

**IMPACT OF METHANE FERMENTATION WASTE FLUID UTILIZATION
ON CROPS AND THE FATE OF NITROGEN UNDER DIFFERENT
SOIL CONDITIONS**

Doctoral Dissertation

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ABSTRACT

Methane fermentation waste fluid (MFW) is increasingly used as a fertilizer in agricultural 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 under different cultivation conditions. This study aimed to elucidate the effects of MFW application rates on crop growth and inorganic N pathways in soils with contrasting textures to maximize crop productivity while minimizing environmental N loss during submerged and upland cultivation.

Two-year pot experiments were conducted using sandy loam (SL) and clay loam (CL) soils with rice and maize, which were grown under submerged and upland conditions, respectively. Five treatments were applied with four replicates: a chemical fertilizer (1N-CHEM) (NH_4Cl , 200 mg N pot^{-1} – 100 kg N ha^{-1} equivalent for rice and 750 mg N pot^{-1} – 150 kg N ha^{-1} equivalent for maize), three MFW rates of total N were calculated as a half (0.5N-MFW), equal (1N-MFW), and double (2N-MFW) amount compared to chemical fertilizer, and no-fertilizer as 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.

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 the 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 90 kg N-MFW ha^{-1} equivalent for both soils, beyond which further yield increases were accompanied by substantial N leaching and emissions.

By contrast, maize biomass and yield were significantly enhanced in SL soil than in CL soil. Increasing MFW rates improved maize yield in 2021 but showed reduced effectiveness under limited irrigation in 2022. Despite lower Ndfif retention in SL soil, total Ndfif recovery (soil and plant) was significantly

higher compared to CL soil, which was strongly influenced by MFW rates and water availability. Ndfif losses were more pronounced in CL soil due to NH_3 volatilization and $\text{NO}_3\text{-N}$ leaching, especially at higher MFW rates. Although N recovery from MFW was lower than that of chemical fertilizer at equivalent N levels, MFW provided sufficient N for maize yield. Considering yield and environmental impact, the optimum MFW application rate was estimated at $150 \text{ kg N pot}^{-1}$ equivalent for SL soil and $133 \text{ kg N pot}^{-1}$ equivalent for CL soil under controlled water management.

These findings highlighted that MFW is highly effective in clay soil under submerged conditions for rice cultivation, while it was more efficient in sandy soils under upland conditions for maize cultivation. The application of MFW requires careful management to mitigate N losses through volatilization and leaching, especially under high water regimes in upland conditions. By tailoring MFW rates to soil texture and water regimes, it is possible to enhance crop productivity and reduce environmental impacts via N loss, supporting sustainable nutrient management strategies in agricultural systems. These findings emphasize the importance of soil texture in determining optimal nutrient management for sustainable crop production.

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CHAPTER I

INTRODUCTION

1.1. Introduction

Methane fermentation (or anaerobic digestion) technology has contributed to mitigating greenhouse gas emissions, providing green renewable energy, and benefiting the economy, environment, and climate (Scarlat et al. 2018). Instead of discharge to the environment, most of the wastes from human activities, such as livestock waste, agricultural waste, wastewater, food waste, etc., can be utilized as feedstocks of the methane fermentation system (Frac and Ziemiński 2012). The technology can be used for single digestion with mono feedstock or co-digestion with two or more substrates put into the system (Möller and Müller 2012; World Biogas Association 2019; Jimenez et al. 2020). Methane was produced by the systems that occupied approximately 60% of the total gases (Möller and Müller 2012), which had been utilized for cooking at the house and producing electricity, and were improved as biofuel for vehicles by industrial plants (World Biogas Association 2019).

The waste of an anaerobic digestion process is called anaerobically digested or methane fermentation waste, which can be separated into solid organic waste and liquid organic waste (called methane fermentation waste fluid - MFW) (Logan and Visvanathan 2019). Depending on various feedstocks and fermentation methods, MFWs contain high pH, high $\text{NH}_4\text{-N}$, low organic matter content, and a C/N ratio higher than the original feedstock or manure (Tampio et al. 2016; Iocoli et al. 2019; Jimenez et al. 2020) (Table 1.1). MFW has a pH of 7.5 – 8.8, with 1% to 59% solid biomass. The $\text{NH}_4\text{-N}$ content occupies up to 90% of total N, but $\text{NO}_3\text{-N}$ is negligible. The $\text{NH}_4\text{-N}$ content in waste was high in the co-digestion system combining manure and food waste or residue (47 – 77%).

However, it has widely changed with single manure digestion or co-digestion of household waste, wastewater, or multiple solid waste (Jimenez et al. 2020) (Table 1.1). The C/N ratio of MFW, summarized from previous publications (Table 1.1), ranges from 0.5 to 25, which was slightly lower than the original manure input (Abubaker et al. 2013; Grave et al. 2018; Iocoli et al. 2019). In addition, many researchers indicated that the MFW contained fewer harmful fungi or bacteria, weed seeds, or heavy metals than manure (Voća et al. 2005; Möller and Müller 2012; Seadi and Lukehurst 2012; Cb et al. 2018; O'Brien et al. 2020). Therefore, MFW has been recognized as a potentially sustainable alternative to conventional inorganic and other undigested organic fertilizers (Abubaker et al. 2015; Möller 2015; Baştabak and Koçar 2020).

Table 1.1 Characteristics of digested fluids from different sources and the methane fermentation (MF) system compared with several unfermented feedstocks.

Feedstocks	Materials	MF method			Characteristics												Reference
		MS or TS	^o C	HRT (days)	Water content (%)	pH	Total C (g/kg; g/L)	Total N (g/kg; g/L)	org. N g/kg; g/L	NH4 (g/kg; g/L)	NO3 (g/kg; g/L)	% NH4/ Total N	Total P (g/kg; g/L)	K2O (g/kg; g/L)	C:N ratio	COD (g/kg; g/L)	
Agricultural residues mix with food waste	Wheat straw silage; Sudan grass; Ley silage; and source- separated organic household waste	MS		20 – 100	94.1 - 95.8	7.6 - 8.7	14 - 24	2.8 - 5.3	1-2	0.65 - 3.3	0.015	22,7 - 67.2	0.18 - 0.77	1.1 - 3.7	4.8 - 6.0		Voća et al. 2005; Ghoneim et al. 2008; Abubaker et al. 2013; Jimenez et al. 2020
Animal manure	Chicken manure; Cow manure; Pig manure; Poultry litter.	MS/ TS	22 - 55	15 – 60	83 - 97	6.2 - 8.65	2.16 - 32.91	0.3 - 25.85	0.15 - 2.6	0.2 - 6.8	0 - 0.02	3.72 - 85.9	0.02 - 5.36	0.06 - 21.22	1.6 – 8.9		Voća et al. 2005; Win et al. 2010; Chen et al. 2012; Pastorelli et al. 2013; Sawada and Toyota 2015; Chen et al. 2017; Cheng et al. 2018; Grave et al. 2018; Iocolli et al. 2019; Wang et al. 2019; Cheng et al. 2020; Jimenez et al. 2020; Du et al. 2021;
Animal mix food waste	Pig manure and vegetable wastes of 70:30; 80% cattle manure and 20% food waste	MS			98	7-6 - 8.6		1.12 - 10.8		0.5 - 5.4	0.04 - 0.53	46.7 - 50	0.48 - 7.67	1-9			Li et al. 2003; Nakamura et al. 2014; Kathijotes et al. 2015
Household waste/ Food waste	Domestic food waste; Slaughterhouse and source-	MS/ TS	35 - 55	26 - 58	70.7 - 98.3	7.6 - 8.5	6.8 - 109.6	4.7 - 9	0.6	0.8 - 4.5		9.7 - 87.1	0.2 - 5	1 - 9.3	1.4 - 12.1		Abubaker et al. 2013; Tampio et al. 2016

	separated organic household wastes																
Manure mix residue	Cattle, chicken, pig, and agricultural residues; organic household waste	MS/TS	22 - 56	16 - 60	77 - 99.52	7.6 - 8.9	3.74 - 56	2.2 - 115		0.69 - 87.5		32.2 - 77.3	0.04 - 10.7		1.4 - 16.5	26.7 - 1400	Wulf et al. 2002; Watanabe et al. 2012; Sawada and Toyota 2015; Tampio et al. 2016; Iocolli et al. 2019; Wang et al. 2019; Jimenez et al. 2020
Organic fraction of municipal solid waste		MS/TS		20 - 60	47 - 94	8.3	0.1 - 10.3	0.2 - 29.8		0.1 - 34.7		0.3 - 71.1	0.046 - 18	0.01 - 16.1	0.2 - 2.3	0.8 - 1497	Tampio et al. 2016; Cb et al. 2018; Stanley et al. 2019; Jimenez et al. 2020
Wastewater	Wastewater treatment sludge; digestate of wastewater treatment sludge (1/3) and green wastes (2/3)	MS		20	41 - 78			26.4 - 40.6		0.92 - 10.8	0.58	3.5 - 26.8	16.2 - 20.4		2.2 - 4.8		Jimenez et al. 2020
Unfermented (for reference)	Cattle slurry	None			95.00	7.60	21.00	3.00	1.40	1.60		53.33	0.40	3.00	7.00		Abubaker et al 2013
	Cattle manure	None				9.40	304.00	10.09		0.52	0.03	5.11	5.30		30.14		Iocolli et al. 2019
	Composted swine slurry	None			70.90		317.00	16.60	15.10	1.20	0.30	7.23			19.10		Grave et al. 2018
	Pig slurry	None				5.10	28.82	3.13		2.24	0.05	71.54	0.46		9.20		Iocolli et al. 2019
	Poultry litter	None				9.30	343.30	13.18		1.84	0.04	13.96	19.40		26.04		Iocolli et al. 2019
	Swine slurry	None			92.60		29.00	4.40	1.70	2.70		61.36			6.59		Grave et al. 2018

Note: MS: Mesophilic; TS: Thermophilic; HRT: Hydraulic retention time.

Numerous studies have reported the positive effects of the MFW on the growth of many crops, such as wheat (Šimon et al. 2016), wheat-maize rotation (Du et al. 2019), vegetables (Kathijotes et al. 2015; Iocoli et al. 2019), grassland (Wulf et al. 2002; Tampio et al. 2016), maize (Pastorelli et al. 2013; Cb et al. 2018), and mainly in rice (Minamikawa et al. 2006; Hou et al. 2007; Ghoneim et al. 2008; Hong et al. 2010; Nishikawa et al. 2014; Hu et al. 2021). The efficiency of MFW on the growth and yield of crops could be comparable to other fertilizers. For example, the MFW application was comparable to chemical fertilizer in rice cultivation (Nishikawa et al. 2014; Tanaka et al. 2017). Du et al. (2019) reported that even though MFW reduced the yield of wheat and maize by 23-30%, it was not significantly different compared to chemical fertilizer. Herrmann et al. (2012) reported that MFW had a similar impact on maize yield as pig slurry or cattle slurry, while Riva et al. (2016) said that MFW gave the same maize yield as urea. Ronga et al. (2019) indicated that MFW can enhance the biomass compared to a standard solution in a hydroponic system. However, there were some reports indicating that crops yield from MFW application were significantly lower than chemical treatments, such as on maize (Wolf et al. 2014) or vegetables (Iocoli et al. 2019), suggesting that MFW should be applied with high amounts based on $\text{NH}_4\text{-N}$ content for the plant. In terms of the rapid expansion of biogas plants worldwide (Scarlat et al. 2018), MFW could be a cheaper fertilizer compared to chemical fertilizers (Snapp et al. 2023).

