

Article

Effects of Different Long-Term Fertilizer Management Systems on Soil Microbial Biomass Turnover in a Double-Cropping Rice Field in Southern China

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Abstract: Soil microbial biomass content is usually regarded as an early indicator of changes in soil quality and soil fertility in paddy fields. Soil microbial biomass turnover is mainly influenced by the application of different fertilizer management systems. However, there is still a need to further investigate the effects of different long-term fertilizer management systems on soil microbial biomass turnover in paddy fields under the double-cropping rice (*Oryza sativa* L.) system. Therefore, the effects of different long-term (36 years) fertilizer practices on soil microbial biomass carbon (SMBC) and soil microbial biomass nitrogen (SMBN) contents, and the flux turnover rates of SMBC and SMBN at the 0–10 cm and 10–20 cm layers in a double-cropping rice field in southern China were investigated in the present paper. The field experiment included four different fertilizer treatments: MF, RF, OM, and CK. The results showed that SMBC and SMBN contents at the 0–10 cm and 10–20 cm soil layers with RF and OM treatments were increased compared with the MF and CK treatments. Compared with the CK treatment, SMBC contents at the 0–10 cm and 10–20 cm soil layers with RF and OM treatments increased by 35.72% and 50.28%, and 32.29% and 42.77%, respectively. SMBN contents at the 0–10 cm and 10–20 cm soil layers with RF and OM treatments increased by 15.52% and 22.70%, and 16.32% and 21.49%, respectively. The fluxes of SMBC and SMBN at the 0–10 cm and 10–20 cm soil layers with RF and OM treatments were significantly higher than those of the CK treatment. This result indicated that the flux turnover rates of SMBC and SMBN at the 0–10 cm and 10–20 cm soil layers with the MF, RF, and OM treatments were significantly higher than those of the CK treatment. Compared with the CK treatment, the flux turnover rates of SMBC and SMBN at the 0–10 cm and 10–20 cm soil layers with OM treatment increased by 46.10% and 48.59%, and 73.39% and 116.67%, respectively. SMBC and SMBN contents, and the flux turnover rates of SMBC and SMBN at the 0–10 cm layer were higher than those of the 10–20 cm layer under the same fertilizer treatment condition. Early rice and later rice yields with RF and OM treatments were significantly higher than those of the MF and CK treatments. As a result, the combined application of crop residue and organic manure with inorganic fertilizer management is a beneficial practice for increasing soil nutrients and rice yield under the double-cropping rice system in southern China.

Keywords: fertilizer; soil microbial biomass carbon; soil microbial biomass nitrogen; microbial biomass turnover



Citation: Tang, H.; Xu, Y.; Li, C.; Shi, L.; Wen, L.; Li, W.; Cheng, K.; Xiao, X. Effects of Different Long-Term Fertilizer Management Systems on Soil Microbial Biomass Turnover in a Double-Cropping Rice Field in Southern China. *Agriculture* **2022**, *12*, 1662. <https://doi.org/10.3390/agriculture12101662>

Academic Editor: Riccardo Scotti

Received: 9 September 2022

Accepted: 4 October 2022

Published: 11 October 2022

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1. Introduction

Soil microbial biomass (SMB) plays an important role in regulating soil nutrient, including soil carbon (C), nitrogen (N), phosphorus (P), sulfur (S) and so on [1]. It is generally believed that the SMB content is regarded as an early indicator of changes of soil quality and soil fertility as it is sensitive to external environmental change [1]. The SMB content (soil microbial biomass carbon (SMBC) and soil microbial biomass nitrogen (SMBN)) is more sensitive to variation of the soil environment [2], which are frequently

influenced by different field management systems, such as cropping systems, tillage, crop residue, fertilizer regimes, and so on [3,4].

