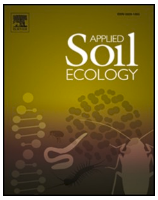
Applied Soil Ecology 189 (2023) 104945

Contents lists available at ScienceDirect

Applied Soil Ecology

journal homepage: www.elsevier.com/locate/apsoil



Dose effect of pig manure addition on *cbbL*-harboring bacterial community in a paddy soil

Jiangbing Xu [a], Jingyi Luo [a], Yaqian Chen [a], Zhen Yu [a], Guoyi Zhou [a], Jianbo Fan [b,c], Wenjing Qin [d], Jia Liu [d], Xiaoli Liu [b,c], Ling Chen [b,c], Meng Wu [b,c], Xiaoyan Ma [e,*], Ming Liu [b,c,**]

[a] *Institute of Ecology, Jiangsu Key Laboratory of Agricultural Meteorology, School of Applied Meteorology, Nanjing University of Information Science and Technology, Nanjing 210044, China*
[b] *State Key Laboratory of Soil and Sustainable Agriculture, Institute of Soil Science, Chinese Academy of Sciences, Nanjing 210008, China*
[c] *University of Chinese Academy of Sciences, Beijing 100049, China*
[d] *National Engineering and Technology Research Center for Red Soil Improvement, Soil and Fertilizer & Resources and Environment Institute, Jiangxi Academy of Agricultural Sciences, Nanchang 330200, China*
[e] *Key Laboratory of Molecular Medicine and Biotherapy, School of Life Science, Beijing Institute of Technology, Beijing 100081, China*

ARTICLE INFO

Keywords:
Autotrophic microorganisms
Organic fertilizer
Alpha diversity
Community structure
Metal

ABSTRACT

CO_2 fixation by autotrophic microbes has a significant effect on C cycle in agricultural filed. Organic fertilization is an eco-friendly strategy for environmental protection and agricultural sustainability via ameliorating soil microbial community. However, the information of the dose effect of organic fertilizer on soil CO_2 fixing microbes has been less documented. In this study, the *cbbL* gene, a key gene in the Calvin-Benson-Bassham cycle, was used to characterize the CO_2-fixing microbes under the gradient rates of pig manure (PM) addition (0, 1400, 2800, 5600, 11,200, 22,400, and 44,800 kg ha^{-1}) in a 5-year field experiment with a double-rice cropping system in the red soil region of China. The results showed that Proteobacteria and Actinobacteria accounted for ~99.4 % of total *cbbL*-harboring bacteria in average. High PM doses (22,400 and 44,800 kg ha^{-1}), rather than Low PM doses (11,200 kg ha^{-1} and less), would significantly change the *cbbL*-harboring bacterial community attributes. Specifically, high PM doses decreased the alpha-diversity of *cbbL*-harboring bacterial community and the relative abundances of some lineages belonging to Proteobacteria and Actinobacteria, such as *Bradyrhizobium Methylibium, and Variovorax*, but increased the proportions of some stress-tolerant taxa (i.e. *Hydrogenophaga* and *Methyloferula*). Redundancy analysis (RDA) and partial least squares path modeling (PLS-PM) showed that pH, AP, and metals (Cu and Zn) negatively influenced the *cbbL*-harboring bacterial diversity and components through PM addition. Collectively, our study demonstrated that high PM addition would potentially threaten the soil *cbbL*-harboring microbes, and a rational application of manure-derived organic fertilizer should be addressed in agriculture for maintaining soil bio-diversity in future.

1. Introduction

Autotrophic microorganisms are ubiquitously distributed in environment. They play an essential role in global C cycle by assimilating CO_2 into organic carbon, a vital pathway regulating the atmospheric CO_2 concentration (Šantrůčková et al., 2018; Stein et al., 2005). Recently, it has been reported that autotrophic microbes are found in the upland soil of wetland soil, grassland soil, forest soil, paddy soil, and even desert soil, converting soil CO_2 into soil organic carbon (SOC) at a rate of 5.57–85.1 mg C m^{-2} d^{-1} using C fixation pathways and increasing the SOC pool (Liao et al., 2023; Tang et al., 2022; Yuan et al., 2012; Zheng et al., 2022). In temperate forest soils, autotrophic microorganisms have been estimated to fix about 0.3 Pg C yr^{-1}, corresponding to 3–6 % of annual soil CO_2 emissions (Akinyede et al., 2022). Six pathways capable of fixing atmospheric CO_2 for autotrophic microorganisms have been reported so far, the Calvin-Benson-Bassham (CBB) cycle being the most ubiquitous and dominant pathway in soils across the globe (Liao et al., 2023). This path is performed by multiple bacteria

---

* Correspondence to: X. Ma, Key Laboratory of Molecular Medicine and Biotherapy, School of Life Science, Beijing Institute of Technology, 5 South Zhongguancun Street, Haidian District, Beijing 100081, China.
** Correspondence to: M. Liu, Institute of Soil Science, Chinese Academy of Sciences, East Beijing Road 71, Nanjing 210008, China.
E-mail addresses: xyma@bit.edu.cn (X. Ma), mliu@issas.ac.cn (M. Liu).

https://doi.org/10.1016/j.apsoil.2023.104945
Received 8 December 2022; Received in revised form 16 March 2023; Accepted 30 April 2023

and protist organisms. In the CBB cycle, ribulose-1,5-bisphosphate carboxylase/oxygenase (RubisCO) is responsible for catalyzing the first rate-limiting step for autotrophic CO_2 fixation (Fuchs, 2011). There have been four RubisCO forms (forms I–IV) detected with different structure and O_2 sensitivity, among which form I RubisCO is of the most abundant type (Wang et al., 2021a; Zhao et al., 2018). The *cbbL* gene encodes the large subunit of form I RubisCO, and is thus commonly used as a phylogenetic marker to investigate the autotrophic bacterial community (Li et al., 2022).

It has been reported that agricultural soil comprises a large quantity of *cbbL-harboring* autotrophic microorganisms, with 3×10^8–8×10^8 copies of *cbbL* genes per gram soil (Yuan et al., 2015; Yuan et al., 2012). Agronomic managements, such as tillage, irrigation, cropping system, and fertilization, would influence the abundance, diversity or structure of *cbbL*-harboring microbial community (Tang et al., 2022; Wu et al., 2015; Wu et al., 2017; Yuan et al., 2012), and consequently the C storage in soil (Wang et al., 2021a). However, inconsistent results have been achieved for different agricultural practices. Taking the organic fertilization for example, either positive (Wang et al., 2021a; Yan et al., 2021) or neutral (Liao et al., 2020) impact of organic fertilizer has been documented on the diversity of *cbbL*-harboring community, depending on the fertilizer types (i.e. livestock manures, biochars, sewage sledges, commercial organic fertilizers). For those cases, the differences in the intrinsic traits of organic fertilizers or soil would be the primary reason. Note that the applying rate of organic fertilizer is often distinct in literatures, which hinders the parallel comparison between different experiments. In fact, a previous study has investigated the effect of chemical N gradients (from 0 to 150 kg N ha^{-1} yr^{-1}) on *cbbL*-harboring community (Qin et al., 2021). It shows that high N dose significantly decreases the diversity of *cbbL*-harboring community, while no significant changes were detected under low N doses. Whether a similar pattern exists under the organic fertilizer gradient remains unclear, and analyses of the determinants shaping the *cbbL*-harboring microbial community are missing entirely.

Paddy soil is widespread across all continents, from temperate to tropical climates, but mainly in Asia (Liu et al., 2021b). It has been found paddy soils support a larger proportion of obligate bacterial and protist autotrophs, displaying four-fold of CO_2 fixation rates over upland and forest soils (Liao et al., 2023), with CBB cycle being the most abundant pathway (Xiao et al., 2021). As such, paddy soil could be an ideal ecosystem for studying the response of *cbbL*-harboring community to organic fertilizer gradients. To this end, a 5-year field experiment was conducted by applying pig manure (PM) at different doses to a red paddy soil in this study. The soil *cbbL*-harboring bacterial community was determined by high-throughput sequencing. Soil physicochemical properties were used for statistical analyses to identify the factors influencing the *cbbL*-harboring bacterial community. Based on those, we tried to answer the following two scientific questions to understand the CO_2-fixing bacterial community change rules and provide reference for scientific fertilization system in the paddy soil region: (i) Is there any difference in the characteristics of *cbbL*-harboring microorganisms among PM addition rates. (ii) What is the relationship between *cbbL*-harboring bacteria, PM dose, and soil physicochemical properties? We hypothesized that, with the increasing dose of PM, the diversity of *cbbL*-harboring bacteria would gradually decrease, and some soil physicochemical properties, such as pH, labile nutrients or heavy metals, might be determinants for *cbbL*-harboring bacterial community changes.

