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Effect of Microbial Fuel Cell, fertilizer, and plant spacing on nitrogen dynamics in paddy soil

Syahrul Efendi[1]*, Komariah[2], Jauhari Syamsiyah[2], Widyatmani Sih Dewi[2], Ken Hiramatsu[3], Adhia Azhar Fauzan[3]

[1] Magister Program of Soil Science, Faculty of Agriculture, Universitas Sebelas Maret, Surakarta, Indonesia
[2] Department of Soil Science, Faculty of Agriculture, Universitas Sebelas Maret, Surakarta, Indonesia
[3] Faculty of Applied Biological Sciences, Gifu University, Japan

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***** *Corresponding Author*
Email address:
syahrul.efendi22@student.uns.ac.id

ABSTRACT

Nitrogen is one of the primary nutrients required for growing rice. Still, the efficiency of urea fertilizer application is very low (20-40%) due to the nitrogen loss process, one of which is denitrification. This study aims to determine the effects of combining Microbial Fuel Cell (MFC), plant spacing, and fertilization on nitrogen dynamics in paddy fields. The combination of treatments are expected to reduce the nitrogen loss in paddy fields, and plants can absorb it efficiently. A total of six treatments included Microbial Fuel Cell (MFC) (2 levels: without MFC and with MFC), plant spacing (2 levels: conventional spacing 25 cm × 25 cm and *jajar legowo* spacing 25 cm × 12.5 cm × 50 cm), and fertilization (2 levels: without fertilizer and with 500 kg ha^{-1} of NPK fertilizer), with three replications for each combination. The observed parameters included total soil nitrogen, nitrate, nitrogen uptake, chlorophyll, nitrogen-fixing and denitrifying bacteria, and N_2O gas emissions. The results showed that combining MFC, conventional spacing, and NPK fertilizer in the paddy fields resulted in a high total soil nitrogen (0.44%). The results showed different effects on total soil nitrogen in the MFC and fertilization treatments, leading to increased nitrate levels, nutrient uptake, and chlorophyll. Increasing total soil nitrogen significantly contributes to leaf development and significantly aids photosynthesis. The integration of MFC and fertilization observed in this study resulted in a real impact on nitrogen dynamics in paddy fields. This combined treatment effectively reduces total nitrogen loss due to denitrification in paddy fields, thereby increasing the efficiency of uptake by plants.

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1. INTRODUCTION

Nitrogen plays a crucial role in promoting the growth and success of paddy fields, as it facilitates essential processes (Mahmud et al., 2021). Ranging from supporting leaf development and photosynthesis through its involvement in chlorophyll to reinforce stem and root growth, particularly in waterlogged conditions (Mu & Chen, 2021). Nevertheless, a prevalent issue lies in the ineffective utilization of nitrogen-based fertilizers and nitrogen loss from the soil, including gaseous forms (Guo et al., 2019; Liu et al., 2020). Hence, the management of soil nitrogen dynamics takes center stage in ensuring sustainable and productive rice plants. Maintaining optimal soil nitrogen levels through efficient fertilization strategies is paramount for maximizing plant growth, particularly in paddy fields.

Fertilizers can substantially influence nitrogen dynamics within the soil and affect rice plant growth (Yang et al., 2021). When NPK-based fertilizers are applied correctly (right type, right dose, right time, right method, and right target), they supply essential nutrients that promote plant development (Khalida & Lontoh, 2019; Sethy et al., 2020). Nevertheless, excessive fertilizer use can lead to environmental concerns such as greenhouse gas emissions (Munawaroh et al., 2022; Zhang et al., 2020). Exercising prudent nitrogen fertilizer management becomes crucial to optimize nitrogen efficiency, boost crop yields, and reduce adverse environmental consequences.

Efforts to enhance nitrogen absorption efficiency and minimize nitrogen loss from the soil can be achieved through the optimization of plant spacing. Plant spacing is pivotal in nitrogen utilization within rice plants, ultimately influencing overall plant growth and performance (Jiang et al., 2013; Magfiroh et al., 2017). Striking the right balance in plant spacing is important, particularly by implementing plant spacing *jajar legowo*, which entails alternating rows of wide and narrow rice plants (Hatta, 2012). Little rows foster healthy competition among plants for nitrogen resources, potentially enhancing nutrient utilization (Dass et al., 2016). Conversely, wider rows permit improved light penetration, thus supporting photosynthesis and growth (Suhendrata, 2018). This approach might contribute to a more sustainable and efficient nitrogen utilization in paddy fields.

MFC harnesses microbial activity for electricity. One of them is nitrogen-fixing bacteria. These bacteria convert atmospheric nitrogen into a more accessible form for rice plants. Applying Microbial Fuel Cell (MFC) technology can also positively impact nitrogen availability and cycling within paddy fields (Saito et al., 2011; Zhang et al., 2022). Furthermore, MFC can mitigate *denitrification* by effectively utilizing electrons produced during microbial metabolism, offering an innovative means to steer microbial activity (Wetser et al., 2015). Despite being nascent, this technology represents an eco-friendly avenue for enhancing nitrogen dynamics in paddy fields and potentially curbing the environmental repercussions of excessive synthetic nitrogen fertilization.

Effective management of nitrogen fertilizer is of utmost importance in paddy fields, enhancing rice yields and mitigating greenhouse gas emissions. The incorporation of MFC technology, alongside optimal planting strategies, offers the potential to curtail denitrification and improve the efficacy of nitrogen fertilization in paddy fields. Prior studies, such as the work conducted by Ranatunga et al. (2018) have demonstrated the promise of MFC technology in regulating denitrification in submerged paddy fields. Nevertheless, there remains a scarcity of research examining the combined impact of MFC treatment, plant spacing, and fertilization on the dynamics of total soil nitrogen. This research explores the influence of MFC combined with plant spacing and fertilization practices on nitrogen dynamics within paddy fields.

