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Responses of Labile Organic Carbon and Extractable Cadmium Fractions in an Agricultural Soil Following Long-Term Repeated Application of Pig Manure and Effective Microbes

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Abstract

Long-term pig manure addition has been widely applied in red soil to improve soil fertility. However, the influence of combined utilization of pig manure and effective microbes (EM) on soil organic carbon (SOC) and Cd are not well understood. This study conducted a 23-year (1996–2019) long-term fertilization field trial to investigate the changes of different fractions of SOC and Cd under chemical fertilization (CF), pig manure (PM), and pig manure with effective microbes (PM + EM) treatments in an agricultural soil of Jiangxi Province, South China. The results showed that the pig manure addition significantly enhanced the contents of SOC and Cd in the soils compared with the CF treatments. Furthermore, with the increment of SOC, the PM + EM treatment significantly increased the contents of soil microbial biomass carbon, dissolved organic carbon and easily oxidizable carbon compared with the pig manure application alone. Meanwhile, compared with the CF treatments, the EM addition significantly enhanced the exchangeable and oxidizable fractions of Cd, thus the potential Cd environment risk due to pig manure application should be carefully assessed.

Keywords Pig manure · Effective microbes · Soil labile organic carbon · Cadmium fraction · Long-term repeated fertilization

Manure addition, especially combination with other effective materials, is among the most important measures for improving soil fertility and sustainability (Wang et al. 2019; Qaswar et al. 2021). Wang et al. (2019) and Choudhary et al. (2021) reported that 21-year long-term balanced application of pig manure with common nitrogen, phosphorus and potassium compound fertilizer (NPK) could improve SOC content, SOC mineralization rate, soil microbial biomass and enzyme activities, and crop yields compared with other fertilization strategies in red soil. In addition, other concoctions, such as pig manure combined with both straw and NPK (Tong et al. 2014) or with biochar particles (Shin et al. 2019) could also efficiently improve SOC sequestration, soil fertility and crop yields (Shin et al. 2019; Qaswar et al. 2021).

Effective microbes (EM) are generally a mixed culture of naturally occurring beneficial microorganisms comprising *Rhizobium*, photosynthetic bacteria, *Acetobacter*, *Lactobacillus*, *Bacillus*, and *Actinomycetes* (Chen et al. 2018). Combining EM with pig manure application in soils can boost crop production and mitigate soil-borne diseases (Xiong et al. 2014). In addition, soil microbial communities related to element cycling and plant growth increased significantly under EM application (Chen et al. 2018). Since microbial carbon has been identified as a key factor that regulating SOC accumulation in cropland (Wang et al. 2021), EM can theoretically promote microbial derived carbon content and the contents of soil labile carbon fractions, such as soil dissolved organic carbon (DOC), microbial biomass carbon (MBC) and easily oxidized organic carbon (EOC), which can be effectively mineralized and used by crops. Moreover, due to the long-term pig manure application, cadmium (Cd) pollution has been a serious issue in manure fertilized

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croplands (Zhou et al. 2021). Excessive inputs of pig manure may increase environmental risks of heavy metals and thus threaten crop safety (Wang et al. 2020; Gao et al. 2021). Although either SOC sequestration or heavy metal contamination by manure application is well documented (Craig et al. 2021; Wang et al. 2021), the responses of long-term pig manure mixed with EM treatment on changes of both SOC and Cd in soils remain unclear.

Red soils are distributed mainly in warm, humid climate regions (West et al. 1997). Due to intense leaching together with desilicification and allitization, red soils are highly weathered and usually have low SOC content and soil fertility (Tian et al. 2021). The application of pig manure combination of EM is a promising fertilization method to improve soil quality and increase crop yields (especially peanuts) in the red soil region (Chen et al. 2018; Wang et al. 2020). The aims of this study are to investigate the effects of pig manure application with EM on the variations of 1) the contents of SOC and labile carbon (DOC, MBC and EOC) and 2) the Cd concentrations and its fractions in red soil. We conducted a 23-year long-term fertilization of chemical fertilizer (CF), organic pig manure fertilizer (PM), and together with effective microbes (PM + EM) in a typical red soil field trial located in southern China. The results will improve our understandings of how the long-term pig manure mixed with EM affects the labile carbon and Cd fractions in soils.