In recent years, it has mainly been accepted that SMB content is closely related with its turnover in agricultural soil, which is mainly influenced by the application of different types of fertilizer management [5]. In order to improve soil productivity in paddy fields, soil microbial processes play vital roles in increasing C and N converted into soil organic matter (SOM) under different fertilizer conditions [6]. In a previous study, research showed that SMBC and SMBN contents were increased under the application of a mineral fertilizer condition [7], but other studies indicated that SMB content was not significantly affected by mineral fertilizer treatment, and even that soil microbial diversity was negatively impacted by N fertilizer treatment [8]. Moreover, many results indicated that SMBC and SMBN contents were increased through the application of organic manure or organic manure with inorganic fertilizer, and even that soil microbial diversity was positively impacted by organic manure or organic manure with inorganic fertilizer treatments [6,9]. Many studies were conducted to explore the effects of different fertilizer management practices on SMBC and SMBN contents in paddy fields [10,11], but there is still a need to further study the effects of different long-term fertilizer treatments on SMB content and its turnover in the double-cropping rice field.

The double-cropping rice (*Oryza sativa* L.) system (early rice and late rice) is the mainly cropping system in southern China [12]. It is a beneficial practice for maintaining or increasing soil quality and fertility in paddy fields via the combined application of organic fertilizer and inorganic fertilizer [5]. In a previous study, our results indicated that soil organic carbon (SOC) and SMB content at the soil plough layer were clearly influenced by the use of different fertilizer management systems [10,13]. However, there is little information about the effects of different long-term fertilizer management systems on SMB content and its turnover at the 0–10 cm and 10–20 cm soil layers in southern China. We hypothesized that: (i) the SMB content is increased via the application of organic matter with chemical fertilizer; and (ii) the flux and turnover rate of SMBC and SMBN in paddy fields are changed by the combined application of organic matter with chemical fertilizer. Therefore, the aims of this study were: (1) to analysis the changes of SMB content at the 0–10 cm and 10–20 cm soil layers with different fertilizer treatments; and (2) to investigate the effects of different fertilizer treatments on the flux and turnover rate of SMBC and SMBN in the double-cropping rice field.

2. Materials and Methods

2.1. Sites and Cropping System

The field experiment began in 1986. It was located in Ningxiang City (28°07' N, 112°18' E) in Hunan Province, China. More detailed information about the climatic conditions during the field experiment, the region, and the soil physical and chemical properties at the 0–20 cm layer at the beginning of this field experiment and the cropping system was described by Tang et al. (2018) [14].

2.2. Experimental Design

The field experiment included four fertilizer treatments: chemical fertilizer alone (MF), rice straw and chemical fertilizer (RF), 30% organic manure and 70% chemical fertilizer (OM), and without fertilizer input as a control (CK). A randomized block design was applied with each plot, with three replications of each fertilizer treatment. The area of each plot was 66.7 m² (10 m × 6.67 m), and the distance between different plots was 20 cm. The cropping system of this experimental field was barley–early rice–late rice. During the early rice growth period, the total amount of N, phosphorus pentoxide (P₂O₅), and potassium oxide (K₂O) for MF, RF, and OM treatments was 142.5, 54.0, and 63.0 kg ha⁻¹. During the barley and late rice growth periods, the total amount of N, P₂O₅, and K₂O for the MF, RF, and OM treatments was 157.5, 43.2, and 81.0 kg ha⁻¹, respectively. The kind of organic manure for the OM treatment was decomposed chicken manure. More detail

information about fertilizer management and other field management was described by Tang et al. (2018) [14].

2.3. Soil Sampling

Soil samples were collected at the maturity stage of late rice in October 2021. Soil samples close to rice plants were sampled at the 0–10 cm and 10–20 cm soil layers in the paddy field. Correspondingly, one soil composite from ten points was collected from each plot. Thus, three soil composite samples were collected for each fertilizer treatment. The fresh soil sample was transported to the laboratory. After removing the visible organic material, stones, and rice root by hand, the soil samples were then air dried at room temperature and sieved through a 2 mm sieve for chemical analysis in the laboratory.

2.4. Soil Laboratory Analysis

2.4.1. Soil Bulk Density

Soil bulk density (BD) at the 0–10 cm and 10–20 cm layers was determined by using a metallic core of a known volume (having a 10 cm internal diameter and a 20.0 cm length). These soil samples were oven dried at 105 °C for 24 h to a constant weight for calculating the dry weight of the soil samples [15].

