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# Enhancing soil carbon and rice dry biomass with microbial fuel cells, optimal spacing, and fertilizer in rice fields

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**Abstract.** *Nurhutami SR, Sudadi, Komariah, Dewi WS, Fauzan AA. 2024. Enhancing soil carbon and rice dry biomass with microbial fuel cells, optimal spacing, and fertilizer in rice fields. Biodiversitas 25: 3331-3338.* Microbial fuel cell (MFC) uses electroactive anode bacteria to compete in rice fields without disrupting cultivation or altering diversity. Therefore, this research aimed to explore the combination of MFC, different spacing methods (*Jajarlegowo* and conventional), and NPK (Nitrogen, Phosphorus, and Potassium) fertilizer affecting soil carbon dynamics and rice dry biomass. The key parameters included bacterial community, soil respiration, microbial biomass carbon, Soil Organic Carbon (SOC), Carbon to Nitrogen (CN) Ratio, and rice dry biomass. The results showed that there were no significant differences across the parameters when combined. However, MFC increased soil respiration (0.618 $CO_2$.day$^{-1}$) and microbial biomass carbon (0.121 µg. g$^{-1}$) but decreased SOC and rice dry biomass (0.039% and 3.978 g. clump$^{-1}$). *Jajarlegowo* plant spacing enhanced soil respiration (0.583 $CO_2$.day$^{-1}$), microbial biomass carbon (0.122 µg. g$^{-1}$), and rice dry biomass (10.635 g. clump$^{-1}$). Meanwhile, NPK fertilizer enhanced microbial biomass carbon (0.170 µg. g$^{-1}$), soil respiration (0.615 $CO_2$.day$^{-1}$), and rice dry biomass (5.993 g.clump$^{-1}$) but lowered CN Ratio by 3.471. A positive correlation was also observed between soil carbon and rice dry biomass. These emphasized the need for a holistic method to develop MFC technology in rice paddy soils. Further research was suggested on the role of Electroactive Anodes Bacteria (EAB) in organic matter decomposition over multiple rice growing seasons.

**Keywords:** Bacterial community, conventional spacing, *Jajarlegowo* spacing, NPK, soil respirations

**Abbreviations**: MFC: Microbial Fuel Cell; EAB: Electroactive Anodes Bacteria; SOC: Soil Organic Carbon; C: Ratio-Carbon to Nitrogen Ratio; NPK: Nitrogen, Phosphorus and Potassium (Fertilizer)

## INTRODUCTION

The Microbial Fuel Cell (MFC) is an innovative technology for generating electricity from Electroactive Anodes Bacteria (EAB). The cell receives electrons released by EAB and transfers the particle to a cathode through an electrical circuit to provide current. This cell can be applied to rice fields to minimize oxygen competition (Wetser 2016) and the organic matter in the soil serves as a substrate for EAB (Rizzo et al. 2013; Fakhirruddin et al. 2018; Pham et al. 2019). In addition, MFC has the potential to serve as an alternative method for increasing carbon issues in rice fields (Kouzuma et al. 2014; Zhi et al. 2014) without disrupting cultivation (Kabutey et al. 2019).

Several research studies have identified various bacterial genera capable of performing intercellular electron transfer. Dominant EAB genera include *Geobacter, Anaeromyxobacter, Enterobacter, Desulfovibrio, Proteobacteria,* and *Acidobacteria* (Lu et al. 2019; Rubaba et al. 2013; Zhang et al. 2015) with sulfate-reducing, iron-reducing, and non-ferrous properties (Konovalova et al. 2017). The genera dominate bacterial diversity in rice fields by competing for organic acids with others (Liu et al. 2022). Moreover, bacterial diversity as well as other biotic and abiotic soil characteristics are altered ( Wang et al. 2015a; Gustave et al. 2019). These changes influence respiration, microbial biomass carbon, and Soil Organic Carbon (SOC). According to Orrell and Bennett (2013); Tamburini et al. (2016); Turner et al. (2017); Dewi et al. (2022a), bacteria play a role in soil complexity and dynamics, affecting plant biomass in line with the concept of underground conditions positively supporting above-ground growth.

Changes in the characteristics of rice fields soil affect quality and health, modifying the ecosystem services. These services include the capacity to sustain food production, support nutrient cycling, as well as regulate carbon and climate. Additionally, the services contribute to the filtration and neutralizing of harmful chemical compounds. These ecosystems are crucial for the sustainability of rural and urban communities.