Materials and Methods

The long-term fertilization experiment was performed at the Ecological Experimental Station of Red Soil, Chinese Academy of Sciences, which is located in Yingtan, Jiangxi Province, China (28°15′ 20″ N, 116° 55′ 30″ E). The soil is classified as Typic Plinthudult (Ultisols) according to the USDA soil taxonomy, with soil pH 3.9, organic carbon 4.19 g kg^{-1}, total nitrogen 0.6 g kg^{-1}, total phosphorus 0.2 g kg^{-1}, total potassium 14.5 g kg^{-1}.

The study included three fertilizing treatments, namely, chemical fertilizer (CF, as a control), pig manure (PM) and PM mixed with EM (PM + EM). In the chemical fertilizer plot (CF), nitrogen, phosphorus and potassium source were applied as urea, calcium magnesium phosphate, and potassium chloride, respectively, and the final contents of these three nutrients were 45, 45, and 135 kg ha^{-1}, respectively. In the PM plots, pig manure purchased from a pig farm in the vicinity was used annually after fermentation. The average composition of the pig manure was (in g kg^{-1}) organic carbon 349.2, nitrogen 26.7, phosphorus 18.3, potassium 53.1, with a pH of 8.5. The application dosage of pig manure was 1.69 t ha^{-1} (dry weight basis). In the PM + EM plots, the application amount of EM was 20.55 L ha^{-1} (1×10^9 cfu mL^{-1}). Because the total phosphorus and potassium contents of pig manure were insufficient to reach the final rates of phosphorus and potassium in the chemical fertilization, an extra 14 kg phosphorus ha^{-1} of calcium magnesium phosphate and 45 kg potassium ha^{-1} of potassium chloride were added to the PM and PM + EM plots as well. The fertilization experiment was conducted in 1996, and the fertilizers were applied before sowing each year.

In March 2019, the test soils were collected before the peanuts were sown from each plot using a 5-cm-diameter auger. Five soil samples were randomly taken from each plot to make one composite sample (approximately 1 kg). Each soil sample was divided into two parts: one part was stored at 4 °C, and the other part was air-dried and passed through a 2 mm sieve after removal of impurities.

Soil organic carbon (SOC) was measured via a CNS element analyzer (Elementar, vario MACRO cube, Germany). Soil pH was measured in a 1:2.5 (w/v) aqueous suspension by a digital pH meter (pHS-3C, Shanghai INESA Scientific Instrument Co., Ltd., China). The total nitrogen (TN) was determined by the Kjeldahl method, the total phosphorus (TP) was determined by H_2SO_4-$HClO_4$ digesition (Yang et al. 2022). DOC was extracted by deionized water with the soil to water ratio of 1:5 and measured by the TOC analyzer (TOC-VCPH, Shimadu, Japan). MBC was determined using the chloroform fumigation extraction methods and calculated as DOC differences between fumigated and non-fumigated samples divided by a factor of 0.45 (Jenkinson and Powlson, 1976). EOC was measured using the 333 mmol L^{-1} $KMnO_4$ oxidation method and calculated the consumption of $KMnO_4$ by using a spectrophotometer at 565 nm (Blair et al. 1995).

The total soil Cd content was determined following the HNO_3–$HClO_4$–HF digesition (Byrnes et al. 2018). A three-step sequential extraction BCR method (Rauret et al. 2000) was used to analyze Cd distribution in treated soils. Four operationally defined fractions were determined for Cd inculding exchangeable and acid-soluble fraction (F1), reducible fraction (F2), oxidizable fraction (F3) and residual fraction (F4). The concentrations of Cd in the extracted solutions were measured using an inductively-coupled plasma mass spectrometry (ICP-MS; PerkinElmer Ltd., ELAN DRC II, USA).

