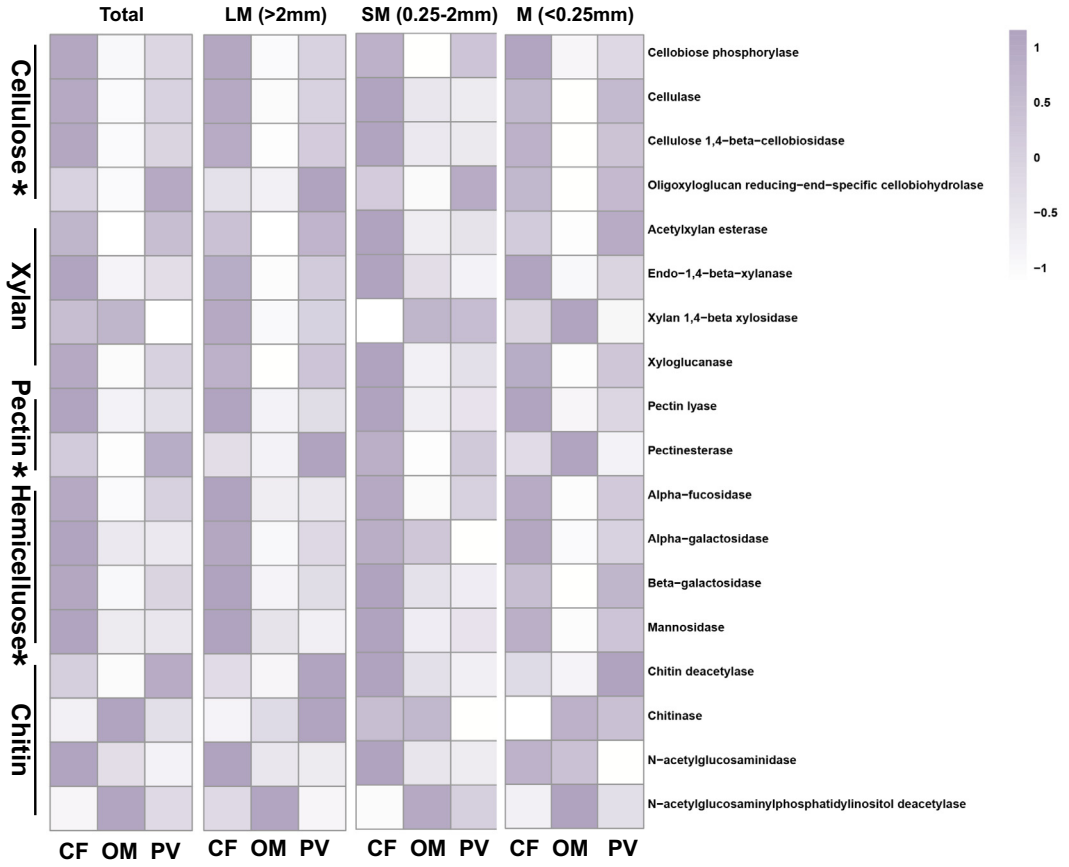

Fig. 4. Heatmap showing the normalized abundances of CAZyme genes in the metagenomes obtained for the aggregate size classes (LM, SM and M) of each fertilizer treatment (CF, OM, and PV). CAZyme modules are classified according to target carbohydrate: cellulose, xylan, pectin, hemicellulose, and chitin. The asterisks aside CAZyme modules indicate that CAZyme genes affiliated with particular modules (cellulose, pectin, hemicellulose) were significantly enriched in the CF treatment relative to the OM treatment ($P_{FDR} < 0.05$). The abundance of genes affiliated with particular CAZyme modules (cellulose, pectin, hemicellulose) did not significantly differ under PV treatment to those in the CF and OM treatments. CF: chemical fertilizer; OM: organic manure plus chemical fertilizer; PV: peat-vermiculite plus chemical fertilizer.