In contrast, excessive application of MFW resulted in N loss to the environment, led to increasing greenhouse gas in the air (Nakamura et al. 2014), and eutrophication in underground water (Carpenter 2005; Du et al. 2019; Malone and Newton 2020). Möller (2015) reviewed that high pH and ammonium concentration in MFW promoted high NH_3 volatilization and increased potential N leaching. In the upland condition, using MFW has been reported to have a high potential of N_2O and NH_3 emissions compared to chemical fertilizers application, such as calcium nitrate (Wolf et al. 2014); while it had less NH_3 emission than urea (Verdi et al. 2019). Meanwhile, Singla and Inubushi (2014) reported that MFW significantly reduced N_2O emissions compared to ammonium fertilizer in a paddy field. However, the previous reports did not clarify the direct contribution of N from MFW in each pattern, which could be important to manage and utilize MFW, balancing with environmental impact. Therefore, it is required to further understand the fate of N and determine the optimum MFW supply to the different soil-

plant systems that is to improve nitrogen use efficiency (NUE) in the agricultural field, as well as adapt to environmental standards and economic values (Nkoa 2014; Baştabak and Koçar 2020).

The fate of N from fertilizer application was defined, which was taken up by the plant, immobilized in the soil, and lost to the environment, including leaching to underground water, run off to the water surface, and emission to the air (Allison 1966; Jokela and Randall 1997; Quan et al. 2021). The information on the N fate of fertilizer application to crops provides a practical solution to improve fertilizer use efficiency (FUE) and minimize the loss of N to the environment (Anas et al. 2020). To identify the pathway of N from fertilizer and soil, two common direct methods have been used for tracing the distribution, which were the total N difference method and the ^{15}N isotope dilution method (Liu et al. 2016). The total N difference method involves comparing the total nitrogen content or uptake in a fertilized or N-fixing plot to that of an unfertilized or non-N-fixing control plot. Using ^{15}N labelled-organic N and ^{15}N labelled-inorganic N can directly determine the fate of inorganic N and organic N from fertilizers (Sørensen et al. 1994; Ding et al. 2019). In addition, the indirect method could be considered to identify the N contribution from organic sources in the plant-soil system (Stevenson et al. 1998). Unfortunately, there were few studies conducted to evaluate the fate of N from MFW application on agricultural land. Chen et al. (2012) compared the effects of an anaerobically digested pig slurry and urea fertilizer on rice at 270 kg N ha^{-1} . By using total N balance, the result showed that the fate of N in MFW treatment contributed by $41.3 \pm 5.1 \%$ N uptake, $16.4 \pm 3.7 \%$ NH_3 volatilization, $0.26 \pm 0.15 \%$ N_2O emission, $1.9 \pm 0.5 \%$ N leached, $0.70 \pm 0.15 \%$ runoff, and $39.4 \pm 8.4 \%$ remained in the soil or N_2 emission. Sharifi et al. (2019) designed greenhouse and incubation studies to evaluate the N dynamics from MFW of co-fermentation (manure mixed with plant residue) with ryegrass in silty clay loam soil. The results showed that N recovery increased linearly with MFW application rates, ranging between 37% and 63% of total MFW - organic N was mineralized. Ghoneim et al. (2008) were successful with a direct method using ^{15}N isotope to determine the N fate of inorganic N-MFW in a pot experiment with rice cultivation. The result indicated that N recovery by rice from MFW was only 50%, which was lower than that from chemical fertilizer (70%). However, he found that even though N loss from MFW to the environment was high (approximately 30%), N from MFW

remaining in the soil was double that from chemical fertilizer. This variability of N fractions and their underlying factors in the soil-plant system is crucial for the effective management of N and achieving sustainable development. Quan et al. (2021) and Li et al. (2023) summarized that the fate of N from fertilizer on crops strongly depends on environmental conditions such as soil properties, techniques, types of fertilizers, and cultivation methods. Unfortunately, the above results were reported with a single soil, providing limited information on the mechanism of N from MFW application.

Soil texture refers to the proportion of sand, silt, and clay-sized particles that make up the mineral fraction of the soil. It is one of the most important factors influencing N mineralization, driving N dynamics and plant growth (Aulakh et al. 1996; Cassity-Duffey et al. 2020; Anaba et al. 2020; Li et al. 2023). Clay content in soil was known to be a factor in protecting organic matter, promoting absorption and desorption of N, and water holding capacity (Hassink et al. 1993; Nieder et al. 2011; Paz-Ferreiro et al. 2018). High clay content in soil induced N leaching, NH_3 volatilization, and increased N uptake and retention, which led to enhanced plant growth (Castellano, Lewis, and Kaye 2013; Cambouris et al. 2016; Dou et al. 2016; Anaba et al. 2020). However, too high a clay content also reduced the available N in the soil, which impacts the crop yield. Luce et al. (2014) indicated that net N mineralization in the sandy loam (678, 165, and 157 g kg^{-1} of sand, silt, and clay) was more significant than in the clay soil (295, 295, and 410 g kg^{-1} of sand, silt, and clay). Soenne et al. (2020) indicated that increasing soil clay content from 30% to 78% decreased N mineralization and reduced plant yield. Meanwhile, low clay content, such as sandy soil or coarse-textured soil, caused a high potential of N leaching (Pandey et al. 2023) and NH_3 volatilization (Duan and Xiao 2000). Rigby and Smith (2013) suggested that the interaction of soil texture and digestate stability on denitrification in digestate-amended soils urgently needs investigation to ensure appropriate management practices minimize greenhouse gas emissions from land-applied biowastes.

Moreover, the crop system is widely cultivated under either submerged (anaerobic) or upland (aerobic) conditions. Those conditions significantly influenced the impact of soil textures on N dynamics through the nitrification and denitrification processes. Submerged conditions, such as those found in paddy fields and wetlands, are ideal for the denitrification process. That slows down the conversion of $\text{NH}_4\text{-N}$ to $\text{NO}_3\text{-N}$ while

increasing the potential for NH_3 volatilization (Shan et al. 2018). By contrast, upland conditions were widely known as upland field conditions, preferred for nitrification processes, which increased the potential of NO_3^- -N release and N_2O emissions (Subbarao et al. 2009). Aulakh et al. (1996) reported that nitrification was better in clay soil than in sandy soil. It was rapid in upland conditions but slow in flooded conditions, resulting in reduced NH_3 volatilization in upland conditions and increased NO_3^- leaching in flooded conditions. Because MFW was commonly known as liquid waste with high pH and NH_4 -N content, it directly influences the mechanisms of nitrification and denitrification after being applied to soil. Abubaker et al. (2015) reported that MFW had a strong negative effect on ammonia oxidation and denitrification in a short-term incubation test using clay soil. Sawada and Toyota (2015) reported that the nitrification rate increased rapidly when MFW was applied to both paddy and upland soils under aerobic conditions, which was significantly related to the increase in soil pH resulting from MFW application. Therefore, the fate of N from the submerged and upland soil could be contrasted by using MFW.

The gaps in the mechanism of plant growth and N distribution are unknown when raising the MFW application rates in different soil textures across cultivation conditions (under submerged or upland conditions). Soil texture could cause the various impacts of MFW on different crop cultivation conditions. Meanwhile, the high potential for N loss from MFW can be easily detected through emission, which may be significantly influenced by soil texture and cultivation conditions. Thus, determining the optimum rate of MFW application that balances the ensuring yield index and mitigates environmental impact is requires a thorough understanding of the fate of N.

Therefore, this study aims to further understand the effects of MFW resulting from the co-digestion of manures and food waste on N dynamics and crop productivity under different soil textures and cultivation conditions, leading to improved management of MFW application to maximize crop production, while minimizing the environmental impact.

1.2. Objectives of this study

To achieve the overall aim and to answer the gaps, three objectives are:

- Clarify the effect of soil texture and various MFW rates on the growth of rice and maize, and inorganic N fate in submerged and upland conditions, accordingly.

- Compare the efficacy of equal-N MFW vs chemical fertilizer (CHEM) on rice and maize growth and inorganic N fate in varying soil conditions.
- Propose optimal MFW rates that match soil texture and crop to maximize yield and minimize environmental impact from inorganic N loss.

To achieve the objective, I used the ^{15}N -enriched inorganic fertilizer at various MFW rates and chemical fertilizer under contrasting soil textures for rice under submerged conditions and maize under upland conditions.

1.3 Frame of the dissertation

The dissertation consists of the following chapters: Chapter II presents the methodology and materials for this study. Chapter III clarifies the impact of soil texture and MFW levels on growth and the fate of inorganic N in rice cultivation. Chapter IV focuses on evaluating the impact of soil texture and MFW levels on growth and the fate of inorganic N in an experiment with maize cultivation. Chapter V summarizes all of the results of both experiments and provides a general discussion. Chapter VI includes the final conclusion and future potential study needs.

CHAPTER II

MATERIALS PREPARATION

2.1. Soil preparation

Two paddy fields were selected from Kyoto city (Figure 2.1), located at Shugakuin farm (35.05258, 135.80209), Sakyo-ku, and Ishiwari farm (34.90286, 135.77360), Fushimi-ku, in February 2021 and April 2022. After collection, organic residues were removed from the soil before air drying in the Kyoto University greenhouse. A part of the soil was sieved by a 2mm sieve for analyzing physical and chemical characteristics (Table 2.1).



Figure 2.1: The location of sampling fields

2.1.1. Soil physical analysis

Soil texture was analyzed using the hydrometer method. Soil bulk density was determined by the cylindrical vessel method. Soil organic matter (SOM) and moisture content were measured by loss on drying in the oven at 105°C and 550°C, respectively.

$$\text{Moisture (\%)} = (W_{\text{Airdried}} - W_{105}) * 100 / W_{105}$$

$$\text{SOM (\%)} = (W_{105} - W_{550}) * 100 / W_{105}$$

2.1.2. Soil chemical analysis

a. Soil pH and EC

Two batches of air-dried soil were shaken with distilled water (1:5) for 1 hour, then pH and EC were detected by a portable pH-EC meter F74BW (HORIBA, Japan) with a pH electrode 9615S model and an EC electrode 9382-10D model. In addition, soil pH_{KCl} was

determined after extracting 5g of air-dried soil with 1 M KCl at a ratio of 1:5 (w/v) by pH meter 9615S model (HORIBA, Japan).

b. Cation Exchangeable Capacity (CEC)

The CEC was determined by the summation of exchangeable bases (Ca, Mg, Na, K). After being extracted with 1M ammonium acetate, the extracted concentration of exchangeable bases was determined by atomic absorption spectrophotometry (AAS) (Shimadzu, AA-7000, Japan). To determine cation exchange capacity (CEC), the residual soils were washed with ethanol after ammonium acetate extraction, and the retained NH_4^+ was subsequently displaced with 10% sodium chloride (NaCl). The displaced NH_4^+ was quantified using a Flow Injection Analyzer (Aqualab Co., Ltd, Japan). The CEC was calculated from the sum of the base cations.

c. Inorganic N in the soil ($\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$)

5g of air-dried soil was extracted with 25 mL of 2M KCl using a shaker at 120 rpm for 30 min. After filtering with a 5C filter paper, the content of inorganic N in the solution was determined by the colorimetry method via an FIA system (AQLA-700, Aqualab, Tokyo, Japan).

d. Total soil C and total soil N

An amount of air-dried soil was mixed well, and ball-milled at 450 rpm in 2 minutes (RETSCH PM 100, USA), then oven-dried at 105 °C for 8 hours before being analyzed by the EA IsoLink CN-Isotope Ratio MS (Thermo Fisher Scientific, Massachusetts, USA).

Soil available P and K: The available phosphorus (P) was determined by the Truog method. After extracting 1g with sulfuric acid 0.002 N (100 mL) in 30 minutes, the solution was filtered by 5C before being colored by the Molybdenum blue method and analyzed at 660 nm by UV-VIS Spectrometer 1240 model (Shimadzu, Kyoto, Japan). The available potassium (K) was determined similarly to CEC analysis. The final solution was detected by an atomic absorption spectrometer (AAS, Shimadzu, Kyoto, Japan).

Table 2.1 Soil properties of SL and CL soils used in the experiment.

Properties	Shugakuin farm		Ishiwari farm	
	2021	2022	2021	2022
Soil texture	Sandy loam (SL) (10% clay, 71% sand)		Clay loam (CL) (30% clay, 13% sand)	
pH (H₂O)	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
NH₄-N (mg N kg⁻¹)	7.2	14.6	5.6	6.3
NO₃-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

2.2. Methane fermentation fluid waste preparation

The 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).