All data were analyzed using one-way analysis of variance (ANOVA) at a significance level of $p < 0.05$ using SPSS version 23.0. Duncan's post-hoc test was performed to detect significant differences between the means of different treatments.

Results and Discussion

Pig Manure Combined with Effective Microbes Increased SOC, Nitrogen, Phosphorus and pH

Over two decades of PM and PM + EM treatments significantly increased the SOC and nutrient contents compared to the CF plots (Table 1). The effects under long-term PM + EM treatment were more significant than those in PM plots. The SOC contents in the soils were ranked as PM + EM > PM > CF. Compared with CF, SOC were improved by 17.5% and 25.0% in PM and PM + EM treatments, respectively. There were two main ways to increase SOC by long-term applications of animal manure or combined with mineral fertilizers: by adding organic matter directly and by increasing organic matter in crop residues due to higher crop yields indirectly (Tong et al. 2014; Chen et al. 2021; Mustafa et al. 2021). On the one hand, organic fertilizers as carbon source materials annually input into the soil over 23 years had continuously greater positive effects than the control plots on SOC accumulation. On the other hand, previous studies showed that pig manure amendments could significantly improve crop yields (Du et al. 2020). As Chen et al. (2018) recorded in the same study area, the peanut crop yields under continuous PM + EM application were significantly higher than that in the CF and PM plots. Thus, more crop biomass would undoubtedly result in more residues return to the soils of organic fertilization-treated plots. However, the differences in SOC between PM and PM + EM were insignificant, indicating that pig manure was the major contributor to SOC accumulation in the soil. Although there were not significant differences of soil C:N ratios among fertilization treatments due to the study plots had uniform geomorphologic conditions, they gradually decreased from CF, PM to PM + EM treatments (Table 1). This result suggested that the rate of SOC decomposition was slower than its accumulation in organic fertilizers with EM application (Luo et al. 2020; Qaswar et al. 2021), and indicated that the PM + EM treatment may be a more effective method for SOC sequestration than the PM treatment. Additionally, the data in this study also showed that soil pH, TP and TN increased progressively from CF and PM to PM + EM (Table 1). The results were consistent with previous studies (Du et al. 2020; Li et al. 2020), indicating that pig manure fertilizers could make the soil more fertile than chemical fertilizers and the EM had a more apparent influence.

Effects of Pig Manure with Effective Microbes on the Labile Organic Carbon Fractions in Soil

The contents of all three labile carbon fractions decreased gradually from PM + EM, PM to CF (Fig. 1). Continuous pig manure combined with EM fertilization significantly increased both the contents of labile carbon fractions (MBC, DOC and EOC) and their proportions in SOC (Fig. 1; Table 2). Continuous organic fertilization of pig manure with and without EM considerably increased the MBC content in soil by 96.2% and 168.4% compared to the CF plot (Fig. 1a). Meanwhile, the PM + EM plot had 36.8% higher MBC than the PM treated soils. The reasons behind the increment of MBC may be attributed to two aspects: (1) the addition of EM may be directly contributed to part of the microbial biomass, and (2) more nutrients (e.g. phosphorus) can stimulate soil microbes survival and plant growth, thus promoting the accumulation of MBC in the PM + EM treated soil (Tamilselvi et al. 2015). The contents of DOC in the soils from the CF, PM and PM + EM treatments were 11.79, 89.99 and 112.82 mg kg^{-1}, which accounted for 0.17%, 1.08% and 1.29% of SOC, respectively (Table 2).

Compared to the CF treated soils, the DOC proportions in SOC increased approximately six-fold in the PM and PM + EM treatments (Fig. 1b). A significant positive correlation between the contents of DOC and the SOC was observed ($r = 0.80$, $p < 0.01$), implying that DOC was probably a determinant of the production of SOC, which was consistent with the findings of Xu et al. (2018). Moreover, DOC is mainly derived from the decomposition of soil organic matter driven by soil microbes (Li et al. 2018). In this study, the higher MBC content in PM + EM was in accordance with its higher DOC content, which increased by 23.4% compared with the PM treatment.