Planting rice in specific patterns to optimize space and sunlight exposure, combined with appropriate fertilization practices can improve seedling growth and rice yields. The *Jajarlegowo* planting space used by Javanese farmers in Indonesia with the increased number of clumps and open rows, can significantly maximize sunlight intensity to enhance growth and yields compared to conventional methods

(Kusyaeri et al. 2014). This method provides a richer source of organic material for EAB than traditional planting spaces. Since there is no research supporting the context, further analyses are needed to confirm the potential.

Several research studied have attempted to develop MFC by integrating specific cultivation practices, such as adding fertilizers or exploring EAB consortia. The primary aims are to enhance ecological benefits in energy production, reduce greenhouse gas emissions, and improve rice yields. However, no optimal combination has been identified to meet the challenges. For instance, Amin and Djoyowasito (2017) successfully optimized electrical voltage, while Pham et al. (2019) reported a significant reduction in rice yields. In this context, further research is needed to enhance rice yields and understand the impact on SOC dynamics. In addressing the challenge, optimal plant spacing and fertilization practices may offer significant potential.

This study aims to investigate the combined effects of MFC, planting space, and NPK on soil carbon dynamics, including soil respiration, microbial biomass carbon, and SOC, as well as rice dry biomass weight. A deeper understanding is required since the combination of treatments has not been extensively explored.

## MATERIALS AND METHODS

### Study area

This research was undertaken in Pojok Villages, Tawangsari District, Sukoharjo District, Central Java, Indonesia (7°43'21.0" S 110°47'39.6" E) from June 2022 to June 2023 during dry months. The research area has an average annual rainfall of 2790 mm, temperature ranging from 23°C to 34°C, and an average annual humidity of 77%.

### Experimental design

The experiment uses a Strip Plot design with three factors: Microbial Fuel Cell (MFC vs. non-MFC), planting space (*Jajarlegowo* vs. conventional), and fertilizer application (fertilized vs. non-fertilized). The rice fields were partitioned into 24 experimental plots, including eight treatments and three repetitions, measuring 3 meters by 2.5 meters. The planting pit received three rice seedlings of the Pak Tiwi variety per the Indonesian Minister of Agriculture Decree No. 2434/Kpts/SR.120/7/2012. This variety is commonly used by farmers and the research follows the established agricultural practices. NPK 15:15:15 with a dosage of 500 kg.ha$^{-1}$ was applied only to the plants in the fertilized group. Additionally, no organic material was added to the treatments in line with local farmer practices. *Jajarlegowo* and the conventional plant spacing are reported in Figure 1.

### Procedures

*Pre-transplanting*

This stage includes plot preparation, MFC assembly, and rice seed sowing. Soil preparation includes clearing and burying weeds into the soil, plowing, puddling, and rinsing with water to a depth of 10-15 cm from the soil surface. Sowing is carried out in paddy fields using Pak Tiwi-1 rice. The MFC consists of an anode made of carbon graphite felt with dimensions of 10x10x1 cm³, and a graphite rod measuring 10 cm in length with a diameter of 0.5 cm as the cathode. The anode is connected to the cathode using an external circuit cable with a resistance of 330 Ω as reported in Figure 2.

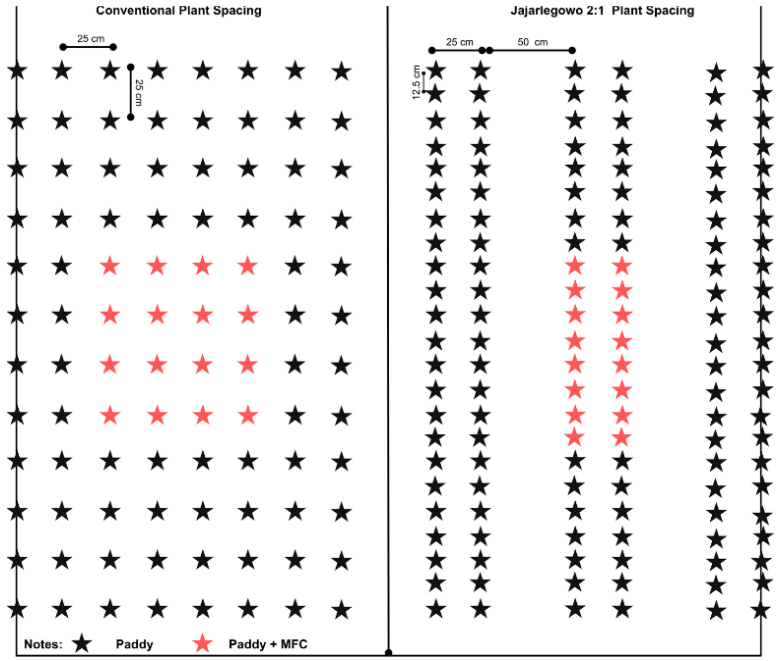


**Figure 1**. In conventional plant spacing, plants are arranged in a grid of 25 cm. In the *Jajarlegowo*, the spacing is different since there is an empty corridor of 25 cm for every two rows of plants spaced 50 cm apart. Within each row, plants are spaced 12.5 cm apart. The red and black stars represent rice with MFC and Non MFC treatment, respectively