2. Materials and methods

2.1. Experimental site

A field experiment was carried out in a double rice cropping system (early and late season rice) (*Oryza sativa* L.), from 2013 to 2017 at Yujiang county, Yingtan, Jiangxi, China (28°13′44″ N, 116°53′52″ E). The climate at the experimental site is subtropical, humid, monsoonal, warm and rainy. The average annual sunshine hours, air temperature, and precipitation are 1739.4 h, 17.6 °C, and 1750 mm, respectively.

2.2. Experimental design and soil sampling

At the beginning of each cropping season, chemical fertilizers, together with different doses of pig manure (PM), were applied to each plot (9 m × 3 m). The rates for chemical fertilizers were 75 kg N ha^{-1}, 37.5 kg P_2O_5 ha^{-1}, 75 kg K_2O ha^{-1} in the forms of urea, calcium magnesium phosphate, and potassium chloride, respectively. The composted pig manure (PM) was applied under gradient doses, i.e. 0, 1400, 2800, 5600, 11,200, 22,400, and 44,800 kg ha^{-1} (wet-weight base, ~70 % water content). There were seven treatments in total with three replicates for each treatment. The highest dose of 44,800 kg ha^{-1} represented extreme condition of manure addition in the region (Li et al., 2020c). The composted manure contained 260.33 g kg^{-1} total organic C, 20.97 g kg^{-1} total N (TN), 22.47 g kg^{-1} total P (TP), and 9.55 g kg^{-1} total potassium (TK) based on the dry weight, and had a C/N ratio of 14.48 and a slightly alkaline pH of 7.43. A randomized complete block design was arranged for all plots. Rice was cultivated successively in every cropping season (approximately May for early-season rice and August for late-season rice every year). Agricultural practices, i.e. subsurface drainage, irrigation, herbicide, and pesticide use, were identical for all treatments.

After 10 seasons of successive rice cultivation, soil samples of 20-cm depth were collected in December 2017 (one month after second season rice harvested). Within each plot, a homogeneous composite sample was collected by mixing thoroughly five separate soil cores (6-cm in diameter). Then the soil samples were put into a portable fridge (4 °C) immediately and transported to the laboratory within 24 h. In laboratory, each sample was separated into two parts, a subsample (air-dried, ground and sieved through a 2-mm mesh) for measuring physical and chemical properties, and the other for molecular analysis (−40 °C).

2.3. Analysis of soil physiochemical properties

Soil physiochemical properties were determined using the methods described by Lu (1999). Soil pH was assayed using a pH meter with a 1:2.5 soil:water suspension. Cation exchange capacity (CEC) was qualified by ammonium acetate method. Soil organic carbon (SOC) was determined using the sulfuric acid-potassium dichromate oxidation method. TN and available nitrogen (AN) were measured as Kjeldahl-N. TP and available P (AP) were assayed using HF-$HClO_4$ digestion and sodium bicarbonate extraction (molybdenum blue method), respectively. TK and available K (AK) were determined by HF-$HClO_4$ digestion and ammonium acetate extraction (flame emission spectrometry). Soil ammonium (NH_4-N) and nitrate nitrogen (NO_3-N) were extracted with 2 mol L^{-1} KCl and assessed by using a continuous flow analyzer (Skalar, Breda, Netherlands). Furthermore, soil samples were digested with a mixture of $HNO_3/HClO_4$ (5/1, v/v) for measuring the total contents of Cu, Zn, Cd, and As using an inductively coupled plasma-mass spectrometry analyzer (7500A, Agilent, USA).

2.4. Soil DNA extraction, amplification, Illumina sequencing, and sequence data processing

Soil DNA was extracted from 0.5 g of soil (fresh weight) using a Fast®DNA SPIN Kit (MP Biomedicals, CA, USA) and then subsequently purified using a PowerClean® DNA Clean-Up Kit (MoBio, CA, USA) according to the manufacturers' instructions. The concentration and quality of the extracted DNA were measured using a NanoDrop ND-1000 spectrophotometer (Thermo Fisher, Waltham, MA, USA).

According to the concentration, DNA was diluted to 1 ng ml^{-1} using sterile water. The *cbbL* gene primer K2f/V2r, 5′-ACCAYCAAGCCSAAGCTSGG-3′/5′-GCCTTCSAGCTTGCCSACCRC-3′, was used to amplify the autotrophic bacterial community (Tolli and King, 2005).

The 5 bp barcoded oligonucleotides were fused to the forward primer. All PCR were carried out in 50 μl reactions with 25 μl of Taq DNA polymerase (TaKaRa, Japan), 0.2 mmol L^{-1} of forward and reverse primers, and 10 ng of template DNA. Thermal cycling consisted of initial denaturation at 98 °C for 1 min, followed by 30 cycles of denaturation at 98 °C for 10 s, annealing at 50 °C for 30 s, and elongation at 72 °C for 60 s. The PCR products remained at 72 °C for 5 min, and then the same volume of 1 × loading buffer was mixed with the PCR products and analyzed by electrophoresis on 2 % agarose gel. The PCR products were then mixed in equivalent ratios and purified with QIAquick® PCR purification Kit (Qiagen, Germantown, MD, USA) and quantified using a NanoDrop ND-1000 spectrophotometer. Amplified products were sent to Shanghai Genesky Biotechnologies Inc. (Shanghai, China) for sequencing using the Illumina MiSeq platform.

2.5. Data analysis

High throughput sequencing data were analyzed using the Quantitative Insight into Microbial Ecology (QIIME) 1.9.0 pipeline (https://www.qiime.org). Sequences with lengths <200 bp were discarded, and chimaeras were filtered with UPARSE. Then all samples were rarefied to an equal number of 36,900 reads for the downstream analyses. Operational taxonomic units (OTUs) were delineated using a 97 % similarity threshold, and taxonomy was determined using Silva 132 (https://www.arb-silva.de) for the bacterial community. Raw sequences were submitted to the NCBI Sequence Read Archive (SRA) under SRA accession number PRJNA899344.

All bioinformatics analyses, data visualization, and statistical analyses were performed in R 4.2.1 (R Core Team, 2022). The statistical significance was determined using a one-way analysis of variance (ANOVA) and a Tukey's test for confirming the significant difference in the variables. Statistical significance was set at $P < 0.05$ unless otherwise stated.

Principal coordinates analysis (PCoA) was used to evaluate the Bray-Curtis distances of *cbbL*-harboring bacterial community using the R package vegan. The taxonomic differences were analyzed by using metastat approach implemented in the microeco (v0.11.1) package (Liu et al., 2021a; White et al., 2009). Pairwise PERMANOVA was conducted using the pairwiseAdonis package (Permutations = 999, $P < 0.05$). Bonferroni *P*-value correction was applied as default for multiple corrections. The interplay relationships between *cbbL*-harboring bacteria and environmental factors were calculated using a correlation heatmap and redundancy analysis (RDA) by using the R package microeco (Liu et al., 2021a). The key environmental factors were then included in the partial least squares path modeling (PLS-PM) to further identify the possible pathways by which factors controlled the *cbbL*-harboring microbial properties. Variables with loadings < 0.7 was removoed, and goodness-of-fit index (GOF) and R^2 were used to estimate model performance. The PLS-PM was performed using the plspm package (Sanchez et al., 2015).

3. Results

3.1. Soil physicochemical properties

Soil physicochemical properties showed distinct tendencies under different doses of PM addition (Table 1). With PM dose increasing, pH, SOC, TK, CEC, Cu, and Zn increased steadily, with the significantly higher values under the doses of 22,400 and 44,800 kg ha^{-1} than those under control ($P < 0.05$). Other traits, such as NO_3-N, NH_4-N, TN, TP, TK, and AP, did not show significant changes among all treatments ($P > 0.05$). Further correlation analysis showed that pH, SOC, TN, TP, AP, AK, CEC, Cu, and Zn had close affinities with PM dose (Table S1, $P < 0.05$).

3.2. Alpha-diversity of cbbL-harboring bacterial community

The alpha-diversity indices of *cbbL*-harboring bacterial community, i.e. Observed OTUs, Shannon index, and Chao1 index, displayed similar trends with the increase of PM dose (Fig. 1). For the observed OTUs, the doses of 11,200 kg ha^{-1} and less did not lead to significant changes compared to control, with OTUs ranging from 428 to 504. By contrast, the doses of 22,400 and 44,800 kg ha^{-1}, significantly lowered the observed OTUs compared to the other treatments ($P < 0.05$). Consistently, with PM dose increasing, the values of Shannon and Chao1 indices showed the decreasing trends, with significantly lower values under the doses of 22,400 kg ha^{-1} as compared to those under control ($P < 0.05$). Such features indicated the negative impacts of overdosed PM (22,400 kg ha^{-1} and high) on the alpha-diversity of *cbbL*-harboring bacterial community.