The characteristics of MFW were analyzed per the standard wastewater method (APHA, 1989). The flowchart of MFW analysis is presented in Figure 2.1. The pH and electrical conductivity (EC) were measured directly in the sample using a portable pH-EC meter F74BW (HORIBA, Japan). The total solid in the MFW fluid was measured by the loss of weight in the oven method at 105 °C (W_{s-105}). The dried MFW samples were continuously burned at 550 °C for 4 hours (W_{s-550}) to determine the volatile solid weight based on the following formula:

$$\text{Total solid (g/kg)} = W_{s-105} * 1000 / W_{s-org.}$$

$$\text{Volatile solid (g/kg)} = (W_{s-105} - W_{s-550}) * 1000 / W_{s-org.}$$

To evaluate total C and N, a part of the original MFW was extracted with 2M KCl and centrifuged at 2000 rpm for 20 minutes before separating the solid and solution parts with a 0.45 µm filter (Akhiar 2017). The substantial part was washed with distilled water until clean, then dried in the oven at 105 °C. The oven-dried samples were mixed well and

ball-milled at 450 rpm for 2 minutes. Total C and total N in the solid part were measured by using the EA IsoLink CN-Isotope Ratio MS (Thermo Fisher Scientific, Massachusetts, USA). The liquid part was used to analyze the content of inorganic N by the colour method with the FIA system (AQLA-700, Aqualab, Tokyo, Japan), and the total dissolved organic carbon and total dissolved organic nitrogen were analyzed using a total organic carbon-total nitrogen (TOC-TN) analyzer (TOC_TNM- Shimadzu, Kyoto, Japan). The whole C and N were lump sums of the corresponding C and N contents in the solid and liquid parts.

To determine the total content of P and K, 50mg of air-dried MFW was digested using the nitric acid and sulfuric acid digestion method. The final solution was diluted in distilled water before analysis. Total P was determined by the molybdate-blue method at 880nm by spectrometry (Shimadzu, Japan), and total K was measured by an atomic absorption spectrometer (AAS, Shimadzu, Japan).

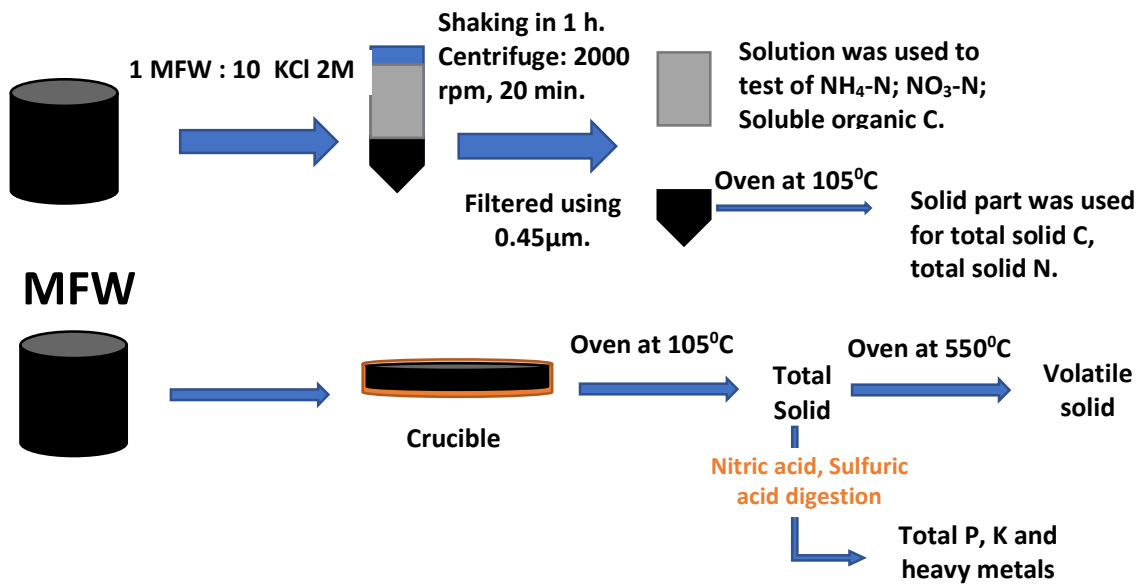


Figure 2.2. Flow chart of analyzing characteristics of methane fermentation waste (MFW)

The characteristic of MFW is shown in Table 2.2. Depending on the sampling time, the measured parameters in MFW varied slightly. According to the two sampling years, MFW had low biomass (3-4.6%), high pH (7.9-8.8), and a low C: N ratio (3.8-6). Total N in MFW was approximately 3000 mg kg⁻¹ FW, with 70-80% present as NH₄-N, and negligible amounts as NO₃-N form. The total volatile solids in MFW ranged from 11 to

15 g kg⁻¹ fresh weight (FW), while the total P content was 0.2 to 0.4 g kg⁻¹ FW. Total K was a high concentration compared to P, which occupied 2.5 g kg⁻¹ FW.

Table 2.2 Characteristics of methane fermentation waste fluid applied during two growing 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
NH₄-N (mg kg⁻¹ FW)	2083	2519
NO₃-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 ratio	6.0	3.8

Note: w/w: Weight per weight; FW: Fresh weight; n.a: None analysis

CHAPTER III

IMPACTS OF SOIL TEXTURE AND MFW RATES ON THE GROWTH OF RICE AND THE FATE OF N UNDER SUBMERGED CONDITIONS

3.1. Background

Paddy rice is one of the most important crops in the world, as half of the world's population eats rice every day. It accounts for 20% of the world's total dietary energy, compared to 19% from wheat and 5% from maize. In many Asian countries, rice makes up over 70% of the daily calorie intake (Bin Rahman and Zhang 2023). Rapid population growth leads to high food demand, resulting in increased fertilizer use for rice production. Nitrogen (N) is the critical nutrient for improving the yield and production of rice. Therefore, reducing inorganic N fertilizer use by replacing it with organic or biowaste in rice production is key for sustainable rice production. 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 et al. 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).

The impact of soil texture on rice growth and N dynamics has been reported. Clay particles protect organic matter and adsorb $\text{NH}_4\text{-N}$ (Hassink et al. 1993; Nieder et al. 2011), resulting in higher available N and greater rice yields in clay soils than in sandy soils (Ichinose et al. 2023; Dou et al. 2016). In contrast, sandy soils have higher potential for inorganic N leaching (Hansen et al. 2000; Zhou et al. 2006) and NH_3 volatilization (Duan and Xiao 2000; Fan et al. 2011). However, sandy soils may enhance the N harvest index and physiological N use efficiency compared to clay 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 NH_4^+ -N retention, NO_3^- -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 NH_4^+ -N constitutes 70–80% of total N in MFW. By quantifying N uptake by plants, retention in soil, and loss through leaching and volatilization, the author aim to provide actionable insights for optimizing MFW application rates in paddy fields with different soil textures. Its ultimate goal is to enhance rice yield while minimizing adverse environmental impacts.

To achieve these goals, I 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.

3.2. Materials and Methods

3.2.1. Soil and MFW

The materials for the experiment were described in Chapter II.

3.2.2. 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. I selected $^{15}\text{NH}_4\text{Cl}$ as a tracer because NH_4^+-N accounted for more than 99% of the inorganic N fraction in the MFW used in this study (Table 2.2). 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 $\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}$ and KCl, respectively (Table 3.1). 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. The schedule of rice experiments was presented in Table 3.2.

Table 3.1 Fertilizer treatments for Rice experiments (mg pot⁻¹)

	N-NH ₄ Cl	20% ¹⁵ N-NH ₄ Cl	N-MFW	P	K
0N	-		-	200	0.27
1N-Chem	160	40	-	200	0.27
0.5N-MFW		14	86	200	0.27
1N-MFW		28	172	200	0.27
2N-MFW		56	344	200	0.27

Table 3.2 Schedule of the rice experiment

Details	2021	2022
Basal application and water flooded immediately, NH ₃ trap	June 11 th	June 2 nd
Gas sampling, transplanting, the 1 st leachate sampling	June 12 th	June 3 rd
Gas sampling, measure growth, and leachate sampling	Jun 13 th ~ October 5 th	June 4 th ~ September 26 th
The last leachate sampling,	October 5 th	September 26 th
Harvesting	October 12 th	October 3 rd
Soil sampling, gas sampling (fallow period)	October 12 th	October 3 rd ~

3.2.3. 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 3.1). 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 CuBr_2 ($1 \text{ mg L}^{-1} \text{ Cu}$) for inhibiting microbial contamination, filtered using a $0.45\text{-}\mu\text{m}$ 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 ($\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$) concentrations were examined using a flow injection analysis (FIA) method (AQLA-700, Aqualab, Tokyo, Japan).

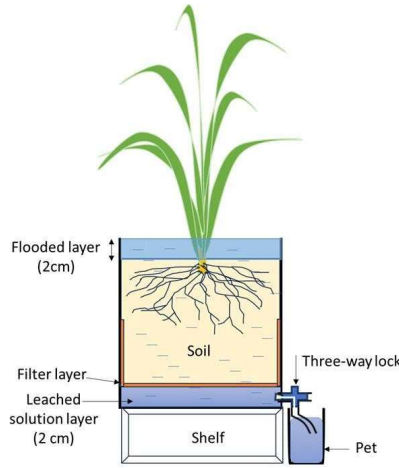


Figure 3.1. The design of the experimental pot for collecting daily leachate solution

3.2.4 Gas sampling

a. NH_3 volatilization

NH_3 volatilization was measured using the vented chamber method (Wang et al. 2004; Chen et al. 2012) after fertilizer application and later at intervals of 2-14 days. 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 NH_3 . 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 $0.45 \mu\text{m}$ membrane filter for analyzing $\text{NH}_4\text{-N}$ content via the FIA method (AQLA-700, Aqualab, Tokyo, Japan). The NH_3 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 $\text{NH}_3\text{-N}$ collected by the vented chamber (mg), A is the cross-sectional area of the round chamber (m^2), and D is the sampling duration (hour).

b. 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 days 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 an 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).

3.2.5. Plant performance and N of plant and soil

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

3.2.6. ¹⁵N analysis and calculation

Next, ¹⁵N-NH₄ and ¹⁵N-NO₃ isotopes in the samples were converted to N₂O using chemical conversion (Liu et al. 2014) and denitrification methods (Sigman et al. 2001), respectively. The ratio of ¹⁵N excess in N₂O gas of these samples was determined via gas chromatography-mass spectrometry (QP2020; Shimadzu, Japan). Excess of ¹⁵N in soil and in plant samples were analyzed via EA IsoLink CN-Isotope Ratio MS (Thermo Fisher Scientific, Massachusetts, USA) after ball milling and drying at 105 °C and 70 °C,

respectively. The following formulas were used to calculate the N derived from inorganic fertilizer (Ndfif) in the samples based on the percentage of ^{15}N :

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

${}^{15}\text{N}_{\text{atm}}$: represents N from the air; ${}^{15}\text{N}_0$: represents N from ^{15}N NH_4Cl .

% N excess in a sample from inorganic fertilizer:

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

Amount of N derived from inorganic fertilizer in samples:

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

Amount of N derived from unlabeled source (Ndfun):

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

N recovery from inorganic N upon fertilizer application:

$$\% \text{N}_{\text{recovery}} = \frac{\text{Ndfif}_{\text{sample}}}{\text{inorg. N}_{\text{rate}}} * 100 \quad (5)$$

$$\text{Total} \% \text{N}_{\text{recovery}} = \% \text{N}_p + \% \text{N}_s \quad (6)$$

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

$$\text{N fate} (\%) = \% \text{N}_p + \% \text{N}_L + \% \text{N}_E + \% \text{N}_S + \% \text{N}_{\text{uno}}. \quad (7)$$

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

3.2.7. Statistical analysis

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

3.3. Results

3.3.1. Rice growth under different MFW treatments

Table 3.3 Key agronomic characteristics of rice at harvest under MFW treatments in SL and CL soils across two growing seasons

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

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

Soil texture and MFW significantly influenced rice performance indicators, and their interactions substantially affected rice biomass in the 2022 growing year (Table 3.3). In 2021, rice was transplanted late, with older seedlings and lower initial soil N levels (Table 2.1), potentially contributing to reduced rice performance compared to 2022 (Table 3.3). Soil texture significantly affected rice height, panicle number, total biomass, and yield in

both years, except for panicle number in 2022, with rice plants growing better in the CL soil than in the SL soil. Rice height increased moderately with elevated MFW application rates in both soil types, and this effect was significant only in 2022. Increasing MFW application rates significantly enhanced the panicle 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.