Effective management of nitrogen fertilizer is of utmost importance in paddy fields, enhancing rice yields and mitigating greenhouse gas emissions. The incorporation of MFC technology, alongside optimal planting strategies, offers the potential to reduce denitrification and improve the effectiveness of nitrogen fertilization in rice fields. Previous studies, such as those conducted by Ranatunga et al. (2018). have shown the promise of MFC technology in regulating denitrification in flooded rice fields. Thus, the novelty of this study is to examine the combined impact of MFC treatment, plant spacing, and fertilization on total soil nitrogen dynamics. Thus, this study aims to explore the effect of MFC combined with plant spacing and fertilization practices on nitrogen dynamics in rice fields.

2. MATERIAL AND METHODS

2.1. Research location

The research was carried out in Sukoharjo, Central Java (7°43'55.9'' S 110°47'42.3'' E), and soil analysis was carried out at the collaboration laboratory of Gifu University and the Soil Chemistry and Fertility Laboratory of Universitas Sebelas Maret during July 2022 to March 2023. The soil type at the research site is classified as Inceptisols, with soil pH and nitrogen ranging from 6.01-6.73 and 0.30-0.45%, respectively.

Table 1. Combination treatment description

Combination Treatment Code	Description
A0B0C0	Non-MFC; Conventional spacing; Non fertilizer
A0B0C1	Non-MFC; Conventional spacing; Fertilizer
A0B1C0	Non-MFC; *Jajar legowo* spacing; Non-Fertilizer
A0B1C1	Non-MFC; *Jajar legowo* spacing; Fertilizer
A1B0C0	MFC; Conventional spacing; Non-Fertilizer
A1B0C1	MFC; Conventional spacing; Fertilizer
A1B1C0	MFC; *Jajar legowo* spacing; Non fertilizer
A1B1C1	MFC; *Jajar legowo* spacing, Fertilizer

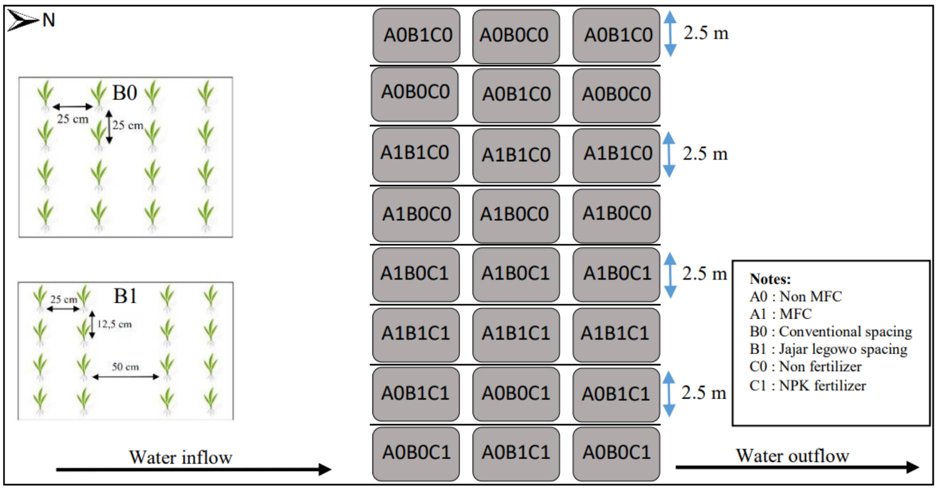

Figure 1. Research plot

2.2. Experimental design

The study employed a factorial field experiment with three factors, namely Microbial fuel cell, abbreviated as MFC (factor A), plant spacing (factor B), and fertilization (factor C). Factor A consisted of 2 levels, including A0 (Without MFC) and A1 (MFC). Factor B also consisted of 2 levels, namely B0 (Conventional spacing 25 cm × 25 cm) and B1 (*jajar legowo* spacing 25 cm × 12.5 cm × 50 cm). Factor C was conducted in 2 levels, i.e., C0 (without fertilizer) and C1 (fertilizer with NPK: nitrogen, phosphorus, and potassium). The fertilizer application in C1 was 400 kg ha^{-1} (in the 4^{th} week after transplanting), with a total of 500 kg ha^{-1}. The experiment was carried out using the IR64 rice variety with a strip plot design arranged for eight combination treatments (Table 1) with three replications, where each experiment plot size was 3 × 2.5 m^2 (Fig. 1).

The irrigation system on the land is carried out using rainwater and flowing water from the reservoir to the land. The direction of the water flows is in accordance with Figure 1 because the water moves from south to north. Therefore, to avoid homogeneity of the treatments given to plants, the research design was created using a strip plot design to avoid the influence of other treatments given.

2.3. Sampling procedures and observation parameters

Soil samples were taken at a depth of 20 cm in a composite manner, then part of the sample was air-dried, pounded, and sieved using a 0.5 mm sieve for analysis of total soil nitrogen, and part of it was left fresh for analysis of nitrate and bacteria. Soil pH was measured weekly by weighing 5 g of soil samples and placing them in a shaking bottle, adding 25 ml of ion-free water, and shaking for 30 minutes. Soil suspension was measured with a pH meter. Total soil nitrogen and plant nitrogen uptake was measured once a week using the Kjeldahl method (Kirk, 1950). 1 g of soil sample was digested with 3 ml of concentrated H_2SO_4 and 1 g of catalyst. Then, the solution was added with 50 ml of distilled water and 10 ml NaOH 40%, then distilled and titrated (Tan, 2005).

The determination of nitrate (NO_3^-) levels was carried out using the spectrophotometer method at a wavelength (λ) of 494 nm (Brake et al., 1958). Plant samples were taken at the maximum vegetative period and the end of the growing period. The chlorophyll extraction process was performed using spectrophotometer (Comar & Zscheile, 1942). 0.1 g of rice leaves were ground, and 10 ml of acetone 80% was added. The solution was filtered with filter paper and read with a UV spectrophotometer at wavelengths (λ) 646 nm and 663 nm, with each sample repeated three times.