3.3. Structure of cbbL-harboring bacterial community

Clustering analysis, followed by PCoA based on Bray-Curtis distance, was used to profile the structure of *cbbL*-harboring bacterial communities under different doses of PM addition (Fig. 2). It was evident that all samples could be grouped into three categories, i.e. dose zero (annotated as control hereafter), the doses of 11,200 kg ha^{-1} and less (annotated as Low hereafter), and the doses of 22,400 kg ha^{-1} and more (annotated as High hereafter) (Fig. 2A). We then presented the shifts of

Table 1
Soil physicochemical properties in different treatments.

PM dose (kg ha^{-1})	0	1400	2800	5600	11,200	22,400	44,800
pH	5.64 ± 0.10c	5.68 ± 0.04c	5.84 ± 0.25c	6.11 ± 0.24bc	6.43 ± 0.21ab	6.65 ± 0.14a	6.81 ± 0.16a
SOC (g kg^{-1})	24.06 ± 2.25bc	23.26 ± 0.96c	25.13 ± 1.10abc	24.6 ± 1.25abc	27.96 ± 1.21abc	29.16 ± 2.49a	28.63 ± 2.33ab
TN (g kg^{-1})	2.57 ± 0.40a	2.56 ± 0.16a	2.77 ± 0.16a	2.67 ± 0.10a	2.96 ± 0.09a	3.05 ± 0.22a	3.05 ± 0.23a
TP (g kg^{-1})	0.81 ± 0.16c	0.80 ± 0.14c	1.10 ± 0.18c	1.07 ± 0.13c	1.53 ± 0.11b	2.02 ± 0.06a	2.05 ± 0.14a
TK (g kg^{-1})	6.91 ± 0.57a	6.96 ± 0.35a	6.96 ± 0.15a	6.78 ± 0.03a	7.11 ± 0.24a	6.9 ± 0.08a	6.71 ± 0.36a
AN (mg kg^{-1})	229.08 ± 21.22a	204.58 ± 12.91a	233.98 ± 11.81a	215.6 ± 9.25a	233.98 ± 11.81a	241.33 ± 14.85a	221.73 ± 16.57a
AP (mg kg^{-1})	70.79 ± 18.32a	72.77 ± 13.56a	82.29 ± 8.64a	90.69 ± 17.54a	85.61 ± 6.97a	120.45 ± 6.07a	142.46 ± 13.72a
AK (mg kg^{-1})	54.17 ± 1.44a	52.5 ± 5.00a	54.17 ± 3.82a	51.67 ± 5.20a	52.5 ± 2.50a	69.17 ± 8.04a	85.83 ± 19.42a
NO_3^--N (mg kg^{-1})	7.65 ± 3.10a	15.58 ± 11.01a	7.56 ± 3.80a	9.92 ± 4.62a	12.25 ± 8.47a	13.72 ± 9.08a	12.74 ± 8.18a
NH_4^+-N (mg kg^{-1})	7.29 ± 0.94a	6.84 ± 1.19a	7.05 ± 0.72	7.32 ± 1.30a	6.61 ± 1.12a	6.52 ± 0.29a	7.02 ± 0.33a
CEC (cmol kg^{-1})	11.1 ± 0.79bc	10.83 ± 0.23c	12.03 ± 1.07bc	11.47 ± 0.91bc	12.77 ± 0.32ab	14 ± 0.62a	14.17 ± 0.23a
Cu (mg kg^{-1})	23.41 ± 2.27c	22.03 ± 1.24c	25.94 ± 3.28c	26.63 ± 2.14bc	32.06 ± 1.84b	39.09 ± 2.41a	41.56 ± 0.38a
Zn (mg kg^{-1})	88.46 ± 18.30c	85.41 ± 11.41c	105.05 ± 19.04c	111.41 ± 10.25bc	145.96 ± 8.19b	236.16 ± 9.06a	266.57 ± 8.84a
Cd (mg kg^{-1})	0.26 ± 0.04a	0.25 ± 0.07a	0.23 ± 0.06a	0.2 ± 0.02a	0.24 ± 0.02a	0.24 ± 0.01a	0.22 ± 0.01a
As (mg kg^{-1})	3.86 ± 0.12a	3.51 ± 0.40a	4.39 ± 1.04a	3.7 ± 0.25a	5.98 ± 2.35a	3.6 ± 0.22a	3.7 ± 1.32a

Values are mean ± standard deviation. Different letters in the same row indicate the significant differences among treatments. PM: pig manure; SOC: soil organic carbon; TN: total nitrogen; TP: total phosphorus; TK: total potassium; AN: available nitrogen; AP: available phosphorus; AK: available potassium; CEC: cation exchange capacity.

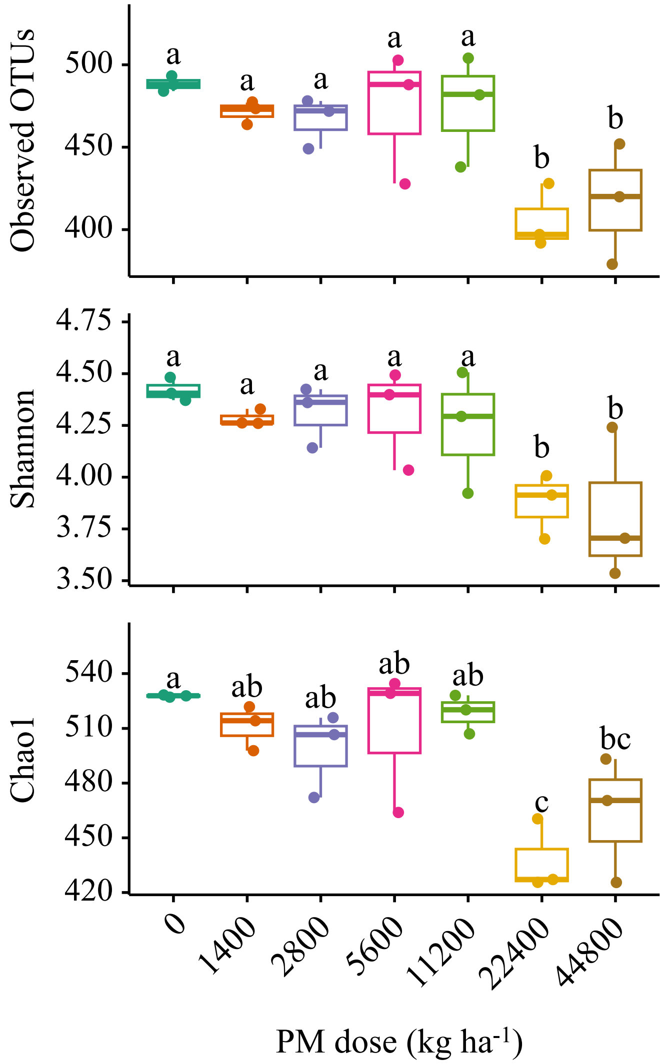
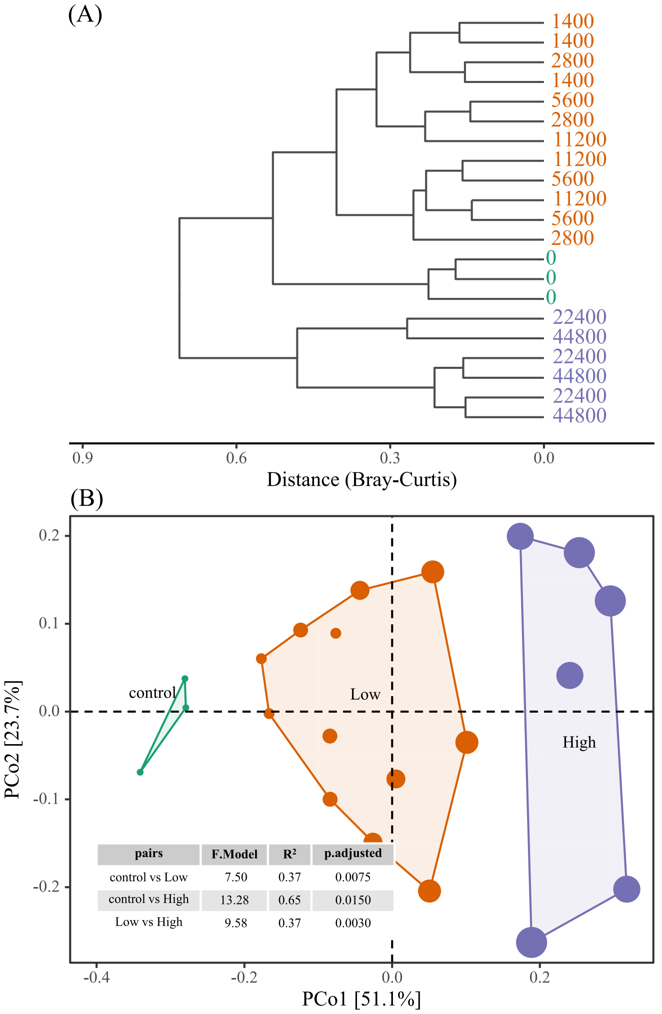


Fig. 1. Alpha-diversity indices, i.e. Observed OTUs, Shannon, and Chao1, of *cbbL*-harboring bacterial community under different doses of PM additions. Different letters above the boxes indicate significant differences among different treatments. ($P < 0.05$).

cbbL-harboring community based on those three categories in the following section.

