

Soil texture affects the nitrogen fate in the methane fermentation digestate applied to paddy soil

Short running title:

Effect of soil texture on N fate from MFW in paddy

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Abstract

Purpose: Waste fluid (digestate) from methane fermentation (MFW) is increasingly used as a fertilizer in paddy fields to reduce greenhouse gas emissions and mitigate climate change. However, the influence of soil texture on the fate of nitrogen (N) following MFW application remains unclear. This study aimed to elucidate the effects of MFW application rates on rice growth and N pathways in soils with contrasting textures to minimize environmental N loss during rice cultivation.

Materials and methods: Two-year pot experiments were conducted using sandy loam (SL) and clayey loam (CL) soils. Five treatments were applied, each with four replicates: a chemical fertilizer (NH_4Cl , 200 mg N pot^{-1}), three MFW rates of total N (100, 200, and 400 mg N pot^{-1}), and a no-fertilizer control. To trace N derived from the inorganic fertilizer (Ndfif), $^{15}\text{NH}_4\text{Cl}$ was added to account for 20% of the inorganic N in each treatment, while maintaining total N input constant.

Results: Increasing MFW application rates significantly enhanced the rice growth and yield, with a more pronounced effect in CL soil compared to SL soil. Higher MFW application rates further increased Ndfif uptake by plant, soil N retention, and NH_3 volatilization in CL soil, whereas SL soil showed more significant Ndfif leaching. Chemical fertilizers showed significantly higher Ndfif recovery but were more prone to leaching in SL soil compared to MFW-derived N. Based on normalized comparisons of rice yield and N loss, optimal MFW application rates were estimated at approximately 180 mg N-MFW pot^{-1} for both soils, beyond which further yield increases were accompanied by substantial N leaching and emissions.

Conclusions: Soil texture critically affects the fate of N following MFW application, especially regarding NH_3 volatilization in CL soils and N leaching in SL soils. Optimizing MFW application rates is crucial for achieving enhanced rice yields with minimal environmental N loss, thereby facilitating sustainable rice production. These findings emphasize the importance of soil texture in determining optimal nutrient management for sustainable rice production.

Keywords: biogas waste, clay content, leaching, rice growth, volatilization

1. Introduction

Proper fertilization is essential for enhanced yield and crop quality (Ritter and Chirnside 1987; Chaney 1990; Flores, Castellar, and Navarro 2005). However, excessive use of fertilizers, especially nitrogen (N), can lead to nutrient loss, soil degradation, and reduced crop yield and quality, resulting in significant nutrient loss and environmental pollution (Anas et al., 2020; Gu et al. 2021). Nitrogen losses such as NH_3 volatilization, $\text{NO}_3\text{-N}$ leaching, and N_2O emissions are major contributors to environmental problems. NH_3 volatilization and $\text{NO}_3\text{-N}$ leaching have serious adverse effects on ecosystems and human health, including the loss of plant diversity, eutrophication and acidification of aquatic ecosystems, and respiratory issues in human (Zilio et al. 2021; de Vries 2021; Nieder and Benbi 2022). Additionally, N_2O is well known as a potent greenhouse gas contributing to global climate change, with a warming potential 273 times greater than CO_2 over a 100-year period (Hu et al. 2012; Butterbach-Bahl et al. 2013). Many studies are focusing on substituting chemical fertilizers with organic alternatives to sustain high crop yields and safeguard the environment (Geng et al. 2019).

Currently, a large environmental burden arises from the incineration or landfill disposal of organic wastes, necessitating their effective utilization (Abdel-Shafy and Mansour 2018). Organic waste, originating from the agricultural, livestock, forestry, and fisheries, can be scientifically converted and utilized for agricultural purposes, offering valuable plant nutrients, enhancing crop growth, and improving soil fertility (Odlare et al. 2011). A key approach to using organic waste in agriculture is the sustainable cycle in which the biodegradable organic portion of the waste is transformed into beneficial manure or fertilizer through composting or methane fermentation (anaerobic digestion), yielding compost and slurry for agricultural applications (Sharma et al. 2019).

Compared with composting, methane fermentation is a promising technique for managing organic waste, enabling both the stabilization of organic material and the recovery of energy (Ferguson, Coulon and Villa 2016). A significant byproduct of this process, methane fermentation waste fluid (MFW), also known as anaerobic digestate, can substitute for chemical fertilizers (Tambone et al.

2010; Geng et al. 2019). MFW has a high $\text{NH}_4\text{-N}$: total N ratio and a lower C/N ratio than
 undigested manure because complex organic N compounds are mineralized to $\text{NH}_4\text{-N}$ during
 fermentation (Möller and Müller 2012). This high $\text{NH}_4\text{-N}$ content allows for greater degradability
 and reduced application rates to meet crop N requirement (Möller and Müller 2012; Albuquerque
 et al. 2012; Risberg et al. 2017; Iocoli et al. 2019; Panuccio et al. 2021). Additionally, MFW
 contains negligible amounts of harmful fungi, bacteria, weed seeds, or heavy metals, making it safer
 than untreated manures (Voća et al. 2005; Möller and Müller 2012; Seadi and Lukehurst 2012; Cb,
 Ho, and Go 2018; O’Brien, Neher, and Roy 2020). Previous studies have demonstrated that MFW
 improves N availability, enhances crop yield (de Boer, 2008), and stabilizes soil microbial diversity
 (Abubaker et al. 2013; Coelho et al. 2020) more effectively than undigested manure, making it a
 cost-effective alternative to chemical fertilizers (Oritate 2016).

Many studies have explored MFW applications for sustainable N management, particularly in rice
 cultivation, focusing on application methods (Watanabe et al. 2012; Du et al. 2019; Hu et al. 2021),
 long-term effects (Nishikawa et al. 2012; Tanaka et al. 2017), application rates (Li, Inamura, and
 Umeda 2003; Nishikawa, Li, and Inamura 2014; Tanaka et al. 2017), and gas emissions (Wulf et al.
 2002; Hou et al. 2007; Win et al. 2014; Singla and Inubushi 2014; Tanaka et al. 2017; Wang et al.
 2019). These studies demonstrate that rice yield with MFW is comparable to that with chemical
 fertilizers, with outcomes strongly influenced by factors such as application timing (Watanabe et al.
 2012), water levels (Win et al. 2010), and the materials combined with MFW (Win et al. 2010, Hu
 et al. 2021).

Soil texture is a critical factor in N dynamics and plant growth (Dixon 1991; Grant et al. 2012;
 Hamoud et al. 2019) and affects the fate of fertilizer N in soils (Allison 1966). Clay particles protect
 organic matter and adsorb $\text{NH}_4\text{-N}$ (Hassink et al. 1993; Nieder, Benbi, and Scherer 2011), resulting
 in higher available N and greater rice yields in clayey soils than in sandy soils (Ichinose et al. 2023;
 Dou et al. 2016). In contrast, sandy soils have higher potentials for inorganic N leaching (Hansen et
 al. 2000; Zhou et al. 2006) and NH_3 volatilization (Duan and Xiao 2000; Fan, Li, and Alva 2011).

However, sandy soils may enhance the N harvest index and physiological N use efficiency compared to clayey soils (Ye et al. 2007). Given that MFW contains not only $\text{NH}_4\text{-N}$ but also organic N and diverse bacteria (Abubaker et al. 2013), its impact on N fate may differ from that of chemical fertilizers (Rigby and Smith 2013) or untreated manure (Nicholson et al. 2017; Risberg et al. 2017) across different soil textures.

The information on N fate from fertilizers is critical for improving N use efficiency and minimizing environmental losses (Anas et al. 2020). While a few studies have investigated N fate from MFW in plants, soil, and the environment during rice cultivation (e.g., Ghoneim et al. 2008; Chen et al. 2012), these studies were limited to single soil types. This leaves a gap in understanding how MFW interacts with different soil textures. Clarifying these interactions could help optimize MFW use as a fertilizer, reduce reliance on chemical fertilizers, and support sustainable agricultural practices. Therefore, this study aimed to elucidate how MFW application rates influence key N dynamics, including $\text{NH}_4^+\text{-N}$ retention, $\text{NO}_3^-\text{-N}$ leaching, and NH_3 volatilization, in soils with contrasting textures. Specifically, this research investigates the short-term N fate of inorganic N in MFW, given that $\text{NH}_4^+\text{-N}$ constitutes 70–80% of total N in MFW. By quantifying N uptake by plants, retention in soil, and loss through leaching and volatilization, we aim to provide actionable insights for optimizing MFW application rates in paddy fields with different soil textures. Our ultimate goal is to enhance rice yield while minimizing adverse environmental impacts.

To achieve these goals, we focused on three specific objectives: (1) compare the effects of different application rates of MFW and the effects of MFW versus chemical fertilizer on rice growth in contrasting soil textures, (2) quantify the fate of inorganic N from MFW and chemical fertilizers in rice cultivation using the ^{15}N isotope tracer method, and (3) recommend appropriate MFW application rates for rice cultivation in contrasting soil textures.

2. Materials and Methods

2.1. Soil and MFW

Two soils with different textures were collected from the Shugakuin paddy field (sandy loam [SL] soil) and the Ishiwari paddy field (clayey loam [CL] soil) in Kyoto, Japan. The soils were freshly sampled in February 2021 and April 2022, respectively, from the same field lots to ensure consistency. After collection, the soil was sieved through a 4-mm sieve for planting and 2-mm sieve to examine its physical and chemical characteristics. All soil properties are presented in Table 1. MFW was collected annually from Yagi's Bio-Ecology Center (Kyoto, Japan) and stored in a refrigerator at -10°C until use. The raw materials include dairy cattle manure (75–80%), discarded soy milk, and soybean refuse (okara), which were co-digested at 55°C for approximately 33 days (Nishikawa et al., 2014; Japan Organics Recycling Association, 2023). Fundamental characteristics of the MFW were analyzed using the standard method for wastewater analysis (APHA, 1989) and are listed in Table 2.

2.2. Experimental design

A pot (1/5,000a) experiment was carried out over two years, from 13th June to 13th October 2021 and from 2nd June to 2nd October 2022, under greenhouse conditions at Kyoto University. In 2021, 2.5 kg of dry soil was added to each pot. In 2022, the amount of SL soil was adjusted to match the volume of 2.5 kg of CL soil, in order to standardize the soil layer depth between the two soil types. Five fertilizer treatments, each with four replicates, were designed: 200 mg N of NH_4Cl pot^{-1} (100 kg N ha^{-1} equivalent; 1N-Chem), three doses of MFW based on total N (equivalent to 100, 200, and 400 mg N pot^{-1} ; 0.5N-MFW, 1N-MFW, and 2N-MFW, respectively) and a control treatment without N application (0N). To trace the fate of inorganic N, $^{15}\text{NH}_4\text{Cl}$ (99.1% atom) was applied at a rate of 20% of inorganic N in 1N-Chem or MFW treatments, as described by Ghoneim et al. (2008), while keeping the total N amount consistent across treatments. We selected $^{15}\text{NH}_4\text{Cl}$ as a tracer because $\text{NH}_4^{+}\text{-N}$ accounted for more than 99% of the inorganic N fraction in the MFW used in this study (Table 2). Thus, NH_4^{+} was the dominant form of mineral N in the digestate, and $^{15}\text{NH}_4\text{Cl}$ appropriately represents the inorganic N component for tracking its fate in the plant–soil system.

Equal amounts of P (100 kg P ha⁻¹) and K (135 kg K ha⁻¹) were applied in all the treatments based on the content of P and K in MFW treatments and supplements using Ca(H₂PO₄)₂ · H₂O and KCl, respectively. All fertilizers were applied as a basal layer by thoroughly mixing the top layer of the soil in the pots, followed by the immediate establishment of waterlogged conditions. Three young Nikomaru rice plants (28 days old in 2021 and 21 days old in 2022) were transplanted into each pot one day after fertilizer application. The continued flood condition was maintained with a 2 cm water layer until five days before harvesting.

2.3. Leachate sampling

Water was allowed to percolate through the soil layer and a geotextile layer before accumulating at a 2 cm height in the space at the bottom of each pot (Figure 1). Leachate solution was collected daily using a valve connected to the bottom of each pot. To simulate the actual percolation rate observed in the Shugakuin field (SL soil), the daily leachate collection was limited to a maximum of 400 mL/day for both soil types, corresponding to an approximate infiltration rate of 2 cm/day. To maintain the waterlogged conditions required for paddy rice cultivation, additional water was carefully added during the collection process up to four times daily. After collection, the pH of the leachate was measured (Horiba portable pH meter). The collected leachate was treated with CuBr₂ (1 mg L⁻¹ Cu) for inhibiting microbial contamination, filtered using a 0.45-μm membrane filter (Sartorius, Minisart RC 0.45), and stored at 4°C until further analysis (Shibata et al., 2017). Total dissolved N (TDN) concentrations were measured using a total organic carbon-total nitrogen (TOC-TN) analyzer (TOC_TNM- Shimadzu, Kyoto, Japan). Inorganic N (NH₄-N and NO₃-N) concentrations were examined using a flow injection analysis (FIA) method (AQLA-700, Aqualab, Tokyo, Japan).

2.4. Gas sampling

2.4.1 NH₃ volatilization

NH₃ volatilization was measured using the vented chamber method (Wang et al. 2004; Chen et al. 2012) after fertilizer application and later at intervals of two–14 d. A column (15 cm height and 5 cm diameter) was placed at a depth of 5 cm in the soil. A sponge containing 3 mL of phosphate glycerol (50 mL of phosphoric acid: 40 mL of glycerol in 1 L of solution) was inserted into the middle of the column to collect the volatile NH₃. A second sponge was used to cover the top of the column for 24 hours to prevent air contamination. Subsequently, the sponge was shaken with 100 mL 1 M KCl for 1 h and filtered using a 0.45 µm membrane filter for analyzing NH₄⁺-N content via the FIA method (AQLA-700, Aqualab, Tokyo, Japan). The NH₃ volatilization flux was calculated using the following equation (Xu et al. 2012):

$$\text{NH}_3 \text{ flux (mg N m}^{-2} \text{ h}^{-1}) = \frac{M}{(A \times D)}$$

where M is the amount of NH₃-N collected by the vented chamber (mg), A is the cross-sectional area of the round chamber (m²), and D is the sampling duration (hour).

2.4.2 N₂O emission

N₂O gas was collected using the closed chamber method (Cai et al. 1997) between 8:00–12:00 and 17:00–20:00 at intervals of 2–14 d until harvest and then monthly during the fallow stage. The chamber (50–120 cm in height and 25 cm in diameter) was equipped with a fan (12 V), 1 L airbag, and auto thermometer (RH-KN Lab., Inc, Japan). Gas (40 mL) was collected at 0, 15, 30, and 45 min after closing the chamber and stored in glass vials (Nichiden-Rika Glass Co., Ltd., Japan). The presence of N₂O gas was determined using gas chromatography (GC-2014; Shimadzu, Japan) equipped with an Electron Capture Detector (ECD-14) using ⁶³Ni. The N₂O flux rate was calculated according to Murphy et al. (2022):

$$\text{N}_2\text{O-N flux (mg N m}^{-2} \text{ h}^{-1}) = \frac{\Delta C}{\Delta t} \times \frac{V}{A} \times \frac{P \times M_{\text{gas}}}{R \times T} \times 10^{-5}$$

where $\Delta C/\Delta t$ is the rate of change in N₂O concentration (ppb h⁻¹), V is chamber volume (cm³), A is chamber base area (cm²), P is atmospheric pressure (101.325 kPa), R is gas constant (8.3145 J mol⁻¹ K⁻¹), M_{gas} is the molar mass of the gas (28 g N₂O-N mol⁻¹), and T is air temperature (K).

203

204 **2.5. Plant performance and N of plant and soil**

205 Plant height, tiller number, and soil plant analysis development chlorophyll (SPAD) values were
206 measured every two weeks after transplanting. Total biomass and rice yield were measured after
207 drying at 70°C until a constant weight was achieved. Root and soil samples were separated using a
208 2 mm sieve at the end of the experiment. After washing, root samples were dried at 70°C until a
209 constant weight was reached in the oven. The total N, inorganic N, and ¹⁵N contents of the plant and
210 soil samples were analyzed for comparison with the different treatments.