N_2O gas emissions were measured using an Electron Capture Detector (ECD) detector (Bramston-Cook, 2008) at the end of the planting period. Gas samples are taken using a chamber (Fig. 2) every 15 minutes starting from the 0^{th} minute, 15^{th} minute, 30^{th} minute, 45^{th} minute, and 60^{th} minute, with a total of 5 takes. Denitrifying bacteria and Nitrogen-fixing bacteria were analyzed using the Full Length 16S Barcoding for Metagenomics using Oxford Nanopore Platform (Santos et al., 2020; Sedlar, 2018).

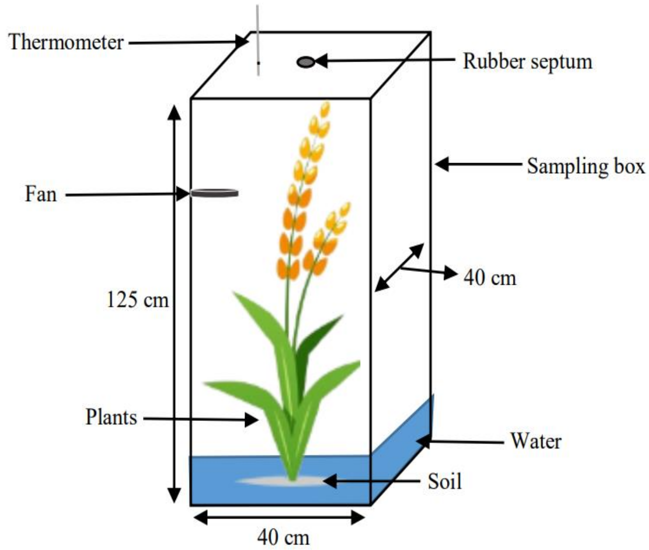

Figure 2. Chamber for gas sampling

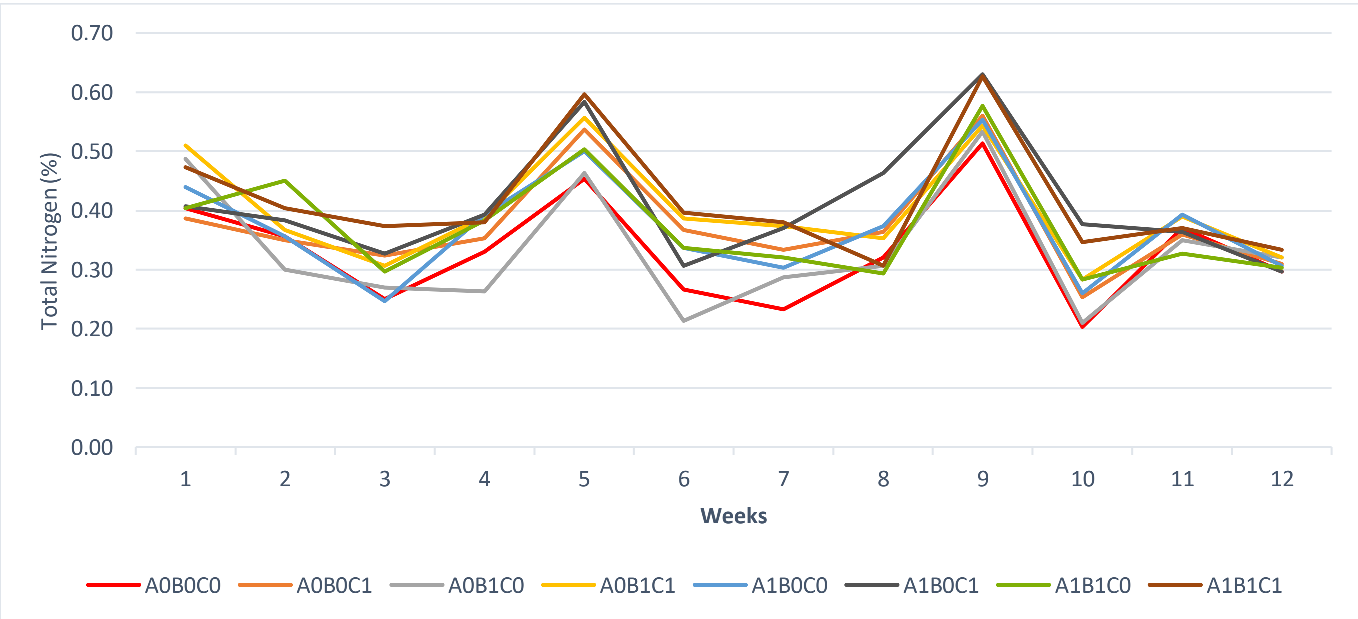

Figure 3. Weekly total soil nitrogen in every phase

2.4. Data analysis

Data from observations and laboratory results were analyzed using ANOVA (Analysis of Variance) with a confidence level of 95%, and Pearson's correlation test was carried out to determine the correlation between the observed variables to explain the effect of treatments on the dynamics of total soil nitrogen. Stepwise regression was used to help identify the most appropriate model for predicting treatments by repeatedly testing various treatment combinations and assessing their performance on total soil nitrogen. All data analysis was performed using R Studio software version 4.3.0

3. RESULTS

3.1. Nitrogen dynamics

The 12-week observations revealed fluctuations in total soil nitrogen concentration in the study area (Fig. 3). In the early stages of planting, all treatment combinations showed a decrease in total soil nitrogen content until the 3rd week, except in the A1B1C0 treatment, which increased in the 2nd week, which could occur due to low nitrogen uptake by plants and bacteria working on MFC by increasing nitrogen. Furthermore, in the 4th to 5th week there was an increase in total soil nitrogen due to the application of fertilizer. However, in the treatment without fertilization, the total soil nitrogen value also increased due to the overflow of irrigation water, which caused homogeneity in the fertilizer content in the 5th week.