PCoA results revealed that the soil *cbbL*-harboring bacterial communities were markedly affected by PM doses (Fig. 2B). The higher the PM dose was, the greater the distance was, hence the different structures among the treatments. As shown in Fig. 2B, control, Low, and High groups were separated along horizontal axis, suggesting the effect of PM gradient. Pairwise PERMANOVA results further showed that the differences among those three categories were significant ($P < 0.05$, the inserted table in Fig. 2B).



pairs	F.Model	R²	p.adjusted
control vs Low	7.50	0.37	0.0075
control vs High	13.28	0.65	0.0150
Low vs High	9.58	0.37	0.0030

Fig. 2. Clustering analysis of the relative abundance of *cbbL*-harboring bacteria in each treatment (A). The closer the distance, the shorter the branch length, the more similar the two *cbbL*-harboring community are. (B) Principal coordinates analysis (PCoA) representation of soil *cbbL*-harboring bacterial community under the different doses of PM addition based on Bray-Curtis anisotropy. Three groups of PM addition, i.e. control, Low, and High, are categorized based on the clustering analysis (A). The sizes of the dots in PCoA plot are in according with the doses of PM addition. PairwiseAdonis (the inserted table in B) is used to test whether the between-group differences are significantly greater than the within group differences and thus to determine whether the groupings are significant. *P*-values are calculated using a permutation test to indicate whether a significant difference exists (permutations = 999).

3.4. Composition of cbbL-harboring bacterial community

All OTUs across 21 soil samples were assigned to 6 phyla, 13 classes, 35 orders, 54 families, and 98 genera. At the phylum level, Proteobacteria accounted for 86.9 % in average, with the maximum of 97.4 %. The average relative abundance of Actinobacteria was 12.5 %, with the maximum of 20.3 % (Fig. 3A). The rare phyla (<0.5 %) included Chloroflexi (0.01 % ~ 0.97 %), Cyanobacteria (0.07 % ~ 0.42 %), Verrucomicrobia (0.01–0.08 %), and Firmicutes (0–0.04 %). When the PM dose increased, the relative abundance of Proteobacteria increased simultaneously, showing a positive relation between them (Table S2, $P < 0.001$, $r = 0.77$). By contrast, Actinobacteria showed an opposite trend with the increasing PM dose (Table S2, $P < 0.001$, $r = -0.75$). Among the dominant classes (data not shown), Betaproteobacteria accounted for the largest proportion (38.5 % in average), followed by Alphaproteobacteria (36.8 % in average), and Gammaproteobacteria (11.1 % in average). Nevertheless, only Betaproteobacteria (Table S2, $P = 0.006$, $r = 0.58$) and Gammaproteobacteria (Table S2, $P = 0.003$, $r = 0.61$) positively correlated with PM dose.

At the genus level, different trends were detected with the increase of PM dose. The relative abundances of top 15 genera significantly changed among treatments were illustrated in Fig. 4 based on the metastat analysis. In comparison with control, the High group significantly decreased the relative abundances of *Variovorax* (Betaproteobacteria), *Rhodomicrobium* (Alphaproteobacteria), *Methylonatrum* (Gammaproteobacteria), *Nocardia* (Actinobacteria), *Bradyrhizobium*

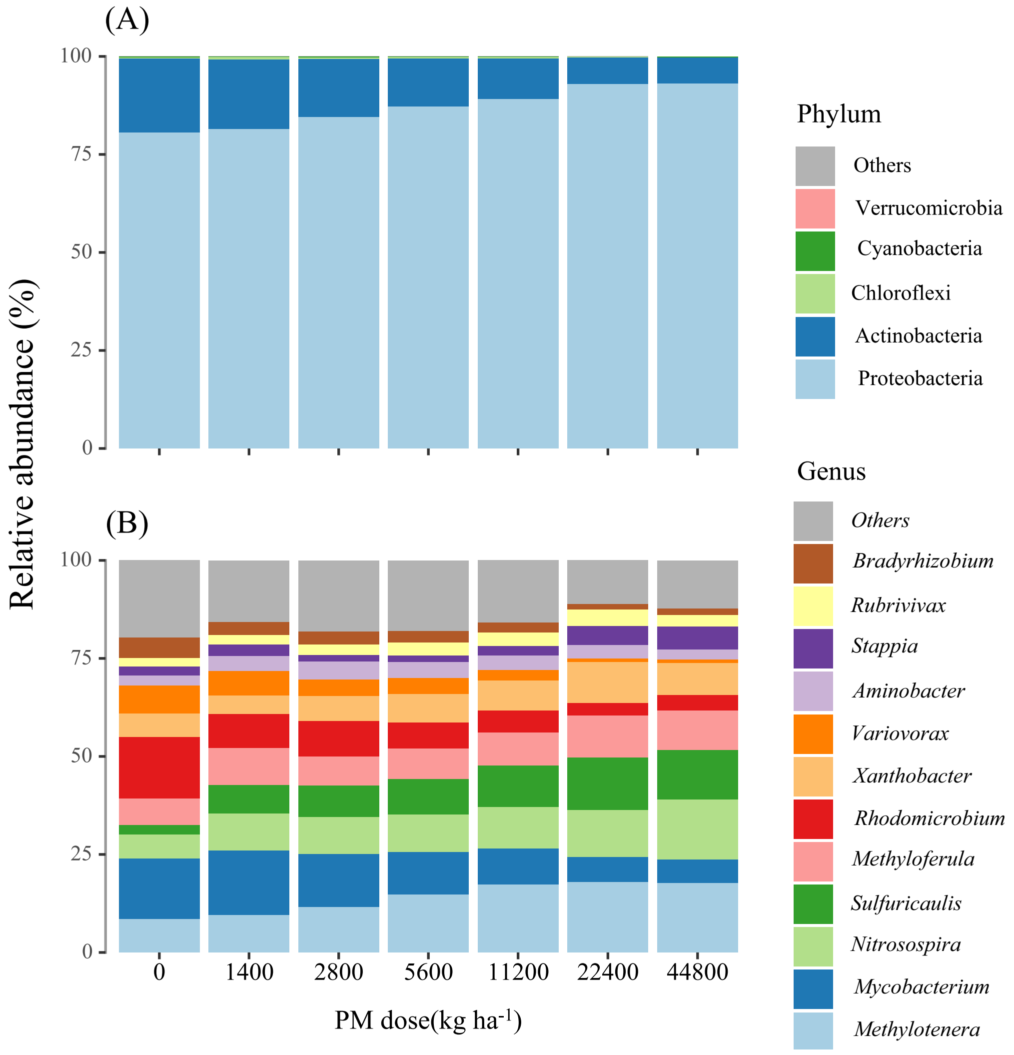


Fig. 3. Relative abundances of the *cbbL*-harboring bacterial phyla (A) and genera (B) under different treatments.

(Alphaproteobacteria), *Brevirhabdus* (Alphaproteobacteria), *Methylibium* (Alphaproteobacteria), *Afipia* (Alphaproteobacteria), *Kouleothrix* (Chloroflexi), *Mycobacterium* (Actinobacteria), and *Hoeflea* (Alphaproteobacteria), but significantly increased those of *Hydrogenophaga* (Betaproteobacteria), *Methyloferula* (Alphaproteobacteria), and *Sulfuricaulis* (Gammaproteobacteria) ($P < 0.05$, Fig. 4). By contrast, *Cupriavidus* (Betaproteobacteria) was found to be increased by the Low group as compared to control ($P < 0.05$, Fig. 4).