2.4.2. Soil Microbial Biomass Content

Soil microbial biomass carbon (SMBC) and soil microbial biomass nitrogen (SMBN) contents were measured using the fumigation–extraction method as described by Wu et al. (1990) [16]. Briefly, each soil sample was divided into two parts; one part was fumigated for 24 h with ethanol-free chloroform, and the other part was unfumigated as a control. The fumigated and unfumigated soil samples were shaken for 1 h with 0.5 M K₂SO₄ and then centrifuged and filtered.

2.4.3. Flux Turnover Rate of SMBC and SMBN

The flux turnover rates of SMBC and SMBN with all soil sample were calculated according to the following equation:

$$\text{Flux of SMBC (SMBN)} \text{ (g m}^{-2}\text{a}^{-1}\text{)} = \frac{\text{SMBC (SMBN)} \times \text{BD} \times \text{depth coefficient}}{\text{Turnover time}}$$

$$\text{Flux turnover rate of SMBC (SMBN)} \text{ (a}^{-1}\text{)} = \frac{\text{Sum of dynamic decrease (or increase) of SMBC (SMBN) in a year}}{\text{Average value of SMBC (SMBN) in a year}}$$

$$\text{Fluxturnover time of SMBC (SMBN)} \text{ (a}^{-1}\text{)} = \frac{1}{\text{Flux turnover rate of SMBC (SMBN)}}$$

where the depth coefficient every 10 cm of sampling depth was 1.

2.4.4. Yield of Early Rice and Later Rice

At the maturity stages of early rice and late rice, rice yields were investigated from a 1 m² sampling area in each plot at harvest and were expressed as rough (unhulled) rice at 14% moisture content.

2.5. Statistical Analysis

The data of each of the measured items with different fertilizer treatment means were compared by using a one-way analysis of variance (ANOVA) following standard procedures at the 5% probability level. All the statistical analyses in the present paper were conducted using the SAS 9.3 software package [17]. The results of each measured item with all fertilizer treatments were expressed as mean and standard error.

3. Results

3.1. SMBC and SMBN Contents

Compared with the MF and CK treatments, the SMBC contents at the 0–10 cm and 10–20 cm soil layers with RF and OM treatments were significantly increased ($p < 0.05$). At the 0–10 cm and 10–20 cm soil layers, the SMBC contents with the MF, RF and OM treatments were significantly higher ($p < 0.05$) than those of CK treatments. However, there were no significant ($p > 0.05$) differences in SMBC contents at the 0–10 cm and 10–20 cm soil layers between RF and OM treatments. The result indicated that the SMBC contents at the 0–10 cm soil layer with the MF, RF, OM and CK treatments were higher than those of the 10–20 cm layer. Compared with the CK treatment, the SMBC contents at the 0–10 cm and 10–20 cm soil layers with RF and OM treatments increased by 35.72% and 50.28%, and 32.29% and 42.77%, respectively (Figure 1a).