211

212 **2.6. ¹⁵N analysis and calculation**

213 Next, ¹⁵N-NH₄ and ¹⁵N-NO₃ isotopes in the samples were converted to N₂O using chemical
214 conversion (Liu et al., 2014) and denitrification methods (Sigman et al., 2001), respectively. The
215 ratio of ¹⁵N excess in N₂O gas of these samples was determined via gas chromatography-mass
216 spectrometry (QP2020; Shimadzu, Japan). Excess of ¹⁵N in soil and plant samples were analyzed
217 via EA IsoLink CN-Isotope Ratio MS (Thermo Fisher Scientific, Massachusetts, USA) after ball
218 milling and drying at 105 and 70 °C, respectively. The following formulas were used to calculate
219 the N derived from inorganic fertilizer (Ndfif) in the samples based on the percentage of ¹⁵N:

$$220 \quad \Delta^{15}\text{N} = {}^{15}\text{N} / ({}^{14}\text{N} + {}^{15}\text{N}) = \frac{\left(\frac{{}^{15}\text{N}_0}{1000} + 1\right) * {}^{15}\text{N}_{\text{atm}}}{\left[\left(\frac{{}^{15}\text{N}_0}{1000} + 1\right) * {}^{15}\text{N}_{\text{atm}}\right] + 1} \quad (1)$$

221 ${}^{15}\text{N}_{\text{atm}}$: represents N from the air; ${}^{15}\text{N}_0$: represents N from ¹⁵N NH₄Cl.

222 % N excess in a sample from inorganic fertilizer:

$$223 \quad {}^{15}\text{Ndfif}_{\text{excess}} \% = (\Delta^{15}\text{N}_{\text{sample}} - \Delta^{15}\text{N}_{\text{control}}) \times 100 \quad (2)$$

224 Amount of N derived from inorganic fertilizer in samples:

$$225 \quad \text{Ndfif}_{\text{sample}} (\text{mg}) = \text{Total g N in sample} * (\% {}^{15}\text{N}_{\text{excess in samples}} / \% {}^{15}\text{N}_{\text{excess in fertilizer}}) * 10^{-1} \quad (3)$$

226 Amount of N derived from unlabeled source (Ndfun):

$$227 \quad \text{Ndfun}_{\text{sample}} (\text{mg}) = \text{Total N}_{\text{sample}} - \text{Ndfif}_{\text{sample}} \quad (4)$$

228 N recovery from inorganic N upon fertilizer application:

$$229 \quad \%N_{\text{recovery}} = \frac{N_{\text{dif sample}}}{\text{inorg.}N_{\text{rate}}} * 100 \quad (5)$$

$$230 \quad \text{Total}\% N_{\text{recovery}} = \%N_p + \%N_s \quad (6)$$

231 The fate of inorganic N upon fertilizer application was evaluated using the following equation:

$$232 \quad N \text{ fate } (\%) = \% N_p + \%N_L + \% N_E + \% N_S + \%N_{\text{uno}}. \quad (7)$$

233 P: Plant uptake; L: leaching; E: Emission; S: remain in the soil; uno: unobserved

234

235 **2.7. Statistical analysis**

236 Statistical Package for the Social Sciences software (IBM SPSS Statistics version 22) was used for
237 all statistical analyses. One-way analysis of variance (ANOVA) was used to test the differences in
238 fertilizer applications in each soil texture at 95%. The differences among different fertilizers or soil
239 texture groups and interactions between the two factors were assessed using two-way ANOVA.
240 Tukey's test was used to evaluate the significant differences among the different treatments or
241 groups. Statistical significance was set at $P < 0.05$.

242

243 **3. Results**

244 **3.1. Rice growth under different MFW treatments**

245 Soil texture and MFW significantly influenced rice performance indicators, and their interactions
246 substantially affected rice biomass in the 2022 growing year (Table 3). In 2021, rice was
247 transplanted late, with older seedlings and lower initial soil N levels (Table 1), potentially
248 contributing to reduced rice performance compared to 2022 (Table 3). Soil texture significantly
249 affected rice height, panicle number, total biomass, and yield in both years, except for panicle
250 number in 2022, with rice plants growing better in the CL soil than in the SL soil. Rice height
251 increased moderately with elevated MFW application rates in both soil types, and this effect was
252 significant only in 2022. Increasing MFW application rates significantly enhanced the panicle
253 number, total dry biomass, and yield in both seasons. Specifically, 2N-MFW treatments

consistently showed higher biomass and yield than the other treatments across all soil textures and seasons.

3.2. Changes in the N pathway in plants, soil, leachate, and gas emission in different MFW treatments and contrasting soil textures

To evaluate Ndfif with varying MFW application rates in contrasting soil textures, we measured the total N (TN) and Ndfif contents in whole plants, soil, leachate solution (Table 4), and gas emissions (Figure 2). The TN content and Ndfif of plants were substantially affected by the soil texture and MFW application rate. With increasing MFW application rates, the TN and Ndfif in rice increased significantly for both soil texture types. However, the interaction effect of soil texture and MFW application rates on TN and Ndfif in plants was statistically significant in 2022, indicating that increasing MFW application rates will improve plant N uptake in CL soils more than in SL soils. Increasing MFW application rates significantly enhanced Ndfif in the soil; 2N-MFW demonstrated the greatest effect, followed by 1N-MFW and 0.5N-MFW, for both soil textures and growth years. Ndfif in the CL soil was significantly higher than that in the SL soil in both years. Ndfif increased with increasing MFW application rates, and the effect was more rapid in CL soil than in SL soil. Increasing the MFW application rates significantly increased the leached TDN and Ndfif for both soil textures, with the SL soil being more susceptible than the CL soil in both years (Figure 4). The results revealed that when MFW increased, Ndfif increased dramatically for both soil textures. NH_3 volatilization increased linearly with MFW application rates in both soil textures (Figure 2a). A significant difference was observed only in the 2N-MFW treatments compared with the other treatments for each soil type. In addition, NH_3 volatilization in the CL soil was significantly higher than that in the SL soil (Figure 2a). Increasing the MFW application rate significantly enhanced NH_3 -Ndfif emissions in both soil textures, which were significantly higher in CL soil than in SL soil (Figure 2b). N_2O emissions were negligible in all treatments, accounting for less than 1% of Ndfif, and were not influenced by MFW application rates in either soil texture (Figure 2c and d).

3.3. Comparison of chemical fertilizer and MFW in contrasting soil textures

The effects of chemical fertilizer (1N-Chem) and MFW application (1N-MFW) on rice performance were comparable for both soil textures (Table 5). Except for panicle numbers in 2022, all agronomy indicators for both fertilizer types were significantly higher in the CL soil than in the SL soil. In both years, the Ndfif from chemical fertilizer and MFW application was strongly affected by soil texture (Figure 3). Plant N uptake, including both Ndfif and Ndfun, was significantly higher in 1N-Chem than in 1N-MFW in 2021 (Figure 3a); however, this was not the case in 2022 (Figure 3e). Conversely, the Ndfif from 1N-Chem was significantly higher than that from 1N-MFW for both types of soil textures in both years. Plant N uptake was significantly lower in the SL soils than in the CL soils in both years (Figure 3a and e). In addition, the remaining soil Ndfif from 1N-Chem was significantly higher than that from 1N-MFW for both soil textures across the two years (Figure 3b and f) and was significantly lower in SL soils than in CL soils. Thus, Ndfif recovered by plants and soil from chemical fertilizers was higher than that recovered from MFW, with a more pronounced effect in CL soil than in SL soil. TDN and inorganic Ndfif leached from 1N-Chem were significantly higher than those leached from 1N-MFW in both soil textures (Figure 3c and g), except for the CL soil in 2022. These losses were more pronounced in the SL soils than in the CL soils. The higher dissolved organic N (DON) in 2022, particularly in the SL soil, could be attributed to the higher initial TN in the SL soil than in the CL soil (Table 1). However, there was no significant difference between the DON leached from the two fertilizer types over the two years. Consequently, MFW application significantly reduced TDN and Ndfif compared to chemical fertilizers, especially in the SL soil compared to the CL soil. Gas emissions from 1N-MFW were comparable to those from 1N-Chem in both years. N₂O emissions in 1N-MFW and 1N-Chem were not significantly different in both years and soil textures (Figure 3d and h).

3.4. Distribution of inorganic N from both chemical fertilizer and MFW in contrasting soil textures

Chemical fertilizers provided the highest inorganic N recovery (in both plants and soil) compared with MFW applications for both soil textures (Table 6). Although 1N-Chem significantly increased the proportion of N in the leachate, it markedly decreased the unobserved N compared to the 1N-MFW application. Moreover, the soil texture and MFW application rate independently affected the proportion of N in all observed samples. Except for unobserved N in 2021, N recovery by plants and soil was significantly greater in CL soil than in SL soil, whereas N loss (the sum up of leaching and gaseous emissions) and unobserved N were significantly higher in SL soil than in CL soil. Increased MFW application rates significantly enhanced the N-use efficiency (plant uptake) in CL soil in 2022, while it decreased in SL soil in both seasons. In contrast, increasing the MFW application rate significantly decreased the percentage of N retained in the soil and significantly increased the proportion of N lost through leachate, especially in SL soil.

4. Discussion

4.1. Effects of soil textures on rice growth and inorganic N fate in MFW

Our results demonstrated that rice growth was significantly better in CL soils compared to SL soils (Table 3), consistent with previous findings by Dou et al. (2016). Rice growth improved with increasing MFW application rates in both soil textures, but the contribution of $\text{NH}_4\text{-N}$ from MFW to rice N uptake and soil retention was higher in CL soil due to reduced N leaching compared to SL soil (Tables 4 and 6) (Zhou et al. 2006; Ji et al. 2011; Nguyen et al. 2020). This difference can be attributed to clay particles to preserve organic matter and retain $\text{NH}_4\text{-N}$ (Hassink et al. 1993; Nieder, Benbi, and Scherer 2011; Partey et al. 2015), which enhances rice performance and yield in CL soils (Alhaj et al. 2019).

Increased MFW application rates led to higher total biomass and N uptake in CL soil compared to SL soil, as rice grown in CL soil developed more branches and stored more N (Tables 3 and 4). In

the 2022 experiment, better rice growth improved N retention in both soils, reducing N losses from MFW application. This result was associated with a higher shoot-to-root ratio (Figure S2), as reported by Kanbar, Toorchi, and Shashidhar (2009), and an uptake of 44–52% from MFW applications (Table 6), consistent with findings by Ghoneim et al. (2008), who reported 51% $\text{NH}_4\text{-N}$ uptake (Table S1).

In contrast, the 2021 experiment exhibited lower panicle numbers, reduced N uptake, and lower plant Ndfif uptake, primarily due to late-season transplantation with older seedlings (Baloch, Awan, and Hasan 2006). This likely contributed to the absence of significant differences in Ndfif uptake between the two soil types in 2021 (Table 4 and Table 6). Despite these limitations, the Ndfif uptake in 2021 remained within the typical range reported for inorganic fertilizers (Craswell and Vlek 1983) and exceeded the values commonly observed for manure composts (Nishida, Tsuchiya, and Yamamuro 2004; Nishida, Sumida, and Kato 2008) (Table S1), which generally contain low nutrient concentrations [1–2% N, <1% P] (Sikora and Enkiri 1999). Interestingly, although the inorganic N content of MFW increased by 10% in 2022 (Table 2), the Ndfif in the leachate was lower than that in 2021 (Table 4), leading to a reduced proportion of MFW-derived N in the leachate (Table 6). Additionally, unobserved N in 2022 was significantly lower than that in 2021, correlating with improved N uptake and yield, especially in CL soil. These results indicate the improved plant performance in 2022 - combined with the higher nutrient retention capacity of CL soil - enhanced N recovery. Overall, our findings suggest that under greenhouse conditions with basal MFW application and a high leaching rate (2 cm/day), rice yield and N recovery benefit more from MFW application in finer-textured (CL) soils than in coarser-textured (SL) soils.

4.2. Effects of soil textures on N emissions from MFW

Our results revealed lower Ndfif in NH_3 across all treatments compared to previous studies using urea or manure mixed with urea (Chen et al. 2012; Zhao et al. 2009; Yu et al. 2019; Chen et al. 2012) (Figs. 1a, b; Table S1). This reduction can be attributed to the combination of basal

application with deep-placement mixing method used in this study, which is known to mitigate NH_3 volatilization (Hayashi, Nishimura, and Yagi 2006). However, contrary to previous studies suggesting higher clay content reduces NH_3 volatilization (Duan and Xiao 2000; Fan et al. 2011), our results indicated higher volatilization in CL soil, particularly at higher MFW application rates. This could be attributed to increased floodwater pH and NH_4^+ -N concentrations in CL soil (pH 6.4–9.4) compared to SL soil (pH 6.4–7.4) during the midday period (Figure S1a, b), consistent with previous studies (Battye et al. 1994; He et al. 1999; Rochette et al. 2013; Möller and Müller 2012; Maris et al. 2021).

Flooding conditions reduced N_2O emissions in all treatments, consistent with previous studies (Sasada et al. 2011; Singla and Inubushi 2014; Win et al. 2014). Anaerobic conditions also favored anaerobic ammonium oxidation (anammox), converting NH_4 -N to N_2 (Hu et al. 2011; Shen, Zheng, and Ma 2016). Given the optimal pH and high NH_4^+ concentrations, anammox may have contributed to higher unobserved N in MFW treatments compared to chemical fertilizer treatments (Yang et al. 2015; Table 6). However, the presence of organic matter in MFW (Table 2) might have suppressed anammox activity to some extent, as suggested by Shan et al. (2018). Therefore, the exact role of anammox under these conditions remains uncertain and warrants further investigation. The lack of $^{15}\text{NO}_3$ in leachate (data not shown) suggests oxygen-depleted conditions in the pots, likely enhancing denitrification processes (Stepanauskas, Davidsson, and Leonardson, 1996). Therefore, the elevated unobserved N levels in MFW treatments is most likely attributed to N_2 emissions via complete denitrification, with a possible contribution from anammox under anaerobic conditions (Yang et al. 2015)

4.3. Comparison of MFW and chemical fertilizers in contrasting soil textures

Our findings align with previous studies by Ghoneim et al. (2008), Nishikawa et al. (2012), Chen et al. (2012), and Tanaka et al. (2017), demonstrating that MFW can achieve rice yields comparable to chemical fertilizers. Unlike studies that base MFW application rates solely on NH_4 -N (Nishikawa et

al., 2014; Möller et al., 2015), we used total N as the basis for application, with the digestate in our study containing 70–80% $\text{NH}_4^+\text{-N}$ in MFW (Table 2). This approach resulted in reduced inorganic N leaching in SL soil in both years and in CL soil in 2021 (Figs. 2c and g), while maintaining rice yield.

We acknowledge that the $\text{NH}_4^+\text{-N}$ content of digestates can vary considerably depending on feedstock composition and processing conditions (Tampio, Salo, and Rintala 2016; Jimenez et al. 2020), sometimes being as low as 50–60% (Japan Organics Recycling Association, 2023). However, recent studies (e.g., Ran et al. 2022) have shown that even digestates with lower $\text{NH}_4^+\text{-N}$ proportions, when applied based on total N, can achieve comparable agronomic performance to urea. Therefore, while our findings may not apply universally to all digestates, they support the potential of co-digested MFW—particularly those with a relatively high inorganic N content—to mitigate N leaching without compromising rice productivity.

Higher unobserved N in MFW treatments than in chemical fertilizers suggests greater N emissions via unmeasured gaseous pathways (Ghoneim et al. 2008), particularly in SL soils. Meanwhile, the amount of N_2O detected in gaseous forms from both MFW and chemical fertilizers was negligible (Fig. 3d, h). This evidence suggests that MFW application may have induced more N_2 emission than chemical fertilizers, with SL soil showing a greater susceptibility to such losses than CL soil.

4.4. Optimal MFW application rates for different soil textures

Rice yield significantly increased with MFW application rates from 0.5N-MFW to 2N-MFW in both soil textures (Table 3). However, yields plateaued beyond the 1N-MFW, except for SL soil in 2021. This suggests that exceeding 1N-MFW could lead to unnecessary N losses and environmental risks, especially in SL soils with higher leaching potential (Table 4).

In CL soil, as a fine-textured soil, MFW application quickly achieved optimal yields with lower application rates, whereas SL soil required higher rates to achieve comparable yields (Table 3).

However, higher MFW application rates in SL soil significantly increased both N leaching—which

may contribute to eutrophication (Smith et al., 1999; Malone and Newton, 2020)—and NH_3 volatilization (Figure 1a). While digestates generally enhance N availability and crop yields better than untreated animal manure (de Boer 2008), sustainably improving rice yield in SL soil or coarse-textured soils may require long-term enhancement of soil organic matter to increase N retention and reduce leaching (Shan et al. 2018).

Our findings revealed that Ndfif leaching increased linearly with MFW application rates in 2021 and quadratically in 2022, with steeper increases observed in SL soils (Figure 4). NH_3 Ndfif emissions also increased exponentially with higher MFW rates (Figure 2b), although the absolute values were not critical. These trends emphasize the need for careful management of MFW application rates, particularly in sandy soils with high leaching susceptibility.