The PV soil received a single treatment with peat-vermiculite in October 2014, in addition to the seasonal input of plant residues. In contrast to other treatments, the PV soil showed the highest soil fertility and crop productivity (Wu et al., 2021). Despite PV amendment resulting in the highest SOC level, microbial communities thrived under labile carbon deficiency as peat is well known to be recalcitrant against microbial decomposition (Kirchman et al., 2004). Indeed, CF and PV treatments did not significantly differ in the accumulation of microbial carbon and nitrogen (Pan et al., 2020), indicating that peat is too recalcitrant to be broken down (Kirchman et al., 2004). The particular physico-chemical properties of peat-vermiculite, however, lead to a decrease in bulk density but increase in soil aeration (Huat et al., 2011). The further consequence is an increased carbon accumulation across all soil aggregate size classes; in particular compared to CF treatment (Fig. S1). Among the three fertilizer regimes, the PV treatment presumably offered the most diverse microhabitats for colonization, including *K*-selected oligotrophs (e.g., *Acidobacteria*). However, the decrease in bulk density but increase in aeration altered, relative to the CF treatment, the community composition towards enrichment of *r*-selected copiotrophs (i.e., *Actinobacteria*, *Mortierellomycota*), thereby leading to a more efficient decomposition of complex plant residues. Indeed, members of *Actinobacteria* have a high genetic potential to decompose recalcitrant polymeric compounds (Fierer et al., 2012; Lewin et al., 2016), which explains well the significant association of this phylum with soil CSE under PV treatment (Fig. 2). The positive effects of the decrease in bulk density but increase in aeration even may have been greater for fungi, but in particular for members of the order *Mortieralles*. These saprophytic fungi are common inhabitants of forest and agricultural soils and grow well in environments rich in nutrients such as simple sugars, but are also able to efficiently degrade hemicelluloses and chitin to get sugars for their growth. In particular, *Mortierellomycota* have been shown to be involved in the early stages of organic residue decomposition (Koechli et al., 2019; Clocchiatti et al., 2020). Compared to CF treatment, the *Mortieralleles* fungi were significantly increased in relative abundance under both OM and PV treatments (Fig. S5), but their functional role may have differed between the two treatments. While the *Mortieralleles* fungi primarily utilized labile carbon under OM treatment, they may have been majorly involved in the hydrolysis of complex carbon under PV treatment. Indeed, fungi are well known to act as biological binding agents that can drive the process of soil aggregate formation and stabilization (Daynes et al., 2012; Duchicela et al., 2013). Enriched by either the combination of labile and complex carbon (OM treatment) or the particular physico-chemical properties of peat-vermiculite, the biological traits of saprophytic *Mortierellomycota* explain well why this fungal group was both strongest predictor for and positively associated with soil CSE under OM and PV treatments ($P_{FDR} < 0.05$) (Fig. 2).

4.2. Linking microbial life history strategies to SOC storage

Microbial life history strategies are known to be highly correlated with SOC turnover (Docherty and Gutknecht, 2019; Trivedi et al., 2017). Our taxonomic analysis corroborates a *K*-selected life style of the CF microbiome. *Gemmatimonadetes* a phylum defined by slow-growing bacteria that thrive in various soils (Docherty and Gutknecht, 2019; Zhao et al., 2021), were significantly enriched in the CF treatment in comparison to the OM and PV treatments, and their relative abundance was negatively correlated with both SOC and CSE (Malik et al.,