3.5. Relationships between soil physicochemical properties and cbbL-harboring bacterial community

RDA was used to show the relationships between the environmental factors and the *cbbL*-harboring bacterial community (Fig. 5A). RDA1 and RDA2 components accounted for 91.6 % of the variation in the *cbbL*-harboring community. Among the measured factors, pH, TP, AP, CEC, Cu, and Zn had significantly positive correlations with the *cbbL*-harboring bacterial community, implying their roles in shaping the structural characteristics of CO_2-fixing microbes ($P < 0.05$, Table S3).

Heatmap plot was then used to show the correlations between physicochemical properties and some *cbbL*-harboring bacterial genera (Fig. S1). In general, pH, TP, AP, CEC, Cu, and Zn were significantly negatively correlated with the relative abundances of *Variovorax*, *Rhodomicrobium*, *Methylonatrum*, *Nocardia*, *Bradyrhizobium*, *Brevirhabdus*, *Methylibium*, *Afipia*, *Kouleothrix*, *Mycobacterium*, and *Hoeflea*, but significantly positively correlated with those of *Hydrogenophaga*, *Methyloferula*, and *Sulfuricaulis* ($P < 0.05$).

The PLS-PM further refined the measured physicochemical traits to several dominant factors influencing the *cbbL*-harboring bacterial community attributes (Fig. 5B). PM dose determined the *cbbL*-harboring community through its positive effect on soil pH ($r = 0.85$, $P < 0.001$), AP ($r = 0.90$, $P < 0.001$) and metals (Cu and Zn, $r = 0.95$, $P < 0.001$). Soil pH and AP had significantly negative effects on the composition of *cbbL*-harboring community ($r = -0.88$, $P = 0.002$; $r = -0.55$, $P = 0.02$, respectively). By contrast, the significantly negative effects of pH, AP and metals were revealed on the diversity of *cbbL*-harboring community ($r = -0.72$, $P = 0.024$; $r = -0.57$, $P = 0.049$; $r = -0.91$, $P = 0.046$, respectively).

4. Discussion

4.1. High dose of PM reduced the alpha-diversity of cbbL-harboring bacterial community

Autotrophic microorganisms are important for C decomposition, transformation, and stabilization of soil organic C. The investigation of microorganisms possessing the CO_2-fixing ability is conducive to understanding the C cycling in soil under the background of climate change. From both theoretical and empirical perspectives, soil microbial alpha-diversity indices serve as feasible parameters for predicting

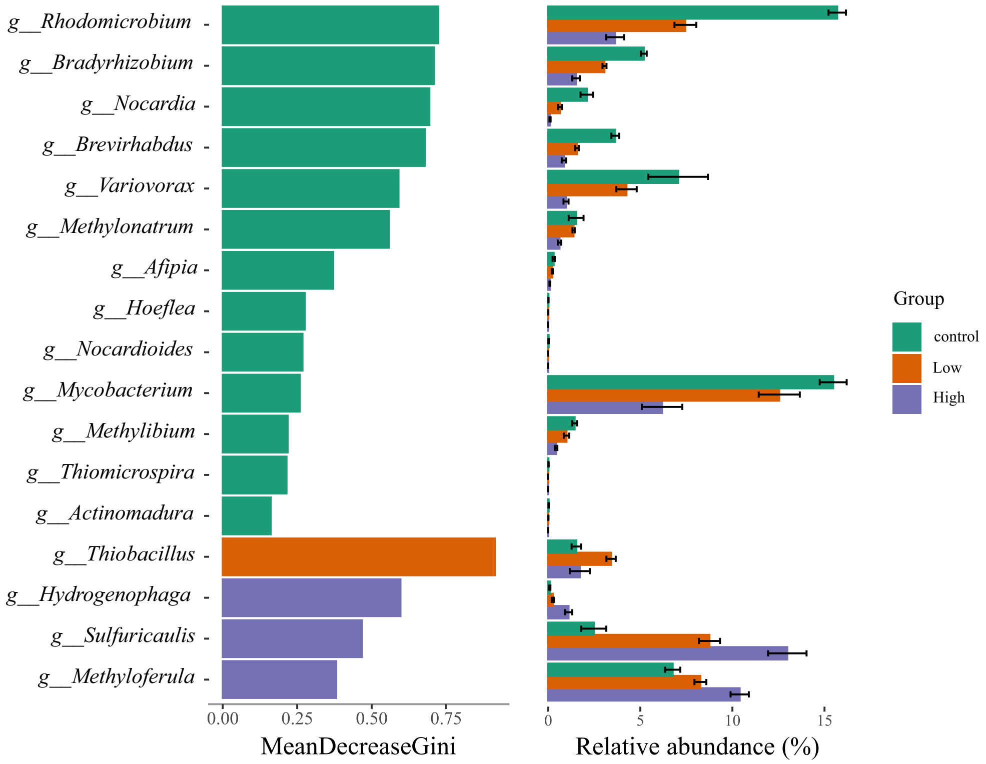


Fig. 4. Representative *cbbL*-harboring genera significantly changed by different treatments.

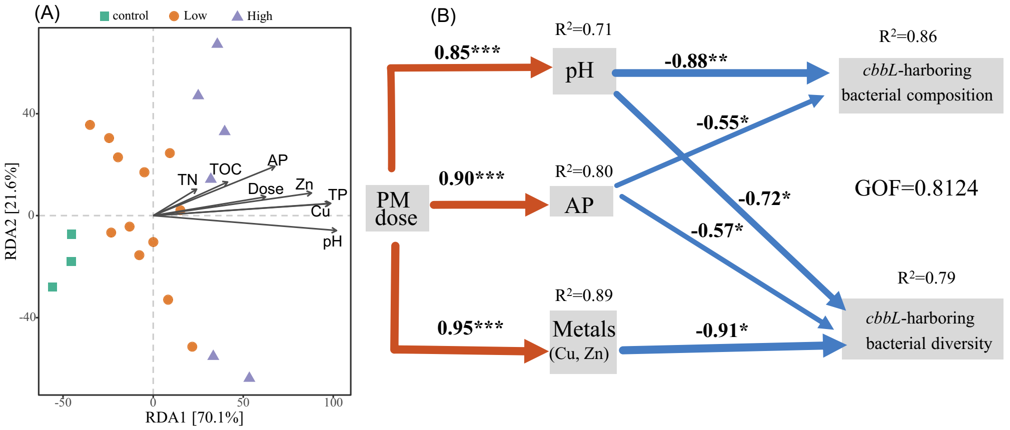


Fig. 5. Redundancy analysis (RDA) results of the *cbbL*-harboring bacterial community structure containing the *cbbL* gene with environmental factors (A). Partial least squares path modeling (PLS-PM) showing the effects of the pH, AP, and metals (Cu and Zn) on the composition and alpha-diversity of *cbbL*-harboring bacterial community (B). The non-significant relationships are not showed in the pathways ($P > 0.05$). Numbers adjacent to the arrows indicate standardized path coefficients (red and blue arrows indicate positive and negative effects, respectively), and R^2 values are the determination coefficients of the variables in the inner model. *$P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

multiple ecological functions of the community (Wang et al., 2021a). More diverse microbial community could support microorganisms to adapt environmental changes and conserve energy (Thingstad et al., 2014).