To determine the optimal MFW application rates, we applied a min–max normalization method, where the normalized value x' was calculated as: $x' = (x_i - \min[x]) / (\max[x] - \min[x])$ (Zhou and Butterbach-Bahl 2014; Zheng et al. 2019). Here, x_i represents each observed value (e.g., yield or N loss), and $\min[x]$ and $\max[x]$ are the minimum and maximum values in the data group, respectively. This approach allowed us to evaluate the trade-off between rice productivity and environmental N losses on a comparable scale. Based on the 2022 data, the optimal MFW application rates were estimated to be 183 mg N pot^{-1} for SL soil and 175 mg N pot^{-1} for CL soil, both closely aligning with the 1N-MFW application rate (Figure 5). These rates optimize yield while minimizing environmental impacts.

4.5. The limitations of this study

This study focused on the short-term fate of inorganic N in MFW. While organic N in MFW likely contributes to long-term N availability through mineralization (Sharifi et al. 2019), its role could not be evaluated within the scope of this study. Comparison between 1N-Chem and 1N-MFW showed no significant differences in plant Ndfun or DON in leachate (Figure 2), suggesting minimal short-term impact of organic N under experimental conditions. However, in actual field conditions,

organic N may persist in the soil, gradually influencing the long-term fate of N through mineralization (Ueno and Yamamuro 2001; Takakai et al. 2018). Therefore, future studies should explore the long-term effects of both organic and inorganic N components in MFW. Pot experiments, while offering the advantage of controlled conditions, inherently limit the extent to which field environments—such as variable water regimes and percolation rates—can be replicated (de Vries 1980). In this study, percolation was fixed across all treatments, which may have obscured natural differences in infiltration between sandy and clayey soils. Consequently, key N transformation processes such as nitrification, denitrification, and anammox could not be fully assessed under soil-specific hydrological conditions (Zhou et al. 2011; Zhou et al. 2012). In addition, leaching in clayey soils may have been overestimated, as clayey soils generally have lower infiltration rates than sandy soils under field conditions (Gärdenäs et al., 2005). Although this study represents an early attempt to evaluate the fate of inorganic N from basal MFW application across all pathways (plants, soil, leachate, and gas emissions) in soils of contrasting textures, further validation under field conditions is required to confirm these findings. In particular, for understanding how a more realistic assessment of how soil-specific infiltration and water management practices affect inorganic N distribution is necessary. This aspect warrants further investigation to resolve the uncertainties in the behavior of unaccounted-for N. Additionally, because fertilizers were mixed into the soil immediately after application, NH_3 volatilization was likely suppressed in the experiment of this study. In practical field situations where digestate may remain on the soil surface before incorporation, NH_3 emissions could be higher. Therefore, our measured NH_3 losses may underestimate volatilization under typical field conditions.

5. Conclusions

This study demonstrated the significant influence of soil texture on rice growth and N dynamics following the MFW application and chemical fertilizers. Rice growth and N uptake were

consistently higher in CL soil than in SL soil, highlighting the role of soil texture in determining the fate of applied N. While MFW enhanced rice yields across both soil types, its environmental impact varied. In SL soil, higher MFW application rates led to significantly increased N leaching, whereas in CL soil, NH_3 volatilization increased at elevated MFW rates, likely due to higher floodwater pH and NH_4^+ concentrations. Using the min–max normalization approach, we identified the optimal MFW application rate as approximately 90 kg N ha⁻¹ equivalent. This rate maximized the difference between normalized rice productivity and total N loss, thereby achieving a balance between agronomic performance and environmental sustainability. Notably, MFW achieved comparable or even better rice yields than chemical fertilizers under short-term, controlled conditions. However, distinct management strategies are necessary: mitigating NH_3 volatilization in CL soils and N leaching in SL soils. These findings emphasize the need for soil texture-specific nutrient management when applying MFW. Future research should evaluate the long-term effects of organic N in MFW, including its mineralization dynamics and contribution to sustainable agricultural systems. Field-based studies are also essential to validate these results under diverse environmental conditions and to refine MFW application strategies for broader agricultural adoption.

Data availability statement

Data supporting the results of this study are available upon request from the corresponding authors.

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Disclosure statement

The authors report there are no competing interests to declare.

489 **References**

- 490 Abdel-Shafy, H. I., and M. S. M. Mansour. 2018. "Solid Waste Issue: Sources, Composition,
491 Disposal, Recycling, and Valorization". *Egyptian Journal of Petroleum* 27 (4): 1275–1290.
492 <https://doi.org/10.1016/j.ejpe.2018.07.003>.
- 493 Abubaker, J., H. Cederlund, V. Arthurson, and M. Pell. 2013. "Bacterial Community Structure and
494 Microbial Activity in Different Soils Amended with Biogas Residues and Cattle Slurry".
495 *Applied Soil Ecology* 72 (October):171–180. <https://doi.org/10.1016/j.apsoil.2013.07.002>.
- 496 Alburquerque, J. A., C. de la Fuente, M. Campoy, L. Carrasco, I. Nájera, C. Baixauli, F. Caravaca,
497 A. Roldán, J. Cegarra, and M. P. Bernal. 2012. "Agricultural Use of Digestate for
498 Horticultural Crop Production and Improvement of Soil Properties". *European Journal of*
499 *Agronomy* 43 (November):119–128. <https://doi.org/10.1016/j.eja.2012.06.001>.
- 500 Allison, F. E. 1966. "The Fate of Nitrogen Applied to Soils". In *Advances in Agronomy*, edited by
501 A. G. Norman, 18:219–58. Academic Press. [https://doi.org/10.1016/S0065-2113\(08\)60651-](https://doi.org/10.1016/S0065-2113(08)60651-3)
502 [3](https://doi.org/10.1016/S0065-2113(08)60651-3).
- 503 Anas, M., F. Liao, K. K. Verma, M. A. Sarwar, A. Mahmood, Z. L. Chen, Q. Li, X. P. Zeng, Y.
504 Liu, and Y. R. Li. 2020. "Fate of Nitrogen in Agriculture and Environment: Agronomic,
505 Eco-Physiological and Molecular Approaches to Improve Nitrogen Use Efficiency".
506 *Biological Research* 53 (1): 47. <https://doi.org/10.1186/s40659-020-00312-4>.
- 507 APHA, American Water Works Association, and Water Pollution Control Federation. 1989.
508 *Standard Methods for the Examination of Water and Wastewater. 17th Edition*. American
509 Public Health Association. <https://tamug-ir.tdl.org/handle/1969.3/24401>.
- 510 Baloch, M. S., I. U. Awan, and G. Hassan. 2006. "Growth and Yield of Rice as Affected by
511 Transplanting Dates and Seedlings per Hill under High Temperature of Dera Ismail Khan,
512 Pakistan". *Journal of Zhejiang University. Science. B* 7 (7): 572–579.
513 <https://doi.org/10.1631/jzus.2006.B0572>.

514 Battye, R., W. Battye, S. Fudge, and C. Overcash. 1994. “*Development and Selection of Ammonia*
515 *Emission Factors*”. Final Report, Atmospheric Research and Exposure Assessment
516 Laboratory, U.S. Environmental Protection Agency Research Triangle Park, North Carolina
517 27711, 111 pages.

518 Boer, H. C. de. 2008. “Co-Digestion of Animal Slurry Can Increase Short-Term Nitrogen Recovery
519 by Crops”. *Journal of Environmental Quality* 37 (5): 1968–73.
520 <https://doi.org/10.2134/jeq2007.0594>.

521 Butterbach-Bahl, K., E. M. Baggs, M. Dannenmann, R. Kiese, and S. Zechmeister-Boltenstern.
522 2013. “Nitrous Oxide Emissions from Soils: How Well Do We Understand the Processes
523 and Their Controls?”. *Philosophical Transactions of the Royal Society B: Biological*
524 *Sciences* 368 (1621): 20130122. <https://doi.org/10.1098/rstb.2013.0122>.

525 Cai, Z., G. Xing, X. Yan, H. Xu, H. Tsuruta, K. Yagi, and K. Minami. 1997. “Methane and Nitrous
526 Oxide Emissions from Rice Paddy Fields as Affected by Nitrogen Fertilisers and Water
527 Management”. *Plant and Soil* 196 (1): 7–14. <https://doi.org/10.1023/A:1004263405020>.

528 Cb, O., S. Ho, and A. Go. 2018. “Effect of Liquid Digestate on Agricultural Soil-II: Microbial
529 Population Dynamics”. *Applied Microbiology: Open Access* 04 (1): 1000145
530 <https://doi.org/10.4172/2471-9315.1000145>.

531 Chaney, R. L. 1990. “Twenty Years of Land Application Research”. *BioCycle (USA)* 31 (9): 6.

532 Chen, D., L. Jiang, H. Huang, K. Toyota, R.A. Dahlgren, and J. Lu. 2012. “Nitrogen Dynamics of
533 Anaerobically Digested Slurry Used to Fertilize Paddy Fields”. *Biology and Fertility of*
534 *Soils*, 49 (6): 647-659. <https://doi.org/10.1007/s00374-012-0752-8>.

535 Coelho, J. J., A. Hennessy, I. Casey, C. R. S. Bragança, T. Woodcock, and N. Kennedy. 2020.
536 “Biofertilisation with Anaerobic Digestates: A Field Study of Effects on Soil Microbial
537 Abundance and Diversity”. *Applied Soil Ecology* 147 (March):103403.
538 <https://doi.org/10.1016/j.apsoil.2019.103403>.

539 Craswell, E., and P. Vlek. 1983. "Fate of Fertilizer Nitrogen Applied to Wetland Rice". *Gaseous*
540 *Loss of Nitrogen from Plant-Soil Systems*, January, 237–264. [https://doi.org/10.1007/978-](https://doi.org/10.1007/978-94-017-1662-8_10)
541 [94-017-1662-8_10](https://doi.org/10.1007/978-94-017-1662-8_10).

542 Dixon, J. B. 1991. "Roles of Clays in Soils". *Applied Clay Science* 5 (5): 489–503.
543 [https://doi.org/10.1016/0169-1317\(91\)90019-6](https://doi.org/10.1016/0169-1317(91)90019-6).

544 Dou, F., J. Soriano, R. E. Tabien, and K. Chen. 2016. "Soil Texture and Cultivar Effects on Rice
545 (Oryza Sativa, L.) Grain Yield, Yield Components and Water Productivity in Three Water
546 Regimes". *PLoS ONE* 11 (3): e0150549. <https://doi.org/10.1371/journal.pone.0150549>.

547 Du, H., W. Gao, J. Li, S. Shen, F. Wang, L. Fu, and K. Zhang. 2019. "Effects of Digested Biogas
548 Slurry Application Mixed with Irrigation Water on Nitrate Leaching during Wheat-Maize
549 Rotation in the North China Plain". *Agricultural Water Management* 213: 882–893.
550 <https://doi.org/10.1016/j.agwat.2018.12.012>.

551 Duan, Z. H., and H. L. Xiao. 2000. "Effects of Soil Properties on Ammonia Volatilization". *Soil*
552 *Science and Plant Nutrition* 46 (4): 845–852.
553 <https://doi.org/10.1080/00380768.2000.10409150>.

554 Fan, X. H., Y. C. Li, and A. K. Alva. 2011. "Effects of Temperature and Soil Type on Ammonia
555 Volatilization from Slow-Release Nitrogen Fertilizers". *Communications in Soil Science and*
556 *Plant Analysis* 42 (10): 1111–1122. <https://doi.org/10.1080/00103624.2011.566957>.

557 Ferguson, R. M. W., F. Coulon, and R. Villa. 2016. "Organic Loading Rate: A Promising Microbial
558 Management Tool in Anaerobic Digestion". *Water Research* 100: 348–356.
559 <https://doi.org/10.1016/j.watres.2016.05.009>.

560 Flores, P., I. Castellar, and J. Navarro. 2005. "Nitrate Leaching in Pepper Cultivation with Organic
561 Manure and Supplementary Additions of Mineral Fertilizer". *Communications in Soil*
562 *Science and Plant Analysis* 36 (19–20): 2889–2898.
563 <https://doi.org/10.1080/00103620500306072>.

564 Gärdenäs, A. I., P. J. Hanson, D. E. Todd, and J. M. Warren. 2005. "Influence of soil texture and
565 precipitation on soil carbon and nitrogen dynamics in southern Appalachian forests." *Forest
566 Ecology and Management* 220 (1–3): 301–314. <https://doi.org/10.1016/j.foreco.2005.08.001>

567 Geng, Y., G. Cao, L. Wang, and S. Wang. 2019. "Effects of Equal Chemical Fertilizer Substitutions
568 with Organic Manure on Yield, Dry Matter, and Nitrogen Uptake of Spring Maize and Soil
569 Nitrogen Distribution". *PLOS ONE* 14 (7): e0219512.
570 <https://doi.org/10.1371/journal.pone.0219512>.

571 Ghoneim, A., H. Ueno, A. Ebid, N. Asagi, and I. Abou El. 2008. "Analysis of Nitrogen Dynamics
572 and Fertilizer Use Efficiency in Rice Using the Nitrogen¹⁵ Isotope Dilution Method
573 Following the Application of Biogas Slurry or Chemical Fertilizer". *International Journal of
574 Soil Science* 3: 11–19. <https://doi.org/10.3923/ijss.2008.11.19>.

575 Grant, C. A., R. Wu, F. Selles, K. N. Harker, G. W. Clayton, S. Bittman, B. J. Zebarth, and N. Z.
576 Lupwayi. 2012. "Crop Yield and Nitrogen Concentration with Controlled Release Urea and
577 Split Applications of Nitrogen as Compared to Non-Coated Urea Applied at Seeding". *Field
578 Crops Research* 127: 170–180. <https://doi.org/10.1016/j.fcr.2011.11.002>.

579 Gu, B., D. Chen, Y. Yang, P. Vitousek, and Y. Zhu. 2021. "Soil-Food-Environment-Health Nexus
580 for Sustainable Development". *Research* 2021: 9804807.
581 <https://doi.org/10.34133/2021/9804807>.

582 Hamoud, Y. A., X. Guo, Z. Wang, H. Shaghaleh, S. Chen, A. Hassan, and A. Bakour. 2019.
583 "Effects of Irrigation Regime and Soil Clay Content and Their Interaction on the Biological
584 Yield, Nitrogen Uptake and Nitrogen-Use Efficiency of Rice Grown in Southern China".
585 *Agricultural Water Management* 213 (March): 934–946.
586 <https://doi.org/10.1016/j.agwat.2018.12.017>.

587 Hansen, B., E. S. Kristensen, R. Grant, H. Høgh-Jensen, S. E. Simmelsgaard, and J. E. Olesen.
588 2000. "Nitrogen Leaching from Conventional versus Organic Farming Systems - a Systems

589 Modelling Approach”. *European Journal of Agronomy* 13 (1): 65–82.
590 [https://doi.org/10.1016/S1161-0301\(00\)00060-5](https://doi.org/10.1016/S1161-0301(00)00060-5).

591 Hassink, J., L. A. Bouwman, K. B. Zwart, J. Bloem, and L. Brussaard. 1993. “Relationships
592 between Soil Texture, Physical Protection of Organic Matter, Soil Biota, and C and N
593 Mineralization in Grassland Soils”. *Geoderma, Soil Structure/Soil Biota Interrelationships*,
594 57 (1): 105–128. [https://doi.org/10.1016/0016-7061\(93\)90150-J](https://doi.org/10.1016/0016-7061(93)90150-J).

595 Hayashi, K., S. Nishimura, and K. Yagi. 2006. “Ammonia Volatilization from the Surface of a
596 Japanese Paddy Field during Rice Cultivation”. *Soil Science and Plant Nutrition* 52 (4):
597 545–555. <https://doi.org/10.1111/j.1747-0765.2006.00053.x>.

598 He, Z., A. Kumar, D. Calvert, and D. Banks. 1999. “Ammonia Volatilization from Different
599 Fertilizer Sources and Effects of Temperature and Soil pH”. *Soil Science* 164
600 (October):750–758. <https://doi.org/10.1097/00010694-199910000-00006>.

601 Hou, H., S. Zhou, M. Hosomi, K. Toyota, K. Yosimura, Y. Mutou, T. Nisimura, M. Takayanagi,
602 and T. Motobayashi. 2007. “Ammonia Emissions from Anaerobically-Digested Slurry and
603 Chemical Fertilizer Applied to Flooded Forage Rice”. *Water, Air, and Soil Pollution* 183
604 (1): 37–48. <https://doi.org/10.1007/s11270-007-9353-9>.

605 Hu, B., L. Shen, X. Xu, and P. Zheng. 2011. “Anaerobic Ammonium Oxidation (Anammox) in
606 Different Natural Ecosystems”. *Biochemical Society Transactions* 39 (6): 1811–1816.
607 <https://doi.org/10.1042/BST20110711>.