In the 6th to 8th week, total soil nitrogen decreased again. However, there are different graphs in the 7th and 8th weeks, which occur due to the solubility of the previous fertilizer, the presence of MFC, which increases nitrogen, and the homogeneity of fertilizer caused by irrigation water. In the 9th week, another round of fertilization resulted in an increase in total soil nitrogen content, followed by a gradual decrease until the 10th week. All treatments experienced an increase in total soil nitrogen when the plants entered the 11th week. During the harvest, treatments involving the addition of MFC showed more consistent total soil nitrogen content with a smaller reduction rate than treatments without MFC. These dynamics indicate nitrogen fluctuations that are influenced by plant absorption processes.

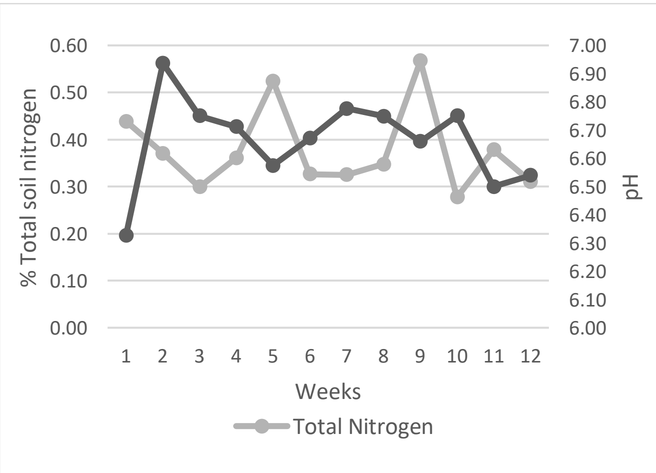

Figure 4. Weekly total soil nitrogen and pH

Table 2. Total soil nitrogen content and soil nitrate

Treatment	Total Soil Nitrogen (%)	Nitrate (me l^{-1})
A0B0C0	0.33a	0.056a
A0B0C1	0.38b	0.065bc
A0B1C0	0.33a	0.059a
A0B1C1	0.41b	0.064ab
A1B0C0	0.38b	0.062ab
A1B0C1	0.42b	0.076e
A1B1C0	0.38b	0.061a
A1B1C1	0.44c	0.069d

Notes: A0: Non MFC, A1: MFC, B0: conventional spacing, B1: *jajar legowo* spacing, C0: non fertilizer, C1: fertilizer. Number followed by the same letter are not significantly different.

Table 3. The significance of total soil nitrogen and nitrate under each factor of MFC plant spacing, and fertilization

Treatment	Total soil nitrogen	Nitrate
MFC		
Non MFC	0.36a	0.061a
MFC	0.40b	0.067b
Plant Spacing		
Conventional	0.38a	0.065a
Jajar legowo	0.39a	0.063a
Fertilizer		
Non fertilizer	0.35a	0.059a
Fertilizer	0.41b	0.068b

Notes: Number followed by the same letter are not significantly different.

Figure 4 shows the weekly dynamics of total soil nitrogen and pH in the soil, which are generally inversely proportional to the soil pH. The interaction between nitrogen and soil pH is evident in the treatment with the addition of fertilizer inputs, which decreased soil pH in 5th week and 9th week. The soil nitrogen content in 5th week increased from 0.36% to 0.52%, with a decrease in soil pH from 6.75 to 6.57. In the 9th week, the total soil nitrogen value also increased from 0.33% to 0.57%, which was accompanied by a decrease in soil pH from 6.75 to 6.66, although the difference is not significant.

3.2. Soil Nitrogen Content

Table 2 shows that the combination of MFC, *jajar legowo* plant spacing, and fertilizer, as indicated by the notation resulting from ANOVA analysis, has a significantly higher total soil nitrogen value compared to other treatments. Although the range of total soil nitrogen values is not too high, this combination still yields higher total soil nitrogen than the other treatments. This can occur because the provision of MFC in paddy fields acts as a binder of nitrogen nutrients through the tethering of nitrogen nutrients by bacteria. On the other hand, the addition of NPK fertilizer also increases the content of nitrogen elements in the soil. Meanwhile, the lowest total soil nitrogen was found in the treatment without

MFC, with *jajar legowo* spacing, and without fertilization, with an average total soil nitrogen of 0.32%. Providing fertilizer and MFC treatment, according to Anova analysis, showed higher soil nitrogen results compared to treatment without them while providing plant spacing treatment had no significant effect on total soil nitrogen values (Table 2). However, applying *jajar legowo* spacing in combination with MFC, plant spacing, and fertilization factor provided higher results than conventional spacing, although the results obtained were not significant.

Table 3 shows that factor MFC and factor fertilizer resulted in significantly higher total soil nitrogen and nitrate according to ANOVA analysis. Total soil nitrogen with MFC (0.40%) is higher than without MFC (0.36%). Accordingly, soil nitrate was also higher in factor MFC treatment (0.067 me l^{-1}) than without MFC (0.061 me l^{-1}). NPK fertilizer application also contributed a positive impact by resulting in high total soil nitrogen (0.41%) and nitrate (0.068 me l^{-1}). However, plant spacing did not contribute to total soil nitrogen and nitrate; it can be seen from the notation of the results of the ANOVA analysis, which shows that conventional plant spacing and *jajar legowo* are not significantly different.

3.3. Nitrogen affecting bacteria

Table 4 presents the nitrogen-fixing and denitrifying bacteria population in each treatment. It is confirmed from Table 4 that combining MFC with NPK fertilizer and *jajar legowo* spacing (A1B1C1) leads to the highest nitrogen-fixing bacteria population (863 $\times 10^6$ CFU) but, in opposite, resulted in the lowest denitrifying bacteria population (298 $\times 10^6$ CFU). Table 4 also presents that the implementation of MFC combined with NPK fertilizer is significantly different in producing more nitrogen than other treatment combinations because the number of available nitrogen-providing bacteria is greater than the number of denitrifying bacteria so that it can suppress the nitrogen loss process through denitrification.