In this study, the alpha-diversity of *cbbL*-harboring community was not markedly changed under the Low dose (11,200 kg ha^{-1} and less) of PM, but was significantly decreased under the High dose (22,400 kg ha^{-1} and more). Divergences exist between our result and those in literatures. For instance, biochar amendment leads to a more diverse *cbbL*-harboring bacterial community even at the dose of 1.2 % or 6 % (ca. 27,000 kg ha^{-1}) (Li et al., 2020b). By contrast, no profound shifts of the alpha-diversity indices were detected after the horse manure addition at a dose of 18,600 kg ha^{-1} (ca. 75 kg N ha^{-1}) (Liao et al., 2020), a pattern resembling to the Low group in this study. Three potential explanations were proposed here. Firstly, the difference in the organic fertilizer properties is the primary reason. Taken manure and biochar for example, the labile nutrients, such as inorganic N and dissolved C, were less abundant in the latter (Yan et al., 2021). Less fast-acting nutrients in fertilizer might stimulate the proliferation of *cbbL*-harboring bacteria to increase C input, leading to a more diverse community (Qin et al., 2021). Secondly, the dose of organic fertilizer applied to soil is another reason. Labile nutrients could promote plant growth (evidenced by rice grain yields in this study, Table S4), resulting in microorganisms not receiving distinguishable amount of nutrients for their own reproduction. As such, when the organic fertilizer input is below a threshold (such as PM dose of 11,200 kg ha^{-1} in this study), the available nutrients might not be adequate for inducing significantly changes of *cbbL*-harboring bacterial diversity. When the dose of organic fertilizer continues to increase (or excessive dose), the available nutrients in soil would increase simultaneously, resulting in the decreased diversity of *cbbL*-harboring bacterial community (Qin et al., 2021). As a proof, the significantly higher TP and AP contents, but the lower *cbbL*-harboring bacterial diversity, were found in the High group than in the other groups in this study (Fig. 1 and Table 1), supporting our hypothesis. Thirdly, the livestock manure-derived organic fertilizer would unintentionally introduce metals (such as Cu and Zn in Table 1) or antibiotics into soil. As evidenced, soil Cu and Zn increased significantly with the increase of PM addition (Table 1), since heavy metal-additives (Cu, Zn, and others) are frequently added to animal feed in breeding farms (Li et al., 2020c). The additives result in excessive amounts of heavy metal excreted with manure, accumulate in soils, and subsequently inhibit the proliferation of some microbes (Rajapaksha et al., 2004). Our PLS-PM results provided the direct evidence by showing the negative effect of metals on the *cbbL*-harboring bacterial diversity (Fig. 5B).

4.2. PM addition changed the composition of *cbbL*-harboring bacterial community

A wide spectrum of autotrophic microbes in soil are facultative autotrophs, capable of both chemolithoautotrophic (via the Calvin cycle) and heterotrophic (by accumulating polyhydroxyalkanoates) metabolic pathways and reactions (Akinyede et al., 2022; Xiao et al., 2014; Zhou et al., 2019). Such aspects highlight the role of nutrient supply in shaping the *cbbL*-harboring bacterial community. Proteobacteria and Actinobacteria account for a majority of *cbbL*-harboring bacteria, as shown in both this study and others (Li et al., 2022; Liao et al., 2020; Wang et al., 2021a). Proteobacteria contain the representatives of autotrophic bacteria, using reduced TCA cycle or reductive acetyl-CoA pathway to fix CO_2 (Hugler et al., 2005). They are rich in species and genetic diversity (e.g. aerobic and anaerobic, autotrophic and heterotrophic and both phototrophic and chemotrophic types). For instance, some Proteobacteria can decompose litter as food or use soil available N and obtain CO_2 from the environment as C source to synthesize organic matter for their growth (Li et al., 2022). In this regard, more labile sources from PM would motivate the growth of Proteobacteria, enhancing concurrently the relative abundance of *cbbL*-harboring Proteobacteria in soil. Actinobacteria play an important role in decomposing organic matter, and accelerating the decomposition of various residues (Feng et al., 2022). However, the *cbbL*-harboring Actinobacteria prefer oligotrophic condition (Zhao et al., 2018), as proved by the negative correlation between their proportion and PM dose in this study (Table S2). Noteworthy that no marked correlations were found between PM dose and the relative abundance of Proteobacteria or Actinobacteria based on whole bacterial community analysis (16S rRNA gene sequencing) for the same samples (Li et al., 2020c), which implied that the *cbbL*-harboring groups was more sensitive to PM addition. At the genus level, divergent changes of *cbbL*-harboring lineages were found with the increasing PM dose. For example, the relative abundances of some Proteobacterial genera were significantly decreased by high PM addition, such as *Variovorax*, *Rhodomicrobium*, *Methylonatrum*, *Bradyrhizobium*, *Brevirhabdus*, *Methylibium*, *Afipia*, and *Hoeflea*, while those of *Hydrogenophaga*, *Methyloferula*, and *Sulfuricaulis* were increased. Some genera affiliated to Actinobacteria were also decreased, including *Nocardia* and *Mycobacterium* under the overdosed PM addition. Most of those bacteria were previously reported as facultative autotrophs (Liao et al., 2020), functioning in degrading C-containing organic compounds, and playing crucial roles in N or S cycling as well (Guo et al., 2015; Wang et al., 2021a; Yuan et al., 2013). For example, *Bradyrhizobium* not only harbors the ability of CO_2- and N_2-fixing (containing *cbbL* and *nifH* genes, respectively) (Liao et al., 2020; Liu et al., 2022), but also can use NH_4^+, NO_2^-, S^{2-}, H_2S, H_2, and Fe^{2+} as energy sources for autotrophic growth (Kojima et al., 2016; Wang et al., 2021b). Besides, *Bradyrhizobium* prefers acidic environment and is more abundant at acidic pH, despite its versatility in metabolisms (Fan et al., 2018). Given the contrasting pH values of the tested soil and the PM (5.64 and 7.43, respectively), more PM addition would lead to a more neutral environment (especially for the High group), which might suppress the *Bradyrhizobium* growth. The similar shift of *Bradyrhizobium* has been reported in a long-term experiment as well (Liu et al., 2022). The *cbbL*-harboring *Variovorax* is also common inhabitants in soil (Tang et al., 2022). It involves in both CO_2-fixing and denitrification (Im et al., 2010; Liu et al., 2013), and some species in *Variovorax* are capable of degrading diverse organic carbons including starch, cellulose, gelatin, chitin, and humic acids (Guo et al., 2015), indicating their high metabolic versatility. *Methylibium* is a typical methylotroph (Zhao et al., 2018). It can not only participate in assimilating CO_2 using energy from the oxidation of inorganic sulfur compounds (Kojima et al., 2016), but also reduce iron and volatile compounds in micro-aerobic environments (Boada et al., 2020; Kappler et al., 2012). Despite their metabolic versatility, a trait shared by *Methylibium* and *Variovorax* is their preference of oligotrophic condition (Zhou et al., 2019), which explained their decreased abundance in the overdosed PM treatments. Noteworthy that the High dose of PM addition enhanced the proportions of some *cbbL*-harboring stress-tolerant microorganisms. *Hydrogenophaga* is reported to adapt to the As- and Cd-polluted environment and may be metal-resistant (Garcia-Dominguez et al., 2008; Qiu et al., 2020). *Methyloferula* is found to be tolerant to abiotic stress (Kumar et al., 2019) or sulfonamides (Li et al., 2020a). The increased relative abundances of those lineages might signify the unfavorable environment caused by the overdosed PM addition.

Together, our results demonstrated that chemoautotrophs and mixotrophs dominated the *cbbL*-harboring autotrophic bacterial communities in the tested paddy soil. The High dose of PM might restrain the proliferation of some *cbbL*-harboring lineages specializing in element cycling, and increase some stress-tolerant bacteria, which subsequently changed the soil multiple ecological functions.

4.3. Physicochemical factors influencing the *cbbL*-harboring bacterial community

Both RDA and PLS-PM pathway analysis showed that pH, AP, and metals (Cu and Zn) had close affinities with the *cbbL*-harboring bacterial community attributes (Fig. 5B). Due to a narrow pH range for optimal growth of soil bacteria and the changes in nutrient availability (Liao et al., 2020), soil pH has been reported to be the crucial driver regulating the whole bacterial and fungal community (Lauber et al., 2009), or the functional groups (Fan et al., 2018). In this study, a much neutral pH was expected under the high PM dose addition because of the contrasting pH of the soil and the manure. Besides, as an essential nutrient for microbial growth, P (either TP or AP) is more important for the tested soil owing to its typical P-deficiency. In this sense, the elevated pH and AP would stimulate the expansion of the fast-growing species and competitive exclusion of others (Liao et al., 2020). Given that most of *cbbL*-harboring bacterial lineages that had significantly decreased proportions prefer oligotrophic condition, such changes in the environment would not be conducive for enhancing *cbbL*-harboring bacterial community attributes, i.e. diversity and components. Indeed, several researches have already revealed that the dramatically increased pH and P content (TP or AP) may limit or co-limit soil autotrophic microorganisms (Li et al., 2022; Li et al., 2020c; Liao et al., 2020; Liu et al., 2020; Yuan et al., 2015), consistent with the present results. Note that there is also result showing the absent effect of AP on *cbbL*-harboring bacterial community, such as in Entisol (Zhou et al., 2019), which might be ascribed to the niches difference among different studies.

In line with the hypothesis, our results further revealed that metals, i.e. Cu and Zn, had significantly negative influences on the diversity of *cbbL*-harboring community. The potential explanation for this might be related to the fact that Cu and Zn contribute to the disruption of physiological functions, denaturation of proteins and destruction of cellular membranes of microorganisms (Wyszkowska et al., 2013). In support, Holanda et al. (2016) find some *cbbL*-harboring bacteria are sensitive to metals, including Zn.

