



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

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ARTICLE INFO

Keywords:

Nitrogen dynamics
Nitrogen loss
N₂O gas emission
Soil nitrate

Article history

Submitted: 2024-04-30

Accepted: 2024-06-05

Available online: 2024-06-30

Published regularly:

June 2024

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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 N₂O 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.

How to Cite: Efendi, S., Komariah, Syamsiyah, J., Dewi, W.S., Hiramatsu, K., Fauzan, A.A. (2024). Effect of Microbial Fuel Cell, fertilizer, and plant spacing on nitrogen dynamics in paddy soil. *Sains Tanah Journal of Soil Science and Agroclimatology*, 21(1): 83-94. <https://doi.org/10.20961/stjssa.v21i1.86312>

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

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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; <i>Jajar legowo</i> spacing; Non-Fertilizer
A0B1C1	Non-MFC; <i>Jajar legowo</i> spacing; Fertilizer
A1B0C0	MFC; Conventional spacing; Non-Fertilizer
A1B0C1	MFC; Conventional spacing; Fertilizer
A1B1C0	MFC; <i>Jajar legowo</i> spacing; Non fertilizer
A1B1C1	MFC; <i>Jajar legowo</i> spacing, Fertilizer

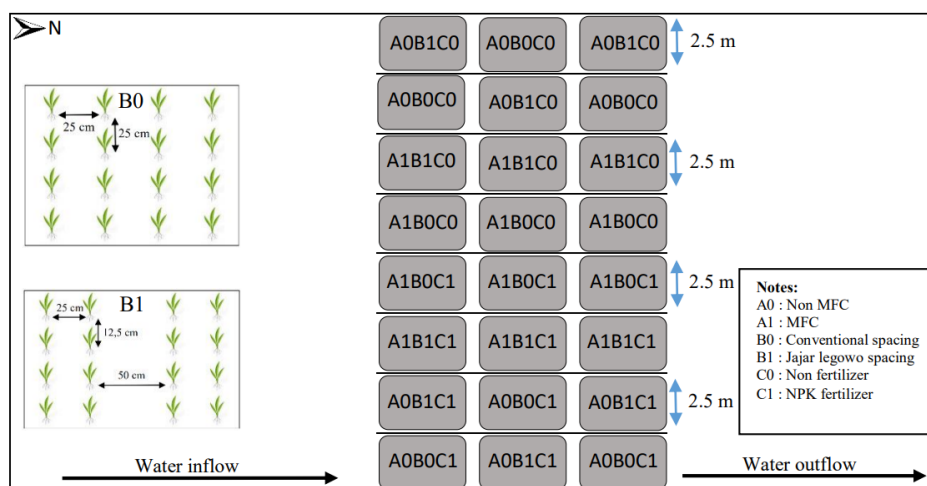


Figure 1. Research plot

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 4th 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 m² (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 H₂SO₄ 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 (NO₃⁻) 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.

N₂O 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 0th minute, 15th minute, 30th minute, 45th minute, and 60th 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).