3.3.2. 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, I measured the total N (TN) and Ndfif contents in whole plants, soil, leachate solution (Table 3.4), and gas emissions (Figure 3.2). 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 (Table 3.4). The results revealed that when MFW increased, Ndfif increased dramatically for both soil textures.

NH₃ volatilization increased linearly with MFW application rates in both soil textures (Figure 3.2a). A significant difference was observed only in the 2N-MFW treatments compared with the other treatments for each soil type. In addition, NH₃ volatilization in the CL soil was significantly higher than that in the SL soil (Figure 3.2a). Increasing the MFW application rate significantly enhanced NH₃-Ndfif emissions in both soil textures, which were significantly higher in CL soil than in SL soil (Figure 3.2b). N₂O 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 3.2c and d).

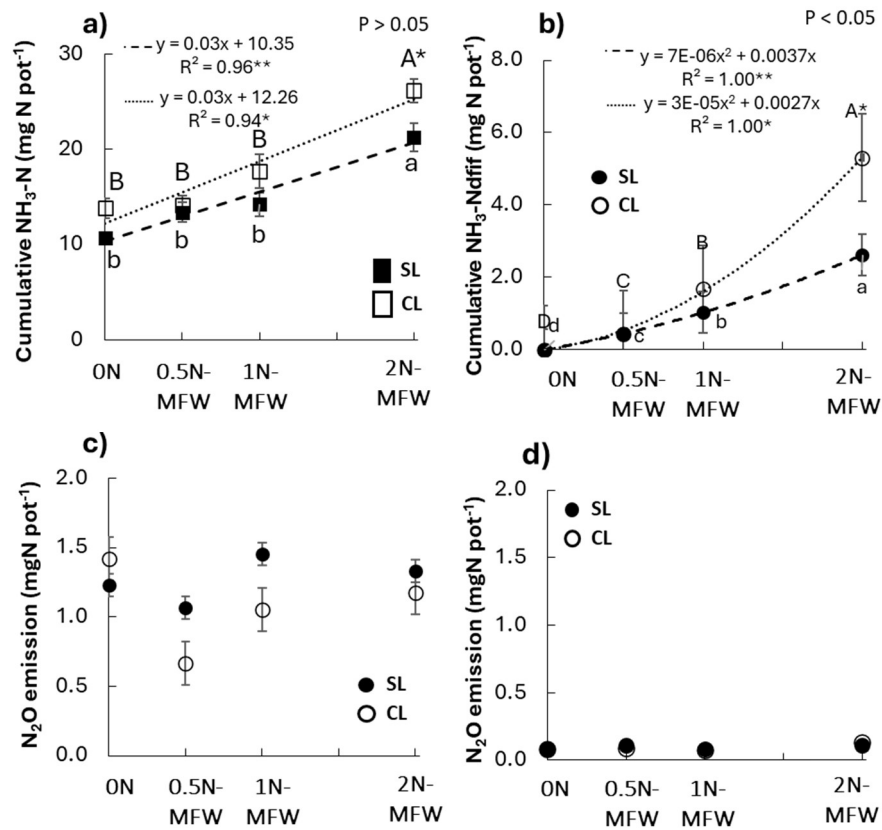


Figure 3.2. Comparison of different methane fermentation waste fluid (MFW) treatments in contrasting soil textures (sandy loam, SL; clay loam, CL) for (a) NH_3 volatilization in 2022, (b) $\text{NH}_3\text{-N}$ derived from inorganic N of MFW ($\text{NH}_3\text{-Ndfif}$) in 2022, (c) N_2O emission in 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 ($P < 0.05$).

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

3.3.3. 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 3.5). 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.

Table 3.5 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)
Mean		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)
Mean		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.*

In both years, the Ndfif from chemical fertilizer and MFW application was strongly affected by soil texture (Figure 3.3). 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 3.3e). 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 3.3a 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 3.3b 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.

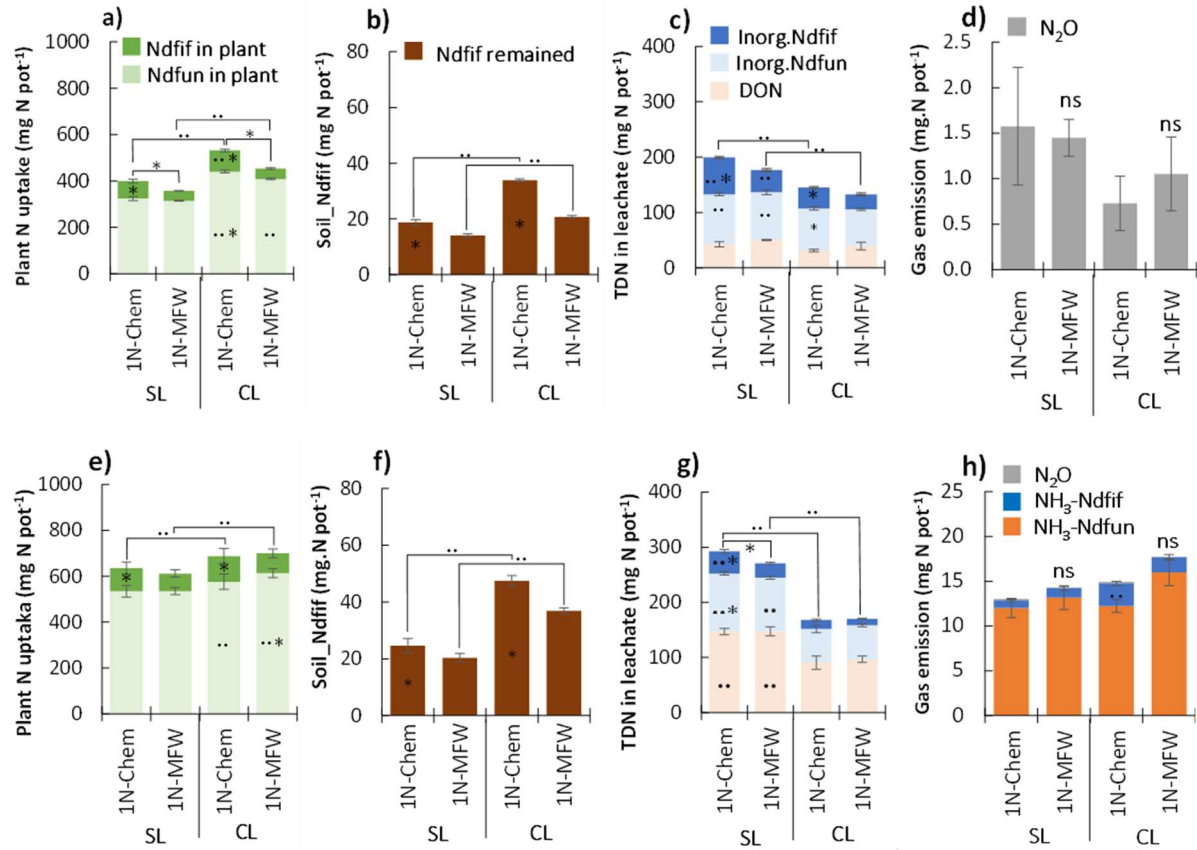


Figure 3.3. Comparison of nitrogen (N) content in plant N uptake (a, e), soil N derived from inorganic fertilizer (b, f), total dissolved N (TDN) in leachate (c, g), and gas emissions (d, h) between 1N-Chem and 1N-MFW under contrasting soil textures (SL and CL) in 2021 (a–d) and 2022 (e–h). Ndfif: Nitrogen 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.

TDN and inorganic Ndfif leached from 1N-Chem were significantly higher than those leached from 1N-MFW in both soil textures (Figure 3.3c 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 2.1). 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 3.3d and h).

3.3.4. 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 3.6). 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 and significantly increased the proportion of N lost through leachate, especially in SL soil.

Table 3.6 Distribution of Ndfif in plant, soil, and leachate under MFW treatments in SL and CL soils across two growing seasons.

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

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

3.4. Discussion

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

My results demonstrated that rice growth was significantly better in CL soils compared to SL soils (Table 3.3), 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 3.4 and 3.6) (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 et al. 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 S3.2), as reported by Kanbar et al. (2009), and an uptake of 44–52% from MFW applications (Table 3.6), consistent with findings by Ghoneim et al. (2008), who reported 51% $\text{NH}_4\text{-N}$ uptake (Table S3.1).

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 et al. 2006). This likely contributed to the absence of significant differences in Ndfif uptake between the two soil types in 2021 (Table 3.4 and Table 3.6). 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 et al. 2004; Nishida et al. 2008) (Table S3.1), 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 2.2), the Ndfif in the leachate was lower than that in 2021 (Table 3.4), leading to a reduced proportion of MFW-derived N in the leachate (Table 3.6). 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, my 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.

3.4.2. Effects of soil textures on N emissions from MFW

My results revealed lower Ndfif in NH₃ 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. 3.1a, b; Table S3.1). 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 NH₃ volatilization (Hayashi et al. 2006). However, contrary to previous studies suggesting higher clay content reduces NH₃ volatilization (Duan and Xiao 2000; Fan et al. 2011), my results indicated higher volatilization in CL soil, particularly at higher MFW application rates. This could be attributed to increased floodwater pH and NH₄⁺-N concentrations in CL soil (pH 6.4-9.4) compared to SL soil (pH 6.4-7.4) during the midday period (Figure S3.1a, 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 N₂O emissions in all treatments, consistent with previous studies (Sasada et al. 2011; Singla and Inubushi 2014; Win et al. 2014). Submerged conditions also favored anaerobic ammonium oxidation (anammox), converting NH₄-N to N₂ (Hu et al. 2011; Shen et al. 2016). Given the optimal pH and high NH₄⁺ concentrations, anammox may have contributed to higher unobserved N in MFW treatments compared to chemical fertilizer treatments (Table 3.6) (Yang et al. 2015). However, the presence of organic matter in MFW (Table 2.2) 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 ¹⁵NO₃ in leachate (data not shown) suggests oxygen-depleted conditions in the pots, likely enhancing denitrification processes (Stepanauskas et al. 1996). Therefore, the elevated unobserved N levels in MFW treatments are most likely attributed to N₂ emissions via complete denitrification, with a possible contribution from anammox under submerged conditions (Yang et al. 2015).

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

The 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 $\text{NH}_4\text{-N}$ (Nishikawa et al., 2014; Möller et al., 2015), I used total N as the basis for application, with the digestate in My study containing 70–80% $\text{NH}_4^+\text{-N}$ in MFW (Table 2.2). This approach resulted in reduced inorganic N leaching in SL soil in both years and in CL soil in 2021 (Figs. 3.2c and g), while maintaining rice yield.

I acknowledge that the $\text{NH}_4^+\text{-N}$ content of digestates can vary considerably depending on feedstock composition and processing conditions (Tampio et al. 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 my 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 (Figures 3.3d and 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.

3.4.5. 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 3.3). However, yields plateaued beyond the 1N-MFW, except for the 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 3.4).

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 3.3). 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 NH₃ volatilization (Figure 3.1a). 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).

My 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 3.4). NH₃_Ndfif emissions also increased exponentially with higher MFW rates (Figure 3.2b), 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, I 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 3.5). These rates optimize yield while minimizing environmental impacts.