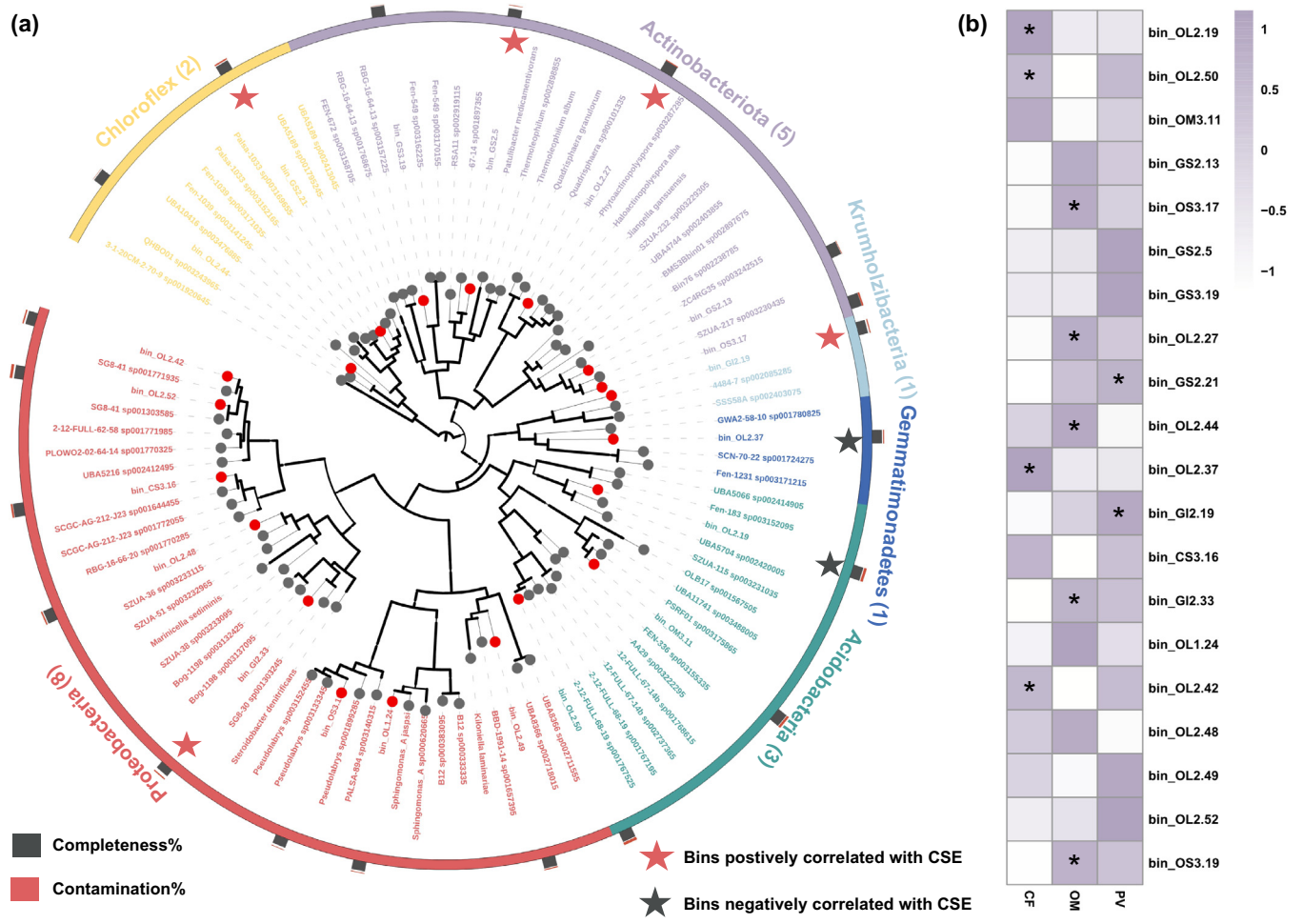

Fig. 5. Maximum-likelihood tree of 20 high-quality MAGs harboring CAZyme genes (a) and their relative abundances across the fertilizer treatments (b). The tree was constructed by using the Fast tree software with the 'WAG 1000's parameters and is based on a concatenated alignment of 120 marker genes from GTDB-Tk. The MAGs obtained in this study are marked by red dots. Further details about the MAGs are given in Fig. S7 and Table S4. Colors on leaf labels indicate the microbial phylum. The completeness of each MAG is displayed by black-red colored bars. The asterisks indicate that the high-quality MAGs were either positively (red) or negatively (black) correlated with soil CSE ($P_{FDR} < 0.05$). The relative abundance of each MAG across the fertilizer treatments was z-scored transformed and is displayed in the format of a heatmap (CF, OM, and PV). The asterisks indicate that the given MAGs were significantly enriched in a particular fertilizer treatment relative to the other two treatments ($P_{FDR} < 0.05$).

2020; Zhao et al., 2021). These slow-growing or oligotrophic bacteria play an important role in SOC decomposition, because they primarily invest in the production of extracellular enzymes for the degradation of complex carbon during periods of resource scarcity. *Cytophagales* (*Bacteroidetes*) and *Bacillales* (*Firmicutes*) were particularly enriched in the OM treatment and displayed a positive but non-significant association with SOC and CSE. These copiotrophic bacteria are typical soil inhabitants and respond fast to nutrient-rich conditions, but are also able to utilize proteins and lipids (microbial components) and to participate in degrading polymers such as cellulose. This further corroborates an *r*-selected life style of the OM microbiome. However, as discussed above, *Actinobacteria* and *Mortierellomycota* were the taxonomic groups most characteristic for the PV treatment, with regard to both their high abundance and their significantly positive correlation with SOC and CSE.