The contents of EOC in the soils from the CF, PM and PM + EM treatments were 1.23, 1.64 and 1.76 mg g^{-1} (Fig. 1c), which accounted for 17.4, 19.7 and 20.0% of the SOC, respectively (Table 2). Compared to the CF treatments, the PM and PM + EM treatments significantly increased approximately 10% of the EOC proportions in SOC. EOC as a component of soil microbial biomass is mainly made up of amino acids, simple carbohydrates, and other simple organic molecules (Li et al. 2018). Because the MBC content was significantly improved in PM + EM treated soils, there was a significant enhancement of EOC content under the EM addition.

Table 1 Soil organic carbon and nutrients contents and pH values under different long-term fertilization treatments

Treatments	SOC (g kg^{-1})	TN (g kg^{-1})	C:N mass ratio	TP (g kg^{-1})	pH (1:2.5 H_2O)
CF	7.08 ± 0.07b	0.82 ± 0.01b	8.63 ± 0.19a	0.34 ± 0.04c	5.13 ± 0.02b
PM	8.32 ± 0.57a	1.02 ± 0.08a	8.16 ± 0.25a	0.88 ± 0.19b	6.30 ± 0.07a
PM + EM	8.85 ± 0.25a	1.11 ± 0.02a	7.97 ± 0.06a	1.13 ± 0.03a	6.35 ± 0.05a

Different letters indicate significant differences among treatments at $p < 0.05$ (Duncan's test)

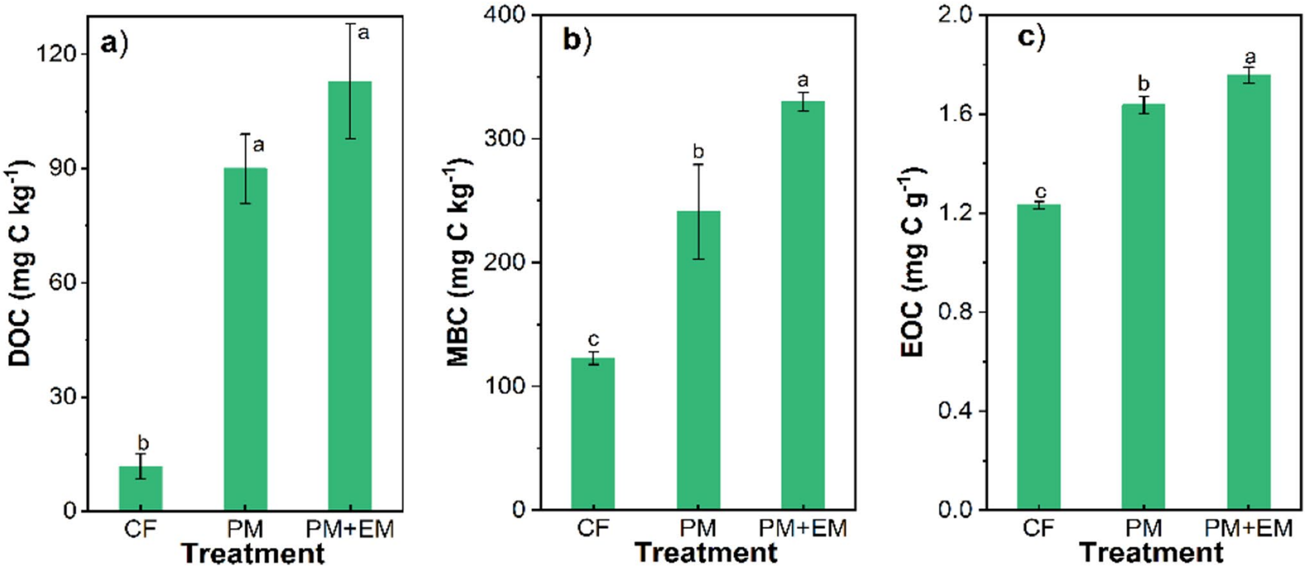

Fig. 1 Contents of labile SOC fractions of **a** DOC (dissolved organic carbon), **b** MBC (microbial biomass carbon), and **c** EOC (easily oxidizable carbon) under different long-term fertilization treatments. Vertical bars denote the standard errors of the mean values (n = 4). Different letters indicate significant differences among treatments at $p < 0.05$ (Duncan's test)