608 Hu, X., H Liu, C. Xu, X. Huang, M. Jiang, H. Zhuang, and L. Huang. 2021. “Effect of Digestate
609 and Straw Combined Application on Maintaining Rice Production and Paddy Environment”.
610 *International Journal of Environmental Research and Public Health* 18 (11): 5714.
611 <https://doi.org/10.3390/ijerph18115714>.

612 Hu, Z., J. W. Lee, K. Chandran, S. Kim, and S. K. Khanal. 2012. “Nitrous Oxide (N₂O) Emission
613 from Aquaculture: A Review”. *Environmental Science & Technology* 46 (12): 6470–6480.
614 <https://doi.org/10.1021/es300110x>.

615 Ichinose, Y., Y. Ise, T. Kanda, Y. Maejima, T. Yagi, Y. Takahashi, S. Nakagawa, et al. 2023.
 616 “Factors Controlling Available Soil Nitrogen in Japanese Paddy Fields”. *Soil Science and*
 617 *Plant Nutrition* 69(5–6), 303–314. <https://doi.org/10.1080/00380768.2023.2231013>.
 618 Iocoli, G. A., M. C. Zabaloy, G. Pasdevicelli, and M. A. Gómez. 2019. “Use of Biogas Digestates
 619 Obtained by Anaerobic Digestion and Co-Digestion as Fertilizers: Characterization, Soil
 620 Biological Activity and Growth Dynamic of *Lactuca Sativa* L.” *Science of The Total*
 621 *Environment* 647 (January): 11–19. <https://doi.org/10.1016/j.scitotenv.2018.07.444>.
 622 Japan Organics Recycling Association (JORA). 2023. “Case studies of utilization of digestate as
 623 fertilizer (in Japanese)”. [https://www.jora.jp/wp-](https://www.jora.jp/wp-content/uploads/2024/04/baioekihijireisyu_hiryo2023_rev.pdf)
 624 [content/uploads/2024/04/baioekihijireisyu_hiryo2023_rev.pdf](https://www.jora.jp/wp-content/uploads/2024/04/baioekihijireisyu_hiryo2023_rev.pdf)
 625 Ji, X. H., S. X. Zheng, L. H. Shi, and Z. B. Liu. 2011. “Systematic Studies of Nitrogen Loss from
 626 Paddy Soils Through Leaching in the Dongting Lake Area of China”. *Pedosphere* 21 (6):
 627 753–762. [https://doi.org/10.1016/S1002-0160\(11\)60179-3](https://doi.org/10.1016/S1002-0160(11)60179-3).
 628 Jimenez, J., M. Grigatti, E. Boanini, D. Patureau, and N. Bernet. 2020. “The impact of biogas
 629 digestate typology on nutrient recovery for plant growth: Accessibility indicators for first
 630 fertilization prediction”. *Waste Management*, 117 (2020): 18-31.
 631 <https://doi.org/10.1016/j.wasman.2020.07.052>
 632 Kanbar, A., M. Toorchi, and H.E. Shashidhar. 2009. “Relationship between Root and Yield
 633 Morphological Characters in Rainfed Low Land Rice (*Oryza Sativa* L.)”. *Cereal Research*
 634 *Communications* 37 (2): 261–268. <https://doi.org/10.1556/CRC.37.2009.2.14>.
 635 Li, K., T. Inamura, and M. Umeda. 2003. “Growth and Nitrogen Uptake of Paddy Rice as
 636 Influenced by Fermented Manure Liquid and Squeezed Manure Liquid”. *Soil Science and*
 637 *Plant Nutrition* 49 (3): 463–467. <https://doi.org/10.1080/00380768.2003.10410033>.
 638 Liu, D., Y. Fang, Ying T., and Y. Pan. 2014. “Chemical Method for Nitrogen Isotopic Analysis of
 639 Ammonium at Natural Abundance”. *Analytical Chemistry* 86 (8): 3787–3792.
 640 <https://doi.org/10.1021/ac403756u>.

641 Malone, T. C., and A. Newton. 2020. “The Globalization of Cultural Eutrophication in the Coastal
642 Ocean: Causes and Consequences”. *Frontiers in Marine Science* 7(670): 1–30.
643 <https://doi.org/10.3389/fmars.2020.00670>.

644 Maris, S. C., F. Capra, F. Ardeni, R. Boselli, D. Pochintesta, G. M. Beone, V. Tabaglio, and A.
645 Fiorini. 2021. “The Interaction between Types of Cover Crop Residue and Digestate
646 Application Methods Affects Ammonia Volatilization during Maize Cropping Season”.
647 *Journal of Environmental Quality* 50 (2): 504–512. <https://doi.org/10.1002/jeq2.20205>.

648 Möller, K., and T. Müller. 2012. “Effects of Anaerobic Digestion on Digestate Nutrient Availability
649 and Crop Growth: A Review”. *Engineering in Life Sciences* 12 (3): 242–257.
650 <https://doi.org/10.1002/elsc.201100085>.

651 Möller, K. 2015. “Effects of Anaerobic Digestion on Soil Carbon and Nitrogen Turnover, N
652 Emissions, and Soil Biological Activity. A Review”. *Agronomy for Sustainable
653 Development* 35 (3): 1021–1041. <https://doi.org/10.1007/s13593-015-0284-3>.

654 Murphy, R., M. Saunders, K. G. Richards, D. Król, A. W. Gebremichael, J. Rambaud, N. Cowan,
655 and G. Lanigan. 2022. “Nitrous oxide emission factors from an intensively grazed temperate
656 grassland: a comparison of cumulative emissions determined by eddy covariance and static
657 chamber methods”. *Agriculture, Ecosystems & Environment*, 324: 107725.
658 <https://doi.org/10.1016/j.agee.2021.107725>.

659 Nguyen, B. T., B.T. Phan, T. X. Nguyen, V. N. Nguyen, T. V. Tran, and Q. V. Bach. 2020.
660 “Contrastive Nutrient Leaching from Two Differently Textured Paddy Soils as Influenced
661 by Biochar Addition”. *Journal of Soils and Sediments* 20 (1): 297–307.
662 <https://doi.org/10.1007/s11368-019-02366-8>.

663 Nicholson, F., A. Bhogal, L. Cardenas, D. Chadwick, T. Misselbrook, A. Rollett, M. Taylor, R.
664 Thorman, and J. Williams. 2017. “Nitrogen Losses to the Environment Following Food-
665 Based Digestate and Compost Applications to Agricultural Land”. *Environmental Pollution
666 (Barking, Essex: 1987)* 228: 504–516. <https://doi.org/10.1016/j.envpol.2017.05.023>.

667 Nieder, R., and D. K. Benbi. 2022. “Reactive Nitrogen Compounds and Their Influence on Human
668 Health: An Overview”. *Reviews on Environmental Health* 37 (2): 229–246.
669 <https://doi.org/10.1515/reveh-2021-0021>.

670 Nieder, R., D. K. Benbi, and H. W. Scherer. 2011. “Fixation and Defixation of Ammonium in Soils:
671 A Review”. *Biology and Fertility of Soils* 47 (1): 1–14. [https://doi.org/10.1007/s00374-010-](https://doi.org/10.1007/s00374-010-0506-4)
672 [0506-4](https://doi.org/10.1007/s00374-010-0506-4).

673 Nishida, M., H. Sumida, and N Kato. 2008. “Fate of Nitrogen Derived from 15N-Labeled Cattle
674 Manure Compost Applied to a Paddy Field in the Cool Climate Region of Japan”. *Soil*
675 *Science & Plant Nutrition* 54 (3): 459–466. [https://doi.org/10.1111/j.1747-](https://doi.org/10.1111/j.1747-0765.2008.00255.x)
676 [0765.2008.00255.x](https://doi.org/10.1111/j.1747-0765.2008.00255.x).

677 Nishida, M., K. Tsuchiya, and S. Yamamuro. 2004. “Fate of N and Relative Efficiency of 15N-
678 Labeled Organic Materials Applied to Transplanted Rice in Northern Kyushu Region of
679 Japan”. *Soil Science and Plant Nutrition* 50 (2): 225–232.
680 <https://doi.org/10.1080/00380768.2004.10408471>.

681 Nishikawa, T., K. Li, and T. Inamura. 2014. “Nitrogen Uptake by the Rice Plant and Changes in the
682 Soil Chemical Properties in the Paddy Rice Field during Yearly Application of
683 Anaerobically-Digested Manure for Seven Years”. *Plant Production Science* 17 (3): 237–
684 244. <https://doi.org/10.1626/pps.17.237>.

685 Nishikawa, T., K. Li, H. Inoue, M. Umeda, H. Hirooka, and T. Inamura. 2012. “Effects of the
686 Long-Term Application of Anaerobically-Digested Cattle Manure on Growth, Yield and
687 Nitrogen Uptake of Paddy Rice (*Oryza Sativa* L.), and Soil Fertility in Warmer Region of
688 Japan”. *Plant Production Science* 15 (4): 284–292. <https://doi.org/10.1626/pps.15.284>.

689 O’Brien, B. J., D. A. Neher, and E. D. Roy. 2020. “Nutrient and Pathogen Suppression Properties
690 of Anaerobic Digestates from Dairy Manure and Food Waste Feedstocks”. *Waste and*
691 *Biomass Valorization* 11 (12): 6565–6573. <https://doi.org/10.1007/s12649-019-00906-4>.

692 Odlare, M., V. Arthurson, M. Pell, K. Svensson, E. Nehrenheim, and J. Abubaker. 2011. "Land
693 Application of Organic Waste – Effects on the Soil Ecosystem". *Applied Energy* 88 (6):
694 2210–2218. <https://doi.org/10.1016/j.apenergy.2010.12.043>.

695 Oritate, F. 2016. "Use of Digested Slurry from Livestock Manure in Paddy Fields and Its
696 Environmental Effect in Southeast Asia". PhD diss., Renewable Energy and Resources Unit,
697 Regional Resources Engineering Division: Kyoto University.
698 <http://doi.org/10.14989/doctor.r13024>.

699 Panuccio, M. R., F. Romeo, C. Mallamaci, and A. Muscolo. 2021. "Digestate Application on Two
700 Different Soils: Agricultural Benefit and Risk". *Waste and Biomass Valorization* 12 (8):
701 4341–4353. <https://doi.org/10.1007/s12649-020-01318-5>.

702 Partey, S., K. Saito, R. Preziosi, and G. Robson. 2015. "Biochar Use in a Legume-Rice Rotation
703 System: Effects on Soil Fertility and Crop Performance". *Archives of Agronomy and Soil
704 Science*, 62(2): 199-215. <https://doi.org/10.1080/03650340.2015.1040399>.

705 Ran, Y., X. Bai, Y. Long, and P. Ai. 2022. "Yield and Quality of Rice under the Effects of
706 Digestate Application". *Agriculture* 12 (4): 514.
707 <https://doi.org/10.3390/agriculture12040514>.

708 Rigby, H., and S. R. Smith. 2013. "Nitrogen Availability and Indirect Measurements of Greenhouse
709 Gas Emissions from Aerobic and Anaerobic Biowaste Digestates Applied to Agricultural
710 Soils". *Waste Management* 33 (12): 2641–2652.
711 <https://doi.org/10.1016/j.wasman.2013.08.005>.

712 Risberg, K., H. Cederlund, M. Pell, V. Arthurson, and A. Schnürer. 2017. "Comparative
713 Characterization of Digestate versus Pig Slurry and Cow Manure – Chemical Composition
714 and Effects on Soil Microbial Activity". *Waste Management* 61: 529–538.
715 <https://doi.org/10.1016/j.wasman.2016.12.016>.

716 Ritter, W. F., and A. E. M. Chirnside. 1987. "Influence of Agricultural Practices on Nitrates in the
717 Water Table Aquifer". *Biological Wastes* 19 (3): 165–178. [https://doi.org/10.1016/0269-
718 7483\(87\)90050-4](https://doi.org/10.1016/0269-7483(87)90050-4).

719 Rochette, P., D. A. Angers, M. H. Chantigny, M. O. Gasser, J. D. MacDonald, D. E. Pelster, and N.
720 Bertrand. 2013. "NH₃ Volatilization, Soil Concentration and Soil pH Following Subsurface
721 Banding of Urea at Increasing Rates". *Canadian Journal of Soil Science* 93 (2): 261–268.
722 <https://doi.org/10.4141/cjss2012-095>.

723 Sasada, Y., K. Win, R. Nonaka, A. Win, K. Toyota, T. Motobayashi, M. Hosomi, D. Chen, and J.
724 Lu. 2011. "Methane and N₂O Emissions, Nitrate Concentrations of Drainage Water, and
725 Zinc and Copper Uptake by Rice Fertilized with Anaerobically Digested Cattle or Pig
726 Slurry". *Biology and Fertility of Soils* 47: 949–956. [https://doi.org/10.1007/s00374-011-
727 0601-1](https://doi.org/10.1007/s00374-011-0601-1).

728 Seadi, T. A., and C. Lukehurst. 2012. "Quality Management of Digestate from Biogas Plants Used
729 as Fertiliser". *Task 37, Energy from Biogas*, 40. IEA Bioenergy

730 Shan, J., P. Yang, X. Shang, M. Rahman, and X. Yan. 2018. "Anaerobic Ammonium Oxidation and
731 Denitrification in a Paddy Soil as Affected by Temperature, pH, Organic Carbon, and
732 Substrates". *Biology and Fertility of Soils* 54 (3): 341–348. [https://doi.org/10.1007/s00374-
733 018-1263-z](https://doi.org/10.1007/s00374-018-1263-z).

734 Sharifi, M., S. Baker, L. Hojabri, and M. Hajiaghahi-Kamrani. 2019. "Short-Term Nitrogen
735 Dynamics in a Soil Amended with Anaerobic Digestate". *Canadian Journal of Soil Science*,
736 99: 173-181. <https://doi.org/10.1139/cjss-2018-0060>.

737 Sharma, B., B. Vaish, Monika, U. K. Singh, P. Singh, and R. P. Singh. 2019. "Recycling of Organic
738 Wastes in Agriculture: An Environmental Perspective". *International Journal of
739 Environmental Research* 13 (2): 409–429. <https://doi.org/10.1007/s41742-019-00175-y>.

740 Shen, L., P. Zheng, and S. Ma. 2016. "Nitrogen Loss through Anaerobic Ammonium Oxidation in
 741 Agricultural Drainage Ditches". *Biology and Fertility of Soils* 52 (2): 127–136.
 742 <https://doi.org/10.1007/s00374-015-1058-4>.

743 Shibata, M., S. Sugihara, A. D. Mvondo-Ze, S. Araki, and S. Funakawa. 2017. "Nitrogen Flux
 744 Patterns through Oxisols and Ultisols in Tropical Forests of Cameroon, Central Africa". *Soil
 745 Science and Plant Nutrition*, 63 (3): 306-317.
 746 <https://doi.org/10.1080/00380768.2017.1341285>.

747 Sigman, D. M., K. L. Casciotti, M. Andreani, C. Barford, M. Galanter, and J. K. Böhlke. 2001. "A
 748 Bacterial Method for the Nitrogen Isotopic Analysis of Nitrate in Seawater and Freshwater".
 749 *Analytical Chemistry* 73 (17): 4145–4153. <https://doi.org/10.1021/ac010088e>.

750 Sikora, L. J., and N. K. Enkiri. 1999. "Growth of Tall Fescue in Compost/Fertilizer Blends". *Soil
 751 Science* 164 (1): 62-69. <https://doi.org/10.1097/00010694-199901000-00009>.

752 Singla, A., and K. Inubushi. 2014. "Effect of Biogas Digested Liquid on CH₄ and N₂O Flux in
 753 Paddy Ecosystem". *Journal of Integrative Agriculture* 13 (3): 635–640.
 754 [https://doi.org/10.1016/S2095-3119\(13\)60721-2](https://doi.org/10.1016/S2095-3119(13)60721-2).

755 Smith, V. H., G. D. Tilman, and J. C. Nekola. 1999. "Eutrophication: Impacts of Excess Nutrient
 756 Inputs on Freshwater, Marine, and Terrestrial Ecosystems". *Environmental Pollution* 100
 757 (1–3): 179–196. [https://doi.org/10.1016/S0269-7491\(99\)00091-3](https://doi.org/10.1016/S0269-7491(99)00091-3).