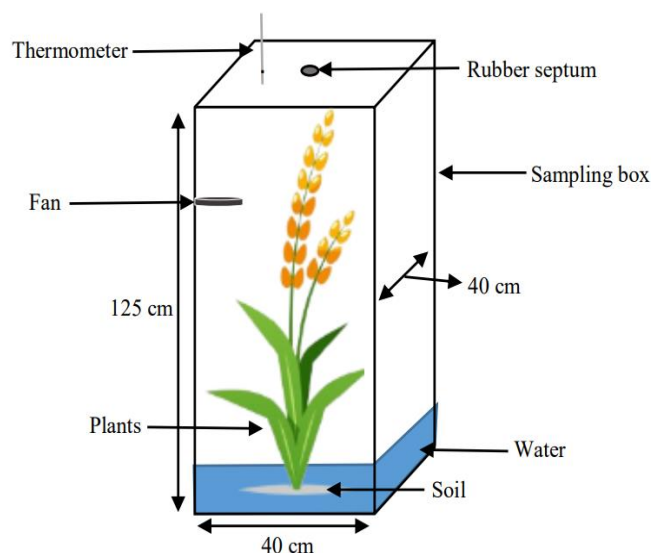


Figure 2. Chamber for gas sampling

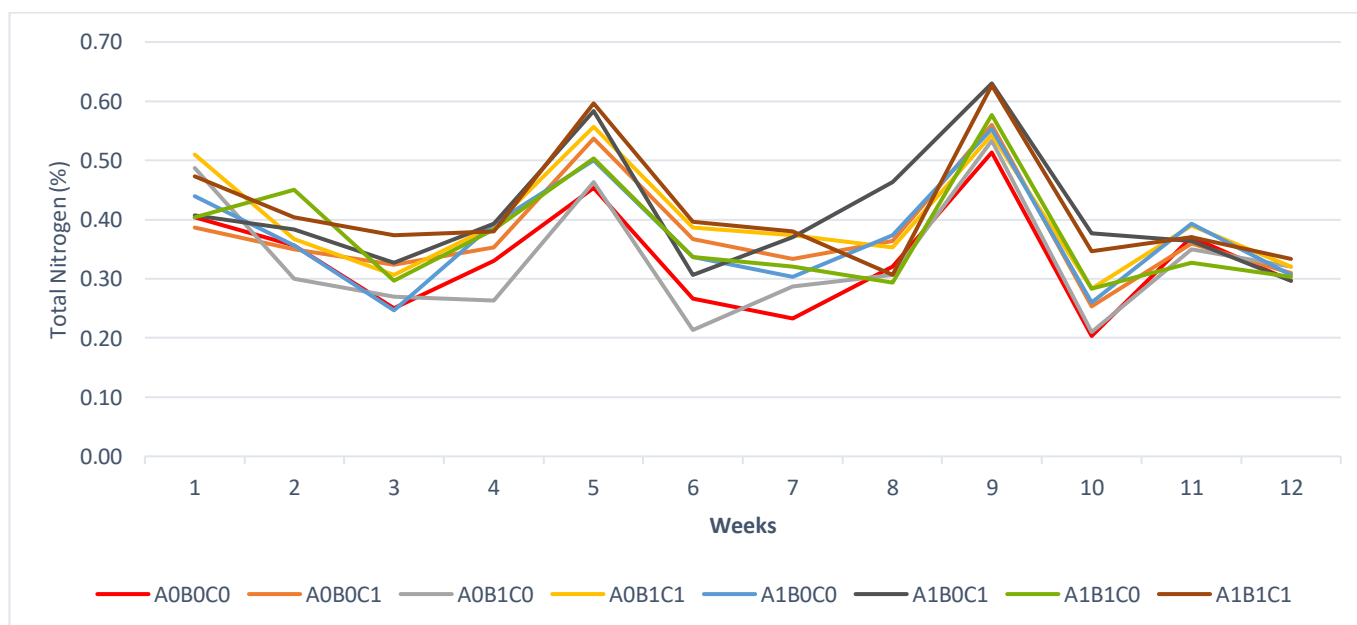


Figure 3. Weekly total soil nitrogen in every phase

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.

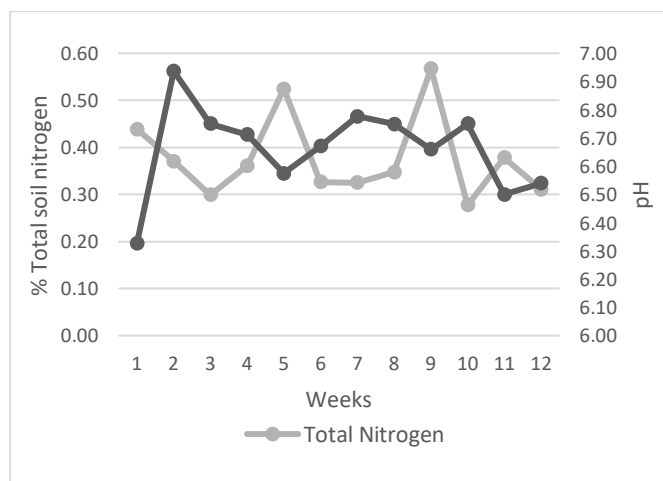


Figure 4. Weekly total soil nitrogen and pH

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
<i>Jajar legowo</i>	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 ×10⁶ CFU) but, in opposite, resulted in the lowest denitrifying bacteria population (298 ×10⁶ 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. N₂O gas emission

Figure 5 shows that the N₂O gas emissions in generally affected by the MFC, where the lowest N₂O emission value (4.04 mg m⁻² N₂O) 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 (×10 ⁶ CFU)	Denitrification Bacteria (×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