5. Conclusions

The results in this study showed that the *cbbL*-harboring bacterial community attributes were changed to a little extent under the Low PM input, but were significantly changed by the High PM addition. High dose of PM decreased the alpha-diversity of *cbbL*-harboring bacterial community and the relative abundances of some lineages belonging to Proteobacteria and Actinobacteria, such as *Bradyrhizobium Methylibium, and Variovorax*, but increased the proportions of some stress-tolerant taxa (i.e. *Hydrogenophaga* and *Methyloferula*). Such changes might be principally driven by the environmental factors such as pH, AP, and metals (Cu and Zn) through PM addition, i.e. the significantly negative influence of those factors on the diversity and components of *cbbL*-harboring bacterial community. Taken together, our study demonstrated the potentially negative impacts of High PM addition on the *cbbL*-harboring microbes, and a rational application of manure-derived organic fertilizer was encouraged for maintaining soil ecological functions and C sequestration from the atmosphere by soil microorganisms.

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.

Data availability

Data will be made available on request.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (grant number 42177294, 42130506), the Jiangxi Modern Agricultural Innovation Project (grant number JXXTCXQN202008), the Key Research and Development Program of Jiangxi Province (grant number 20212BBF63007), and the Jiangxi Province Natural Science Foundation (grant number 20202ACBL213007).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.apsoil.2023.104945.

References

Akinyede, R., Taubert, M., Schrumpf, M., Trumbore, S., Küsel, K., 2022. Dark CO_2 fixation in temperate beech and pine forest soils. Soil Biol. Biochem. 165 https://doi.org/10.1016/j.soilbio.2021.108526.

Boada, E., Santos-Clotas, E., Bertran, S., Cabrera-Codony, A., Martín, M.J., Bañeras, L., et al., 2020. Potential use of *methylibium* sp. As a biodegradation tool in organosilicon and volatile compounds removal for biogas upgrading. Chemosphere 240, 124908. https://doi.org/10.1016/j.chemosphere.2019.124908.

Fan, K., Weisenhorn, P., Gilbert, J.A., Shi, Y., Bai, Y., Chu, H., 2018. Soil pH correlates with the co-occurrence and assemblage process of diazotrophic communities in rhizosphere and bulk soils of wheat fields. Soil Biol. Biochem. 121, 185–192. https://doi.org/10.1016/j.soilbio.2018.03.017.

Feng, H., Wang, Z., Jia, P., Gai, J., Chen, B., Wang, S., 2022. Diversity and distribution of CO_2-fixing microbial community along elevation gradients in meadow soils on the tibetan plateau. Sci. Rep. 12 https://doi.org/10.1038/s41598-022-13183-4, 9621-9621.

Fuchs, G., 2011. Alternative pathways of carbon dioxide fixation: insights into the early evolution of life? Annu. Rev. Microbiol. 65, 631–658. https://doi.org/10.1146/annurev-micro-090110-102801.

Garcia-Dominguez, E., Mumford, A., Rhine, E.D., Paschal, A., Young, L.Y., 2008. Novel autotrophic arsenite-oxidizing bacteria isolated from soil and sediments. FEMS Microbiol. Ecol. 66, 401–410. https://doi.org/10.1111/j.1574-6941.2008.00569.x.

Guo, G., Kong, W., Liu, J., Zhao, J., Du, H., Zhang, X., et al., 2015. Diversity and distribution of autotrophic microbial community along environmental gradients in grassland soils on the Tibetan Plateau. Appl. Microbiol. Biotechnol. 99, 8765–8776. https://doi.org/10.1007/s00253-015-6723-x.

Holanda, R., Hedrich, S., Nancucheo, I., Oliveira, G., Grail, B.M., Johnson, D.B., 2016. Isolation and characterisation of mineral-oxidising "Acidibacillus" spp. From mine sites and geothermal environments in different global locations. Microbiol. Res. 167, 613–623. https://doi.org/10.1016/j.resmic.2016.04.008.

Hugler, M., Wirsen, C.O., Fuchs, G., Taylor, C.D., Sievert, S.M., 2005. Evidence for autotrophic CO_2 fixation via the reductive tricarboxylic acid cycle by members of the epsilon subdivision of proteobacteria. J. Bacteriol. 187, 3020–3027. https://doi.org/10.1128/JB.187.9.3020-3027.2005.

Im, W.-T., Liu, Q.-M., Lee, K.-J., Kim, S.-Y., Lee, S.-T., Yi, T.-H., 2010. *Variovorax ginsengisoli* sp nov., a denitrifying bacterium isolated from soil of a ginseng field. Int. J. Syst. Evol. Microbiol. 60, 1565–1569. https://doi.org/10.1099/ijs.0.014514-0.

Kappler, U., Davenport, K., Beatson, S., Lucas, S., Lapidus, A., Copeland, A., et al., 2012. Complete genome sequence of the facultatively chemolithoautotrophic and methylotrophic alpha proteobacterium *Starkeya novella* type strain (ATCC 8093T). Stand. Genomic Sci. 7, 44–58. https://doi.org/10.4056/sigs.3006378.

Kojima, H., Watanabe, T., Fukui, M., 2016. *Sulfuricaulis limicola* gen. nov., sp. nov., a sulfur oxidizer isolated from a lake. Int. J. Syst. Evol. Microbiol. 66, 266–270. https://doi.org/10.1099/ijsem.0.000709.

Kumar, M., Kour, D., Yadav, A.N., Saxena, R., Rai, P.K., Jyoti, A., et al., 2019. Biodiversity of methylotrophic microbial communities and their potential role in mitigation of abiotic stresses in plants. Biologia 74, 287–308. https://doi.org/10.2478/s11756-019-00190-6.

Lauber, C.L., Hamady, M., Knight, R., Fierer, N., 2009. Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. Appl. Environ. Microbiol. 75, 5111–5120. https://doi.org/10.1128/AEM.00335-09.

Li, M., Rong, L., Zhou, S., Xiao, X., Wu, L., Fan, Y., et al., 2020a. Dissipation of sulfonamides in soil emphasizing taxonomy and function of microbiomes by metagenomic analysis. J. Agric. Food Chem. 68, 13594–13607. https://doi.org/10.1021/acs.jafc.0c04496.

Li, N., Wang, B., Huang, Y., Huang, Q., Jiao, F., An, S., 2022. Response of *cbbL*-harboring microorganisms to precipitation changes in a naturally-restored grassland. Sci. Total Environ. 838, 156191 https://doi.org/10.1016/j.scitotenv.2022.156191.

Li, P., Chen, W., Han, Y., Wang, D., Zhang, Y., Wu, C., 2020b. Effects of straw and its biochar applications on the abundance and community structure of CO2-fixing bacteria in a sandy agricultural soil. J. Soils Sediments 20, 2225–2235. https://doi.org/10.1007/s11368-020-02584-5.

Li, P., Liu, M., Ma, X., Wu, M., Jiang, C., Liu, K., et al., 2020c. Responses of microbial communities to a gradient of pig manure amendment in red paddy soils. Sci. Total Environ. 705, 135884 https://doi.org/10.1016/j.scitotenv.2019.135884.

Liao, H., Hao, X., Qin, F., Delgado-Baquerizo, M., Liu, Y., Zhou, J., et al., 2023. Microbial autotrophy explains large-scale soil CO_2 fixation. Glob. Chang. Biol. 29, 231–242. https://doi.org/10.1111/gcb.16452.

Liao, H., Qin, F., Wang, K., Zhang, Y., Hao, X., Chen, W., et al., 2020. Long-term chemical fertilization-driving changes in soil autotrophic microbial community depresses soil CO_2 fixation in a mollisol. Sci. Total Environ. 748, 141317 https://doi.org/10.1016/j.scitotenv.2020.141317.

Liu, C., Cui, Y., Li, X., Yao, M., 2021a. Microeco: an R package for data mining in microbial community ecology. FEMS Microbiol. Ecol. 97, fiaa255 https://doi.org/10.1093/femsec/fiaa255.

Liu, C., Shi, Y., Chen, J., Qin, H., Liang, C., Fuhrmann, J.J., et al., 2022. Distinct response of soil CO_2- and N_2-fixing bacteria to long-term application of mineral fertilizer combined with manure in a subtropical bamboo forest. J. Soils Sediments. https://doi.org/10.1007/s11368-022-03301-0.

Liu, J., Ma, Q., Hui, X., Ran, J., Ma, Q., Wang, X., et al., 2020. Long-term high-P fertilizer input decreased the total bacterial diversity but not *phoD*-harboring bacteria in wheat rhizosphere soil with available-P deficiency. Soil Biol. Biochem. 149 https://doi.org/10.1016/j.soilbio.2020.107918.