*Transplanting and sampling*

Transplanting is carried out by inserting rice seedlings into the entire experimental plot. In the plots treated with MFC, the seedlings are planted by placing the rice roots above the anode and embedding them into the paddy soil. Plant sampling is conducted by selecting plants located in the center of the plot, which can represent the condition of the plants in each plot (Figure 1). Soil samples are composited across all monthly repetitions and analyzed in the laboratory. Metagenomic include the composite examination of soil and anodes, and evaluating bacterial community structure through sequencing services.

*Parameter*

The parameters observed included rice dry biomass, SOC (%) measured by the Walkey and Black method (Food and Agriculture Organization of The United Nations 2019), Carbon to Nitrogen (CN) Ratio, soil respiration ($CO_2$. $day^{-1}$) determined by the modified Verstraete method (Dermiyati et al. 2017), microbial biomass carbon (µg. $g^{-1}$) assessed through chloroform extraction and fumigation methods (Setia et al. 2012), and metagenome analysis using Oxford Nanopore technology. The procedural steps for metagenomic analysis by the sequencing company included the extraction of genomic DNA using the Macherey-Nagel NucleoSpinSoil & ZymoBIOMICS DNA Miniprep Kit, quality control through NanoDrop spectrophotometer and Qubit fluorometer, as well as library preparation using Oxford Nanopore Technology Kits. Nanopore sequencing was executed with MinKNOW software version 22.05.7, followed by base calling using Guppy version 6.1.5. FASTQ file quality was evaluated with NanoPlot, and quality filtering was implemented using NanoFilt. The classified readings were subjected to analysis using the Centrifuge classifier, with the Bacterial and Archaeal Index referencing the NCBI 16S RefSeq database. Bacterial diversity also included Operational Taxonomy Unit (OTU), as well as Shannon and Simpson index for species and alpha diversity. Meanwhile, beta diversity was assessed using Principal Coordinate Analysis (PCoA) based on Bray Curtis analysis and ANOSIM due to limited funding

**Data analysis**

All data were tested with ANOVA using R Studio 4.3.1 software and followed by an LSD test at a 5% level.

## RESULTS AND DISCUSSION

**MFC, fertilizer, and planting space on soil biology characteristics**

The abundance of bacterial genera varied across treatments. *Arenimonas*, *Clostridium*, *Dyella*, *Geobacter*, *Hyphomicrobium*, *Methylocystis*, *Phenylobacterium*, *Pseudomonas*, *Streptomyces*, and *Thiobacillus* are the 10 most abundant bacterial genera observed in Figure 3. The highest abundance in treatments A0B0C0 (Non-MFC, Conventional plant spacing, Non- Fertilized) and A1B1C0 (MFC, *Jajarlegowo* plant spacing, Non- Fertilized) is the genus *Dyella*. Meanwhile, the highest in treatments A0B0C1 (Non-MFC, Conventional plant spacing, Fertilized), A0B1C0 (Non- MFC, *Jajarlegowo* plant spacing, Non-Fertilized), A0B1C1 (Non- MFC, *Jajarlegowo* plant spacing, Fertilized), A1B0C0 (MFC, Conventional plant spacing, Non-Fertilized), and A1B0C1 (MFC, *Jajarlegowo* plant spacing, Fertilized) is *Clostridium*. In A1B1C1 (MFC, *Jajarlegowo* plant spacing, Fertilized), the highest abundance is *Pseudomonas*. The genera included in the EAB group are *Pseudomonas*, *Geobacter*, *Dyella*, and *Arenimonas* with different abundances across all treatments. A higher abundance of EAB in MFC enhances the degradation of organic carbon, leading to more efficient performance.