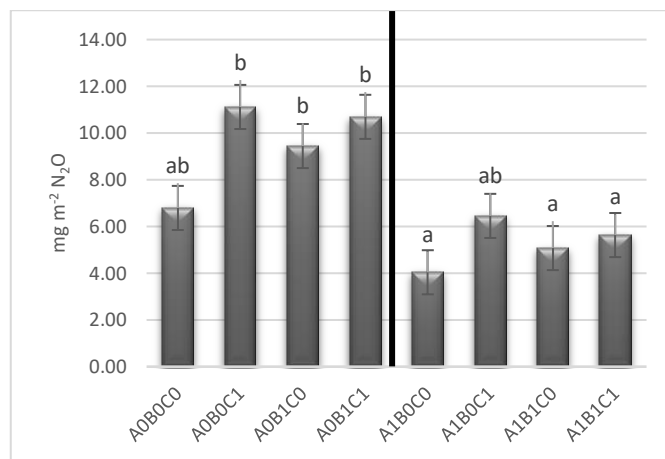


Figure 5. Nitrous Oxide (N₂O) gas emission in treatment

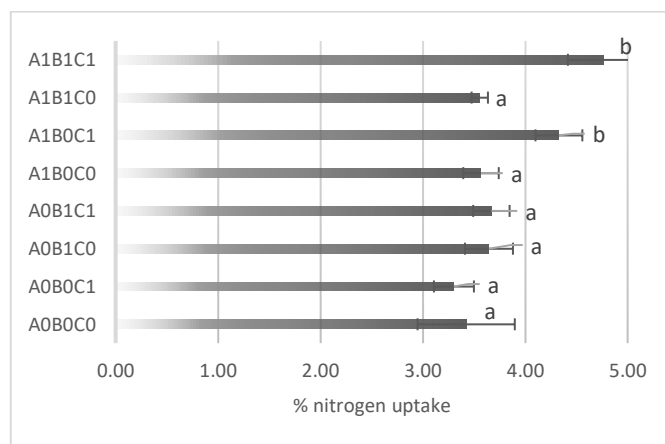


Figure 6. Nitrogen uptake by the plant in each treatment

However, fertilization led to the high N₂O gas emission, as seen in Figure 5, whereas the high N₂O gas emission occurred in the treatment without MFC. The highest N₂O gas emission was observed in the combination treatment of without MFC, conventional spacing, and NPK fertilizer, A0B0C1 (11.12 mg m⁻² N₂O); and without MFC, *jajar legowo* spacing, and NPK fertilizer, A0B1C1 (10.69 mg m⁻² N₂O).

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.

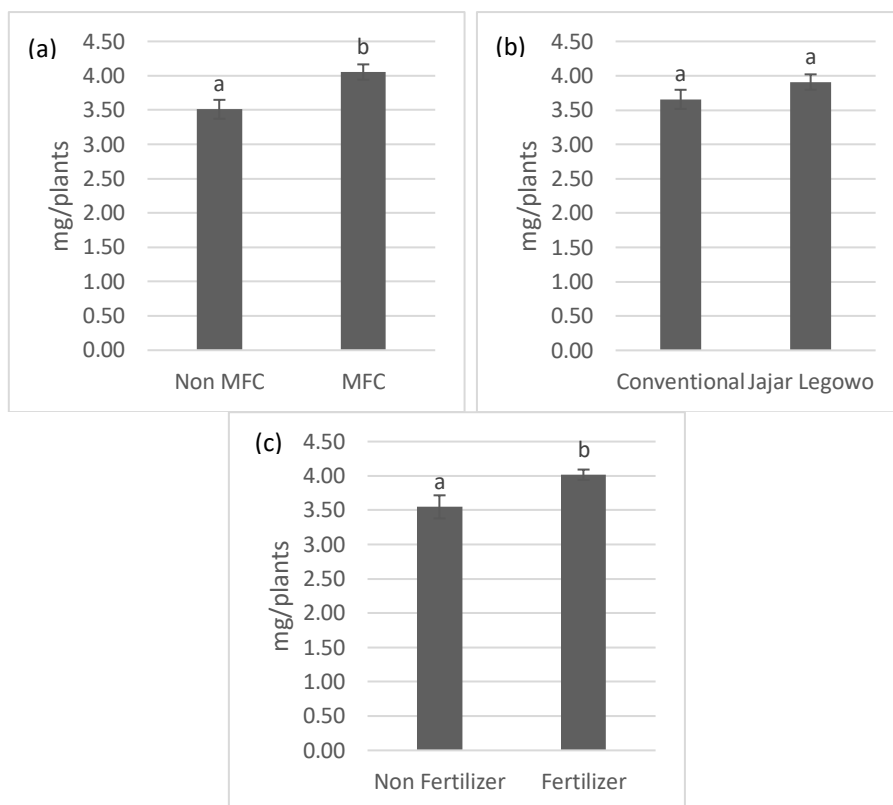


Figure 7. Significance of nitrogen uptake under each factor of MFC (a), plant spacing (b), fertilization (c)
Notes: bars followed by the same letter are not significantly different.

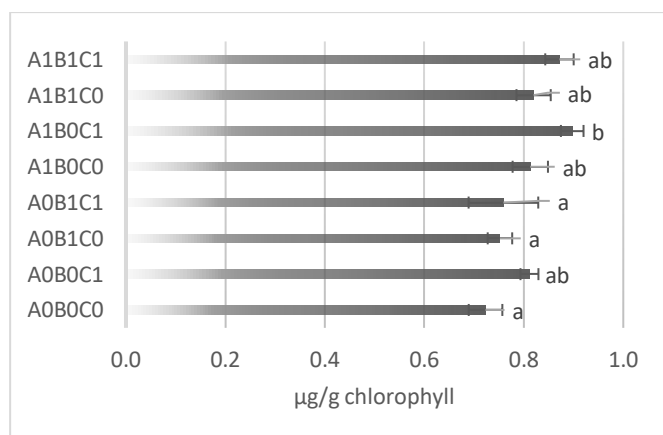


Figure 8. Chlorophyll in plants

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 (Barióg 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 (Nitorisavut & Regmi, 2017). The MFC treatment exhibited a higher presence of nitrogen-fixing bacteria with average 693 ×10⁶ CFU than denitrifying bacteria with average 463 ×10⁶ 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 (N₂) into simpler, non-toxic, and soluble forms, predominantly NH₄⁺ (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	<i>r</i>
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 ₂ O 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 ×10⁶ 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 ×10⁶ CFU. In this scenario, the absence of the MFC function as an electron acceptor restrains the utilization of *nitrogenase* by bacteria. N₂ 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 ×10⁶ 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 H₂O.