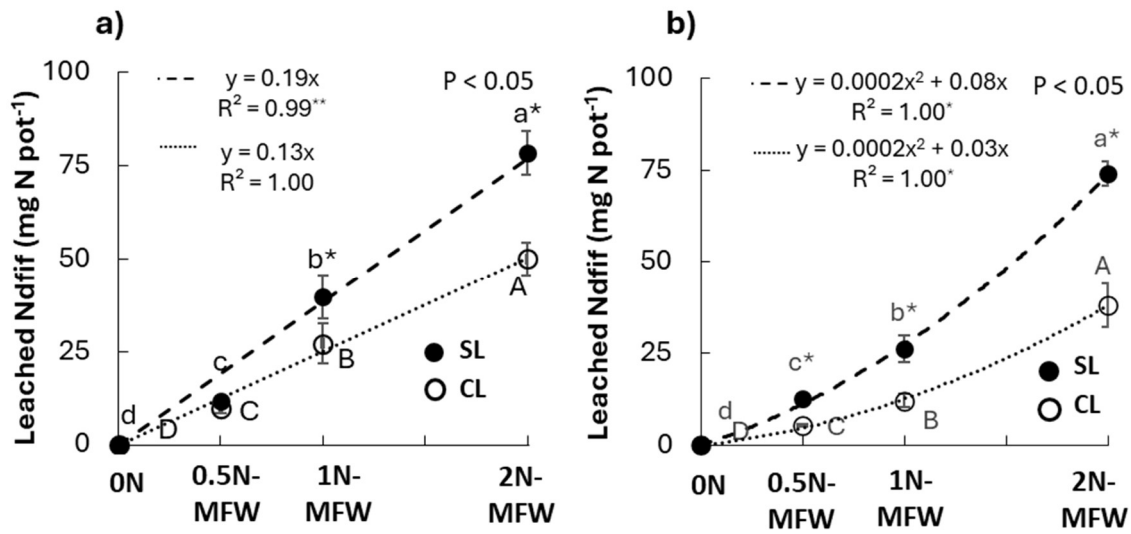


Figure 3.4. Ndfif in leachate under different MFW treatments in 2021 (a) and 2022 (b) for SL and CL soils. 0N: no fertilizer; 0.5N-MFW: 100 mg N as MFW; 1N-MFW: 200 mg N as MFW; 2N-MFW: 400 mg N as MFW. Error bars represent standard error ($n = 4$), except for SL-0N and CL-2N-MFW treatments in 2022 ($n = 3$). 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.

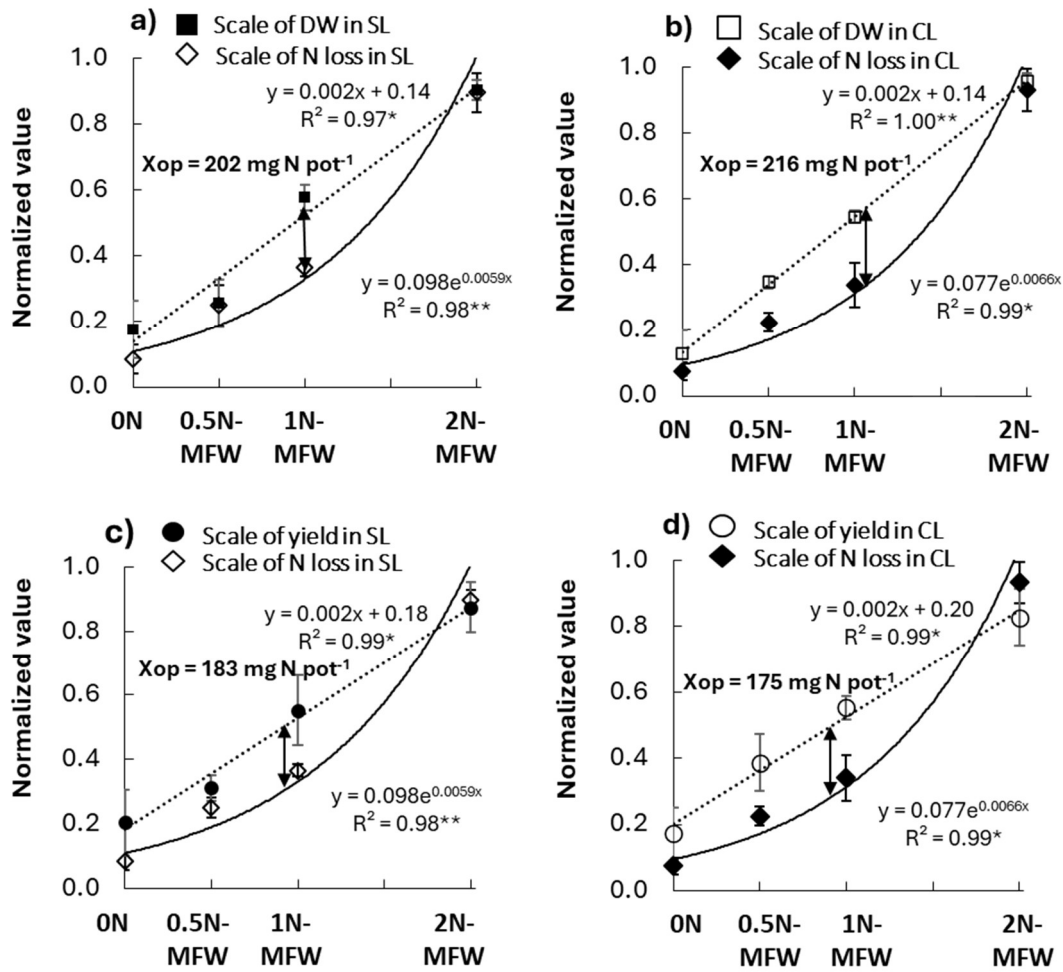


Figure 3.5. Relationship between normalized rice biomass (a, b), yield (c, d), and total N loss under different MFW application rates in SL (a, c) and CL (b, d) soils in 2022. 0N: no fertilizer; 0.5N-MFW: 100 mg N as MFW; 1N-MFW: 200 mg N as MFW; 2N-MFW: 400 mg N as MFW. Dotted lines indicate linear 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 $^{**}P < 0.01$.

3.5. 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, I 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.

CHAPTER IV

IMPACT OF SOIL TEXTURE AND MFW RATES ON THE GROWTH OF MAIZE AND THE FATE OF N UNDER UPLAND CONDITIONS

4.1. Background

Global maize production has increased 104% annually, reaching 121 million tons in 2021 with 205 million ha worldwide (FAO, 2023), reflecting its critical role as a food source, livestock feed, and biofuel raw material (Saldivar and Perez-Carrillo 2016). In regions like Asia, Africa, and Latin America, maize provides more than 30% of caloric intake, underscoring its importance for global food security (Shiferaw et al. 2011). With the demand for maize and other cereals expected to double by 2050 (Nelson et al. 2010), optimizing N management for maize cultivation is essential for ensuring both productivity and environmental sustainability.

Methane fermentation waste fluid (MFW) has been successfully applied to maize cultivation, but its effect on maize growth varies with application rates and environmental conditions. Some studies report no significant differences in maize yield between certain MFW application levels (Pastorelli et al. 2013), while others highlight yield increases with higher application rates (Stanley et al., 2019). Furthermore, MFW can achieve maize yields comparable to chemical fertilizers (Riva et al. 2016; Verdi et al. 2019) or mixed fertilizers like chemical N combined with pig slurry (Sigurnjak et al. 2017). Herrmann et al. (2012) even reported that maximum maize yield could be achieved with MFW application rates lower than those required for mineral N. However, the use of MFW also poses risks, including increased emissions of NH_3 and N_2O , depending on soil type and application methods (Nkoa 2014; Wolf et al. 2014; Dicke et al. 2015; Verdi et al. 2019; Maris et al. 2021). These findings highlight the need to identify optimal MFW application rates that balance crop yield with environmental sustainability.

Furthermore, both maize growth and N dynamics are strongly influenced by soil texture (Aulakh et al. 1996; Li et al. 2023). Fang and Su (2019) demonstrated that high clay content in soil significantly increased maize yield while reducing $\text{NO}_3\text{-N}$ leaching, highlighting the ability of clay soils to retain nutrients more effectively than sandy soils. Consistent with this, Sogbedji et al. (2000) showed that $\text{NO}_3\text{-N}$ leaching was more severe in loamy sand compared to clay loam, emphasizing the vulnerability of sandy soils to

nutrient losses. Furthermore, Feng et al. (2016) reported a relationship between maize root length and N supply was prominent in loamy clay and clay loam soils but not in sandy loam (SL) soils, suggesting that soil texture significantly affects maize nutrient uptake dynamics. However, clay-rich soils also present challenges. A higher clay content induces NH_3 volatilization due to greater N mineralization capacity (Duan and Xiao, 2000; Aulakh et al. 1996), which extends to MFW application (Abubaker et al. 2013). Under upland conditions, Ottaiano et al. (2020) further reported that N_2O emissions increased exponentially with $\text{NO}_3\text{-N}$ levels in soil and were significantly higher in clay soils compared with SL soils. This is exacerbated by the presence of ammonia-oxidizing bacteria (AOB) in MFW, which contributes to elevated N_2O emissions during its application (Law et al. 2011). These findings underscore the potential for severe gaseous N losses, particularly in clay soils treated with MFW. Conversely, sandy soils exhibit distinct advantages and challenges. Makádi et al. (2016) found that MFW application had a more considerable effect on chemical properties and microbial activities of sandy soils than clay soils. This suggests that the fate of N from MFW application differs substantially depending on soil texture, with sandy soils being more responsive to nutrient amendments but more susceptible to leaching losses. Given that the primary N component of MFW is $\text{NH}_4\text{-N}$, its behavior under different soil conditions is highly dependent on soil texture, particularly in the short term. Clay soils tend to retain more nutrients but are prone to gaseous emissions, while sandy soils allow faster N translocation but exhibit higher risks of leaching. These contrasting characteristics underscore the need for soil-specific strategies to optimize MFW application and minimize environmental impacts.

However, there is limited understanding of how soil textures and MFW application rates interact to influence maize growth and N dynamics under aerobic conditions, especially inorganic N, during the initial cultivation season. While previous studies have highlighted the potential of MFW as a biofertilizer, the specific interactions between soil texture and MFW-derived N dynamics have not been thoroughly quantified. This knowledge gap is particularly critical for optimizing MFW utilization to balance agricultural productivity with environmental sustainability. To address this gap, I conducted this study (i) to evaluate the impact of MFW application rates on maize growth and the fate of inorganic N under two contrasting soil texture types with sandy loam (SL) and clay loam (CL) soils using a tracer isotope method, and (ii) to determine the optimum MFW rate for maize

cultivation in each soil texture type, with the goal of maximizing yield while minimizing the environmental impacts in the short term. I hypothesize that increasing MFW application rates will enhance maize yield; however, the response will vary between soil textures due to differences in N dynamics. Specifically, sandy soils are expected to exhibit higher nutrient translocation and leaching risks, while clay soils may retain more N but show increased gaseous losses, such as NH_3 and N_2O under aerobic conditions. By elucidating these mechanisms, this study aims to provide actionable insights into soil-specific nutrient management strategies for sustainable maize production.

4.2. Materials and Methods

4.2.1. Soil and MFW

Those materials were presented in Chapter II.

4.2.2. Experimental design

The experiments were conducted in 1/2000a Wagner pots (Figure S4.1) using sweet corn (*Takii 90 EX*) under greenhouse conditions over two years (2021 and 2022). Fertilizer treatments included three levels of total N-MFW: 375 mg N pot⁻¹ (0.5N-MFW), 750 mg N pot⁻¹ (1N-MFW), and 1500 mg N pot⁻¹ (2N-MFW). These were compared with a chemical fertilizer treatment (1N-Chem) at 750 mg N pot⁻¹ (equivalent to 150 kg N ha⁻¹) based on the average recommended N application rate for maize in Japan (Yagi et al. 2018), and a control treatment without any N fertilizer. All treatments were with four replicates. To trace the fate of N derived from inorganic fertilizer (Ndfif), ¹⁵NH₄Cl (99.1% atom) was mixed with MFW or chemical fertilizer, corresponding to 20% of the inorganic N content, while maintaining the same total N application across all treatments. This tracer was selected because over 99% of the inorganic N in MFW was in the form of ammonium (NH₄⁺-N), making ¹⁵NH₄Cl a suitable analog for tracking the fate of applied inorganic N. The same amounts of P (150 kg P ha⁻¹) and K (200 kg K ha⁻¹) were supplied for all treatments by Ca(H₂PO₄)₂·2H₂O and KCl supplements, respectively (Table 4.1). In 2021, each pot contained 9.0 kg of air-dried soil. In 2022, the soil amount was adjusted based on bulk density to ensure equal volumes for both soil types.