The contributions of distinct microbial life history strategies (oligotrophs vs copiotrophs) to SOC storage/decomposition were further evidenced by reconstructed MAGs. Members of the *Acidobacteria* are known to be specialists in degrading recalcitrant carbon (Trivedi et al., 2016), while *Gemmatimonadetes* are aerobic heterotrophs capable of utilizing multiple substrates (e.g., polypeptone, acetate) (Zhang et al., 2003; Baker et al., 2015). MAGs affiliated with the *Thermoanaerobaculia* (*Acidobacteria*) (bin_OL2.19) and *Gemmatimonadetes* (bin_OL2.37) in the CF treatment showed a high genetic potential for the production of carbohydrate-active enzymes, which are indicative of oligotrophic populations that in our study, mainly contributed to SOC decomposition in the CF treatment. High abundance of *Actinobacteria*-affiliated MAGs was observed in the OM and PV treatments, in good agreement with the positive correlation of the *Actinobacteria* with SOC and CSE. The strong correlation with SOC and soil CSE was observed in particular for MAGs affiliated with two specific subgroups of the *Actinobacteria*, namely *Solirubrobacterales* and *Jiangellales* (bin_GS2.5 and bin_OL2.27). In previous research, *Actinobacteria* with copiotrophic life history strategy were characterized by less extracellular enzyme production but high biomass yield, thus contributing to SOC storage by necromasss accumulation (Liang et al., 2017). Moreover, the assimilation of monomeric substrates into microbial residues would increase SOC stabilization (Malik et al., 2020). Strong accumulation of microbial necromass may thus best explain the increase in soil CSE under OM treatment, while the PV properties led to a decrease in bulk density but increase in soil aeration and carbon accumulation across all aggregate size classes (Fig. S1), thereby resulting in a more efficient decomposition of complex carbon by *Actinobacteria*, and in particular by fungi (*Mortierellomycota*), than under CF treatment. Notably, *Acidobacteria* were generally classified as oligotrophs, while *Bacteroidetes*, *Actinobacteria*, *Alphaproteobacteria*, and *Betaproteobacteria* were defined as copiotrophic organisms in previous research (Fierer et al., 2007; Fierer et al., 2012; Trivedi et al., 2017). This does not imply that every member of these phyla can be distinctly classified

into copiotrophic or oligotrophic microorganisms (Fierer et al., 2007). However, both relative abundance and genetic potential of these taxa respond in a predictable manner to organic materials amendments, and most of their members can be broadly affiliated with a copiotrophic or oligotrophic life style. Taken together, we identified mechanisms underlying the increase in soil CSE as a result of particular organic material amendments. These led to trade-offs in microbial functional traits defined by two distinct microbial life history strategies that in our study, shaped SOC decomposition potential and storage efficiency.

5. Conclusions

Our results showed that sole CF application can stimulate microbial decomposition of SOC, while OM amendment increased microbial diversity but decreased potential for decomposition of polymeric carbon. These phenomena are closely associated with shifts in microbial life history strategies and, in consequence, functional genetic potential. Slow-growing oligotrophs, such as *Gemmatimonadetes* and *Acidobacteria*, are favored by CF amendment, while fast-growing copiotrophs, such as *Cytophagales* and *Bacillales*, proliferate more in response to OM treatment. However, we identified an abundant mix of both oligotrophs (e.g., *Acidobacteria*) and copiotrophs (e.g., *Actinobacteria*, *Mortierellomycota*) under PV treatment. The particular trade-offs in genetic traits between "resource acquisition" and "cellular growth" are also documented by the fact that under PV treatment, the abundance of genes indicative of either life history strategies showed no significant difference to their abundance in either CF or OM treatment, thereby being indicative of a "mixed life history strategy". In consequence, strong accumulation of microbial necromass may be the major explanation for the increase in soil CSE under OM treatment, while the response of the microbial community to the physico-chemical properties of peat-vermiculite is the major explanation for the increase in soil CSE under PV treatment. Taken together, our study illustrates how metabolic trade-offs between life history traits affect soil carbon dynamics, with major implications for sustainable organic carbon storage management.

CRediT authorship contribution statement

Xingjie Wu: Investigation, Methodology, Software, Data curation, Formal analysis, Writing – original draft. **Pengfei Liu:** Formal analysis, Methodology, Writing – review & editing. **Carl-Eric Wegner:** Data curation, Methodology, Formal analysis. **Yu Luo:** Writing – review & editing. **Ke-Qing Xiao:** Methodology, Writing – review & editing. **Zhenling Cui:** Conceptualization, Writing – review & editing. **Fusuo Zhang:** Conceptualization, Writing – review & editing. **Werner Liesack:** Methodology, Writing – review & editing. **Jingjing Peng:** Conceptualization, Methodology, Software, Data curation, Formal analysis, Writing – review & editing, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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