The augmented total soil nitrogen levels, resulting from reduced denitrification, correspond to lower N₂O gas emissions (negative *r*). N₂O gas is generated by converting nitrate into gas, a process known as denitrification (Timilsina et al., 2020). N₂O 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 N₂O 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 N₂O gas emissions in rice cultivation. Fertilization, specifically with traditional and *jajar legowo* spacing without MFC application, resulted in increased N₂O gas production.

The elevated N₂O 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 N₂O gas. MFC notably stands out in curtailing N₂O gas emissions, demonstrating lower N₂O 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 N₂O gas levels than the combination devoid of MFC and fertilization, the utilization of MFC markedly varies in its ability to mitigate N₂O 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 (NO₃⁻) 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 N₂O 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 N₂O 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.

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

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.

References

- Aasfar, A., Bargaz, A., Yaakoubi, K., Hilali, A., Bennis, I., Zeroual, Y., & Meftah Kadmiri, I. (2021). Nitrogen Fixing Azotobacter Species as Potential Soil Biological Enhancers for Crop Nutrition and Yield Stability [Review]. *Frontiers in Microbiology*, 12. <https://doi.org/10.3389/fmicb.2021.628379>
- Adviento-Borbe, M. A. A., & Linqvist, B. (2016). Assessing fertilizer N placement on CH₄ and N₂O emissions in irrigated rice systems. *Geoderma*, 266, 40-45. <https://doi.org/10.1016/j.geoderma.2015.11.034>
- Agegnehu, G., Nelson, P. N., & Bird, M. I. (2016). Crop yield, plant nutrient uptake and soil physicochemical properties under organic soil amendments and nitrogen fertilization on Nitisols. *Soil and Tillage Research*, 160, 1-13. <https://doi.org/10.1016/j.still.2016.02.003>
- Ahmed, M., Rauf, M., Mukhtar, Z., & Saeed, N. A. (2017). Excessive use of nitrogenous fertilizers: an unawareness causing serious threats to environment and human health. *Environmental Science and Pollution Research*, 24(35), 26983-26987. <https://doi.org/10.1007/s11356-017-0589-7>
- Alhammad, B. A., Mohamed, A., Raza, M. A., Ngie, M., Maitra, S., Seleiman, M. F., . . . Gitari, H. I. (2023). Optimizing Productivity of Buffel and Sudan Grasses Using Optimal Nitrogen Fertilizer Application under Arid Conditions. *Agronomy*, 13(8), 2146. <https://doi.org/10.3390/agronomy13082146>
- Anshori, A., Sunarminto, B. H., Haryono, E., & Mujiyo, M. (2018). Potential Production of CH₄ And N₂O in Soil Profiles from Organic and Conventional Rice Fields. *Sains Tanah - Journal of Soil Science and Agroclimatology*, 15(1), 7. <https://doi.org/10.15608/stjssa.v15i1.19324>
- Astuti, H. B., & Wibawa, W. (2014). Penerapan Teknologi Pemupukan Padi Sawah Di Provinsi Bengkulu. *Jurnal AGRISEP: Kajian Masalah Sosial Ekonomi Pertanian dan Agribisnis*, 13(1), 51-59. <https://doi.org/10.31186/jagrisesep.13.1.51-59>
- Barłóg, P., Grzebisz, W., & Łukowiak, R. (2022). Fertilizers and Fertilization Strategies Mitigating Soil Factors Constraining Efficiency of Nitrogen in Plant Production. *Plants*, 11(14), 1855. <https://doi.org/10.3390/plants11141855>