The alpha diversity analysis is measured using several indices: OTU, Chao1, Shannon, and Simpson (Table 1). The highest and lowest OTU values are found in A0B0C1 (Non-MFC, Conventional plant spacing, Non-Fertilized) and A1B1C0 (MFC, Conventional spacing of plant, Fertilized), respectively. Meanwhile, the Chao1 index is highest and lowest in treatment A0B1C1 (Non-MFC, *Jajarlegowo* plant spacing, Fertilized) and A0B0C0 (Non-MFC, Conventional plant spacing, Non-Fertilized), respectively. Analysis using the Shannon index shows that the highest and lowest values are in treatment A0B0C1 (Non-MFC, *Jajarlegowo* plant spacing, Non-Fertilized) and A1B1C0 (MFC, Conventional plant spacing, Fertilized). Conversely, the Simpson index suggests that the lowest value is in treatment A1B1C0 (MFC, Conventional plant spacing, Fertilized).

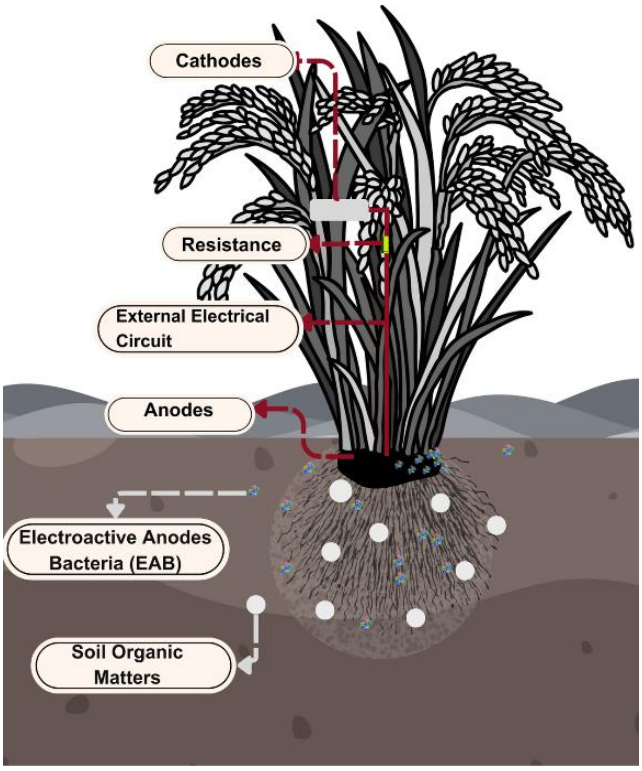


**Figure 2.** MFC in rice plant

Beta diversity analysis using PCoA based on Bray-Curtis dissimilarity and ANOSIM was performed to differentiate between MFC and non-MFC groups (Figure 4). The results reported no significant difference between the groups, with a dissimilarity value of approximately 45.70% (Figure 4.A). The ANOSIM results (Figure 4.B) showed an R-value of -0.031 and a p-value greater than 0.05, indicating no significant difference in beta diversity. Therefore, MFC does not create a distinct difference in microbial community composition or distribution compared to non-MFC groups, as evidenced by the negative ANOSIM R value. The presence or absence of MFC did not lead to meaningful clustering or separation of the microbial communities. Additionally, LEFse analysis (Figure 4.C) identified *Thiobacillus sajanensis sp.nov.* as a significant biomarker with a linear discriminant analysis (LDA) score greater than 0.

### Soil organic carbon, soil respiration, soil microbial biomass carbon, CN ratio dynamic and rice dry biomass weight

Figure 5 shows the monthly dynamics of paddy soil carbon properties affected by the combination of MFC, fertilizer application, and plant spacing. SOC dynamic shown in Figure 5.A confirms the decrease, with the A0B1C1 (Non- MFC, *Jajarlegowo* plant spacing, Fertilized) treatment combination indicating the remaining higher SOC since the second month. The soil respiration tended to increase from the first to third weeks but decreased in the fourth week. A similar pattern is also shown by soil microbial biomass carbon, which increased from the first to the third week and decreased in the fourth week. From Figure 5.D, the CN ratio decreased from the first to the third week and increased in the fourth except for the A0B1C1 combination decreasing from the second to the fourth. Figure 5.E shows that the highest rice dry biomass weight was found in treatment A0B1C1, while the lowest was observed in treatment A1B1C1(MFC, Jajarlegowo plant spacing, Fertilized). Based on a deeper analysis in Table 2, there are significant differences in every single factor.