Table 2 The percentages of labile organic carbon (DOC, MBC and EOC) and extractable cadmium fractions (F1, F2, F3 and F4) in the soil

Treatments	DOC/SOC (%)	MBC/SOC (%)	EOC/SOC (%)	F1 (%)	F2 (%)	F3 (%)	F4 (%)
CF	0.17 ± 0.05b	1.74 ± 0.08c	17.41 ± 0.25c	23.00 ± 1.66b	1.87 ± 0.23a	24.54 ± 1.90a	50.68 ± 3.40a
PM	1.08 ± 0.04a	2.88 ± 0.26b	19.73 ± 0.95ab	46.24 ± 1.65a	1.14 ± 0.19a	9.75 ± 0.95b	42.86 ± 2.79a
PM + EM	1.29 ± 0.20a	3.74 ± 0.15a	19.95 ± 0.85a	43.57 ± 2.73a	1.05 ± 0.18a	11.85 ± 0.61b	43.53 ± 2.98a

Different letters indicate significant differences among treatments at $p < 0.05$ (Duncan's test)

Effects of Pig Manure with Effective Microbes on Total and Extractable Fractions of Cd in Soil

Cd pollution is a serious issue in long-term pig manure fertilized croplands (Zhou et al. 2021). The results clearly showed that the total content of Cd significantly increased in pig manure treated soils (PM and PM + EM) compared with CF (Fig. 2a), exceeding the soil environmental quality risk screening standard (0.3 mg kg^{-1}, $5.5 < pH \leq 7.5$) for soil contamination of agricultural land in China (GB 15618–2018) by 3.3–3.5 times. Wang et al. (2020) also reported that the Cd content (7.91 mg kg^{-1}) in PM applied in 2012 exceeded the Chinese Organic Fertilizer Standard (NY525-2012) by 126%. The long-term pig manure fertilization could be considered as a primary reason for the increase of total Cd in soils due to the significant positive correlation between the contents of Cd and SOC ($r = 0.91$, $p < 0.01$) (Fan et al. 2017). However, the differences in total Cd between PM and PM + EM were insignificant, indicating that pig manure was the major contributor to Cd accumulation in the soil.

Besides the total concentrations, the chemical fractions of Cd were also affected by the SOC content and other soil properties (Yang et al. 2021). The results obtained from the BCR sequential extraction scheme were shown in Fig. 2b. The acid soluble/exchangeable fraction (F1) and reducible fraction (F2) are normally considered as the bioavailable fractions, while the oxidizable fraction (F3) and residual fraction (F4) are generally considered as stable phases (Gao et al. 2021). In PM and PM + EM treated soils, the F1 (~ 0.70 mg kg^{-1}) and F2 (~ 0.01 mg kg^{-1}) fractions accounted for over 40% of the total Cd (Table 2). Meanwhile, the proportions of F1 and F2 in PM and PM + EM dramatically improved by over 90% compared with CF treatments. No significant variations ($p > 0.05$) of these two fractions were observed between PM and PM + EM (Table 2). The results indicated that the long-term application of pig manure could increase Cd bioavailability (Gao et al. 2021), however, the EM amendment may not further stimulate Cd mobility. In contrast, Cd was mainly bound in stable phases (F3 and F4), which occupied more than 50% of the total Cd in all the three treatments. However, compared with the CF