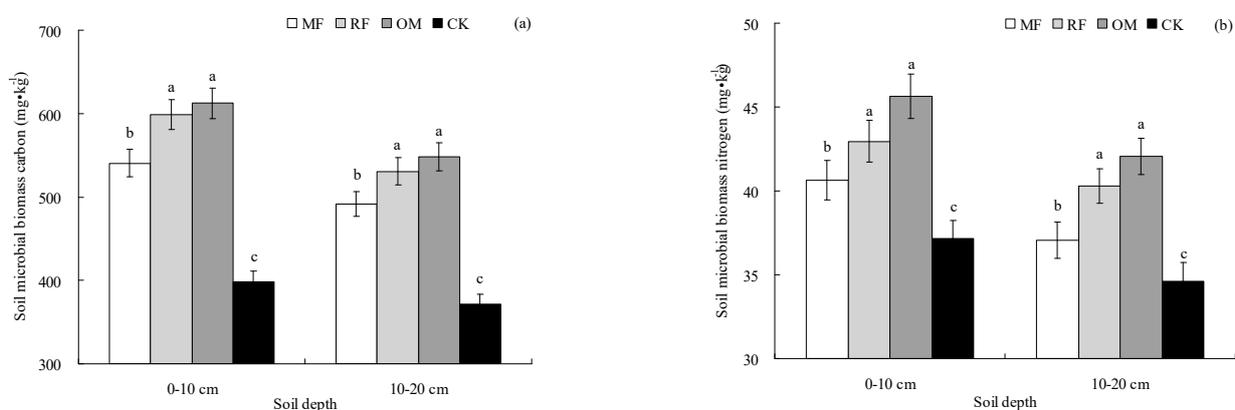


Figure 1. Effects of different long-term fertilizer treatments on soil microbial biomass carbon (a) and nitrogen (b) contents in the double-cropping rice field. MF: chemical fertilizer alone; RF: rice straw residue and chemical fertilizer; OM: 30% organic manure and 70% chemical fertilizer; CK: without fertilizer input as a control. Different lowercase letters indicate significant differences ($p < 0.05$) among different fertilizer treatments.

Compared with the CK treatment, the soil microbial biomass nitrogen (SMBN) content at the 0–10 cm and 10–20 cm soil layers with the MF, RF, and OM treatments were significantly increased ($p < 0.05$). There were no significant ($p > 0.05$) differences in SMBN contents at the 0–10 cm and 10–20 cm soil layers between RF and OM treatments. The result indicated that the SMBN contents at the 0–10 cm soil layer with the MF, RF, OM and CK treatments were higher than those of the 10–20 cm soil layer. Compared with the CK treatment, the SMBN content at the 0–10 cm soil layer with the MF, RF, and OM treatments increased by 9.25%, 15.52%, and 22.70%, respectively. The SMBN content at the 10–20 cm soil layer with the MF, RF, and OM treatments increased by 7.02%, 16.32%, and 21.49%, compared with the CK treatment, respectively (Figure 1b).

3.2. Flux of SMBC and SMBN

Compared with the CK treatments, the fluxes of SMBC at the 0–10 cm and 10–20 cm soil layers with RF and OM treatments were significantly increased ($p < 0.05$). There were no significant ($p > 0.05$) differences in the fluxes of SMBC at the 0–10 cm soil layer between the RF and MF treatments. The result indicated that the fluxes of SMBC at the 0–10 cm soil layer with the MF, RF, OM, and CK treatments were higher than those of the 10–20 cm soil layer. Compared with the CK treatment, the fluxes of SMBC at the 0–10 cm and 10–20 cm soil layers with the RF and OM treatments increased by 7.38% and 11.45%, and 7.79% and 19.39%, respectively (Figure 2a).

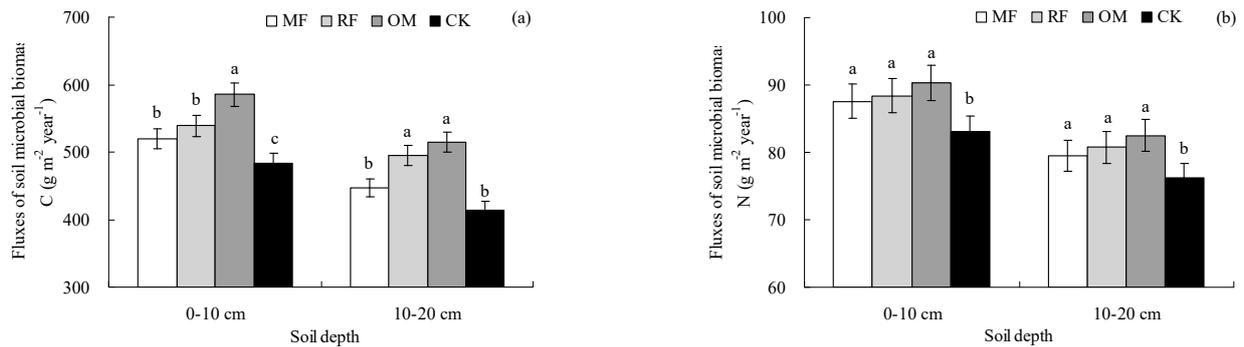


Figure 2. Effects of different long-term fertilizer treatments on the flux of soil microbial biomass carbon (a) and nitrogen (b) in the double-cropping rice field. Different lowercase letters indicate significant differences ($p < 0.05$) among different fertilizer treatments.