Table 4.1 Fertilizer treatments for maize experiments (mg pot⁻¹)

	N-NH ₄ Cl	20% ¹⁵ N-NH ₄ Cl	N-MFW	P	K
0N	-		-	750	1010
1N-Chem	600	150	-	750	1010
0.5N-MFW		59	316	750	1010
1N-MFW		118	632	750	1010
2N-MFW		236	1264	750	1010

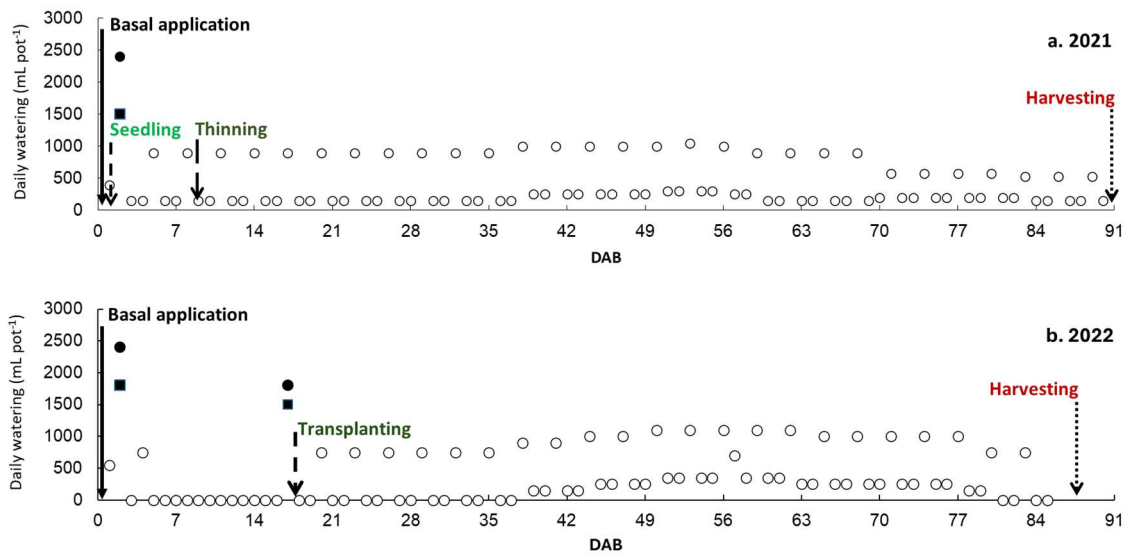


Figure 4.1. Water irrigation schedule and cultivation timeline for 2021 (a) and 2022 (b) across both soil types. Black circles and squares indicate the initial water supply events required to achieve 80% of the water-holding capacity for SL and CL soils, respectively. White dots represent the daily total water supplied throughout the cultivation period. DAB: Days after basal application.

The experimental schedule is presented in Figure 4.1 and Table 4.2. Fertilizers were applied to the pots in a basal layer and mixed well with the top layer (0–10 cm depth) on August 24, 2021 and July 20, 2022. Then, water was slowly supplied to reach 80% of each soil's water-holding capacity. In the 2021 season, three seeds were directly sown into the soil immediately after fertilization, whereas in the 2022 season, young seedlings were transplanted 15 days after fertilization. Daily watering for each pot was determined based on the growing stage using the Blaney-Criddle Method, following the FAO's

irrigation water management guidance (FAO, 1986). Artificial rainfall was applied every three days after transplanting, 15 mm per three days applied over 1.5 hours, referring to the average monthly rainfall during the cultivation season in Kyoto over the past 20 years (2000–2020). During the 2021 season, daily watering and artificial rainfall were initiated immediately after sowing (Figure 4.1a), with a total water supply of 758 mm and 776 mm for SL and CL soils, respectively. In the 2022 season, water was supplied just three days after fertilization, followed by rewetting to 80% of water-holding capacity a day before transplanting (Figure 4.1b). Because artificial rainfall alone maintained sufficient soil moisture content, daily watering began only after the first recorded leaching event. The total water supply in the 2022 season was lower than that in the 2021 season, amounting to 709 mm and 739 mm for SL and CL soils, respectively.

Table 4.2 Schedule of the Maize experiment

Details	2021	2022
Basal application, water supply,	August 24 th	July 20 th
NH ₃ trap, sowing	August 25 th	July 20 th
Gas sampling, NH ₃ trap, leachate sampling	August 25 th ~ September 2 nd	Jul, 21 th ~ August 5 th (No water supply)
Thinning/ Transplanting	September 2 nd (Thinning)	August 5 th (Transplanting)
Gas sampling, water supply, leachate sampling, and growth measurement	September 2 nd ~ November 24 th	August 5 th ~ October 12 th
Harvesting	November 24 th	October 13 th
Gas sampling, soil sampling	November 25 th ~	October 14 th ~

4.2.3. Sampling

a. Growth and biomass

I measured plant height and soil plant analysis development (SPAD) values using SPAD meter (SPAD 502 plus, Minolta, Japan), described by Kandel (2020), every two weeks following transplantation. After harvest, the dry biomass weight was measured by oven-

drying samples at 70 °C until a constant weight was achieved. Root systems were carefully separated from the soil using a 4 mm sieve, thoroughly cleaned, and oven-dried for subsequent analysis. All plant samples were finely ground using a ball-mill at 450 rpm for two minutes prior to N content analysis.

b. Gas sampling

NH₃ gas emissions were collected using the vented chamber method described by Wang et al. (2004) and Chen et al. (2013). A plastic cylinder (15 cm in height and 5 cm in diameter) was used to trap NH₃ gas one day after fertilizer application during the 2021 season and immediately after application in the 2022 season. Sampling was conducted every two days during the first two weeks and every biweekly thereafter until harvest. NH₃-N content was analyzed immediately using the flow injection analysis (AQLA-700, Aqua Lab, Japan) and stored at 4 °C until the ¹⁵N content was determined.

N₂O gas was collected using the closed chamber method (Kim et al. 2021) at 2–4 day intervals over three weeks post-application, biweekly during the growth stage, and monthly after harvest. Chambers consisted of an acrylic column (25 cm diameter, 50 cm height) fitted to the pot size, and installed with a 12V fan, 1 L airbag, and an auto-thermometer. For plants taller than 50 cm, a modified chamber was used (Figure S4.1). Gas was collected at intervals of 0, 15, 30, and 45 minutes after closing the chamber, during two periods: 9:00 a.m.–12:00 p.m. and from 3:00 p.m.–7:00 p.m. Samples were kept in 20 mL glass vials (Nichiken-Rika, Japan) and analyzed for N₂O content using gas chromatography (GC-2014, Shimadzu, Japan).

c. Leachate sampling

Leachate was collected from the bottom of each pot using a capillary fiber, after water had percolated through the soil and a geotextile layer positioned 2 cm above the base of the pot (Figure S4.1). The total soil thickness in each pot was approximately 23 cm, ensuring sufficient rooting depth for maize growth. Sampling was conducted every three days until the flowering stage, and weekly during the maturity stage until harvest. To prevent microbial transformation, the leachate was preserved with CuBr₂ (~1 mg Cu L⁻¹), filtered through a 0.45 µm membrane filter (Sartorius minisart RC 0.45), and stored at 4 °C prior to analysis (Shibata et al. 2017). Total dissolved N was determined using a

TOC analyzer equipped with a TN unit (TOC-L with TNM-L unit, Shimadzu, Japan). Inorganic N (NH_4^+ -N and NO_3^- -N) was analyzed using a flow injection analysis (AQLA-700, Aqualab, Tokyo, Japan). For ^{15}N analysis, 0.5–1 mL of leachate was oven-dried in Sn capsules (Capsule Sn 98, Ludiswiss, Switzerland) at 60 °C prior to isotopic analysis.

d. Soil sampling

Soil samples were collected after harvest, mixed well, oven-dried at 105 °C, and finely ground using a ball-mill for total N content and ^{15}N ratio analysis.

4.2.4. ^{15}N analysis and calculation

The ^{15}N excess in N_2O gas samples was analyzed by gas chromatography-mass spectrometry (GCMS-QP2020, Shimadzu, Japan). For NH_3 samples, the ^{15}N excess was converted to N_2O gas by the chemical conversion method described by Liu et al. (2014) before analyzing the isotope ratio. The ^{15}N excess in leachate, as well as the total N and ^{15}N in soil and plant samples, were analyzed using Isotope Ratio Mass Spectrometry (Delta V Advantage Isotope Ratio MS, Thermo Fisher Scientific Inc.).

The following equations were used to calculate the N derived from inorganic fertilizer (Ndfif) excess in samples based on the percentage of ^{15}N .

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

$^{15}\text{N}_{\text{atm}}$: from the air; $^{15}\text{N}_0$: from $^{15}\text{N}\text{-NH}_4\text{Cl}$

% N excess in a sample from inorganic fertilizer:

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

Amount of Ndfif in the samples:

Ndfif_{sample} (mg) =

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

Amount of N loss from inorganic fertilizer:

Ndfif loss (mg) =

$$\text{Inorganic N input (mg)} - (\text{mg Ndfif in plants} + \text{mg Ndfif in soil}) \quad (4)$$

N recovery from inorganic N fertilizer applications:

$$\% \text{N}_{\text{resub-sample}} = \frac{\text{Ndfif}_{\text{sample}}}{\text{N}_{\text{rate}}} * 100 \quad (5)$$

$$\text{Total\% N}_{\text{recovery}} = \%N_P + \%N_S \quad (6)$$

The fate of inorganic N from fertilizer application is evaluated by:

$$\text{N fate (\%)} = \%N_P + \%N_S + \%N_L \quad (7)$$

P: Plant uptake; S: Remain in the soil; L: Loss to the environment

4.2.5. Statistical analysis

All statistical analyses were performed using the SPSS (IBM version 29). One-way and two-way ANOVA were used to evaluate significant differences among treatments and factor groups, followed by Tukey's post-hoc test for pairwise comparisons. Pearson correlation coefficients were used to test linear correlations between variables.

4.3. Results

4.3.1. Maize growth and yield

Table 4.3 Key agronomic characteristics of maize under different fertilizer treatments and contrasting soil textures (sandy loam - SL; clay loam - CL) across two growing years (2021 and 2022).