Liu, Y., Ge, T., van Groenigen, K.J., Yang, Y., Wang, P., Cheng, K., et al., 2021b. Rice paddy soils are a quantitatively important carbon store according to a global synthesis. Commun. Earth Environ. 2, 154. https://doi.org/10.1038/s43247-021-00229-0.

Liu, Z.-H., Cao, Y.-M., Zhou, Q.-W., Guo, K., Ge, F., Hou, J.-Y., et al., 2013. Acrylamide biodegradation ability and plant growth-promoting properties of *Variovorax boronicumulans* CGMCC 4969. Biodegradation 24, 855–864. https://doi.org/10.1007/s10532-013-9633-6.

Lu, R., 1999. Analytical Methods for Soils and Agricultural Chemistry (in Chinese). China Agricultural Science and Technology Press, Beijing, China.

Qin, J., Li, M., Zhang, H., Liu, H., Zhao, J., Yang, D., 2021. Nitrogen deposition reduces the diversity and abundance of *cbbL* gene-containing CO_2-fixing microorganisms in the soil of the stipa baicalensis steppe. Front. Microbiol. 12, 570908 https://doi.org/10.3389/fmicb.2021.570908.

Qiu, Z., Yinghua, Z., Xiu, Z., Jing, S., 2020. Effects of biochar on bacterial genetic diversity in soil contaminated with cadmium. Soil Use Manag. 37, 289–298. https://doi.org/10.1111/sum.12678.

R Core Team, 2022. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Rajapaksha, R.M.C.P., Tobor-Kapłon, M.A., Bååth, E., 2004. Metal toxicity affects fungal and bacterial activities in soil differently. Appl. Environ. Microbiol. 70, 2966–2973. https://doi.org/10.1128/AEM.70.5.2966-2973.2004.

Sanchez, G., Trinchera, L., Russolillo, G., 2015. plspm: Tools for Partial Least Squares Path Modeling.

Šantrůčková, H., Kotas, P., Bárta, J., Urich, T., Čapek, P., Palmtag, J., et al., 2018. Significance of dark CO_2 fixation in arctic soils. Soil Biol. Biochem. 119, 11–21. https://doi.org/10.1016/j.soilbio.2017.12.021.

Stein, S., Selesi, D., Schilling, R., Pattis, I., Schmid, M., Hartmann, A., 2005. Microbial activity and bacterial composition of H_2-treated soils with net CO_2 fixation. Soil Biol. Biochem. 37, 1938–1945. https://doi.org/10.1016/j.soilbio.2005.02.035.

Tang, H., Li, C., Shi, L., Cheng, K., Wen, L., Li, W., et al., 2022. Effects of different short-term tillage managements on rhizosphere soil autotrophic CO_2-fixing bacteria in a double-cropping rice paddy field. Environ. Microbiol. Rep. 14, 245–253. https://doi.org/10.1111/1758-2229.13044.

Thingstad, T.F., Våge, S., Storesund, J.E., Sandaa, R.-A., Giske, J., 2014. A theoretical analysis of how strain-specific viruses can control microbial species diversity. Proc. Natl. Acad. Sci. U. S. A. 111, 7813–7818. https://doi.org/10.1073/pnas.1400909111.

Tolli, J., King, G.M., 2005. Diversity and structure of bacterial chemolithotrophic communities in pine forest and agroecosystem soils. Appl. Environ. Microbiol. 71, 8411–8418. https://doi.org/10.1128/AEM.71.12.8411-8418.2005.

Wang, J., Xie, J., Li, L., Luo, Z., Zhang, R., Wang, L., et al., 2021a. The impact of fertilizer amendments on soil autotrophic bacteria and carbon emissions in maize field on the semiarid loess plateau. Front. Microbiol. 12, 664120 https://doi.org/10.3389/fmicb.2021.664120.

Wang, X., Li, W., Xiao, Y., Cheng, A., Shen, T., Zhu, M., et al., 2021b. Abundance and diversity of carbon-fixing bacterial communities in karst wetland soil ecosystems. Catena 204, 105418. https://doi.org/10.1016/j.catena.2021.105418.

White, J.R., Nagarajan, N., Pop, M., 2009. Statistical methods for detecting differentially abundant features in clinical metagenomic samples. PLoS Comput. Biol. 5, e1000352.

Wu, X., Ge, T., Wang, W., Yuan, H., Wegner, C.E., Zhu, Z., et al., 2015. Cropping systems modulate the rate and magnitude of soil microbial autotrophic CO_2 fixation in soil. Front. Microbiol. 6, 379. https://doi.org/10.3389/fmicb.2015.00379.

Wu, X., Ge, T., Yan, W., Zhou, J., Wei, X., Chen, L., et al., 2017. Irrigation management and phosphorus addition alter the abundance of carbon dioxide-fixing autotrophs in phosphorus-limited paddy soil. FEMS Microbiol. Ecol. 93, fix154 https://doi.org/10.1093/femsec/fix154.

Wyszkowska, J., Borowik, A., Kucharski, M., Kucharski, J., 2013. Effect of cadmium, copper and zinc on plants, soil microorganisms and soil enzymes. J. Elementol. 18, 769–796. https://doi.org/10.5601/jelem.2013.18.4.455.

Xiao, K.-Q., Bao, P., Bao, Q.-L., Jia, Y., Huang, F.-Y., Su, J.-Q., et al., 2014. Quantitative analyses of ribulose-1,5-bisphosphate carboxylase/oxygenase (RubisCO) large-subunit genes (*cbbL*) in typical paddy soils. FEMS Microbiol. Ecol. 87, 89–101. https://doi.org/10.1111/1574-6941.12193.

Xiao, K.-Q., Ge, T.-D., Wu, X.-H., Peacock, C.L., Zhu, Z.-K., Peng, J., et al., 2021. Metagenomic and ^{14}C tracing evidence for autotrophic microbial CO_2 fixation in paddy soils. Environ. Microbiol. 23, 924–933. https://doi.org/10.1111/1462-2920.15204.

Yan, H., Niu, Q., Zhu, Q., Wang, S., Meng, Q., Li, G., et al., 2021. Biochar reinforced the populations of *cbbL*-containing autotrophic microbes and humic substance formation via sequestrating CO_2 in composting process. J. Biotechnol. 333, 39–48. https://doi.org/10.1016/j.jbiotec.2021.04.011.

Yuan, H., Ge, T., Chen, X., Liu, S., Zhu, Z., Wu, X., et al., 2015. Abundance and diversity of CO_2-assimilating bacteria and algae within red agricultural soils are modulated by changing management practice. Microb. Ecol. 70, 971–980. https://doi.org/10.1007/s00248-015-0621-8.

Yuan, H., Ge, T., Wu, X., Liu, S., Tong, C., Qin, H., et al., 2012. Long-term field fertilization alters the diversity of autotrophic bacteria based on the ribulose-1,5-biphosphate carboxylase/oxygenase (RubisCO) large-subunit genes in paddy soil. Appl. Microbiol. Biotechnol. 95, 1061–1071. https://doi.org/10.1007/s00253-011-3760-y.

Yuan, H., Ge, T., Zou, S., Wu, X., Liu, S., Zhou, P., et al., 2013. Effect of land use on the abundance and diversity of autotrophic bacteria as measured by ribulose-1,5-biphosphate carboxylase/oxygenase (RubisCO) large subunit gene abundance in soils. Biol. Fertil. Soils 49, 609–616. https://doi.org/10.1007/s00374-012-0750-x.

Zhao, K., Kong, W., Wang, F., Long, X.-E., Guo, C., Yue, L., et al., 2018. Desert and steppe soils exhibit lower autotrophic microbial abundance but higher atmospheric CO_2 fixation capacity than meadow soils. Soil Biol. Biochem. 127, 230–238. https://doi.org/10.1016/j.soilbio.2018.09.034.

Zheng, Z., Liu, B., Fang, X., Fa, K., Liu, Z., 2022. Dryland farm soil may fix atmospheric carbon through autotrophic microbial pathways. Catena 214. https://doi.org/10.1016/j.catena.2022.106299.

Zhou, Z.-F., Wei, W.-L., Shi, X.-J., Li, Y.-M., Liu, X.-H., Wang, M.-X., 2019. Twenty-six years of chemical fertilization decreased soil RubisCO activity and changed the ecological characteristics of soil *cbbL*-carrying bacteria in an entisol. Appl. Soil Ecol. 141, 1–9. https://doi.org/10.1016/j.apsoil.2019.05.005.