Compared with the CK treatment, the fluxes of SMBN at the 0–10 cm and 10–20 cm soil layers with the MF, RF, and OM treatments were significantly increased ($p < 0.05$). There were no significant ($p > 0.05$) difference in fluxes of SMBN at the 0–10 cm and 10–20 cm soil layers between the MF, RF, and OM treatments. The results indicated that the fluxes of SMBN at the 0–10 cm soil layer with the MF, RF, OM, and CK treatments were higher than those of the 10–20 cm soil layer. Compared with the CK treatment, the fluxes of SMBN at the 0–10 cm and 10–20 cm soil layers with the MF, RF, and OM treatments increased by 5.40%, 6.39%, and 8.67% and 4.34%, 5.97%, and 8.25%, respectively (Figure 2b).

3.3. Flux Turnover Rate of SMBC and SMBN

Compared with the CK treatment, the flux turnover rates of SMBC at the 0–10 cm and 10–20 cm soil layers with the MF, RF, and OM treatments were significantly increased ($p < 0.05$). There were no significant ($p > 0.05$) differences in flux turnover rates of SMBC at the 0–10 cm and 10–20 cm soil layers between the RF and OM treatments. The results indicated that the flux turnover rates of SMBC at the 0–10 cm soil layer with the MF, RF, OM, and CK treatments were higher than those of the 10–20 cm soil layer. Compared with the CK treatment, the flux turnover rates of SMBC at the 0–10 cm and 10–20 cm soil layers with the MF, RF, and OM treatments increased by 26.62%, 37.66%, and 46.10% and 29.58%, 45.07%, and 48.59%, respectively (Figure 3a).

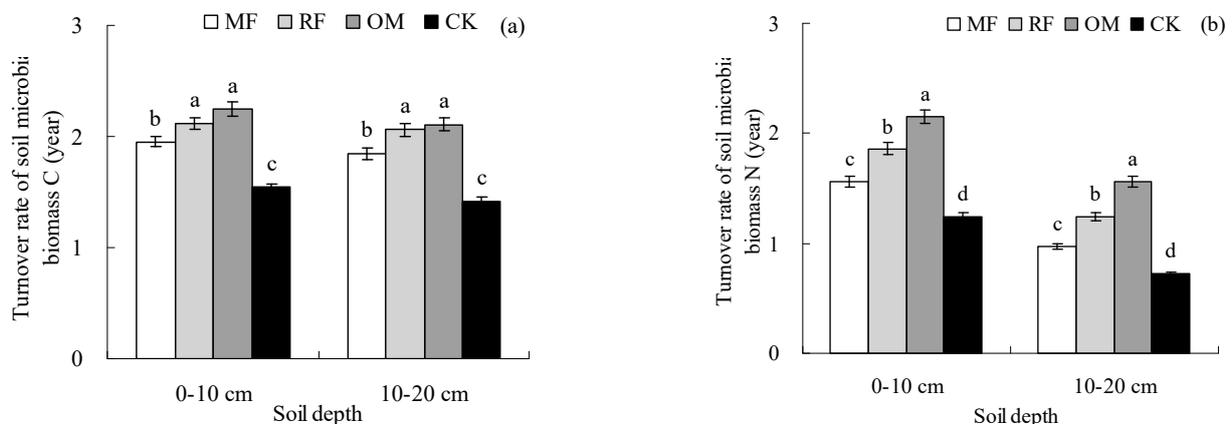


Figure 3. Effects of different long-term fertilizer treatments on flux turnover rates of soil microbial biomass carbon (a) and nitrogen (b) in the double-cropping rice field. Different lowercase letters indicate significant differences ($p < 0.05$) among different fertilizer treatments.