- Basuki, B., & Vega Kartika, S. (2019). Efektivitas Dolomit dalam Mempertahankan PH Tanah Inceptisol Perkebunan Tebu Blimbing Djatiroto. *Buletin Tanaman Tembakau, Serat dan Minyak Industri*, 11(2), 58-64. <https://doi.org/10.21082/btism.v11n2.2019.58-64>
- Bijay, S., & Craswell, E. (2021). Fertilizers and nitrate pollution of surface and ground water: an increasingly pervasive global problem. *SN Applied Sciences*, 3(4), 518. <https://doi.org/10.1007/s42452-021-04521-8>
- Brake, L. D., McNabb, W. M., & Fred Hazel, J. (1958). A spectrophotometric method for the determination of nickel. *Analytica Chimica Acta*, 19, 39-42. [https://doi.org/10.1016/S0003-2670\(00\)88116-7](https://doi.org/10.1016/S0003-2670(00)88116-7)
- Bramston-Cook, R. (2008). New Method for the Determination of Nitrous Oxide in Ambient Air and Vehicle Exhaust Using Gas Chromatography and Electron Capture Detection. EPA/AWMA Symposium on Air Quality Measurement Methods and Technology,
- Brito, J., Valle, A., Almenglo, F., Ramírez, M., & Cantero, D. (2020). Characterization of eubacterial communities by Denaturing Gradient Gel Electrophoresis (DGGE) and Next Generation Sequencing (NGS) in a desulfurization biotrickling filter using progressive changes of nitrate and nitrite as final electron acceptors. *New Biotechnology*, 57, 67-75. <https://doi.org/10.1016/j.nbt.2020.03.001>
- Chen, S.-R., Zhong, H.-L., Chen, H., Wu, G.-K., Zhao, M.-X., Wang, Y., . . . Sun, J.-J. (2023). Bidirectional Electron Transfer and Nitrogen Fixation Performance in Azospirillum Humicireducens Biofilms. Available at SSRN 4572064. <https://doi.org/10.2139/ssrn.4572064>
- Chen, Y., Li, S., Zhang, Y., Li, T., Ge, H., Xia, S., . . . Liu, L. (2019). Rice root morphological and physiological traits interaction with rhizosphere soil and its effect on methane emissions in paddy fields. *Soil Biology and Biochemistry*, 129, 191-200. <https://doi.org/10.1016/j.soilbio.2018.11.015>
- Comar, C. L., & Zscheile, F. P. (1942). Analysis of Plant Extracts For Chlorophylls A and B By a Photoelectric Spectrophotometric Method. *Plant Physiology*, 17(2), 198-209. <https://doi.org/10.1104/pp.17.2.198>
- Danapriatna, N. (2010). Biokimia penambatan nitrogen oleh bakteri non simbiotik. *Cefars: jurnal agribisnis dan pengembangan wilayah*, 1(2), 1-10. <https://jurnal.unismabekasi.ac.id/index.php/cefars/article/view/96>
- Dang, K., Ran, C., Tian, H., Gao, D., Mu, J., Zhang, Z., . . . Guo, L. (2023). Combined Effects of Straw Return with Nitrogen Fertilizer on Leaf Ion Balance, Photosynthetic Capacity, and Rice Yield in Saline-Sodic Paddy Fields. *Agronomy*, 13(9), 2274. <https://doi.org/10.3390/agronomy13092274>
- Dass, A., Chandra, S., Choudhary, A. K., Singh, G., & Sudhishri, S. (2016). Influence of field re-ponding pattern and plant spacing on rice root–shoot characteristics, yield, and water productivity of two modern cultivars under SRI management in Indian Mollisols. *Paddy and Water Environment*, 14(1), 45-59. <https://doi.org/10.1007/s10333-015-0477-z>
- Drescher, G. L., da Silva, L. S., Sarfaraz, Q., Roberts, T. L., Nicoloso, F. T., Schwalbert, R., & Marques, A. C. R. (2020). Available Nitrogen in Paddy Soils Depth: Influence on Rice Root Morphology and Plant Nutrition. *Journal of Soil Science and Plant Nutrition*, 20(3), 1029-1041. <https://doi.org/10.1007/s42729-020-00190-5>