**Table 1.** Bacteria alpha diversity

| Treatments | OTU | Alpha Diversity Indexes | | |
|---|---|---|---|---|
| | | Chao1 | Shannon | Simpson |
| A0B0C0 | 5641 | 8173.75 | 6.56 | 0.99 |
| A0B0C1 | 6548 | 9303.51 | 7.14 | 0.99 |
| A0B1C0 | 5876 | 8711.15 | 6.78 | 0.99 |
| A0B1C1 | 6463 | 9437.08 | 7.08 | 0.99 |
| A1B0C0 | 6283 | 9072.05 | 7.08 | 0.99 |
| A1B0C1 | 6213 | 9089.96 | 7.12 | 0.99 |
| A1B1C0 | 5482 | 8331.33 | 6.20 | 0.97 |
| A1B1C1 | 5758 | 8306.61 | 6.54 | 0.99 |

Notes: Treatment explanations: A0: Non-MFC; A1: MFC; B0: Conventional plant spacing; B1: *Jajarlegowo* plant spacing; C0: Non-fertilized; and C1: Fertilized

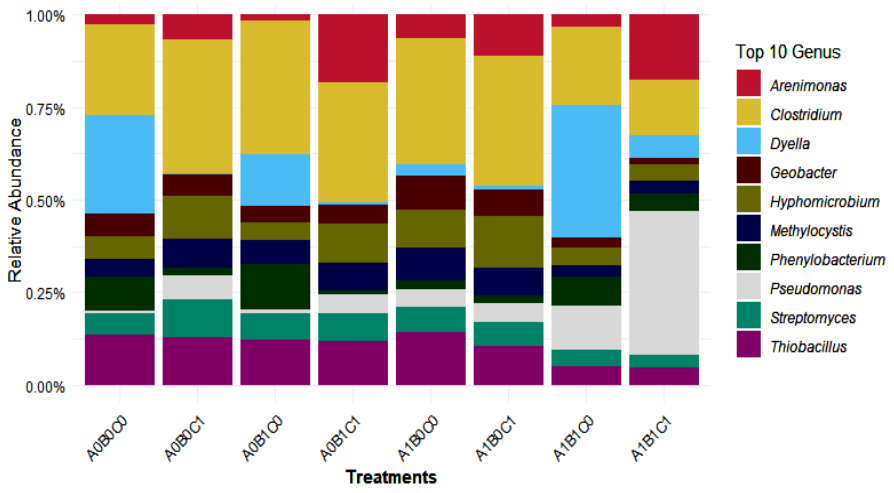


**Figure 3.** Top 10 bacterial genera based on relative abundance. Treatment explanations: A0: Non-MFC; A1: MFC; B0: Conventional plant spacing; B1: *Jajarlegowo* plant spacing; C0: Non-fertilized; and C1: Fertilized

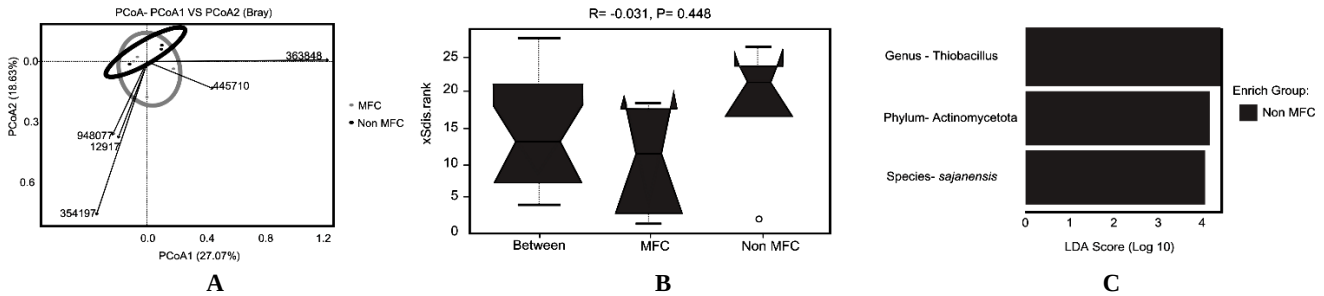


**Figure 4.** PcoA based on A. Bray curtis analysis; B. ANOSIM; C. Bacteria biomarker

Table 2 presents the effects of MFC, fertilizer, and plant spacing on paddy soil carbon and rice dry biomass. MFC significantly resulted in the highest soil respiration and microbial biomass carbon by 0.618 $CO_2$.day$^{-1}$ and 0.121 µg. g$^{-1}$, respectively. However, MFC affected the lower soil organic C (0.039% lower than without MFC) and rice dry biomass (3.978 g. clump$^{-1}$ lower than without MFC). *Jajarlegowo* plant spacing significantly yielded higher soil respiration, microbial biomass carbon, and rice dry biomass by 0.583 $CO_2$.day$^{-1}$, 0.122 µg. g$^{-1}$, and 10.635 g.clump$^{-1}$ than conventional. Similarly, the application of NPK fertilizer significantly produced higher microbial biomass carbon, soil respiration and rice dry biomass by 0.170 µg. g$^{-1}$, 0.615 $CO_2$.day$^{-1}$ and 5.993 g.clump$^{-1}$ higher than without fertilizer, respectively