This result indicated that the flux turnover rates of SMBN at the 0–10 cm and 10–20 cm soil layers with the MF, RF, and OM treatments were significantly higher than those of the

CK treatment (Figure 3b). The order of the flux turnover rates of SMBN at the 0–10 cm and 10–20 cm soil layers with all fertilizer treatments was $OM > RF > MF > CK$. The result showed that the flux turnover rates of SMBN at the 0–10 cm soil layer with the MF, RF, OM, and CK treatments were higher than those of the 10–20 cm soil layer. At the 0–10 cm soil layer, the flux turnover rates of SMBN with the MF, RF, and OM treatments were increased 1.26, 1.50, and 1.73 times more than those of the CK treatments, respectively. At the 10–20 cm soil layer, the flux turnover rates of SMBN with the MF, RF, and OM treatments were increased 1.35, 1.72, and 2.17 times more than those of the CK treatments, respectively.

The results showed that the flux turnover times of SMBC at the 0–10 cm and 10–20 cm soil layers with the CK treatments were significantly ($p < 0.05$) higher than those of the MF, RF, and OM treatments. The result indicated that flux turnover times of SMBC at the 0–10 cm soil layer with the MF, RF, OM, and CK treatments were lower than those of the 10–20 cm soil layer. Compared with the CK treatment, the flux turnover times of SMBC at the 0–10 cm and 10–20 cm soil layers with the MF, RF, and OM treatments decreased by 10.28%, 22.90%, and 42.52% and 13.45%, 22.27%, and 38.66%, respectively (Figure 4a).

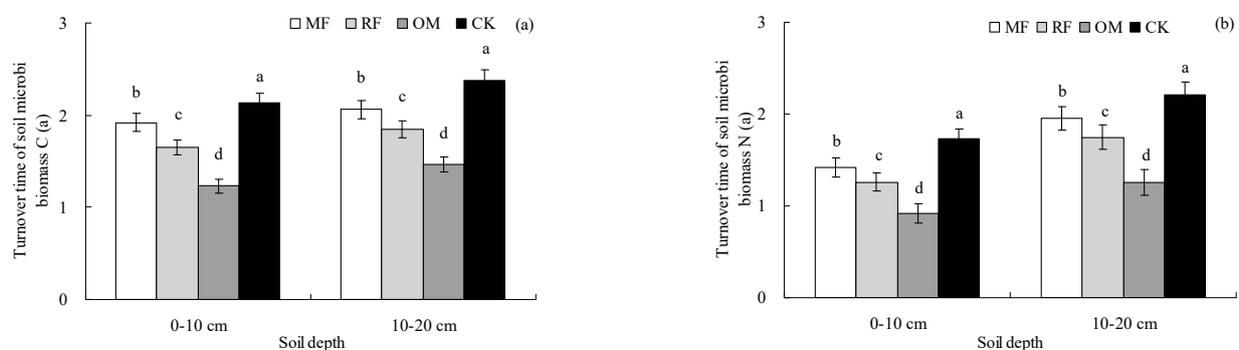


Figure 4. Effects of different long-term fertilizer treatments on flux turnover time of soil microbial biomass carbon (a) and nitrogen (b) in the double-cropping rice field. Different lowercase letters indicate significant differences ($p < 0.05$) among different fertilizer treatments.

Compared with the CK treatment, the flux turnover times of SMBN at the 0–10 cm and 10–20 cm soil layers with the MF, RF, and OM treatments were significantly decreased ($p < 0.05$). The results indicated that flux turnover times of SMBN at the 0–10 cm soil layer with the MF, RF, OM, and CK treatments were lower than those of the 10–20 cm soil layer. Compared with the CK treatment, the flux turnover time of SMBN at the 0–10 cm and 10–20 cm soil layers with the MF, RF, and OM treatments decreased by 18.86%, 28.00%, and 47.43%, and 13.33%, 22.22%, and 44.00%, respectively (Figure 4b).

3.4. Early Rice and Later Rice Yield

This result indicated that early rice and later rice yields with RF and OM treatments were significantly ($p < 0.05$) higher than those of the MF and CK treatments. Compared with the MF treatment, early rice and later rice yields with RF and OM treatments increased by 10.19% and 26.71%, and 11.33% and 6.93%, respectively (Figure 5). Compared with the MF treatment, double-cropping rice yield with RF and OM treatments increased by 10.86% and 15.12%, respectively.