- Fan, L., Dippold, M. A., Ge, T., Wu, J., Thiel, V., Kuzyakov, Y., & Dorodnikov, M. (2020). Anaerobic oxidation of methane in paddy soil: Role of electron acceptors and fertilization in mitigating CH₄ fluxes. *Soil Biology and Biochemistry*, 141, 107685. <https://doi.org/10.1016/j.soilbio.2019.107685>
- Fu, H., Cui, D., & Shen, H. (2021). Effects of Nitrogen Forms and Application Rates on Nitrogen Uptake, Photosynthetic Characteristics and Yield of Double-Cropping Rice in South China. *Agronomy*, 11(1), 158. <https://doi.org/10.3390/agronomy11010158>
- Guo, J. H., Liu, X. J., Zhang, Y., Shen, J. L., Han, W. X., Zhang, W. F., . . . Zhang, F. S. (2010). Significant Acidification in Major Chinese Croplands. *Science*, 327(5968), 1008-1010. <https://doi.org/10.1126/science.1182570>
- Guo, X., Chen, L., Zheng, R., Zhang, K., Qiu, Y., & Yue, H. (2019). Differences in Soil Nitrogen Availability and Transformation in Relation to Land Use in the Napahai Wetland, Southwest China. *Journal of Soil Science and Plant Nutrition*, 19(1), 92-97. <https://doi.org/10.1007/s42729-019-0013-0>
- Gupta, K., Kumar, R., Baruah, K. K., Hazarika, S., Karmakar, S., & Bordoloi, N. (2021). Greenhouse gas emission from rice fields: a review from Indian context. *Environmental Science and Pollution Research*, 28(24), 30551-30572. <https://doi.org/10.1007/s11356-021-13935-1>
- Hachiya, T., & Sakakibara, H. (2016). Interactions between nitrate and ammonium in their uptake, allocation, assimilation, and signaling in plants. *Journal of Experimental Botany*, 68(10), 2501-2512. <https://doi.org/10.1093/jxb/erw449>
- Harter, J., Guzman-Bustamante, I., Kuehfuss, S., Ruser, R., Well, R., Spott, O., . . . Behrens, S. (2016). Gas entrapment and microbial N₂O reduction reduce N₂O emissions from a biochar-amended sandy clay loam soil. *Scientific Reports*, 6(1), 39574. <https://doi.org/10.1038/srep39574>
- Hatta, M. (2012). Uji jarak tanam sistem legowo terhadap pertumbuhan dan hasil beberapa varietas padi pada metode SRI. *Jurnal Agrista*, 16(2), 87-93. <https://jurnal.usk.ac.id/agrista/article/view/291>
- Jiang, W., Wang, K., Wu, Q., Dong, S., Liu, P., & Zhang, J. (2013). Effects of narrow plant spacing on root distribution and physiological nitrogen use efficiency in summer maize. *The Crop Journal*, 1(1), 77-83. <https://doi.org/10.1016/j.cj.2013.07.011>
- Khalida, R., & Lontoh, A. P. (2019). Manajemen Pemupukan Kelapa Sawit (*Elaeis Guineensis* Jacq.), Studi Kasus pada Kebun Sungai Sagu, Riau. *Buletin Agrohorti*, 7(2), 238-245. <https://doi.org/10.29244/agrob.7.2.238-245>
- Kirk, P. L. (1950). Kjeldahl Method for Total Nitrogen. *Analytical Chemistry*, 22(2), 354-358. <https://doi.org/10.1021/ac60038a038>
- Lan, T., Li, M., Han, Y., Deng, O., Tang, X., Luo, L., . . . Gao, X. (2020). How are annual CH₄, N₂O, and NO emissions from rice-wheat system affected by nitrogen fertilizer rate and type? *Applied Soil Ecology*, 150, 103469. <https://doi.org/10.1016/j.apsoil.2019.103469>
- Liu, J., Shu, A., Song, W., Shi, W., Li, M., Zhang, W., . . . Gao, Z. (2021). Long-term organic fertilizer substitution increases rice yield by improving soil properties and regulating soil bacteria. *Geoderma*, 404, 115287. <https://doi.org/10.1016/j.geoderma.2021.115287>
- Liu, S., Xue, H., Wang, M., Feng, X., & Lee, H.-S. (2022). The role of microbial electrogenesis in regulating methane and nitrous oxide emissions from constructed wetland-microbial fuel cell. *International Journal of Hydrogen Energy*, 47(63), 27279-27292. <https://doi.org/10.1016/j.ijhydene.2022.06.063>
- Liu, X., Chen, L., Hua, Z., Mei, S., Wang, P., & Wang, S. (2020). Comparing ammonia volatilization between conventional and slow-release nitrogen fertilizers in paddy fields in the Taihu Lake region. *Environmental Science and Pollution Research*, 27(8), 8386-8394. <https://doi.org/10.1007/s11356-019-07536-2>
- Lv, H., Zhao, Y., Wang, Y., Wan, L., Wang, J., Butterbach-Bahl, K., & Lin, S. (2020). Conventional flooding irrigation and over fertilization drives soil pH decrease not only in the top- but also in subsoil layers in solar greenhouse vegetable production systems. *Geoderma*, 363, 114156. <https://doi.org/10.1016/j.geoderma.2019.114156>
- Ma, W., Tang, S., Dengzeng, Z., Zhang, D., Zhang, T., & Ma, X. (2022). Root exudates contribute to belowground ecosystem hotspots: A review [Review]. *Frontiers in Microbiology*, 13. <https://doi.org/10.3389/fmicb.2022.937940>
- Magfiroh, N., Lapanjang, I. M., & Made, U. (2017). Pengaruh jarak tanam terhadap pertumbuhan dan hasil tanaman padi (*Oryza sativa* L.) pada pola jarak tanam yang berbeda dalam sistem tabela. *Agrotekbis: Jurnal Ilmu Pertanian*, 5(2), 212-221. <http://jurnal.faperta.untad.ac.id/index.php/agrotekbis/article/view/126>
- Mahmud, K., Makaju, S., Ibrahim, R., & Missaoui, A. (2020). Current Progress in Nitrogen Fixing Plants and Microbiome Research. *Plants*, 9(1), 97. <https://doi.org/10.3390/plants9010097>
- Mahmud, K., Panday, D., Mergoum, A., & Missaoui, A. (2021). Nitrogen Losses and Potential Mitigation Strategies for a Sustainable Agroecosystem. *Sustainability*, 13(4), 2400. <https://doi.org/10.3390/su13042400>
- Mania, D., Heylen, K., van Spanning, R. J. M., & Frostegård, Å. (2016). Regulation of nitrogen metabolism in the nitrate-ammonifying soil bacterium *Bacillus vireti* and evidence for its ability to grow using N₂O as electron acceptor. *Environmental Microbiology*, 18(9), 2937-2950. <https://doi.org/10.1111/1462-2920.13124>
- Mu, X., & Chen, Y. (2021). The physiological response of photosynthesis to nitrogen deficiency. *Plant Physiology and Biochemistry*, 158, 76-82. <https://doi.org/10.1016/j.plaphy.2020.11.019>
- Mukherjee, R., & Sen, S. (2021). Agricultural sustainability through nitrogen fixation: approaches and techniques. *Harvest*, 6(1), 48-55. https://papers.ssrn.com/sol3/papers.cfm?abstract_id=3873195