758 Stepanauskas, R., E Davidsson, and L. Leonardson. 1996. "Nitrogen Transformations in Wetland
 759 Soil Cores Measured by ¹⁵N Isotope Pairing and Dilution at Four Infiltration Rates". *Applied
 760 and Environmental Microbiology* 62(7): 2345-2351. [https://doi.org/10.1128/aem.62.7.2345-
 761 2351.1996](https://doi.org/10.1128/aem.62.7.2345-2351.1996)

762 Takakai, F., H. Seiya, Y. Harakawa, K. Hatakeyama, Kentaro Yasuda, T. Sato, K. Kimura, and Y.
 763 Kaneta. 2018. "Fate of Fertilizer-Derived N Applied to Enhance Rice Straw Decomposition
 764 in a Paddy Field during the Fallow Season under Cool Temperature Conditions".
 765 *Agriculture* 8 (4): 50. <https://doi.org/10.3390/agriculture8040050>.

766 Tambone, F., B. Scaglia, G. D'Imporzano, A. Schievano, V. Orzi, S. Salati, and F. Adani. 2010.
 767 "Assessing Amendment and Fertilizing Properties of Digestates from Anaerobic Digestion
 768 through a Comparative Study with Digested Sludge and Compost". *Chemosphere* 81 (5):
 769 577–583. <https://doi.org/10.1016/j.chemosphere.2010.08.034>.
 770 Tampio, E., T. Salo and J. Rintala. 2016. "Agronomic characteristics of five different urban waste
 771 digestates". *Journal of Environmental Management*, 169: 293-
 772 302. <https://doi.org/10.1016/j.jenvman.2016.01.001>
 773 Tanaka, T. S. T., Y. Nitta, K. Kido, T. Nishikawa, T. Matoh, and T. Inamura. 2017. "Effect of the
 774 Long-Term Application of Anaerobically Digested Residual Slurry on Methane Emissions
 775 in a Rice Paddy Field". *Soil Science and Plant Nutrition* 63 (3): 300–305.
 776 <https://doi.org/10.1080/00380768.2017.1322918>.
 777 Ueno, H., and S. Yamamuro. 2001. "Fate of Nitrogen Derived from ¹⁵N-Labeled Plant Residues
 778 and Composts in Rice-Planted Paddy Soil". *Soil Science and Plant Nutrition* 47 (4): 747–
 779 754. <https://doi.org/10.1080/00380768.2001.10408439>.
 780 Voća, N., T. Krička, T. Čosić, V. Rupiće, Ž. Jukić, and S. Kalambura. 2005. "Digested Residue as a
 781 Fertilizer after the Mesophilic Process of Anaerobic Digestion". *Plant, Soil and*
 782 *Environment* 51: 262–266. <https://doi.org/10.17221/3584-PSE>.
 783 Vries, M. P. C. de. 1980. "How Reliable Are Results of Pot Experiments?" *Communications in Soil*
 784 *Science and Plant Analysis* 11 (9): 895–902. <https://doi.org/10.1080/00103628009367090>.
 785 Vries, W. de. 2021. "Impacts of Nitrogen Emissions on Ecosystems and Human Health: A Mini
 786 Review". *Current Opinion in Environmental Science & Health* 21: 100249.
 787 <https://doi.org/10.1016/j.coesh.2021.100249>.
 788 Wang, Y., S. Chikamatsu, T. Gegen, K. Sawada, K. Toyota, S. Riya, and M. Hosomi. 2019.
 789 "Application of Biogas Digestate with Rice Straw Mitigates Nitrate Leaching Potential and
 790 Suppresses Root-Knot Nematode (*Meloidogyne Incognita*)". *Agronomy* 9 (5): 227.
 791 <https://doi.org/10.3390/agronomy9050227>.

792 Wang, Z. H., X. J. Liu, X. T. Ju, F. S. Zhang, and S. S. Malhi. 2004. "Ammonia Volatilization Loss
793 from Surface-Broadcast Urea: Comparison of Vented- and Closed-Chamber Methods and
794 Loss in Winter Wheat–Summer Maize Rotation in North China Plain". *Communications in*
795 *Soil Science and Plant Analysis* 35 (19–20): 2917–2939. [https://doi.org/10.1081/CSS-](https://doi.org/10.1081/CSS-200036499)
796 [200036499](https://doi.org/10.1081/CSS-200036499).

797 Watanabe, S., K. Nakamura, C. S. Ryu, M. Iida, and S. Kawashima. 2012. "Effects of Different
798 Application Timings of Methane Fermentation Digested Liquid to Paddy Plots on Soil
799 Nitrogen and Rice Yield". *Soil Science and Plant Nutrition* 58 (2): 224–237.
800 <https://doi.org/10.1080/00380768.2012.661949>.

801 Win, A. T., K. Toyota, K. T. Win, T. Motobayashi, T. Ookawa, T. Hirasawa, D. Chen, and J. Lu.
802 2014. "Effect of Biogas Slurry Application on CH₄ and N₂O Emissions, Cu and Zn Uptakes
803 by Whole Crop Rice in a Paddy Field in Japan". *Soil Science and Plant Nutrition* 60 (3):
804 411–422. <https://doi.org/10.1080/00380768.2014.899886>.

805 Win, K. T., R. Nonaka, K. Toyota, T. Motobayashi, and M. Hosomi. 2010. "Effects of Option
806 Mitigating Ammonia Volatilization on CH₄ and N₂O Emissions from a Paddy Field
807 Fertilized with Anaerobically Digested Cattle Slurry". *Biology and Fertility of Soils* 46 (6):
808 589–595. <https://doi.org/10.1007/s00374-010-0465-9>.

809 Wulf, S., M. Maeting, and J. Clemens. 2002. "Application Technique and Slurry Co-Fermentation
810 Effects on Ammonia, Nitrous Oxide, and Methane Emissions after Spreading". *Journal of*
811 *Environmental Quality* 31 (6): 1795–1801. <https://doi.org/10.2134/jeq2002.1795>.

812 Xinqiang, L., Y. Junli, H. Miaomiao, L. Hua, L. Liang, and T. Guangming. 2014. "Modeling the
813 Fate of Fertilizer N in Paddy Rice Systems Receiving Manure and Urea". *Geoderma*,
814 Properties, processes and ecological functions of floodplain, peatland, and paddy soils, 228–
815 229: 54–61. <https://doi.org/10.1016/j.geoderma.2013.09.002>.

816 Xu, J., S. Peng, S. Yang, and W. Wang. 2012. "Ammonia Volatilization Losses from a Rice Paddy
 817 with Different Irrigation and Nitrogen Managements". *Agricultural Water Management* 104:
 818 184–192. <https://doi.org/10.1016/j.agwat.2011.12.013>.

819 Yang, X., H. Li, S. Nie, J. Su, B. Weng, G. Zhu, H. Yao, J. A. Gilbert, and Y. Zhu. 2015. "Potential
 820 contribution of anammox to nitrogen loss from paddy soils in southern China". *Applied and*
 821 *Environmental Microbiology*, 81(3): 938-947. <https://doi.org/10.1128/aem.02664-14>

822 Ye, Q. B., H. C. Zhang, H. Y. Wei, Y. Zhang, Benfu Wang, K. Xia, Z. Y. Huo, Q. Dai, and K. Xu.
 823 2007. "Effects of Nitrogen Fertilizer on Nitrogen Use Efficiency and Yield of Rice under
 824 Different Soil Conditions". *Frontiers of Agriculture in China* 1 (1): 30–36.
 825 <https://doi.org/10.1007/s11703-007-0005-z>.

826 Yu, X., Y. Xu, H. Zhu, B. Shutes, B. Yan, X. Chen, and C. Rui. 2019. "Tracking the Fate of
 827 Fertilizer Nitrogen in a Paddy Rice Field Using Isotope Technology". *Polish Journal of*
 828 *Environmental Studies* 29 (1). 419-428. <https://doi.org/10.15244/pjoes/103026>.

829 Zhao, X., Y. X. Xie, Z. Q. Xiong, X. Q. Yan, G. X. Xing, and Z. L. Zhu. 2009. "Nitrogen Fate and
 830 Environmental Consequence in Paddy Soil under Rice-Wheat Rotation in the Taihu Lake
 831 Region, China". *Plant and Soil* 319 (1): 225–234. [https://doi.org/10.1007/s11104-008-9865-](https://doi.org/10.1007/s11104-008-9865-0)
 832 [0](https://doi.org/10.1007/s11104-008-9865-0).

833 Zheng, J., Y. Qu, M. M. Kilasara, W. N. Mmari, and S. Funakawa. 2019. "Nitrate Leaching from
 834 the Critical Root Zone of Maize in Two Tropical Highlands of Tanzania: Effects of
 835 Fertilizer-Nitrogen Rate and Straw Incorporation". *Soil and Tillage Research* 194:104295.
 836 <https://doi.org/10.1016/j.still.2019.104295>.

837 Zhou, J. B., J. G. Xi, Z. J. Chen, and S. X. Li. 2006. "Leaching and Transformation of Nitrogen
 838 Fertilizers in Soil After Application of N with Irrigation: A Soil Column Method1 1Project
 839 Supported by the National Natural Science Foundation of China (Nos. 30230230 and
 840 30370288) and the National Key Laboratory for Soil Erosion and Dryland Farming on the

841 Loess Plateau (No. 10501-116).” *Pedosphere* 16 (2): 245–252.
842 [https://doi.org/10.1016/S1002-0160\(06\)60050-7](https://doi.org/10.1016/S1002-0160(06)60050-7).

843 Zhou, M., and K. Butterbach-Bahl. 2014. “Assessment of Nitrate Leaching Loss on a Yield-Scaled
844 Basis from Maize and Wheat Cropping Systems”. *Plant and Soil* 374 (1): 977–991.
845 <https://doi.org/10.1007/s11104-013-1876-9>.

846 Zhou, S., S. Sugawara, S. Riya, M. Sagehashi, K. Toyota, A. Terada, and M. Hosomi. 2011. “Effect
847 of Infiltration Rate on Nitrogen Dynamics in Paddy Soil after High-Load Nitrogen
848 Application Containing ¹⁵N Tracer”. *Ecological Engineering*, Advances in pollutant
849 removal processes and fate in natural and constructed wetlands, 37 (5): 685–692.
850 <https://doi.org/10.1016/j.ecoleng.2010.04.032>.

851 Zhou, S., Y. Sakiyama, S. Riya, X. Song, A. Terada, and M. Hosomi. 2012. “Assessing nitrification
852 and denitrification in a paddy soil with different water dynamics and applied liquid cattle
853 waste using the ¹⁵N isotopic technique”. *Science of The Total Environment* 430: 93-100.
854 <https://doi.org/10.1016/j.scitotenv.2012.04.056>

855 Zilio, M., A. Pigoli, B. Rizzi, G. Geromel, Erik Meers, O. Schoumans, A. Giordano, and F. Adani.
856 2021. “Measuring Ammonia and Odours Emissions during Full Field Digestate Use in
857 Agriculture”. *Science of The Total Environment* 782:146882.
858 <https://doi.org/10.1016/j.scitotenv.2021.146882>.

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860

861 **Figure legends**

862 **Figure 1.** The design of the experimental pot for collecting daily leachate solution

863 **Figure 2.** Comparison of different methane fermentation waste fluid (MFW) treatments in
864 contrasting soil textures (sandy loam, SL; clayey loam, CL) for (a) NH_3 volatilization in 2022, (b)
865 $\text{NH}_3\text{-N}$ derived from inorganic N of MFW ($\text{NH}_3\text{-Ndfif}$) in 2022, (c) N_2O emission in 2021, and (d)
866 N_2O emission in 2022. 0N: no fertilizer; 0.5N-MFW: 100 mg N as MFW; 1N-MFW: 200 mg N as
867 MFW; 2N-MFW: 400 mg N as MFW. Error bars indicate standard error ($n = 4$), except for SL-0N
868 and CL-2N-MFW treatments in 2022 ($n = 3$). Small and capital letters indicate the significant
869 differences among treatments in SL and CL soils, respectively, as determined by one-way ANOVA
870 ($P < 0.05$). Asterisks indicate the significant difference between two soil textures within the same
871 treatment by Tukey's test ($*P < 0.05$, $**P < 0.01$, and $***P < 0.001$). Significant differences
872 between regression lines for soil textures were computed using the F-test ($P < 0.05$).

873 **Figure 3.** Comparison of nitrogen (N) content in plant N uptake (a, e), soil N derived from
874 inorganic fertilizer (b, f), total dissolved N (TDN) in leachate (c, g), and gas emissions (d, h)
875 between 1N-Chem and 1N-MFW under contrasting soil textures (SL and CL) in 2021 (a–d) and
876 2022 (e–h). Ndfif: Nitrogen derived from inorganic fertilizer. Ndfun: Nitrogen derived from
877 unlabeled sources. Error bars represent standard error ($n = 4$). Double dots represent significant
878 differences between two soil textures within the same treatment for each observed indicator ($P <$
879 0.05). Asterisks indicate significant differences among treatments within each soil texture ($P <$
880 0.05). ns: not significant.

881 **Figure 4.** Ndfif in leachate under different MFW treatments in 2021 (a) and 2022 (b) for SL and CL
882 soils. 0N: no fertilizer; 0.5N-MFW: 100 mg N as MFW; 1N-MFW: 200 mg N as MFW; 2N-MFW:
883 400 mg N as MFW. Error bars represent standard error ($n = 4$), except for SL-0N and CL-2N-
884 MFW treatments in 2022 ($n = 3$). Small and capital letters indicate the significant differences
885 among treatments in SL and CL soils, respectively, as determined by one-way ANOVA ($P < 0.05$).

886 Asterisks indicate the confidence of trendlines: $*P < 0.05$ and $**P < 0.01$. P -values indicate the
887 significant differences between regression lines for SL and CL soils.

888 **Figure 5.** Relationship between normalized rice biomass (a, b), yield (c, d), and total N loss under
889 different MFW application rates in SL (a, c) and CL (b, d) soils in 2022. 0N: no fertilizer; 0.5N-
890 MFW: 100 mg N as MFW; 1N-MFW: 200 mg N as MFW; 2N-MFW: 400 mg N as MFW. Dotted
891 lines indicate linear fits for rice biomass and yield; solid lines indicate exponential fits for total N
892 loss. Normalized values were calculated using the min–max normalization formula: $x' = (x_i - \min$
893 $[x]) / (\max [x] - \min [x])$, where x_i represents each observed value ($n = 4$, unless otherwise noted),
894 $\min [x]$ and $\max [x]$ are the minimum and the maximum values within the group, respectively.
895 Since values shown are treatment means, normalized values may not exactly be 0 or 1 even at 0N or
896 2N-MFW treatments. Xop-values indicate the MFW application rates at which the difference
897 between the normalized rice productivity (biomass or yield) and total N loss is maximized. These
898 values indicate the optimal balance between agronomic performance and environmental impact for
899 each soil texture. Error bars represent the standard error ($n = 4$), except for SL-0N and CL-2N-
900 MFW treatments in 2022 ($n = 3$). Asterisks indicate the confidence of the trendline: $*P < 0.05$ and
901 $**P < 0.01$.

902

903 **Table legends**

904 **Table 1** Soil properties of SL and CL soils used in the experiment.

905 **Table 2** Characteristics of methane fermentation waste fluid applied during two growing seasons.

906 **Table 3** Key agronomic characteristics of rice at harvest under MFW treatments in SL and CL soils
907 across two growing seasons.

908 **Table 4** Total nitrogen content (TN), total dissolved nitrogen (TDN), and nitrogen derived from
909 inorganic fertilizer (Ndfif) in plant, soil at harvest, and leachate under MFW treatments in SL and CL
910 soils across two growing seasons.

1 **Table 1** Soil properties of SL and CL soils used in the experiment.