3.4. N_2O gas emission

Figure 5 shows that the N_2O gas emissions in generally affected by the MFC, where the lowest N_2O emission value (4.04 mg m^{-2} N_2O) was obtained in the MFC treatment without fertilization.

Table 4. Population of nitrogen-fixing bacteria and denitrifying bacteria in each treatment

Treatment	Nitrogen Fixing Bacteria ($\times 10^6$ CFU)	Denitrification Bacteria ($\times 10^6$ CFU)
A0B0C0	578a	473d
A0B0C1	619b	457cd
A0B1C0	722d	624f
A0B1C1	585a	523e
A1B0C0	804e	550e
A1B0C1	710d	352b
A1B1C0	665c	429c
A1B1C1	863f	298a

Notes: A0: Non MFC, A1: MFC, B0: conventional spacing, B1: *jajar legowo* spacing, C0: non fertilizer, C1: fertilizer; number with the same letter are not significantly different at the same parameter

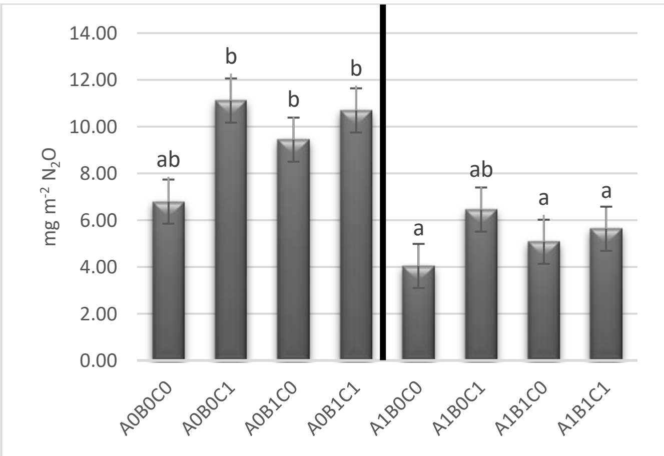

Figure 5. Nitrous Oxide (N_2O) gas emission in treatment

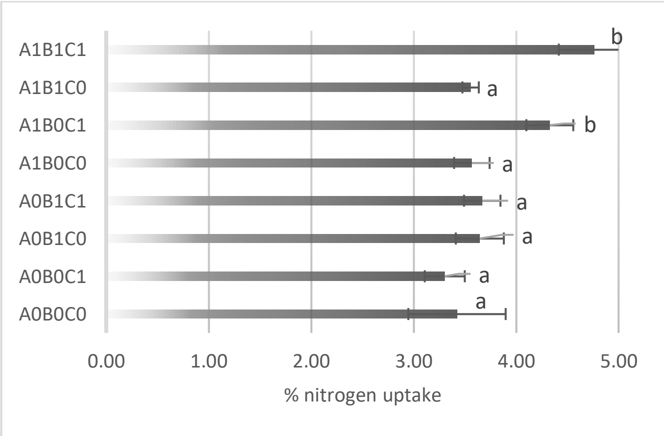

Figure 6. Nitrogen uptake by the plant in each treatment

However, fertilization led to the high N_2O gas emission, as seen in Figure 5, whereas the high N_2O gas emission occurred in the treatment without MFC. The highest N_2O gas emission was observed in the combination treatment of without MFC, conventional spacing, and NPK fertilizer, A0B0C1 (11.12 mg m^{-2} N_2O); and without MFC, *jajar legowo* spacing, and NPK fertilizer, A0B1C1 (10.69 mg m^{-2} N_2O).

3.5. Nitrogen uptake

Figure 6 shows the nitrogen uptake by plants from each treatment. It can be seen in Figure 6 that the significant high nitrogen uptake by plants was found in the combination of MFC and NPK fertilizer regardless of the plant spacing (A1B0C1 and A1B1C1) according to Anova analysis, which were 4.33% and 4.76%, respectively. This is also approved by the analysis shown in Figures 7a, 7b, and 7c.

Figure 7 shows that the nitrogen uptake in plant tissues is contributed by MFC (7a; 4.05 mg $plant^{-1}$) and fertilization application (7c, 4.02 mg $plant^{-1}$), where the means were significantly different with their control (3.51 and 3.55 mg/plant, respectively). Figure 7b also confirmed that plant spacing did not lead to the difference in nitrogen uptake by plants, as shown by the overlap error bars.

Figure 8 shows the chlorophyll levels in rice plant leaves from each treatment. In Figure 8, high levels of chlorophyll in plants are found in the combination of MFC application and NPK fertilization with conventional spacing (A1B0C1) 0.897 µg g^{-1}.