- Munawaroh, U., Komariah, K., Ariyanto, D. P., Zaki, M. K., & Noda, K. (2022). Estimates of methane and nitrous oxide emission from a rice field in Central Java, Indonesia, based on the DeNitrification DeComposition model. *Sains Tanah - Journal of Soil Science and Agroclimatology*, 19(1), 11. <https://doi.org/10.20961/stjssa.v19i1.56928>
- Mussarat, M., Shair, M., Muhammad, D., Mian, I. A., Khan, S., Adnan, M., . . . Khan, F. (2021). Accentuating the Role of Nitrogen to Phosphorus Ratio on the Growth and Yield of Wheat Crop. *Sustainability*, 13(4), 2253. <https://doi.org/10.3390/su13042253>
- Nitorisavut, R., & Regmi, R. (2017). Plant microbial fuel cells: A promising biosystems engineering. *Renewable and Sustainable Energy Reviews*, 76, 81-89. <https://doi.org/10.1016/j.rser.2017.03.064>
- Paśmionka, I. B., Bulski, K., & Boligłowa, E. (2021). The Participation of Microbiota in the Transformation of Nitrogen Compounds in the Soil—A Review. *Agronomy*, 11(5), 977. <https://doi.org/10.3390/agronomy11050977>
- Permatasari, G. Y., Kesumadewi, A. A. I., & Suwastika, A. A. N. G. (2019). Dinamika Amonium dan Nitrat Lahan Sawah Latosol pada Budidaya Konvensional Padi Lokal dan Hibrida di Subak Jatiluwih. *Agrotrop : Journal on Agriculture Science*(2), 135-145%V 139. <https://doi.org/10.24843/AJoAS.2019.v09.i02.p04>
- Rahayu, A., Utami, S. R., & Rayes, M. L. (2017). Karakteristik dan Klasifikasi Tanah Pada Lahan Kering dan Lahan Yang Disawahkan Di Kecamatan Perak Kabupaten Jombang. *Jurnal Tanah dan Sumberdaya Lahan*, 1(2), 79-87. <https://jtsl.ub.ac.id/index.php/jtsl/article/view/115>
- Ranatunga, T., Hiramatsu, K., & Onishi, T. (2018). Controlling the process of denitrification in flooded rice soils by using microbial fuel cell applications. *Agricultural Water Management*, 206, 11-19. <https://doi.org/10.1016/j.agwat.2018.04.041>
- Rashid, M. M., Jahan, M., & Islam, K. S. (2016). Impact of Nitrogen, Phosphorus and Potassium on Brown Planthopper and Tolerance of Its Host Rice Plants. *Rice Science*, 23(3), 119-131. <https://doi.org/10.1016/j.rsci.2016.04.001>
- Read, S. T., Dutta, P., Bond, P. L., Keller, J., & Rabaey, K. (2010). Initial development and structure of biofilms on microbial fuel cell anodes. *BMC Microbiology*, 10(1), 98. <https://doi.org/10.1186/1471-2180-10-98>
- Saito, T., Mehanna, M., Wang, X., Cusick, R. D., Feng, Y., Hickner, M. A., & Logan, B. E. (2011). Effect of nitrogen addition on the performance of microbial fuel cell anodes. *Bioresource Technology*, 102(1), 395-398. <https://doi.org/10.1016/j.biortech.2010.05.063>
- Santos, A., van Aerle, R., Barrientos, L., & Martinez-Urtaza, J. (2020). Computational methods for 16S metabarcoding studies using Nanopore sequencing data. *Computational and Structural Biotechnology Journal*, 18, 296-305. <https://doi.org/10.1016/j.csbj.2020.01.005>
- Sedlar, K. (2018). *Methods for Comparative Analysis of Metagenomic Data* [Doctoral Thesis, Brno University of Technology, Department of Biomedical Engineering]. <https://theses.eurasip.org/theses/799/methods-for-comparative-analysis-of-metagenomic/>
- Sethy, P. K., Barpanda, N. K., Rath, A. K., & Behera, S. K. (2020). Nitrogen Deficiency Prediction of Rice Crop Based on Convolutional Neural Network. *Journal of Ambient Intelligence and Humanized Computing*, 11(11), 5703-5711. <https://doi.org/10.1007/s12652-020-01938-8>
- Sharma, L. K., & Bali, S. K. (2018). A Review of Methods to Improve Nitrogen Use Efficiency in Agriculture. *Sustainability*, 10(1), 51. <https://doi.org/10.3390/su10010051>
- Shetty, R., & Prakash, N. B. (2020). Effect of different biochars on acid soil and growth parameters of rice plants under aluminium toxicity. *Scientific Reports*, 10(1), 12249. <https://doi.org/10.1038/s41598-020-69262-x>
- Singh, B.-. (2018). Are Nitrogen Fertilizers Deleterious to Soil Health? *Agronomy*, 8(4), 48. <https://doi.org/10.3390/agronomy8040048>
- Soumare, A., Diedhiou, A. G., Thuita, M., Hafidi, M., Ouhdouch, Y., Gopalakrishnan, S., & Kouisni, L. (2020). Exploiting Biological Nitrogen Fixation: A Route Towards a Sustainable Agriculture. *Plants*, 9(8), 1011. <https://doi.org/10.3390/plants9081011>
- Stein, L. Y., & Klotz, M. G. (2016). The nitrogen cycle. *Current Biology*, 26(3), R94-R98. <https://doi.org/10.1016/j.cub.2015.12.021>
- Suhendrata, T. (2018). Pengaruh Jarak Tanam Pada Sistem Tanam Jajar Legowo terhadap Pertumbuhan, Produktivitas dan Pendapatan Petani Padi Sawah di Kabupaten Sragen Jawa Tengah. *2018*, 13(2), 7. <https://doi.org/10.20961/sepa.v13i2.21030>
- Syahputra, K., Rusmana, I., & Widyastuti, U. (2011). Isolasi dan karakterisasi bakteri denitrifikasi sebagai agen bioremediasi nitrogen anorganik. *Jurnal Riset Akuakultur*, 6(2), 197-209. <https://doi.org/10.15578/jra.6.2.2011.197-209>
- Tan, K. H. (2005). *Soil sampling, preparation, and analysis*. CRC press. <https://doi.org/10.1201/9781482274769>
- Timilsina, A., Bizimana, F., Pandey, B., Yadav, R. K. P., Dong, W., & Hu, C. (2020). Nitrous Oxide Emissions from Paddies: Understanding the Role of Rice Plants. *Plants*, 9(2), 180. <https://doi.org/10.3390/plants9020180>
- Tiso, M., & Schechter, A. N. (2015). Nitrate Reduction to Nitrite, Nitric Oxide and Ammonia by Gut Bacteria under Physiological Conditions. *PLOS ONE*, 10(3), e0119712. <https://doi.org/10.1371/journal.pone.0119712>
- Ucar, D., Zhang, Y., & Angelidaki, I. (2017). An Overview of Electron Acceptors in Microbial Fuel Cells [Review]. *Frontiers in Microbiology*, 8. <https://doi.org/10.3389/fmicb.2017.00643>
- Van Meter, K. J., Basu, N. B., & Van Cappellen, P. (2017). Two centuries of nitrogen dynamics: Legacy sources and sinks in the Mississippi and Susquehanna River Basins.