Properties	Shugakuin soil – SL		Ishiwari soil - CL	
	2021	2022	2021	2022
Soil texture	Sandy loam (10% clay, 71% sand)		Clayey loam (30% clay, 13% sand)	
pH (H ₂ O)	5.8	5.8	6.8	6.4
pH (KCl)	4.3	4.5	5.5	5.6
CEC (cmol _c kg ⁻¹)	9.0	9.5	22.8	22.0
SOM (%)	5.7	6.8	7.5	7.2
NH ₄ -N (mg N kg ⁻¹)	7.2	14.6	5.6	6.3
NO ₃ -N (mg N kg ⁻¹)	4.7	7.9	0.7	25.8
Total C (g C kg ⁻¹)	17	27	17	19
Total N (g N kg ⁻¹)	1.6	2.3	2.0	2.0
C: N ratio	10.4	11.9	8.3	9.6
Bulk density (g cm ⁻³)		1.1		1.0

3 **Table 2** Characteristics of methane fermentation waste fluid applied during two growing
4 seasons

Parameters	2021	2022
Dry mass % (w/w)	4.6	3.0
pH	8.8	7.9
Volatile solids (g kg ⁻¹ FW)	11	15
NH ₄ -N (mg kg ⁻¹ FW)	2083	2519
NO ₃ -N (mg kg ⁻¹ FW)	0.43	1.3
Total N (mg kg ⁻¹ FW)	3029	3153
Organic N (mg kg ⁻¹ FW)	941	632
Total C (g kg ⁻¹ FW)	19	12
Total P (g kg ⁻¹ FW)	0.2	0.4
Total K (g kg ⁻¹ FW)	2.5	n.a
C/N	6.0	3.8

5 (n.a: not analyzed)

6 **Table 3** Key agronomic characteristics of rice at harvest under MFW treatments in SL and CL
7 soils across two growing seasons

Soil	Fertilizer	Height		Panicle number		Total biomass		Yield	
		(cm)				(mg pot ⁻¹)		(mg pot ⁻¹)	
		2021	2022	2021	2022	2021	2022	2021	2022
SL	0N	97.0	102.0	9.8 ^c	16.0	43.7 ^c	75.6 ^c	4.7 ^b	19.1 ^b
		(2.3)	(3.0)	(0.5)	(0.6)	(0.9)	(2.5)	(0.5)	(0.8)
	0.5N-MFW	95.4	104.0	11.0 ^{bc}	16.8	50.5 ^b	78.0 ^c	5.1 ^b	20.0 ^b
		(2.1)	(1.0)	(0.4)	(1.3)	(1.0)	(2.1)	(0.8)	(0.3)
	1N-MFW	95.4	106.0	11.3 ^{bc}	17.0	53.8 ^b	87.2 ^b	5.3 ^b	21.9 ^{ab}
		(1.2)	(1.0)	(0.3)	(1.1)	(1.6)	(1.1)	(0.6)	(0.9)
	2N-MFW	97.5	109.0	12.8 ^a	19.5	67.8 ^a	96.5 ^a	10.0 ^a	24.4 ^a
		(0.7)	(2.0)	(0.8)	(0.3)	(1.7)	(0.9)	(1.4)	(0.6)
	Mean	96.3	105.3	11.2	17.4	54.0	84.9	6.3	21.5
		(3.4)	(3.9)	(0.4)	(0.6)	(2.4)	(2.3)	(0.7)	(0.6)
CL	0N	103.0	111.0	11.3 ^c	15.5 ^c	64.9 ^c	82.6 ^d	7.7 ^b	20.2 ^c
		(1.0)	(3.0)	(0.5)	(0.5)	(1.3)	(2.6)	(0.6)	(1.1)
	0.5N-MFW	103.0	108.0	13.3 ^{bc}	16.3 ^{bc}	74.2 ^b	91.1 ^c	7.3 ^b	23.0 ^{bc}
		(2.0)	(2.0)	(0.5)	(0.5)	(0.7)	(0.7)	(0.5)	(1.1)
	1N-MFW	105.0	111.0	15.0 ^{ab}	18.8 ^b	80.2 ^b	98.8 ^b	10.5 ^a	25.2 ^{ab}
		(1.0)	(1.0)	(0.4)	(0.6)	(1.1)	(0.7)	(0.5)	(0.5)
	2N-MFW	103.0	116.0	16.0 ^a	22.7 ^a	90.6 ^a	114.6 ^a	10.6 ^a	28.9 ^a
		(2.0)	(2.0)	(0.6)	(0.7)	(2.3)	(1.0)	(0.7)	(1.1)
	Mean	103.5	111.5	13.9	18.0	77.5	95.6	9.0	24.0
		(3.2)	(4.2)	(0.5)	(0.7)	(2.5)	(3.1)	(0.5)	(0.9)
Two-way ANOVA									
Soil		***	***	***	ns	***	***	***	***
MFW levels		ns	*	***	***	***	***	***	***
Soil x MFW levels		ns	ns	ns	ns	ns	*	ns	ns

8 The mean and standard error (SE) have been computed using four replicates. The letters
9 describe the significant difference between treatments in SL or CL computed via one-way
10 analysis of variance (ANOVA). The asterisk represents significant differences: *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$; ns: not significant.

12 **Table 4** Total nitrogen content (TN), total dissolved nitrogen (TDN), and nitrogen derived from inorganic fertilizer (Ndfif) in plant, soil at harvest, and leachate under
13 MFW treatments in SL and CL soils across two growing seasons

Soil	Fertilizer	Plant				Soil				Leachate			
		2021		2022		2021		2022		2021		2022	
		TN (mg pot ⁻¹)	Ndfif (mg pot ⁻¹)	TN (mg pot ⁻¹)	Ndfif (mg pot ⁻¹)	TN (g kg ⁻¹)	Ndfif (mg pot ⁻¹)	TN (g kg ⁻¹)	Ndfif (mg pot ⁻¹)	TDN (mg pot ⁻¹)	Ndfif (mg pot ⁻¹)	TDN (mg pot ⁻¹)	Ndfif (mg pot ⁻¹)
SL	0N	292 ^c (5)	- (-)	529 ^c (31)	- (-)	1.35 (0.03)	- (-)	1.92 ^b (0.02)	- (-)	93 ^d (5)	- (-)	199 ^d (11)	- (-)
	0.5N-MFW	340 ^b (5)	23.9 ^c (0.4)	585 ^{bc} (15)	40.0 ^c (1.3)	1.28 (0.03)	8.1 ^c (0.6)	2.09 ^a (0.05)	11.8 ^c (0.4)	122 ^c (1)	11.6 ^c (0.4)	241 ^c (16)	12.7 ^c (0.6)
	1N-MFW	358 ^b (4)	42.9 ^b (2.6)	612 ^b (8)	76.7 ^b (2.2)	1.35 (0.05)	14.1 ^b (0.6)	2.01 ^{ab} (0.03)	20.7 ^b (0.8)	177 ^b (4)	40.0 ^b (3.0)	271 ^b (8)	26.1 ^b (1.8)
	2N-MFW	455 ^a (12)	90.2 ^a (2.1)	686 ^a (11)	143.9 ^a (3.8)	1.30 (0.04)	24.9 ^a (1.2)	2.05 ^{ab} (0.03)	39.2 ^a (1.2)	239 ^a (2)	78.0 ^a (2.8)	413 ^a (16)	73.6 ^a (1.7)
	<i>Mean</i>	361 (16)	52.3 (8.5)	603 (37)	87.1 (13.1)	1.32 (0.02)	15.7 (2.1)	2.02 (0.02)	23.5 (3.4)	158 (15)	43.2 (4.1)	281 (26)	37.7 (8.0)
CL	0N	419 ^b (9)	- (-)	580 ^b (15)	- (-)	1.65 ^b (0.03)	- (-)	2.02 ^b (0.02)	- (-)	85 ^d (3)	- (-)	137 ^d (3)	- (-)
	0.5N-MFW	443 ^b (6)	25.0 ^c (0.9)	620 ^b (4)	41.0 ^c (0.7)	1.80 ^a (0.04)	13.1 ^c (0.5)	1.89 ^c (0.01)	21.5 ^c (1.0)	114 ^c (5)	9.7 ^c (0.7)	156 ^c (5)	5.4 ^c (0.2)
	1N-MFW	454 ^b (5)	44.7 ^b (1.1)	699 ^{ab} (10)	85.4 ^b (0.6)	1.73 ^{ab} (0.03)	20.8 ^b (0.4)	1.98 ^b (0.01)	37.5 ^b (0.5)	139 ^b (6)	26.9 ^b (2.6)	170 ^b (7)	12.0 ^b (0.9)
	2N-MFW	556 ^a (24)	94.1 ^a (6.9)	840 ^a (27)	168.5 ^a (3.3)	1.70 ^{ab} (0.04)	35.2 ^a (1.7)	2.12 ^a (0.01)	66.9 ^a (1.4)	181 ^a (7)	49.6 ^a (2.2)	243 ^a (9)	37.9 ^a (3.4)
	<i>Mean</i>	438 (22)	54.6 (9.0)	685 (32)	92.1 (16.1)	1.72 (0.02)	23.0 (2.8)	1.99 (0.02)	39.0 (5.6)	130 (11)	28.8 (5.0)	177 (13)	16.7 (4.3)
Two-way ANOVA													
Soil		***	ns	***	***	***	***	ns	***	***	***	***	***
MFW levels		***	***	**	***	ns	***	**	***	***	***	***	***
Soil x MFW levels		ns	ns	**	***	*	*	***	***	***	***	***	***

14 The mean and standard error (SE) have been computed using four replicates (n=4) except for SL-0N and CL-2N-MFW treatments in the 2022 year (n=3). The letters
15 describe the significant difference between treatments in SL or CL computed via one-way analysis of variance (ANOVA). The asterisk represents the significant
16 differences: *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$; ns: not significant.

Table 5 Key agronomic characteristics of rice at harvest under chemical fertilizer and MFW treatments in SL and CL soils across two growing seasons

Soil	Fertilizer	Height (cm)		Panicle number		Total biomass (g pot ⁻¹)		Yield (g pot ⁻¹)	
		2021	2022	2021	2022	2021	2022	2021	2022
SL	1N-Chem	96	107	11.8	19.0	59.7	90.6	6.5	22.9
		(1)	(2)	(0.5)	(0.5)	(1.2)	(1.9)	(1.3)	(1.4)
	1N-MFW	95	106	11.3	17.0	53.8	87.2	5.3	21.9
		(1)	(2)	(0.3)	(1.1)	(1.6)	(1.1)	(1.1)	(1.7)
<i>Mean</i>		96	106	11.5	18.0	56.8	88.9	5.9	22.4
		(2)	(1)	(0.3)	(0.5)	(1.0)	(1.3)	(0.5)	(0.5)
CL	1N-Chem	109	112	15.5	19.8	85.5	103.1	9.9	27.1
		(2)	(2)	(0.5)	(0.6)	(1.8)	(2.9)	(1.6)	(1.1)
	1N-MFW	105	111	15.0	18.8	80.2	98.8	10.5	25.2
		(1)	(1)	(0.4)	(0.6)	(1.1)	(0.7)	(1.0)	(0.9)
<i>Mean</i>		107	111	15.3	19.3	82.8	100.9	10.2	26.2
		(4)	(1)	(0.3)	(0.5)	(1.0)	(1.3)	(0.5)	(0.5)
Two-way ANOVA									
Soil		***	*	***	ns	***	***	***	***
Fer. types		ns	ns	ns	ns	ns	ns	ns	*
Soil x Fer. types		ns	ns	ns	ns	ns	ns	ns	ns

The mean and standard error (SE) have been computed using four replicates. The asterisk represents the significant differences: *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$; ns: not significant.

23 **Table 6** Distribution of Ndfif in plant, soil, and leachate under MFW treatments in SL and CL

24 soils across two growing seasons.

Soil	Fertilizer	Plant		Soil		Leachate		NH ₃ gas	Unobserved	
		%								
		2021	2022	2021	2022	2021	2022	2022	2021	2022
SL	1N-Chem	37.0 ^a	50.5 ^a	9.3 ^b	12.4 ^{ab}	32.7 ^a	20.3 ^a	0.44	21.0 ^c	16.3 ^b
		(1.6)	(2.1)	(0.5)	(0.7)	(1.4)	(1.5)	(0.05)	(2.93)	(1.6)
	0.5N-MFW	34.3 ^{ab}	48.6 ^{ab}	11.6 ^a	14.4 ^a	16.6 ^b	15.4 ^b	0.51	37.5 ^a	21.2 ^a
		(0.6)	(1.6)	(0.8)	(0.5)	(0.6)	(0.8)	(0.06)	(0.9)	(0.9)
	1N-MFW	30.6 ^b	46.5 ^{ab}	10.0 ^b	12.5 ^{ab}	28.3 ^a	15.8 ^b	0.63	31.1 ^b	24.6 ^a
		(1.8)	(1.4)	(0.4)	(0.5)	(2.1)	(1.1)	(0.13)	(1.7)	(0.9)
	2N-MFW	32.2 ^{ab}	43.6 ^c	8.8 ^b	11.9 ^b	27.7 ^a	22.3 ^a	0.79	31.3 ^b	21.4 ^a
		(0.3)	(1.2)	(0.4)	(0.4)	(1.0)	(0.5)	(0.04)	(0.8)	(1.0)
CL	1N-Chem	45.0 ^a	56.1 ^a	16.8 ^a	24.0 ^{ab}	18.7 ^a	8.0 ^b	1.26	19.6 ^b	10.6 ^b
		(1.8)	(2.0)	(0.2)	(0.5)	(1.0)	(0.9)	(0.12)	(1.1)	(1.1)
	0.5N-MFW	36.0 ^b	49.7 ^b	18.7 ^a	26.1 ^a	13.9 ^b	6.5 ^b	0.53	31.4 ^a	17.2 ^a
		(1.3)	(0.8)	(0.7)	(1.2)	(0.9)	(0.2)	(0.12)	(1.3)	(1.2)
	1N-MFW	31.9 ^b	51.8 ^{ab}	14.7 ^b	22.7 ^{bc}	19.1 ^a	7.3 ^b	1.02	34.3 ^a	17.3 ^a
		(0.8)	(0.3)	(0.3)	(0.3)	(1.9)	(0.6)	(0.10)	(1.6)	(0.8)
	2N-MFW	33.6 ^b	51.1 ^{ab}	12.5 ^c	20.3 ^c	17.6 ^{ab}	11.5 ^a	1.61	36.3 ^a	15.6 ^a
		(2.5)	(1.0)	(0.6)	(0.4)	(0.8)	(1.0)	(0.51)	(1.8)	(0.7)
Two-way ANOVA										
Soil		*	**	***	***	***	***	***	ns	**
Fer. Types (1N-Chem vs 1N-MFW)		***	*	ns	ns	ns	*	ns	***	***
Soil x Fer. types		ns	ns	ns	ns	ns	ns	ns	ns	ns
Soil		ns	***	***	***	***	***	**	ns	***
MFW levels		*	ns	***	***	***	***	*	ns	ns
Soil x MFW levels		ns	*	ns	ns	ns	ns	ns	**	**

25 The mean and standard error (SE) have been computed using four replicates ($n=4$) except for
26 SL-0N and CL-2N-MFW treatments in 2022 ($n=3$). The letters describe the significant
27 difference between treatments in SL or CL computed via one-way analysis of variance
28 (ANOVA). The asterisk represents significant differences: *: $P < 0.05$; **: $P < 0.01$; ***: $P <$
29 0.001 ; ns: not significant.

30 **Table S1** Fate of nitrogen from fertilizers on paddy rice from the previous studies

N source	Soil type	Scale	Application method	Plant uptake	Remained in soil	N loss				Calculation method	Author
						<i>N leaching</i>	<i>NH₃</i>	<i>N_xO</i>	<i>Unknown</i>		
Inorganic fertilizers	Unknown	Field/pot	-	7.0-68.0	-		5.0-96.0			Based on Inorganic ¹⁵ N (#): % of N applied	Crasel and Vlek. 1983
Urea	Albic soil	1 m ²	Split	30.3-43.5	13.6-15.7	6.5-10.1	32.7-49.6	-	-		Yu et al. 2019
Urea	Anthrosol	Lysimeter	Split	27.4-29.6	22.0 (#)	6.14-11.7 (#)	6.7-11.8 (#)	0.11-0.12	-		Zhao et al., 2009
NH ₄ Cl	Eutric Fluvisol	Pot	Split	70.3	60.2	-	-	-	23.5	N balance	Ghoneim et al. 2008
MFW				50.5	13.2	-	-	-	36.3		
Urea	Clay loam	1 m ²	Split	36.6	-	2.3	7.5	0.2	53.0		Chen et al. 2012
MFW				41.4	-	1.9	16.4	0.3	39.4		
Cattle manure compost	Unknown	Field	Basal	5.6 - 7.1	59.4-61.9		32.5-33.5			Based on organic ¹⁵ N	Nishida et al. 2004
Poultry manure compost				29.2	52.3		18.5				

Rice straw compost				8.9-11.8	68.4-69.4		19.8-21.7				
Cattle manure compost	Typic Fluvaquents	Pot	Basal	2.8 - 3.0	73.0 - 78.0		20.0 - 25.0				Nishida et al. 2008
Swine manure amended with Urea (15+85%)	Clay loam	Field	Basal with manure and split top dressing for Urea	34.2-39.4	10.9-13.5	8.2-8.5	34.6- 40.1	-	-		
swine manure amended with Urea (30+70%)	Clay loam			37.4-43.2	9.1-11.1	8.9-9.2	32.3- 37.5	-	-	% of N input	Xinqiang et al. 2014
swine manure amended with Urea (45+65%)	Clay loam			38.5-44.5	6.2-9.0	9.0-9.5	32.3- 39.2	-	-		
NH ₄ Cl	Sandy loam	Pot	Basal	37.0-50.5	9.3-12.4	20.3-32.7	0.44	-	16.3-21.0	Based on Inorganic ¹⁵ N	This study
	Clay loam		Basal	45.0-56.1	16.8-24.0	8.0-18.7	1.3	-	10.6-19.6		
MFW	Sandy loam		Basal	30.6-48.6	8.8-14.4	15.4-28.3	0.5-0.8	-	25-35		
	Clay loam		Basal	31.9-51.8	12.5-26.1	6.5-19.1	0.5-1.6	-	20-29		

Table 5 Key agronomic characteristics of rice at harvest under chemical fertilizer and MFW treatments in SL and CL soils across two growing seasons.