Soil	Fertilizers	Height (cm)		SPAD		Dry biomass (g pot ⁻¹)		Maize grain yield (g pot ⁻¹)	
		2021	2022	2021	2022	2021	2022	2021	2022
SL	0N	178b	231	36.9c	47.0	60c	122	15.5c	36.1b
		(3)	(6)	(1.2)	(0.4)	(5)	(3)	(3.0)	(1.1)
	1N-Chem	218ab	232	48.9a	49.9	124a	137	41.7a	49.7a
		(6)	(8)	(0.2)	(0.2)	(6)	(7)	(2.1)	(2.3)
	0.5N-MFW	189ab	225	38.5c	48.5	96b	134	24.4bc	45.4a
		(11)	(6)	(1.9)	(0.4)	(6)	(8)	(3.0)	(1.4)
	1N-MFW	199ab	229	43.7b	49.0	106ab	142	30.2ab	52.4a
		(6)	(1)	(0.2)	(0.6)	(8)	(10)	(6.1)	(1.7)
	2N-MFW	202a	222	49.9a	50.5	131a	130	40.9a	46.2a
		(6)	(1)	(0.5)	(1.0)	(4)	(8)	(7.0)	(1.6)
CL	0N	181b	190b	22.9c	48.9	59c	106.9b	-	24.01b
		(3)	(19)	(1.4)	(0.4)	(2)	(19)	-	(5.9)
	1N-Chem	209ab	228ab	39.6a	47.8	114a	152a	30.9a	44.7a
		(8)	(12)	(1.5)	(1.3)	(4)	(8)	(3.3)	(1.6)
	0.5N-MFW	200ab	235a	25.7bc	48.9	79bc	134ab	-	39.7a
		(7)	(3)	(1.4)	(0.6)	(5)	(3)	-	(2.2)
	1N-MFW	205ab	239a	31.7b	48.3	88ab	151a	0.5b	43.7a
		(10)	(4)	(1.7)	(0.3)	(4)	(12)	0.5b	(1.8)
	2N-MFW	222a	231a	39.6a	48.1	112a	148a	25.4a	42.2a
		(3)	(8)	(1.1)	(0.6)	(10)	(7)	(8.7)	(3.7)
Two-way ANOVA									
Soil		ns	Ns	***	ns	**	ns	***	***
MFW		**	Ns	***	*	***	**	***	***
Soil x MFW		ns	*	ns	ns	ns	ns	ns	*
Chem x MFW		ns	Ns	***	ns	**	ns	***	ns
Soil x Fer. Types		ns	Ns	ns	ns	ns	ns	*	ns

Fertilizer treatments: 0N: no fertilizer; 1N-Chem: 750 mg NH₄Cl-N; 0.5N-MFW: 375 mg N as MFW; 1N-MFW: 750 mg N as MFW; and 2N as MFW: 1500 mg N as MFW. Data represent mean ± standard error (SE) from four replications, except for CL-1N-Chem in 2021 and SL-1N-MFW, SL-2N-MFW, and CL-0N treatments in 2022, with three replications. Significant differences between treatments in SL or CL soil were determined by one-way ANOVA and are indicated by lowercase letters. Height: final height before harvest; SPAD: the highest value measured within the growing stage. *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$; ns: not significant.

The agronomic indicators of maize growth during the 2021 and 2022 seasons are summarized in Table 4.3. Soil texture significantly influenced SPAD values, biomass in 2021, and maize grain yield across both seasons. In 2021, maize height in the 2N-MFW treatments was significantly higher than in control treatments for both soil types; however, no significant differences in height were observed among the MFW application rates, despite a trend of increasing average height with higher application. In 2022, significant differences in height were observed across all MFW treatments in CL soil only, while no significant height differences were observed between treatments or soil textures. SPAD values and dry biomass were significantly higher in SL soil compared to CL soil during the 2021 season. Similarly, maize grain yield was consistently higher in SL soil than in CL soil in both seasons. Notably, maize grain yield in CL soil during the 2021 season was severely reduced across most treatments, with measurable yields only recorded for the 1N-Chem and 2N-MFW treatments. This yield reduction was likely due to limited N availability in the other treatments, a phenomenon not observed in 2022.

MFW application rates significantly influenced most agronomy indicators for both seasons, except for maize height in 2022. In 2021, increasing MFW rates significantly improved maize SPAD values and dry biomass, although height did not show statistically significant differences. Maize grain yield in SL soil increased linearly with MFW rates ($R^2 = 0.99$, $P < 0.05$), whereas in CL soil, yield was constrained due to poor performance in most treatments. In 2022, MFW consistently produced significantly higher yields than control treatments for both soil textures, with the highest yields observed in the 1N-MFW treatments. However, no significant differences in yield were detected among the MFW treatments within each soil type.

When comparing MFW application (1N-MFW) and chemical fertilizer (1N-Chem), significant differences were observed only in SPAD values (both soil types) and yield (CL soil) during the 2021 season. Maize height showed no significant differences between the two treatments in either season. Across soil types, total dry biomass in 1N-Chem was significantly higher than in 1N-MFW in 2021; however, within individual soil textures, no significant differences were observed between these treatments (Table 4.3).

4.3.2. Ndfif distribution

The distribution of Ndfif in plants and soil was analyzed to assess the effects of soil texture and MFW application rates (Table 4.4). Across both seasons, increasing MFW rates significantly enhanced N uptake by plants in both soil textures, with SL soil showing a more pronounced response compared to CL soil. Similarly, Ndfif remaining in the soil increased significantly with higher MFW rates, consistently being higher in CL soil than in SL soil. A significant interaction between soil texture and MFW rate was observed for Ndfif remaining in the soil during the 2022 season. Chemical fertilizers consistently resulted in significantly higher Ndfif uptake by plants compared to 1N-MFW across all soil textures and seasons. However, Ndfif remaining in the soil from 1N-MFW was significantly lower than that from 1N-Chem only in SL soil during the 2021 season.

Table 4.4 and Figure 4.2 summarize Ndfif losses to the environment under different treatments across soil textures. As MFW rates increased, Ndfif losses through leachate and gases (N_2O and NH_3) was significantly higher in CL soil compared to SL soil (Figure 4.2), with the magnitude of loss increasing with MFW application rates. In 2021, Ndfif losses from MFW in leachate accounted for the majority of total Ndfif losses, ranging from 8% to 30% in SL soil and 39% to 51% in CL soil. However, in 2022, NH_3 volatilization became the dominant pathway, contributing 38% to 42% of total Ndfif losses in SL soil and 45% to 75% in CL soil.

Unobserved Ndfif losses were comparable between SL and CL soils across treatments in the 2021 season. However, in 2022, unobserved Ndfif losses were significantly higher in the 1N-MFW treatment for SL soil compared to CL soil. Ndfif losses from 1N-MFW were higher than from 1N-Chem for both soil textures, with a significant difference observed in CL soil in the 2022 season. Conversely, Ndfif losses through leachate were markedly higher from 1N-Chem compared to 1N-MFW in CL soil in the 2021 season and in SL soil across both seasons (Figure 4.2).

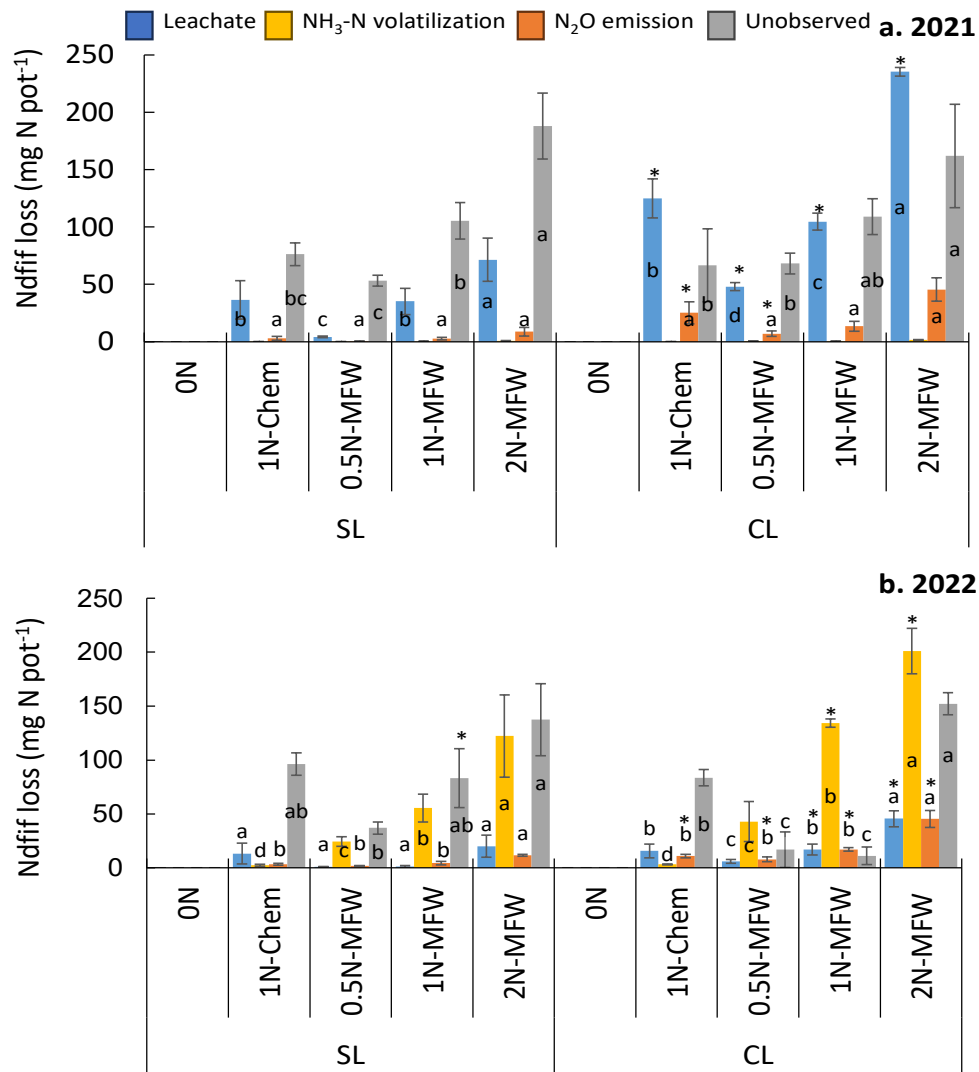


Figure 4.2. N loss derived from inorganic N fertilizer (Ndfif) under contrasting soil textures (sandy loam - SL; clay loam - CL) across growing seasons (a - 2021 and b - 2022). The distribution of Ndfif loss is presented for different fertilizer treatments: 0N: no fertilizer; 1N-Chem: 750 mg NH₄Cl-N; 0.5N-MFW: 375 mg N as MFW; 1N-MFW: 750 mg N as MFW; and 2N as MFW: 1500 mg N as MFW. Data represent the mean $\pm$ standard error (SE) from four replications, except for CL-1N-Chem in 2021 and SL-1N-MFW, SL-2N-MFW, and CL-0N in 2022 with three replications. Asterisks indicate a significant difference between SL and CL soils within the same treatment level, *: $P < 0.05$. Significant differences between treatments in SL or CL soil were determined by one-way ANOVA and are indicated by lowercase letters.

Table 4.4 N derived from inorganic N fertilizer (Ndfif) in plants, soil, and losses after maize harvest under fertilizer treatments and contrasting soil textures (sandy loam - SL; clay loam - CL) across two growing years (2021 and 2022).

Soil	Fertilizers	Ndfif in plants		Ndfif in soil mg N pot ⁻¹		Ndfif loss	
		2021	2022	2021	2022	2021	2022
SL	0N	-	-	-	-	-	-
	1N-Chem	508b (26)	509b (13)	134a (10)	127b (9)	116bc (14)	114bc (14)
	0.5N-MFW	147d (6)	183d (2)	60c (3)	62c (8)	57c (4)	64c (9)
	1N-MFW	289c (18)	359c (17)	97b (8)	115bc (18)	143b (17)	144b (29)
	2N-MFW	623a (17)	736a (44)	164a (10)	210a (27)	269a (18)	289a (17)
	0N	-	-	-	-	-	-
CL	1N-Chem	401a (19)	494b (6)	140b (16)	144b (7)	217bc (24)	112c (11)
	0.5N-MFW	60c (5)	156d (5)	80c (3)	80c (5)	123c (7)	73c (6)
	1N-MFW	153b (8)	298c (15)	149b (3)	140b (7)	227b (12)	179b (9)
	2N-MFW	389a (41)	564a (25)	223a (22)	228a (7)	444a (34)	443a (18)
	0N	-	-	-	-	-	-
	1N-Chem	401a (19)	494b (6)	140b (16)	144b (7)	217bc (24)	112c (11)
Two-way ANOVA							
Soil		***	***	**	***	***	***
MFW		***	***	***	***	***	***
Soil x MFW		**	***	ns	***	*	***
Chem x MFW		***	***	ns	**	ns	**
Soil x Fer. types		ns	ns	*	ns	ns	ns

*Fertilizer treatments: 0N: no fertilizer; 1N-Chem: 750 mg NH₄Cl-N; 0.5N-MFW: 375 mg N as MFW; 1N-MFW: 750 mg N as MFW; and 2N as MFW: 1500 mg N as MFW. Mean and standard error (SE) from four replications except for CL-1N-Chem in 2021; SL-1N-MFW, SL-2N-MFW, and CL-0N treatments in 2022 with three replications. Data represent mean ± standard error (SE) from four replications, except for CL-1N-Chem in 2021 and SL-1N-MFW, SL-2N-MFW, and CL-0N treatments in 2022, with three replications. Significant differences between treatments in SL or CL soil were determined by one-way ANOVA and are indicated by lowercase letters. *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$; ns: not significant.*

4.3.3. Fate of inorganic N from fertilizer application under contrasting soil textures

A summary of Ndfif proportions across treatments is presented in Table 4.5. The highest Ndfif in plants was observed in 1N-Chem, ranging from $67.1 \pm 3\%$ to $67.9 \pm 1.7\%$ in SL soil and from $52.9 \pm 3.3\%$ to $65.9 \pm 0.8\%$ in CL soil, significantly exceeding the values from the MFW treatments across both seasons. In SL soil, Ndfif in plants remained consistent across MFW treatments for both seasons, while in CL soil, it increased significantly during the 2021 season. Maize accumulated $54.6 \pm 3.5\%$ to $59.6 \pm 3.5\%$ of total Ndfif in SL soil and $22.9 \pm 1.8\%$ to $50.6 \pm 1.7\%$ in CL soil. The effects of soil type and MFW rate on Ndfif uptake by plants were significant in both seasons, with a significant interaction between soil texture and MFW rate ($P < 0.01$) in the 2022 season. Ndfif remaining in the soil accounted for less than 30% of the inorganic N input in both seasons and was significantly lower in SL soil compared to CL soil. (Table 4.5). With an increase in MFW rates, the percentage of N retained in the soil decreased significantly, except in SL soil in the 2022 season. Retention of N in 1N-MFW treatments was higher than in 1N-Chem for both soil textures across both seasons, with significant differences observed in CL soil in the 2021 season.