## Correlation between the rice field's carbon characteristic and rice dry biomass

The correlation between soil characteristics and dry biomass of paddy is depicted in a positive relationship. From Table 3, SOC shows correlation coefficients of 0.347, 0.353, and 0.538 with respiration, microbial biomass carbon, and rice dry biomass, respectively. Additionally, microbial biomass carbon reports correlation coefficients of 0.831 and 0.483 with soil respiration and rice dry biomass, respectively. SOC has a correlation coefficient of 0.353 with rice dry biomass.

**Table 2.** The individual effects of MFC treatment, fertilization, and plant spacing on paddy soil carbon and rice dry biomass

| Parameters | Treatments | | | | | |
|---|---|---|---|---|---|---|
| | MFC | | Plant spacing | | Fertilization | |
| | MFC | Without MFC | Conventional | *Jajarlegowo* | Fertilized | Not fertilized |
| SOC (%) | 1.985 [a] | 2.024 [b] | 1.994 [a] | 2.015 [a] | 2.017 [a] | 1.993 [a] |
| Microbial biomass carbon (µg.g$^{-1}$) | 1.703 [b] | 1.581 [a] | 1.582 [a] | 1.703 [b] | 1.727 [b] | 1.557 [a] |
| Soil respiration ($CO_2$. days$^{-1}$) | 11.228 [b] | 10.610 [a] | 10.627 [a] | 11.211 [b] | 11.227 [b] | 10.612 [a] |
| CN ratio | 12.207 [a] | 12.248 [a] | 12.377 [a] | 12.078 [a] | 10.492 [a] | 13.963 [b] |
| Rice dry biomass weight (g.clumps$^{-1}$) | 70.485 [a] | 74.463 [b] | 67.157 [a] | 77.792 [b] | 75.471 [b] | 69.478 [a] |

Notes: Means followed by the same letter in the same column are not significantly different at Alpha = 0.05

**Table 3**. Correlation between the rice field's carbon characteristic and rice dry biomass

| | SOC | CN ratio | Soil respiration | Microbial biomass carbon | Rice dry biomass |
|---|---|---|---|---|---|
| SOC | 1 | | | | |
| CN ratio | .302 | 1 | | | |
| Soil respiration | .347$^{*}$ | -.425$^{*}$ | 1 | | |
| Microbial biomass carbon | .353$^{*}$ | -.562$^{**}$ | .831$^{**}$ | 1 | |
| Rice dry biomass | .538$^{**}$ | -.468$^{*}$ | .388$^{*}$ | .483$^{**}$ | 1 |

Notes: * significant at 0.05 level (1- tailed), ** significant at 0.01 level (1- tailed)