Table 6 Distribution of Ndfif in plant, soil, and leachate under MFW treatments in SL and CL soils across two growing seasons.

Supplementary legends

Figure S1. Floodwater pH (a, b) and temperature (c, d) under different treatments in 2022 for SL (a, c) and CL (b, d) soils. Error bars represent standard error ($n = 4$), except for SL-0N and CL-2N-MFW treatments in 2022 ($n = 3$). All measurements were conducted between 10:00 and 16:00 on gas collection days. DAT: Days after transplanting.

Figure S2. Shoot-to-root ratio of rice at harvest in SL and CL soils during 2021 and 2022. Error bars represent standard error ($n = 4$), except for SL-0N and CL-2N-MFW treatments in 2022 ($n = 3$). P-values indicate significant differences between year (2021 vs. 2022) and soil textures (SL vs. CL).

Figure S3. Inorganic N ($\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$) content in daily leachate for SL and CL soils in 2021 (a–d) and 2022 (e–h). Error bars represent standard error ($n = 4$), except for SL-0N and CL-2N-MFW treatments in 2022 ($n = 3$). DAT: Days after transplanting.

Table S1. Summary of N fate from fertilizers in previous studies on paddy rice.

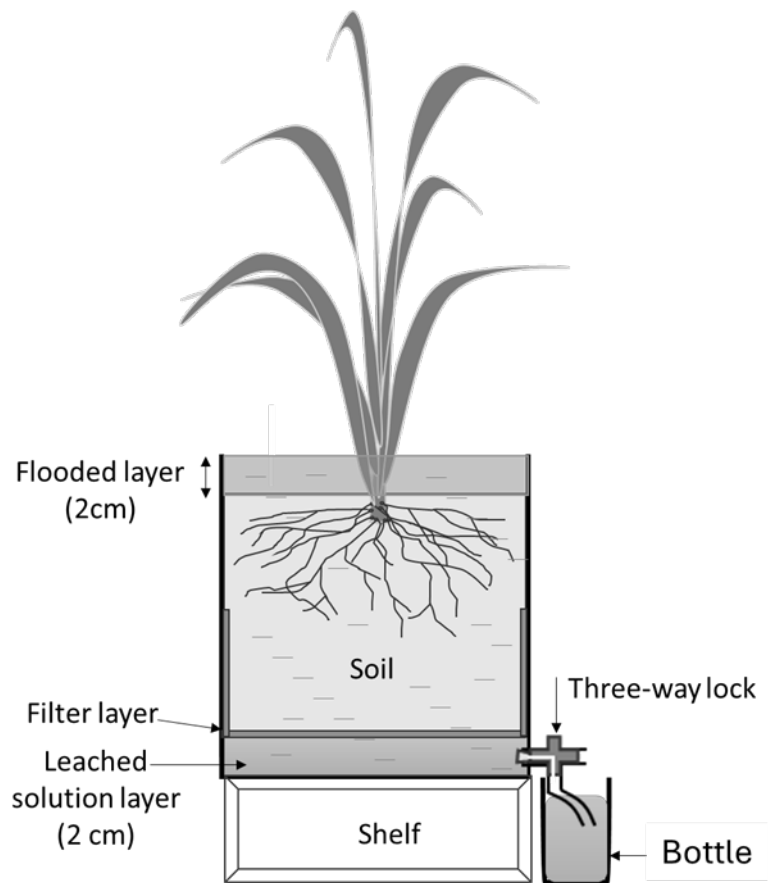


Figure 1. The design of the experimental pot for collecting daily leachate solution

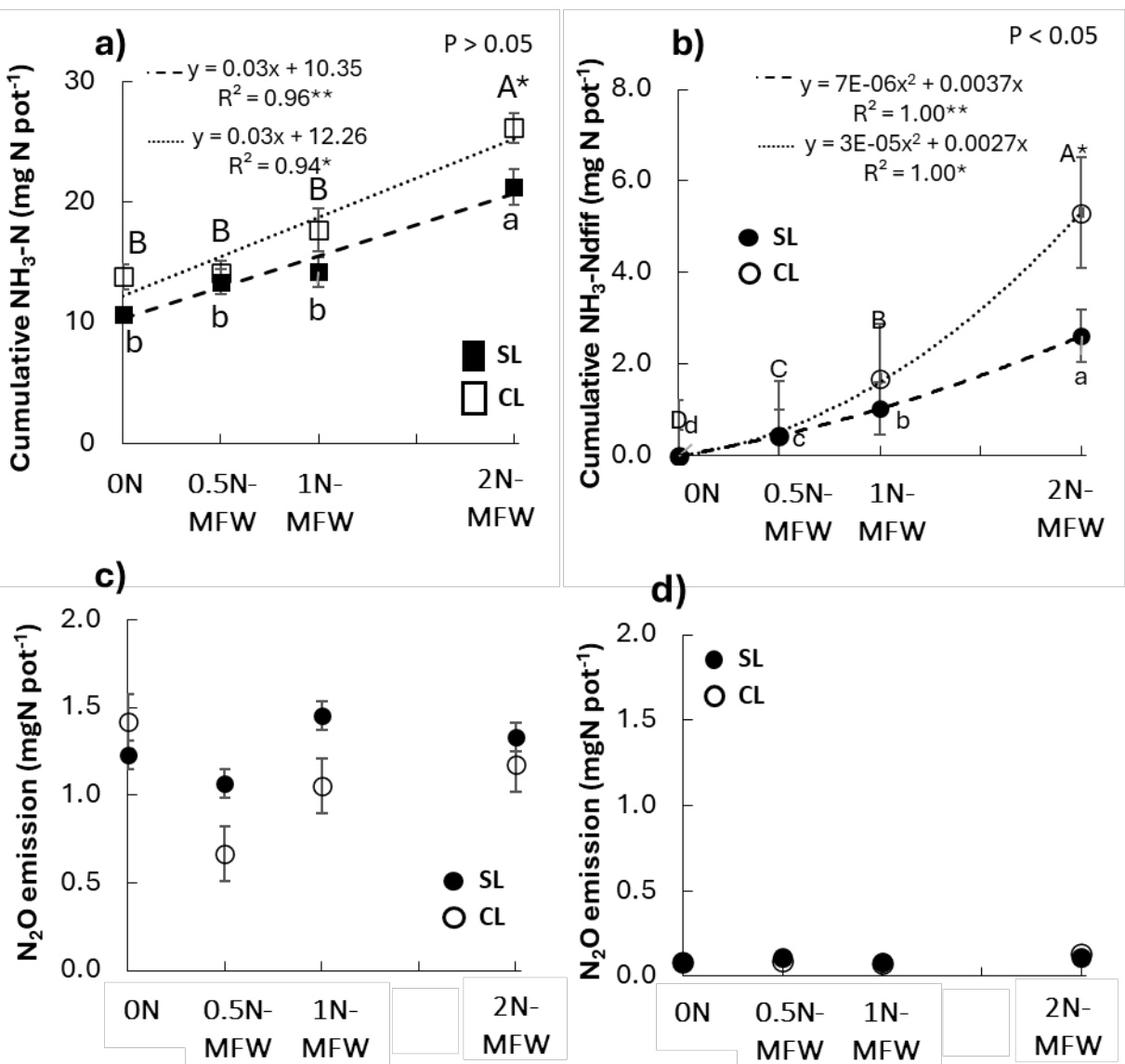
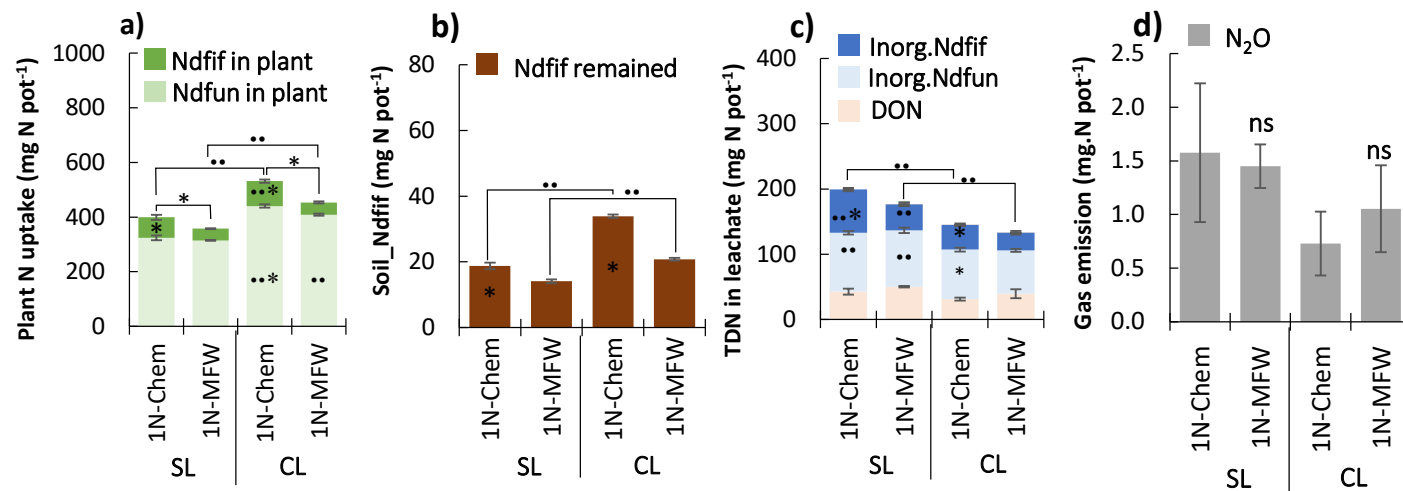


Figure 2. Comparison of different methane fermentation waste fluid (MFW) treatments in contrasting soil textures (sandy loam, SL; clayey loam, CL) for (a) NH_3 volatilization in 2022, (b) $\text{NH}_3\text{-N}$ derived from inorganic N of MFW ($\text{NH}_3\text{-Ndfif}$) in 2022, (c) N_2O emission in 2021, and (d) N_2O emission in 2022. 0N: no fertilizer; 0.5N-MFW: 100 mg N as MFW; 1N-MFW: 200 mg N as MFW; 2N-MFW: 400 mg N as MFW. Error bars indicate standard error ($n = 4$), except for SL-0N and CL-2N-MFW treatments in 2022 ($n = 3$). Small and capital letters indicate the significant differences among treatments in SL and CL soils, respectively, as determined by one-way ANOVA ($P < 0.05$). Asterisks indicate the significant difference between two soil textures within the same treatment by Tukey's test ($*P < 0.05$, $**P < 0.01$, and $***P < 0.001$). Significant differences between regression lines for soil textures were computed using the F-test ($P < 0.05$).

2021



2022

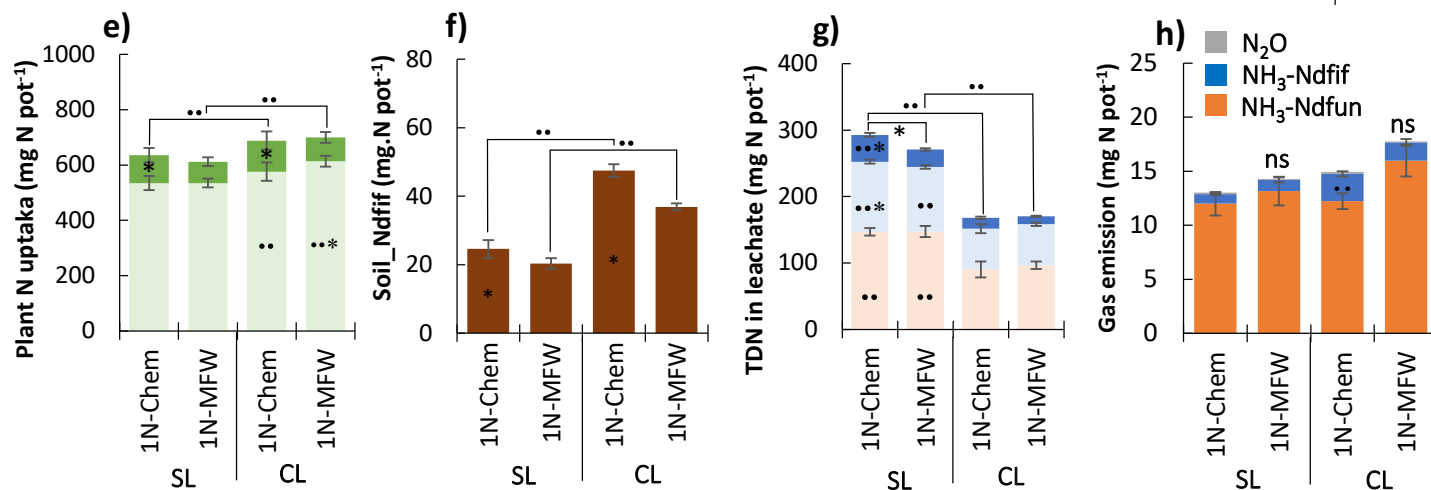


Figure 3. Comparison of nitrogen (N) content in plant N uptake (a, e), soil N derived from inorganic fertilizer (b, f), total dissolved N (TDN) in leachate (c, g), and gas emissions (d, h) between 1N-Chem and 1N-MFW under contrasting soil textures (SL and CL) in 2021 (a–d) and 2022 (e–h). Ndfif: Nitrogen derived from inorganic fertilizer. Ndfun: Nitrogen derived from unlabeled sources. Error bars represent standard error (n = 4). Double dots represent significant differences between two soil textures within the same treatment for each observed indicator (P < 0.05). Asterisks indicate significant differences among treatments within each soil texture (P < 0.05). ns: not significant.

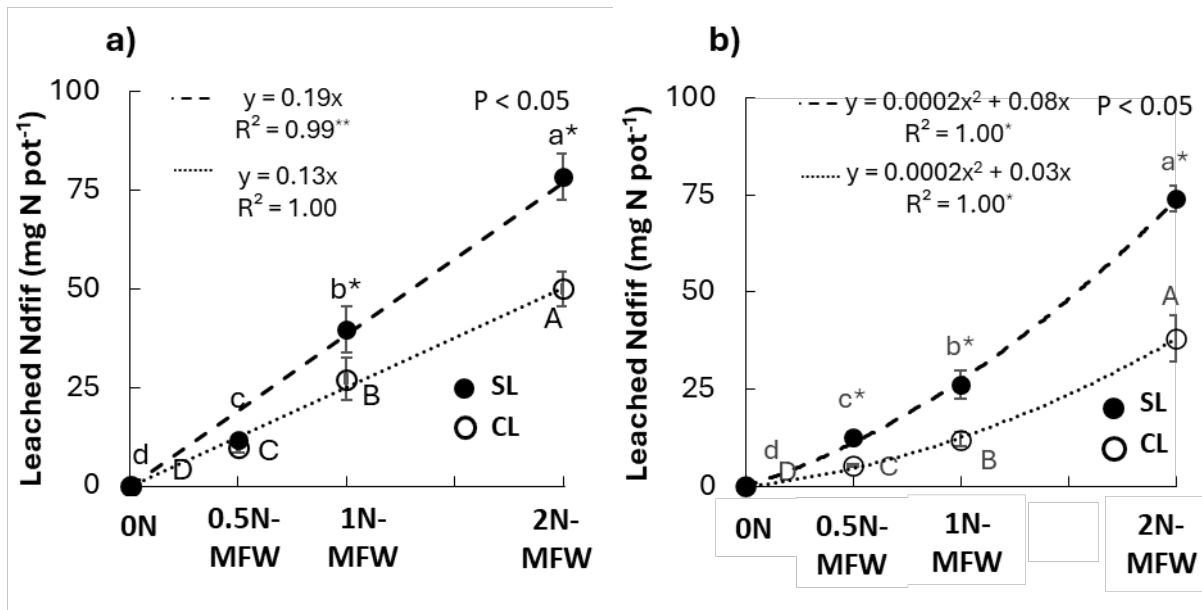


Figure 4. Ndfif in leachate under different MFW treatments in 2021 (a) and 2022 (b) for SL and CL soils. 0N: no fertilizer; 0.5N-MFW: 100 mg N as MFW; 1N-MFW: 200 mg N as MFW; 2N-MFW: 400 mg N as MFW. Error bars represent standard error (n = 4), except for SL-0N and CL-2N-MFW treatments in 2022 (n = 3). Small and capital letters indicate the significant differences among treatments in SL and CL soils, respectively, as determined by one-way ANOVA (P < 0.05). Asterisks indicate the confidence of trendlines: *P < 0.05 and **P < 0.01. P-values indicate the significant differences between regression lines for SL and CL soils.