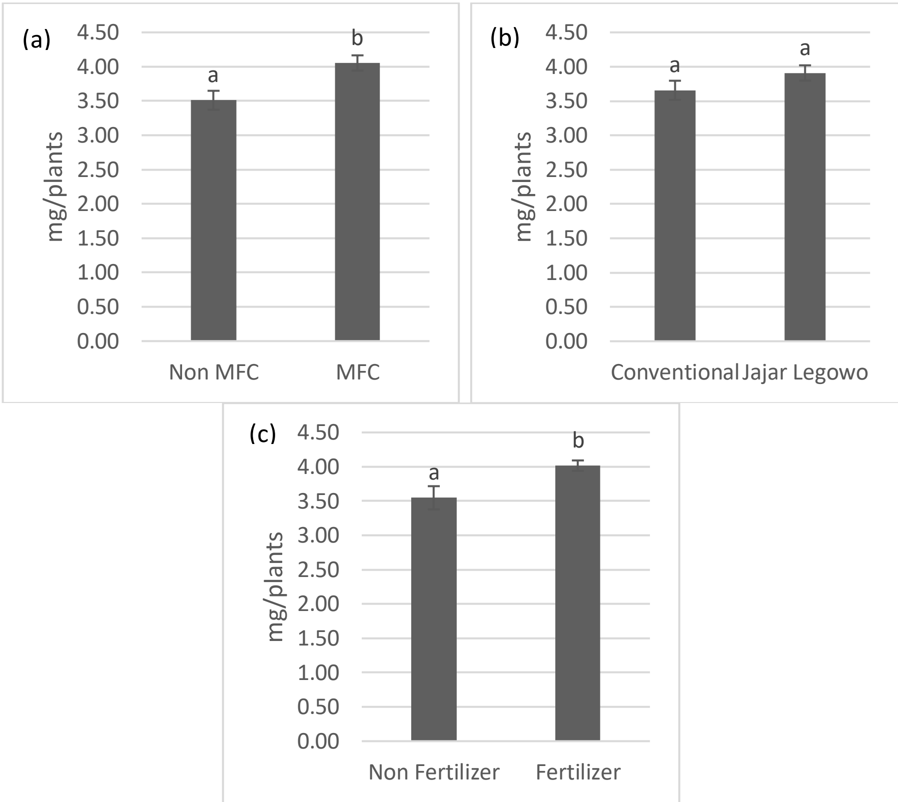

Figure 7. Significance of nitrogen uptake under each factor of MFC (a), plant spacing (b), fertilization (c)
Notes: bars followed by the same letter are not significantly different.

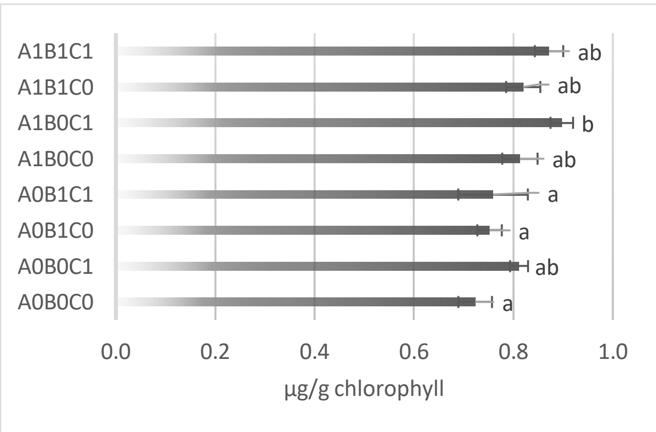

Figure 8. Chlorophyll in plants

4. DISCUSSION

Weekly soil nitrogen dynamics showed that MFC, plant spacing, and fertilization influenced total soil nitrogen. However, among these factors, only MFC and fertilization notably impacted the dynamics of total soil nitrogen. Fertilization is commonly employed to enhance rice productivity by meeting the plant's nitrogen requirements (Yousaf et al., 2017) consequently affecting the overall soil nitrogen conditions (Alhammad et al., 2023). The total soil nitrogen levels in Figure 3 within the fertilizer treatment exhibited lower values than other treatments during weeks 6, 8, and 11 (under 0.47%), attributed to plant nitrogen uptake and nitrogen loss processes. Nitrogen availability in the soil can decrease because plants for growth and experience loss absorb it through various biological and chemical processes. The higher total soil nitrogen content was primarily due to fertilizers supporting plant growth. This is supported by Liu et al. (2021), which states that nitrogen fertilizer is crucial in helping plant growth by providing nutrients. According to Permatasari et al. (2019) and Widodo and Damanhuri (2021), nitrogen fertilizers typically begin hydrolyzing shortly after application, increasing nitrogen levels. However, unabsorbed nitrogen in the soil tends to evaporate and escape into the atmosphere (Gupta et al., 2021; Zhang et al., 2020).

The nitrogen dynamics in rice plants are not only influenced by fertilization but also by MFC. Microbial fuel cell influence the nitrogen cycle in the soil by modulating soil microbial activity. The soil nitrogen dynamics illustrate differences in the total soil nitrogen values between the MFC-treated and untreated (Fig. 3) plots, with the MFC-treated generally displaying higher nitrogen total soil content levels with the highest total soil nitrogen is 0.63%. The impact of MFC on elevating the total soil nitrogen values is evident from the initial weeks. This is attributed to the early stages of the rice plant's growth cycle, from the initial stage to the flowering stage, where the plants tend to generate more root exudates (Xiong et al., 2019). According to Ma et al. (2022), microbes utilize these root exudates as part of their metabolic processes, generating electrons for the MFC mechanism. However, towards the end of the planting period, particularly during weeks 11 and 12, the MFC displayed no significant impact on the total soil nitrogen. The MFC functionality declines at the end of the rice planting period due to a reduction in root exudates (Chen et al., 2019).

MFC has demonstrated its effectiveness in enhancing total soil nitrogen by harnessing highly performing microorganisms (Wang et al., 2015). Within the MFC treatment, it was evident that the factors most influencing the total soil nitrogen were the MFC itself and the addition of fertilizers, while the plant spacing treatment showed no significant impact. The dynamics of the total soil nitrogen can potentially influence the soil pH conditions (Barłóg et al., 2022). Soil pH dynamics exhibit an inverse relationship with nitrogen dynamics. The correlation analysis (Table 5) indicates that a decrease follows an increase in nitrogen in soil pH (negative r). Studies by Guo et al. (2010); Basuki and Vega Kartika (2019) reveal that excessive inorganic chemical fertilizer application leads to a decline in soil pH. This occurs due to the transformation of nitrogen into available forms, releasing H^+ ions and consequently lowering the soil pH (Singh, 2018). According to Lv et al. (2020) a portion of nitrogen-based fertilizer addition undergoes nitrification, leading to the release of H^+. However, waterlogging in saturated paddy fields may elevate the soil pH (Rahayu et al., 2017). Optimal soil pH conditions facilitate the absorption of nutrients into plants (Shetty & Prakash, 2020).