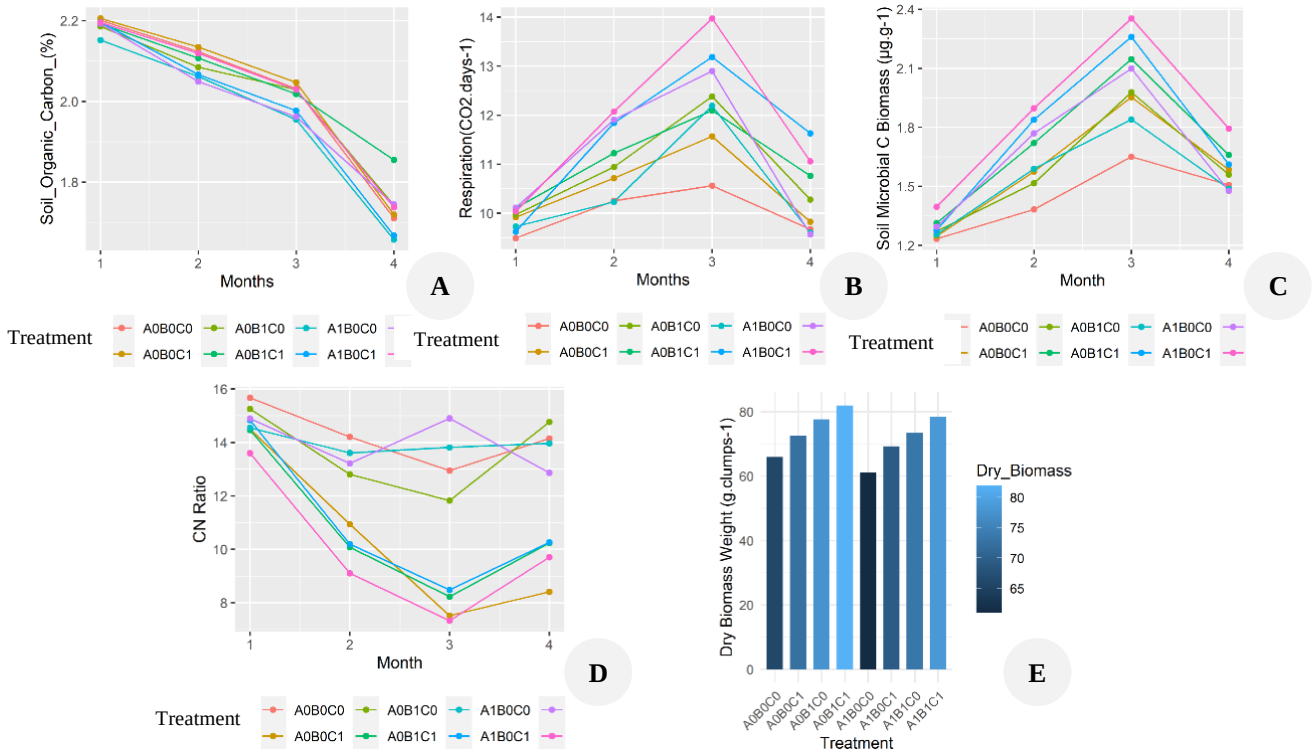


**Figure 5.** Dynamics of paddy soil carbon affected by the combination of MFC, fertilizer application, and plant spacing: A. SOC; B. Soil Respiration; C. Soil Microbial Carbon Biomass; D. CN Ratio; E. Rice dry biomass weight

**Discussion**

The combination of MFC, plant spacing, and fertilizer in rice fields did not lead to significant changes in carbon dynamics (Figure 5) and rice dry biomass (Table 2). Bacterial diversity (Table 1) shows no significant change in the structure of the bacterial community. This is because EAB in MFCs requires a longer time to form a biofilm on the anodes surface and facilitate electron transfer. Buitrón et al. (2017) noted that the process can take 4 to 103 days, depending on the electrode material, operational conditions, and type of EAB. Zhang et al. (2019) added that bacterial inoculation required one to two weeks for biofilm formation in lab-scale MFC. However, bacteria were not inoculated to observe the natural function of MFC. Biofilm formation was less optimal, as indicated by the lack of significant differences in bacterial diversity, which did not significantly influence all parameters. In this context, important changes were observed, particularly in the carbon dynamics and dry weight of rice biomass.

MFC application to rice fields did not cause major changes in the bacterial community, as pure culture were inoculated in rice fields (Fauzan et al. 2022). However, important changes were seen, especially in the increasing abundance of *Dyella*, *Arenimonas*, and *Curvibacter* bacterial Genera (Figure 3). As a result, this causes significant changes in soil respiration and microbial biomass carbon, SOC and rice dry biomass, as shown in Table 2.

EAB inherent to paddy soils is stimulated by the MFC, as reported by Liu et al. (2013); Ranatunga et al. (2018). This research shows a commensurate phenomenon, characterized by a discernible increase in *Arenimonas*, *Curvibacter*, and *Dyella* (depicted in Figure 2), in a line with EAB behavior (Sivasankar et al. 2019; Zhang et al. 2022). *Dyella* assumes an important role in the decomposition of lignin and complex carbon substrates (Zhou et al. 2018), where *Arenimonas* functions as denitrifying bacteria (Zhong et al. 2020), and *Curvibacter* takes on the responsibility for arsenic elimination within the MFC system (Isabel San-Martín et al. 2023). The presence of EAB, such as *Methylocystis*, is effectively restrained, thereby influencing methane formation (Tikhonova et al. 2021). These results fortify the competitive nature of EAB, particularly in proficient competition for acetic acid, as stated by (Inubushi et al. 1997; Liu et al. 2022). Despite the competition, no significant changes were observed in bacterial diversity. These results deviate slightly from those reported by Gustave et al. (2019).