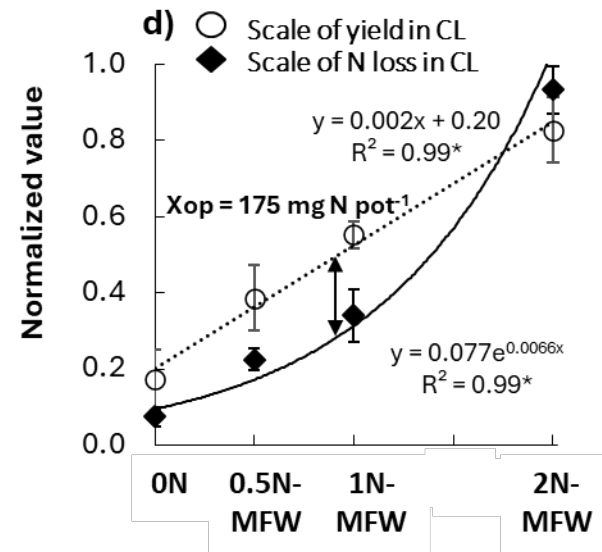
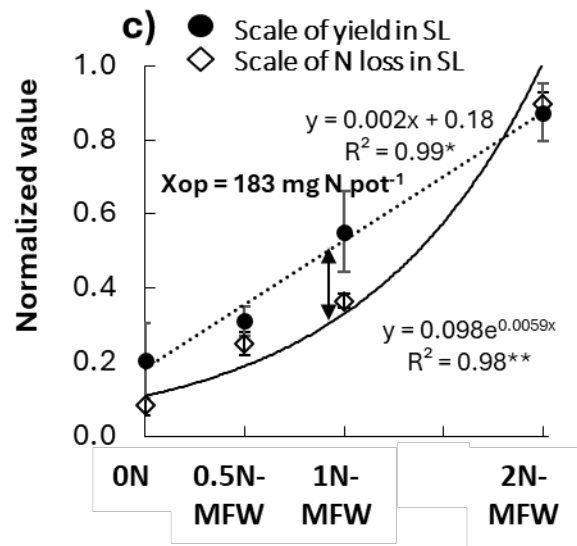
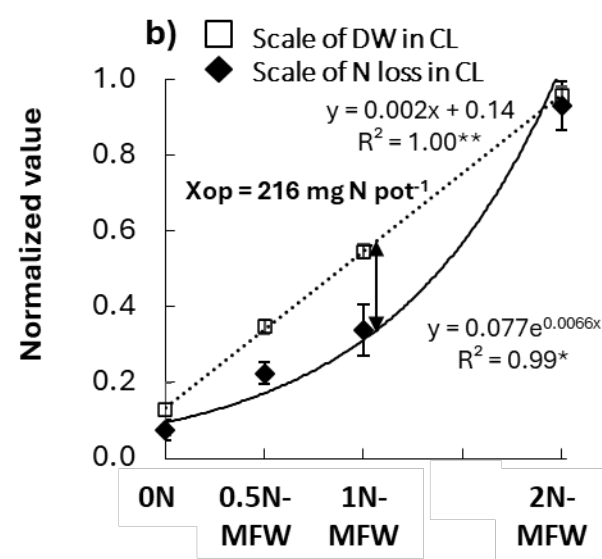
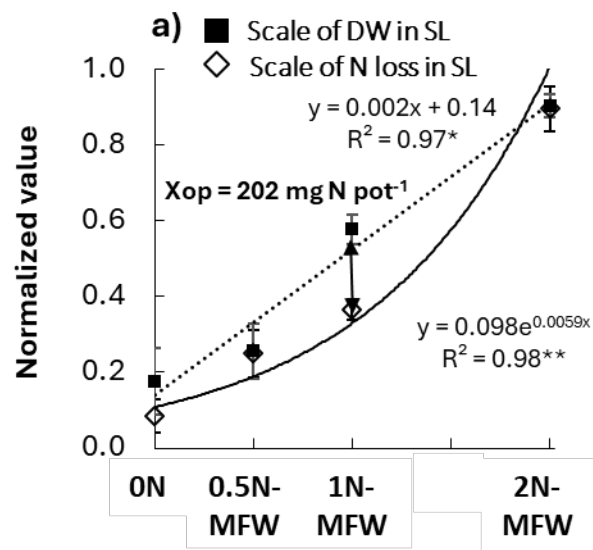


Figure 5. Relationship between normalized rice biomass (a, b), yield (c, d), and total N loss under different MFW application rates in SL (a, c) and CL (b, d) soils in 2022. 0N: no fertilizer; 0.5N-MFW: 100 mg N as MFW; 1N-MFW: 200 mg N as MFW; 2N-MFW: 400 mg N as MFW. Dotted lines indicate linear fits for rice biomass and yield; solid lines indicate exponential fits for total N loss. Normalized values were calculated using the min–max normalization formula: $x' = (x_i - \min [x]) / (\max [x] - \min [x])$, where x_i represents each observed value ($n = 4$, unless otherwise noted), $\min [x]$ and $\max [x]$ are the minimum and the maximum values within the group, respectively. Since values shown are treatment means, normalized values may not exactly be 0 or 1 even at 0N or 2N-MFW treatments. Xop-values indicate the MFW application rates at which the difference between the normalized rice productivity (biomass or yield) and total N loss is maximized. These values indicate the optimal balance between agronomic performance and environmental impact for each soil texture. Error bars represent the standard error ($n = 4$), except for SL-0N and CL-2N-MFW treatments in 2022 ($n = 3$). Asterisks indicate the confidence of the trendline: $^*P < 0.05$ and $^{**}P < 0.01$.

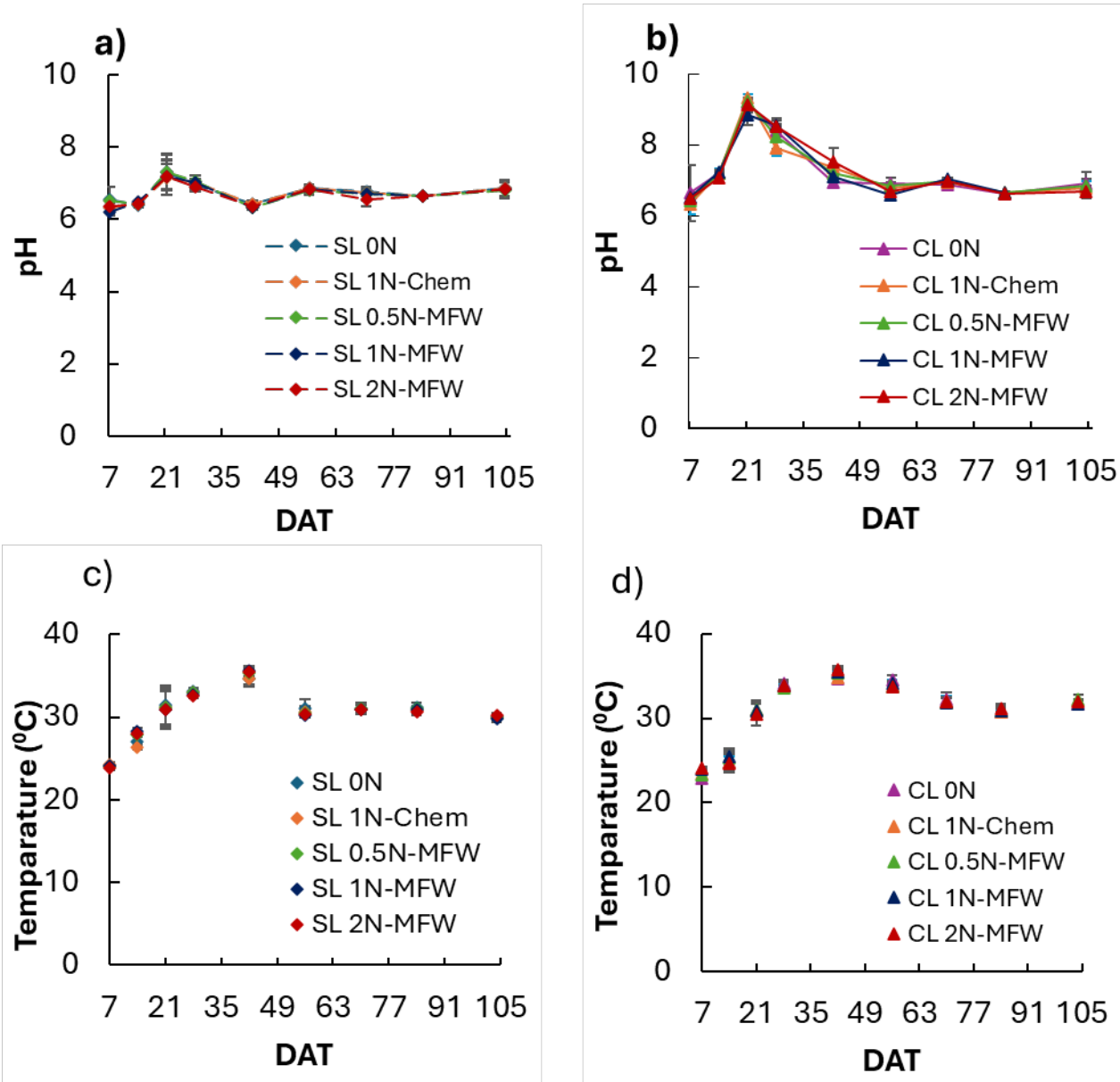


Figure S1. Floodwater pH (a, b) and temperature (c, d) under different treatments in 2022 for SL (a, c) and CL (b, d) soils. Error bars represent standard error (n = 4), except for SL-0N and CL-2N-MFW treatments in 2022 (n = 3). All measurements were conducted between 10:00 and 16:00 on gas collection days.

DAT: Days after transplanting.

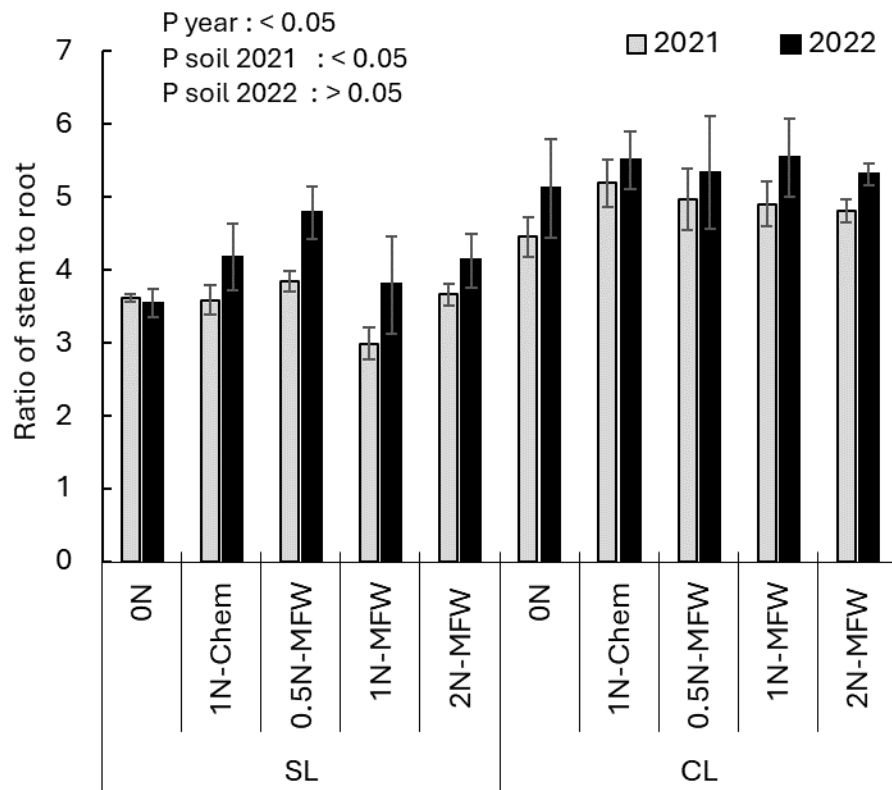


Figure S2. Shoot-to-root ratio of rice at harvest in SL and CL soils during 2021 and 2022. Error bars represent standard error (n = 4), except for SL-0N and CL-2N-MFW treatments in 2022 (n = 3). P-values indicate significant differences between year (2021 vs. 2022) and soil textures (SL vs. CL).

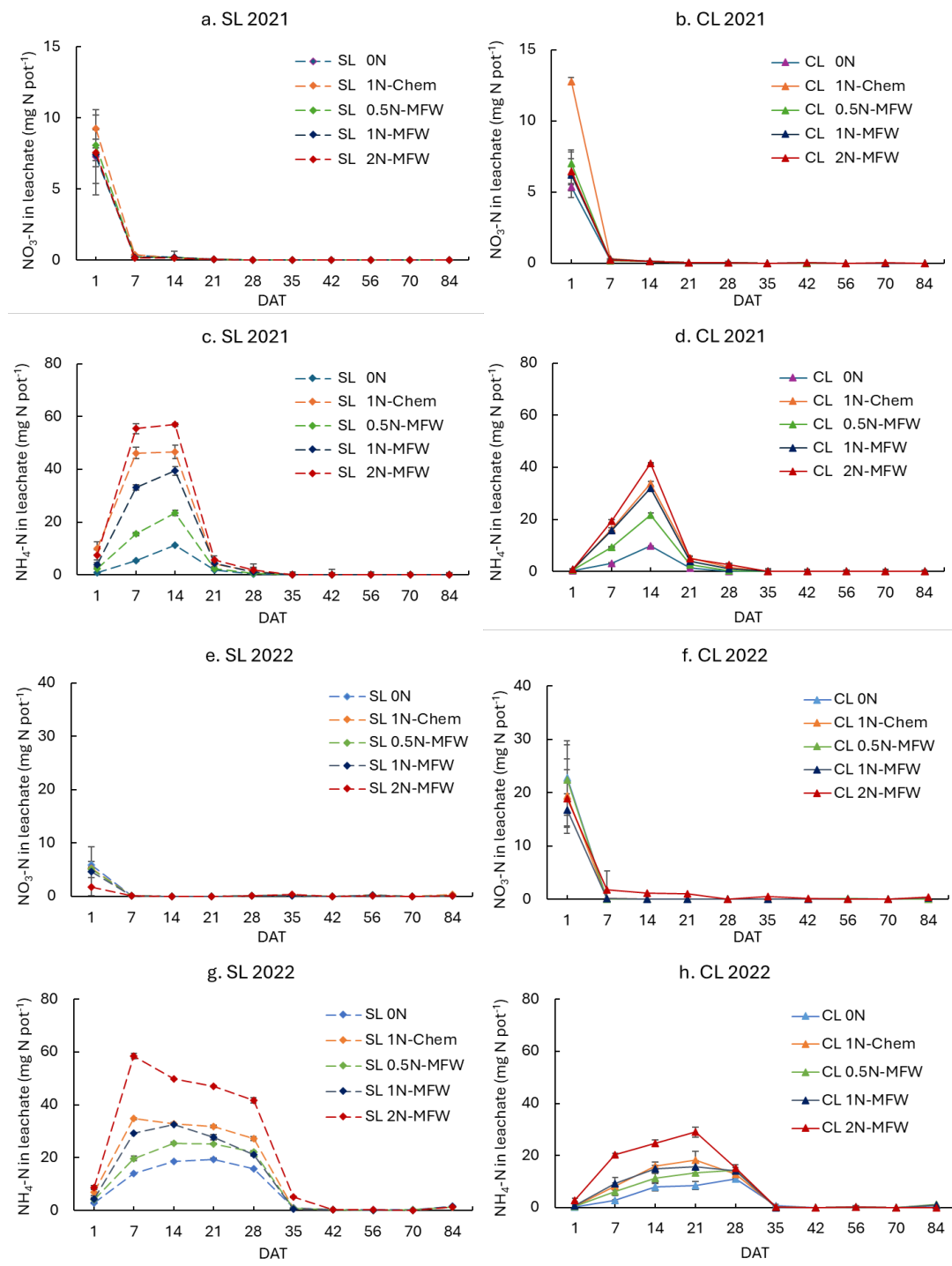


Figure S3. Inorganic N ($\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$) content in daily leachate for SL and CL soils in 2021 (a–d) and 2022 (e–h). Error bars represent standard error ($n = 4$), except for SL-0N and CL-2N-MFW treatments in 2022 ($n = 3$). DAT: Days after transplanting.