- Global Biogeochemical Cycles*, 31(1), 2-23. <https://doi.org/10.1002/2016GB005498>
- Vijay, A., Sonawane, J. M., & Chhabra, M. (2022). Denitrification process in microbial fuel cell: A comprehensive review. *Bioresource Technology Reports*, 17, 100991. <https://doi.org/10.1016/j.biteb.2022.100991>
- Wang, N., Chen, Z., Li, H.-B., Su, J.-Q., Zhao, F., & Zhu, Y.-G. (2015). Bacterial community composition at anodes of microbial fuel cells for paddy soils: the effects of soil properties. *Journal of Soils and Sediments*, 15(4), 926-936. <https://doi.org/10.1007/s11368-014-1056-4>
- Wetser, K., Liu, J., Buisman, C., & Strik, D. (2015). Plant microbial fuel cell applied in wetlands: Spatial, temporal and potential electricity generation of *Spartina anglica* salt marshes and *Phragmites australis* peat soils. *Biomass and Bioenergy*, 83, 543-550. <https://doi.org/10.1016/j.biombioe.2015.11.006>
- Widodo, T. W., & Damanhuri, F. N. U. (2021). Pengaruh Dosis Nitrogen terhadap Pembentukan Tunas dan Pertumbuhan Padi Ratun (*Oryza sativa* L.). *Jurnal Ilmiah Inovasi*, 21(1), 50-53. <https://doi.org/10.25047/jii.v21i1.2635>
- Winarso, S., Mandala, M., Sulistiyowati, H., Romadhona, S., Hermiyanto, B., & Subchan, W. (2020). The decomposition and efficiency of NPK-enriched biochar addition on Ultisols with soybean. *Sains Tanah - Journal of Soil Science and Agroclimatology*, 17(1), 7. <https://doi.org/10.20961/stjssa.v17i1.37608>
- Xiong, L., Liu, X., Vinci, G., Spaccini, R., Drosos, M., Li, L., . . . Pan, G. (2019). Molecular changes of soil organic matter induced by root exudates in a rice paddy under CO₂ enrichment and warming of canopy air. *Soil Biology and Biochemistry*, 137, 107544. <https://doi.org/10.1016/j.soilbio.2019.107544>
- Yang, J., Guo, W., Wang, F., Wang, F., Zhang, L., Zhou, B., . . . Yang, W. (2021). Dynamics and influencing factors of soluble organic nitrogen in paddy soil under different long-term fertilization treatments. *Soil and Tillage Research*, 212, 105077. <https://doi.org/10.1016/j.still.2021.105077>
- Yousaf, M., Li, J., Lu, J., Ren, T., Cong, R., Fahad, S., & Li, X. (2017). Effects of fertilization on crop production and nutrient-supplying capacity under rice-oilseed rape rotation system. *Scientific Reports*, 7(1), 1270. <https://doi.org/10.1038/s41598-017-01412-0>
- Zhang, L., Jiang, M., & Zhou, S. (2022). Conversion of nitrogen and carbon in enriched paddy soil by denitrification coupled with anammox in a bioelectrochemical system. *Journal of Environmental Sciences*, 111, 197-207. <https://doi.org/10.1016/j.jes.2021.03.033>
- Zhang, Z., Gao, S., & Chu, C. (2020). Improvement of nutrient use efficiency in rice: current toolbox and future perspectives. *Theoretical and Applied Genetics*, 133(5), 1365-1384. <https://doi.org/10.1007/s00122-019-03527-6>
- Zhao, H., Zhao, J., Li, F., & Li, X. (2016). Performance of Denitrifying Microbial Fuel Cell with Biocathode over Nitrite [Original Research]. *Frontiers in Microbiology*, 7. <https://doi.org/10.3389/fmicb.2016.00344>