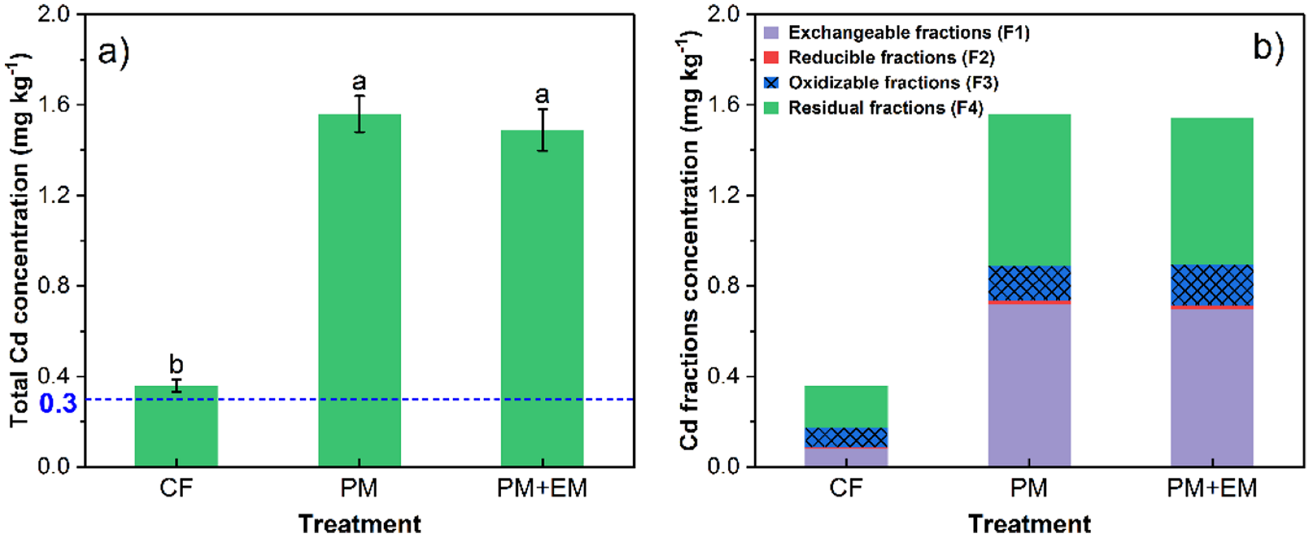

Fig. 2 Concentrations of **a** total Cd and **b** BCR fractions of Cd under different long-term fertilization treatments. Vertical bars denote the standard errors of the mean values (n = 4). Different letters indicate significant differences among treatments at $p < 0.05$ (Duncan's test). The blue dot line in **a** is the soil environmental quality risk screening standard (0.3 mg kg^{-1}, $5.5 < pH \leq 7.5$) for soil contamination of agricultural land in China (GB 15618–2018)

treatment, the contents of F3 and F4 fractions increased by 51.5%–96.0% in PM and PM + EM treated soils. Notably, the F3 proportion under the EM addition was 2.21% higher than the PM treatment (Table 2). This increment was possibly because of the introduction of a large amount of SOC in PM + EM treatment. Organic matter-rich materials could be a Cd stabilizer, and thus, the organic matter-rich materials could be a potentially reasonable choice for the remediation of Cd-contaminated soils (Yang et al. 2022). Pig manure can significantly enhance SOC in red soils, and Gao et al. (2021) also reported that Cd was strongly bound to organic matter in red soil. In this study, Cd content in F3 fraction increased by approximately 105.9% under long-term PM + EM fertilization compared to the CF treatment.

However, a note of caution that the bioavailable Cd fractions (F1 and F2) also greatly increased both the contents (Fig. 2b) and their proportions in total Cd (Table 2). The results indicated that Cd in the pig manure was still highly bioavailable and presented a potential environmental risk. Hence, in order to control Cd accumulation and its entry into the food chain, it is necessary to restrain the source of Cd by pretesting the pig manure at low concentrations for fertilization.

Conclusions

Our study indicated that application of pig manure over two decades could improve the contents of SOC and its labile fractions. Furthermore, the addition of EM to pig manure (PM + EM) was likely to be more efficient than pig manure alone in enhancing the contents of labile organic carbon fractions and promoting SOC sequestration. However, 23 years of pig manure amendment significantly increased soil total Cd and exchangeable and oxidizable Cd fractions. Although EM addition improved the proportion of stable Cd, the Cd environment risk caused by pig manure may hardly be ignored.

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