Plants can uptake nitrate as a form of nitrogen (Hachiya & Sakakibara, 2016). In addition to augmenting the overall soil nitrogen, nitrogen fertilization contributes to the increase in soil nitrate levels (Bijay & Craswell, 2021; Rashid et al., 2016). Table 5 confirms a positive correlation between higher total soil nitrogen and increased soil nitrate content. In addition to fertilization, MFC utilization notably impacts the total soil nitrogen and nitrate levels (Vijay et al., 2022). Applying MFC to rice plants serves as a means of binding nitrogen nutrients (Read et al., 2010) by curbing *denitrification* through electron acceptance (Ranatunga et al., 2018). On the other hand, the spacing between plants did not exhibit a significant influence on the nitrogen and nitrate values.

Fertilization contributes to augmenting nitrogen nutrients in the soil, whereas applying MFC impacts nitrogen dynamics via soil biology (Nitisoravut & Regmi, 2017). The MFC treatment exhibited a higher presence of nitrogen-fixing bacteria with average 693 ×10^6 CFU than denitrifying bacteria with average 463 ×10^6 CFU. As evidenced by the correlation values in Table 5, nitrogen-fixing bacteria display a positive relationship with the total soil nitrogen (r= 0.604). An escalation in nitrogen-fixing bacteria correlates with an increase in total soil nitrogen because these bacteria can supply nitrogen beyond the scope of fertilization. According to Aasfar et al. (2021) nitrogen-fixing bacteria provide soil nitrogen by converting atmospheric nitrogen into a form that plants can absorb. These bacteria naturally convert atmospheric nitrogen (N_2) into simpler, non-toxic, and soluble forms, predominantly NH_4^+ (Chen et al., 2023) which plants utilize, constituting a vital step in the distribution of this essential nutrient within the soil (Mukherjee & Sen, 2021).

Table 5. Pearson correlation of total soil nitrogen with other observed parameters

Parameter	Signification	r
pH	<0.01	-0.672**
Nitrate	<0.01	0.662**
Nitrogen-fixing bacteria	<0.01	0.604**
Denitrifying bacteria	<0.01	-0.622**
Nitrogen uptake	<0.01	0.754**
Chlorophyll	<0.01	0.560**
N_2O gases	0.104	-0.340

The most abundant nitrogen-fixing bacteria were observed in the combined treatment involving MFC, *jajar legowo*, and fertilization have 863 ×10^6 CFU. The heightened presence of nitrogen-fixing bacteria in this setting can be attributed to the continuous transfer of low-potential electrons from the MFC anode to the cathode. These electrons, available at the anode, are readily utilized by *nitrogenase*, which prompts the congregation of numerous nitrogen-fixing bacteria at the MFC anode (Danapriatna, 2010). Conversely, the treatment with the lowest nitrogen-fixing bacteria was the one lacking MFC, conventional plant spacing, and devoid of fertilization with total colony is 578 ×10^6 CFU. In this scenario, the absence of the MFC function as an electron acceptor restrains the utilization of *nitrogenase* by bacteria. N_2 fixation, facilitated by *nitrogenase*, predominantly occurs with most nitrogen-fixing bacteria active under anaerobic conditions (Soumare et al., 2020).

Furthermore, MFC-absorbing electrons produced by denitrifying bacteria tend to reduce nitrogen loss, maintaining a higher total soil nitrogen level (negative r). The treatment without MFC, conventional spacing, and lacking fertilization displayed the highest denitrifying bacteria counts (624 ×10^6 CFU). Interestingly, even in the absence of fertilization, this treatment exhibits a substantial quantity of nitrogen-fixing bacteria, potentially contributing to the elevated nitrogen levels. High nitrogen levels consistently correlate with increased nitrate values (Table 3), which can interact with electrons produced by microbial processes. According to Mahmud et al. (2020) and Stein and Klotz (2016) nitrogen-fixing bacteria increase the nitrogen content in the soil through nitrogen fixation through interaction with electrons produced through their metabolism. In the absence of MFC, nitrate serves as an active electron acceptor, a favorable condition for the proliferation of denitrifying bacteria, thus leading to an increased count of these bacteria (Brito et al., 2020).

The treatment involving MFC and fertilization impacted the reduced presence of denitrifying bacteria, manifesting the lowest count in this particular treatment (Table 4). The heightened nitrate levels observed in the MFC application, along with conventional plant spacing and MFC utilization with *jajar legowo* spacing and fertilization are anticipated to serve as electron acceptors for denitrifying bacteria (Tiso & Schechter, 2015). The existence of MFC renders denitrification inactive, as the system accepts the electrons (Ucar et al., 2017). The diminished denitrifying bacteria levels stem from the suppressed denitrification processes facilitated by the MFC treatment (Zhao et al., 2016). According to Zhang et al. (2022) and Wetser et al. (2015), MFC functions by employing electrons produced by bacteria, including denitrifying bacteria, transferring them from the anode (situated in the soil) to the cathode (interacting with air), where they react with oxygen to form H_2O.

The augmented total soil nitrogen levels, resulting from reduced denitrification, correspond to lower N_2O gas emissions (negative r). N_2O gas is generated by converting nitrate into gas, a process known as denitrification (Timilsina et al., 2020). N_2O gas is deemed a hazardous greenhouse gas because it is a form of nitrogen loss that plants should ideally utilize (Harter et al., 2016; Lan et al., 2020). The research suggests that the fertilization practices aimed at enriching soil nutrients might contribute to heightened N_2O gas levels. Similar studies Adviento-Borbe and Linquist (2016); Anshori et al. (2018), have shown that nitrogen fertilizer significantly contributes to greenhouse gas emissions, particularly N_2O gas emissions in rice cultivation. Fertilization, specifically with traditional and *jajar legowo* spacing without MFC application, resulted in increased N_2O gas production.