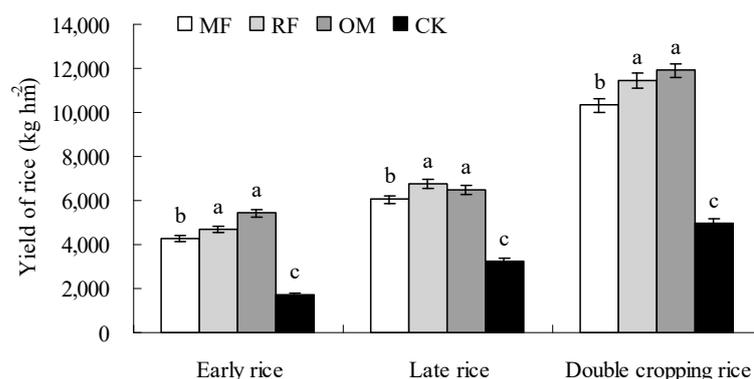


Figure 5. Effects of different long-term fertilizer treatments on yields of early rice and later rice in the double-cropping rice system. Different lowercase letters indicate significant differences ($p < 0.05$) among different fertilizer treatments.

4. Discussion

4.1. Effects of Fertilizer Managements on Soil Microbial Biomass Content

Soil microbial biomass is usually regarded as an early indicator of changes in soil quality and soil fertility in paddy fields [2], which are mainly influenced by the application of different fertilizer practices. In the present study, the results indicated that soil microbial biomass (SMBC and SMBN) contents in the paddy field were noticeably changed under different long-term fertilizer conditions, and the SMBC and SMBN contents in the paddy field with the application of crop residue and organic manure incorporation treatments (RF and OM) were higher than those of chemical fertilizer or without fertilizer input treatments (MF and CK). These results were consistent with hypothesis 1, namely, that SMB contents were increased by the combined application and management of organic matter and chemical fertilizer. The reason may be because organic matter (crop residue and manure) was provided a substrate for soil microbial activities, and the conversion rates of carbon and nitrogen were increased under the conditions of the combined application of organic matter and chemical fertilizer. These results, which also confirmed previous results [12,18], suggest the provision of a suitable, readily available source of a substrate and soil physical environment for soil microorganism activities. In the present study, this result also indicated that SMBC and SMBN contents in the paddy field with the MF treatment were higher than those with the CK treatment (Figure 1), which is in agreement with the previous results [10]. The reason may be attributed to the input of carbon from root/crop residue/rhizodeposition, which may have promoted soil microbial biomass growth with the application of chemical fertilizer. Similar results were also found by Jiang et al. (2006) [19] in China. In the present study, our results showed that rice yield provided the evidence of SMB content change in the paddy field (Figure 5). Rice yield (early rice and late rice) with the RF and OM treatments were higher than those with the MF and CK treatments. SMBC and SMBN contents with the RF and OM treatments were the highest compared with the MF and CK treatments. These results supported that rice yield and soil physicochemical characteristics under a double-cropping rice system were increased by the combined application of crop residue and organic manure with mineral fertilizer management [20].

In the present study, the results showed that the SMBC and SMBN contents at the 0–10 cm soil layer were significantly higher than those of the 10–20 cm soil layer in the paddy field; the reason was mainly attributed to the fact that organic matter (crop residue, organic manure) and chemical fertilizer were incorporated into the 0–10 cm soil layer combined with rotary tillage management when the depth of tillage was 8–10 cm, and the 10–20 cm soil layer in the paddy field was only slightly disturbed in the present study. On the other hand, the decomposition of crop residue and organic manure provided an

abundance of available nutrients in the soil for rice root growth and soil microbial activities at the 0–10 cm soil layer [21].