Several factors contribute to divergent results, including root exudate composition (Berg and Smalla 2009), characteristics of other organic substrates (Dunaj et al. 2012; Wang et al. 2015b), anode accessibility (Kouzuma et al. 2014), and the application of ammonium as a fertilizer (Ding et al. 2014). Microbial biomass carbon and soil respiration in MFC treatment indicated a significant increase in the microbial population in paddy soil. However, the abundance of EAB did not report a significant increase. The CN ratio in the MFC treatment showed no significant difference and only experienced a slight decrease. This suggests that applying MFC effectively increased the decomposition process in paddy soil. Therefore, SOC derived from root exudates reported a decrease.

Dunaj et al. (2012) emphasized that the key to MFC performance was in the quality of organic carbon acting as the substrate for EAB. According to Dewi and Nurhutami (2023), SOC dynamics in flooded rice fields are influenced by various factors, including environmental conditions, substrate quality, and microbial biomass. In the research area, the average temperature ranges from 23℃ to 34℃, and the annual humidity is 77%. Even though the conditions support bacterial growth, Jadhav and Ghangrekar (2009); Behera et al. (2011); found that temperatures of 35°C to 40°C are optimal for MFC performance. The research locations are favorable for bacterial activity but are slightly below the optimal temperature range for maximizing MFC efficiency. Figure 4 shows the SOC dynamic from the first to the fourth month. There is an increase in microbial biomass carbon and soil respiration, but SOC decreases, leading to significantly lower rice dry biomass in the MFC treatment. The MFC treatment reduced rice dry biomass weight by 3.978 g.clump$^{-1}$ compared to the non-MFC treatment (Table 2). Meanwhile, EAB increases organic matter decomposition, as evidenced by the lower CN Ratio and SOC compared to non-MFC treatments. The decrease in rice dry biomass is due to a mismatch between nutrient uptake by rice roots and the availability of nutrients in the flooded paddy soil (Brust 2019). An observation spanning more than a rice growing season is essential to comprehensively assess changes in the decomposition process. A metagenome method is important for analyzing the abundance of other microbes in the MFC system. However, this aspect was not covered in this research due to financial constraints.

The *Jajarlegowo* reports higher soil respiration, microbial biomass carbon, and rice dry biomass than conventional plant spacing. The variation in population numbers due to plant spacing (Figure 1) facilitates a more optimal photosynthesis process, with empty rows allowing leaves to receive more radiation. Therefore, this increases photosynthesis production, which is released into the root area. Plants also play a crucial role in enriching EAB bacterial communities in the rhizosphere anode (Liu et al. 2013) through root exudates to stimulate soil respiration. These results show a positive correlation between soil microbial biomass carbon, SOC, respiration, and rice dry biomass (Table 3). In addition, the research of Orrell and Bennett (2013); Tamburini et al. (2016); Dewi et al. (2022) were confirmed, where soil below and above the ground had a positive interaction.

The application of fertilizers enhances plant tissue growth and development, optimizing photosynthesis and enabling rice plants to release a greater amount of root exudate into the area. Therefore, there is an increase in microbial biomass carbon, soil respiration, rice dry biomass weight, and a significant reduction in CN ratio compared to non-fertilized (Table 2). The increase in the decomposition process leads to a more rapid release of nitrogen and other nutrients into the soil for immediate crop use. The ability of the plant to absorb nutrients is limited, potentially leading to lower rice dry biomass production. Local agricultural practices in Central Java may decrease the possibility of

factors such as the loss of plant nutrients through runoff or volatilization.

In conclusion, the combined application of MFC, plant spacing, and NPK fertilizers in rice fields during a single growing season did not significantly alter soil carbon dynamics or rice dry biomass. However, each factor individually showed notable effects. MFC increased soil respiration (0.618 $CO_2$.day$^{-1}$) and microbial biomass carbon (0.121 μg.g$^{-1}$) but reduced organic carbon (0.039%) and rice dry biomass (3.978 g.clump$^{-1}$). In contrast, *Jajarlegowo* plant spacing also enhanced soil respiration, microbial biomass carbon, and rice dry biomass by 0.583 $CO_2$.day$^{-1}$, 0.122 μg.g$^{-1}$, and 10.635 g.clump$^{-1}$ over conventional spacing. NPK fertilizer significantly improved microbial biomass carbon , soil respiration, and rice dry biomass by 0.170 μg.g$^{-1}$, 0.615 $CO_2$.day$^{-1}$, and 5.993 g.clump$^{-1}$, respectively, respectively. Even though MFC affected soil carbon properties, *Jajarlegowo* plant spacing, and NPK fertilizer were more effective for increasing rice dry biomass. Further research could explore the role of EAB in organic matter decomposition and the effects across multiple rice growing seasons.

## ACKNOWLEDGEMENTS

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