The elevated N_2O gas levels in the treatment lacking MFC are due to bacteria-generated electrons being accepted solely by soil oxidants (Fan et al., 2020). Among these oxidants, nitrates are recognized as ones that can accept electrons (Mania et al., 2016). As per Syahputra et al. (2011), denitrifying bacteria employ nitrate as the ultimate electron recipient, reducing nitrate to nitrite and subsequent conversion into N_2O gas. MFC notably stands out in curtailing N_2O gas emissions, demonstrating lower N_2O levels than treatments without MFC. Through electron absorption, MFC can help reduce nitrogen oxide gas emissions (Liu et al., 2022). Despite the combination of MFC and fertilization displaying higher N_2O gas levels than the combination devoid of MFC and fertilization, the utilization of MFC markedly varies in its ability to mitigate N_2O gas.

The notable surge in nitrogen absorption observed in the MFC and fertilization treatments was also linked to the elevated total soil nitrogen (positive r). As Winarso et al. (2020) and Zhang et al. (2020) indicated, the concentration of nitrogen nutrients within plants mirrors the high presence of nitrogen in the soil. The combined treatment of MFC and fertilization showcases heightened nitrogen and nitrate levels compared to other treatments (Table 3), signifying that elevated nitrate levels correspond to increased nitrogen uptake. According to Paśmionka et al. (2021), in rice plants, absorbed nitrate (NO_3^-) transforms within plant tissues into nitrogen compounds essential for plant growth. With increased soil nitrogen content, more accessible nitrogen becomes available to plants, potentially facilitating their growth and overall development (Agegnehu et al., 2016).

Increased nitrogen uptake can notably influence the chlorophyll status in plants (Drescher et al., 2020; Mussarat et al., 2021), a relationship underscored by the considerable positive correlation between chlorophyll levels and total soil nitrogen (positive r). Findings from the study indicate that MFC and fertilization led to heightened chlorophyll levels in rice plants. Elevated levels of nitrogen within the soil or introduced through fertilization can stimulate chlorophyll production, consequently bolstering the photosynthetic capacity of the plants (Dang et al., 2023). The substantial chlorophyll values in this treatment are attributed to the heightened nitrogen content, resulting in increased nitrate and nitrogen uptake (Guo et al., 2019). Furthermore, as per Mussarat et al. (2021), nitrogen forms a key component of chlorophyll; therefore, an increase in nitrogen supply significantly influences chlorophyll concentration.

The nitrogen dynamics examined in this study can be harmonized through appropriate nitrogen management practices involving fertilizer and MFC treatments, showcasing a positive influence on plant growth. Nitrogen fertilization augments nitrogen availability for plant uptake, fostering increased plant growth and nitrogen utilization (Sharma & Bali, 2018). Nonetheless, excess nitrogen from fertilizers can induce denitrification, leading to nitrogen loss from the soil (Ahmed et al., 2017). In this research, MFC application demonstrated a capacity to mitigate denitrification, consequently positively impacting nitrogen dynamics. The substantial plant uptake of nitrogen and chlorophyll levels and lower N_2O gas emissions corroborate these findings. Elevated chlorophyll values are linked to heightened nitrogen levels, suggesting potential increments in nitrate and nitrogen uptake (Fu et al., 2021). Table 5 presents soil nitrogen's multivariate analysis (stepwise regression) with other observed parameters (such as nitrate, nitrogen-fixing bacteria, denitrifying bacteria, pH, and N_2O gas emissions). Table 3 shows the predominant parameters influencing total soil nitrogen: denitrifying bacteria and soil nitrate.

Stepwise linear regression analysis in Table 3 resulted in the Equation 1 model.

$$Total\ soil\ nitrogen = 0.275 - 0.0003\ denitrifying\ bacteria + 2.92\ nitrate \text{ .... [1]}$$

Equation 1 informs that only two variables synergic ally influenced total soil nitrogen, i.e., denitrifying bacteria and soil nitrate, with the coefficient of determination (R^2) of 0.817 or 81.7%. That means denitrifying bacteria and soil nitrate influenced total soil nitrogen by 81.7%, while 18.3% was explained by other variables that were not included in the currently observed parameters.

The dynamics of nitrogen significantly impact plant growth (Van Meter et al., 2017). The study outcomes indicate distinct effects on total soil nitrogen within MFC and fertilization treatments, leading to heightened levels of nitrate, enhanced nutrient uptake, and elevated chlorophyll. Elevated total soil nitrogen prominently contributes to leaf development and significantly aids photosynthesis (Astuti & Wibawa, 2014) facilitating the production of assimilates that serve as energy sources for growth (Mu & Chen, 2021). The integration of MFC and fertilization observed in this research yields tangible impacts on nitrogen dynamics within rice fields. This combined treatment effectively mitigates total nitrogen loss from denitrification in paddy fields, enhancing efficient absorption by plants. Furthermore, the MFC application in rice fields serves as a technology that actively preserves nitrogen levels within the soil.

5. CONCLUSION

The combination of MFC and fertilizer can increase total soil nitrogen, as seen in the dynamics of total soil nitrogen for 12 weeks. This combination creates a strong correlation between parameters like nitrate, nitrogen-fixing bacteria, nitrogen uptake, and chlorophyll while denitrifying bacteria and soil pH have a strong inverse relationship with total soil nitrogen. Thus, using MFC and fertilization in lowland rice can boost total soil nitrogen by interacting with bacteria to suppress denitrification. Further research on MFCs' role in reducing denitrification is crucial for sustainable agriculture.

Declaration of Competing Interest

The authors declare that no competing financial or personal interests may appear to influence the work reported in this paper.

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