4.2. Effects of Fertilizer Managements on Flux of SMBC and SMBN

In previous studies, the results showed that fluxes of SMBC and SMBN were significantly influenced by the application of different crop residue and organic manure incorporation practices [22]. In the present study, the results indicated that flux and turnover rate of SMBC and SMBN in the paddy field with RF and OM treatments were higher than those of the other fertilizer treatments. These results were consistent with hypothesis 2, namely, that the flux and turnover rate of SMBC and SMBN in the paddy field were changed as a result of different fertilizer management practices. The reason for this may be because organic matter (crop residue, organic manure) and chemical fertilizer were incorporated into the plough soil layer in the paddy field with rotary tillage, providing a larger amount of available substrate and soil nutrients for soil microbial growth and multiplication. On the other hand, the soil active organic pool and soil microorganism metabolism were promoted; therefore, the transformation and supply capacity of soil organic nutrients were improved. Moreover, our results indicated that the flux turnover rates of SMBC and SMBN in the paddy field with the MF treatment were higher than those of the CK treatment, suggesting that rice root growth and the return of residue to the paddy field were increased under the condition of long-term application of chemical fertilizer, the crop residue decomposing and providing available nutrients and substrate for soil microbial growth and multiplication [21].

In the present study, our results indicated that the flux turnover times of SMBC and SMBN in the paddy field with the RF and OM treatments were lower than those of the other fertilizer treatments, which agreed with the previous results [22]. The reason may be because the crop residue and organic manure were incorporated into the ploughed soil layer in the paddy field, suggesting that the protective effect of soil microorganisms and the decomposition rate were increased with the combined application with chemical fertilizer [23]. On the other hand, the SOC and soil nutrient content in the paddy field were increased with RF and OM treatments [10]. Moreover, this result indicated that the flux turnover times of SMBC and SMBN in the paddy field with the MF treatment were lower than those of the CK treatment, suggesting that the return of crop residue and organic manure to the paddy field, and the decomposition of crop residue and organic manure provided a larger amount of available nutrients and substrate for soil microbial growth, and the flux turnover rates of SMBC and SMBN in the paddy field were increased.

In the present study, this result showed that the flux turnover rates of SMBC and SMBN at the 0–10 cm soil layer were higher than those of the 10–20 cm soil layer in the paddy field, but the flux turnover times of SMBC and SMBN at the 0–10 cm soil layer were lower than those of the 10–20 cm soil layer in the paddy field under the same fertilizer conditions; the reasons were mainly attributed to the soil's physical and chemical properties. The soil ecological environment at the 0–10 cm layer was improved with the incorporation of fertilizer under the combined application of rotary tillage practice, rice root exudation, and promotion of root activity. On the other hand, crop residue and organic manure were incorporated into the 0–10 cm soil layer in the paddy field, providing a larger amount of available substrate and soil nutrients for soil microbial growth and multiplication, thus improving the transformation and supply ability of soil organic carbon and nitrogen [13].

5. Conclusions

In the present study, this result indicated that SMBC and SMBN contents and their flux turnover rates at the 0–10 cm and 10–20 cm soil layers in the double-cropping rice field were changed under long-term fertilizer conditions. RF and OM treatments were effective measures for increasing SMBC and SMBN contents at the 0–10 cm and 10–20 cm soil layers. Compared with the MF and CK treatments, the flux turnover rates of SMBC and SMBN were increased with RF and OM treatments. Under the same fertilizer condition,

SMBC and SMBN contents and the flux turnover rates of SMBC and SMBN at the 0–10 cm soil layer were higher than those of the 10–20 cm soil layer, but the flux turnover times of SMBC and SMBN at the 0–10 cm soil layer were lower than those of the 10–20 cm soil layer in the paddy field. Furthermore, RF and OM treatments were beneficial practices for increasing early and later rice yields. As a result, the combined application of crop residue and organic manure with inorganic fertilizer was a beneficial management practice for increasing soil fertility and rice yield under a double-cropping rice system in southern China. However, future research is still needed to explore the effects of different types of long-term fertilizer management on soil carbon and nitrogen fixing bacterial communities under a double-cropping rice system.

Author Contributions: H.T. and X.X. designed the experiments. C.L., K.C., Y.X. and L.W. performed the experiments. L.S. and W.L. analyzed the data, H.T. wrote the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the Hunan Provincial Natural Science Foundation of China (2022JJ30352), Innovative Research Groups of the Natural Science Foundation of Hunan Province (2022CX75), and the National Natural Science Foundation of China (31872851, U21A20187).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data reported in this study are contained within the article.

Acknowledgments: We would like to thank the staff of the Ningxiang Agricultural Technology Extension Center for field management.

Conflicts of Interest: The authors declare no conflict of interest.

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