Overall, N recovery in SL soil was significantly higher than in CL soil across both seasons, with chemical fertilizer achieving the highest N recovery and the lowest N loss in both soil textures (Table 4.5). As MFW rates increased, N recovery decreased for both soil textures, except for CL soil in the 2021 season, though a significant difference was found only in CL soil in the 2022 season.

Table 4.5 Fate of inorganic N from fertilizer treatments under contrasting soil textures (sandy loam - SL; clay loam - CL) across two growing years (2021 and 2022).

Soil	Fertilizer	%N in plant		%N in soil		%N recovery		%N loss	
		2021	2022	2021	2022	2021	2022	2021	2022
SL	0N	-	-	-	-	-	-	-	-
	1N-Chem	67.1 a (3.0)	67.9 a (1.7)	17.9 ab (1.3)	16.9 (1.2)	85.0 a (1.8)	84.7 (1.9)	15.0 b (1.8)	15.3 (1.9)
	0.5N-MFW	55.6 b (2.3)	59.3 ab (0.5)	19.3 a (0.9)	20.2 (2.5)	78.5 b (1.7)	79.4 (3.0)	21.8ab (1.7)	20.6 (3.0)
	1N-MFW	54.6 b (3.5)	58.1 b (2.7)	15.8 ab (1.3)	18.7 (2.0)	73.0 b (3.3)	76.7 (4.6)	27.0 a (2.0)	23.3 (4.6)
	2N-MFW	59.0 ab (1.6)	59.6 ab (3.5)	13.3 b (0.8)	17.0 (1.6)	74.5 b (1.7)	76.6 (1.4)	25.5 a (1.7)	23.4 (1.4)
	0N	-	-	-	-	-	-	-	-
CL	1N-Chem	52.9 a (3.3)	65.9 a (0.8)	18.6 b (1.5)	19.2 b (0.9)	71.4 a (3.1)	85.0 a (1.5)	28.6 b (3.1)	15.0 c (1.5)
	0.5N-MFW	22.9 c (1.8)	50.6 b (1.7)	26.0 a (1.1)	25.8 a (1.6)	53.3 b (2.6)	76.3 b (2.0)	46.7 a (2.6)	23.7 b (2.0)
	1N-MFW	28.9 bc (2.8)	48.4 b (2.4)	24.2 a (0.5)	22.7 b (1.6)	57.1 b (2.3)	71.0 b (1.5)	42.9 a (2.3)	29.0 b (1.5)
	2N-MFW	36.8 b (3.9)	45.7 b (2.0)	18.1 b (1.8)	18.5 b (0.6)	57.9 b (3.2)	64.1 c (1.5)	42.1 a (3.2)	35.9 a (1.5)
	0N	-	-	-	-	-	-	-	-
Two-way ANOVA									
Soil		***	***	***	*	***	**	***	**
MFW		*	***	**	***	ns	***	ns	***
Soil x MFW		ns	**	ns	ns	ns	*	ns	*
Chem x MFW		***	***	**	ns	**	***	**	***
Soil x Fer. Types		ns	ns	*	ns	ns	ns	ns	ns

*Fertilizer treatments: 0N: no fertilizer; 1N-Chem: 750 mg NH₄Cl-N; 0.5N-MFW: 375 mg N as MFW; 1N-MFW: 750 mg N as MFW; and 2N as MFW: 1500 mg N as MFW. Data represent mean ± standard error (SE) from four replications, except for CL-1N-Chem in 2021 and SL-1N-MFW, SL-2N-MFW, and CL-0N treatments in 2022, with three replications. Significant differences between treatments in SL or CL soil were determined by one-way ANOVA and are indicated by lowercase letters. The asterisk represents a significant difference: *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$; ns: not significant.*

4.4. Discussion

4.4.1. Influence of soil texture and MFW rates on maize performance

Maize performance, as indicated by SPAD values, biomass, and yield, was significantly influenced by soil texture and MFW application rates under varying cultivation conditions. SPAD values decreased more rapidly in CL soil than in SL soil during both years (Figure S4.2), especially in 2021, leading to significantly lower yields in CL soil (Table 4.3). SPAD values are positively correlated with chlorophyll content and soil N status (Reis et al. 2009 ; Xiong et al. 2015; Jiang et al. 2017) and serve as a crucial indicator of N availability, which directly influences photosynthesis and plant growth (Palacio et al. 2008; Monostori et al. 2016). The reduced SPAD values in CL soil suggest lower N availability, potentially caused by significant N leaching observed during the early growth stages (0-20 days) in 2021, particularly under high water supply conditions (Figures S4.3, S4.4).

In CL soil, where NO_3^- -N dominated in leachate (Figure S4.5), high leaching was exacerbated by limited root uptake during early germination stages. Additionally, preferential water flow, which is more likely to occur in clay soils due to their structural characteristics (Dekker and Ritsema 1996), may have further contributed to the increased NO_3^- -N leaching in this soil. In contrast, SL soil retained both NH_4^+ -N and NO_3^- -N, resulting in comparatively lower leaching losses. This explains the poor yield performance in CL soil for treatments such as 0.5N-MFW and 1N-MFW in 2021. In 2022, reduced water supply during the germination stage minimized N leaching (Figures S4.2, S4.3e-h, S4.4), leading to improved N uptake and higher maize grain yields in both soil types (Wang et al. 2023) (Table 4.3). These results highlight the critical role of water management in maize performance (Govindasamy et al. 2023), particularly for CL soils, where high clay content limits N availability, contrary to findings from previous studies (Feng et al. 2016; Fang and Su 2019; Ottaiano et al. 2020).

Interestingly, maize grain yield increased linearly with MFW rates for both soil types under high leaching conditions in the 2021 season (Table S4.2), indicating that N availability from MFW effectively enhanced maize grain yield when water was not limiting (Hernández et al. 2015). However, in the 2022 season, under reduced water supply, no significant yield response to increasing MFW rates was observed in CL soil (Table S4.2), indicating diminished efficiency of MFW under controlled water conditions.

4.4.2. Impact of soil textures, fertilizer type, and cultivation conditions on N fate

My results highlighted the complex interaction between soil texture, fertilizer types, MFW application rates, and cultivation conditions in influencing the fate of inorganic N (Table 4.5). This finding is consistent with previous reports that have shown the importance of environmental conditions, management practices, and soil properties in shaping N dynamics in maize cultivation (Nissen and Wander 2003; Sileshi et al. 2010; Quan et al. 2021; Capra et al. 2023).

The highest Ndfif recovery by plants was achieved with chemical fertilizers (1N-Chem) across both soil types and seasons, exceeding values reported in previous studies (Table S4.1). In MFW treatments, Ndfif recovery by plants in SL soil remained relatively stable across application rates and cultivation years ($P > 0.05$), indicating that fertilizer type primarily determines N uptake in sandy soils. Notably, even in CL soil, where Ndfif recovery by plants was lowest (23% in 0.5N-MFW in 2021), these values were still comparable to or higher than those reported for chemical fertilizers in similar studies (Fuhrmann et al. 2018) (Table S4.1). However, in CL soil, N recovery by plants was strongly influenced by water supply, with reduced irrigation in 2022 significantly improving N recovery compared to 2021 (Tables 4.4 and 4.5). This finding aligns with Korsakov et al. (2009), who reported that N uptake is positively correlated with N rates under sufficient water supply but becomes independent of N rates when water is limited. Significant differences in N loss pathways between soil textures were observed. In 2021, high water supply during the initial growth stages caused excessive NO_3^- -N leaching (Figure S4.4), particularly in CL soil. This was likely due to the lower adsorption capacity of negatively charged clay particles for NO_3^- -N (Dixon 1991; Oelmann et al. 2007). Rapid NO_3^- -N accumulation in CL soil following fertilizer application is consistent with Sawada and Toyota (2015), who showed a positive correlation between net nitrification and soil pH after MFW addition. Additionally, higher nitrification rates in clay-rich soils likely accelerated NO_3^- -N accumulation and subsequent leaching (Aulakh et al. 1996; Dixon 1991; Oelmann et al. 2007).

NH_3 volatilization from digestate is known to be particularly high when the digestate is applied to the soil surface without incorporation (Chantigny et al., 2004; Möller, 2015). Field studies have reported NH_3 losses of up to 35% of applied NH_4^+ -N under such conditions. In contrast, incorporation into the soil, either by mixing or injection,

substantially reduces volatilization (Wulf et al. 2002). In this study, the digestate was thoroughly mixed with the soil immediately after application to simulate rapid incorporation and minimize NH_3 losses. Despite these suppression-oriented conditions, NH_3 volatilization was still identified as the dominant N loss pathway in the 2022 season, particularly in CL soil (14-22%) compared to SL soil (8 – 10%) (Table S4.1; Figure S4.6). The relatively high pH and NH_4^+ -N concentration of MFW, coupled with the higher cation exchange capacity (CEC) of CL soil, likely exacerbated NH_3 volatilization, as supported by findings from Duan and Xiao (2000), Maris et al. (2021), and Möller and Müller (2012). Although CEC and organic matter content can buffer against NH_3 loss, Liu et al. (2007) highlighted that soil pH is the dominant factor promoting volatilization. This may explain the elevated NH_3 emissions in CL soil despite its higher CEC. Notably, NH_3 volatilization was significantly lower in chemical fertilizer treatments, highlighting the unique challenges and trade-offs associated with MFW application in relation to environmental N loss.

N_2O emissions were also significantly higher in CL soil than in SL soil, particularly in 2022, and at higher MFW rates (Figure 4.2). This aligns with findings by Groenigen et al. (2004), Rochette et al. (2008), and Ottaiano et al. (2020), who reported that clay-rich soils with higher water-filled pore space promote denitrification. The presence of ammonia-oxidizing bacteria (AOB) in clay-rich soils, under conditions of high pH and water retention, further contributes to N_2O production (Capone and Montoya 2001; Law et al. 2011; Mei et al. 2018). Moreover, excessive water supply in the 2021 season not only increased NO_3^- -N leaching but also enhanced denitrification due to higher water-filled pore space in CL soil, explaining the elevated N_2O emissions compared to 2022 (Rochette et al. 2008). The emission factor observed in my study (e.g., 3.3% for 1N-Chem in 2021) exceeded the average values reported for upland fields in Japan ($0.62 \pm 0.48\%$; Akiyama et al. 2010), likely due to the high soil moisture and clay content under my experimental conditions.

The differences in water regimes between the two years significantly influenced N loss pathways. In 2021, excessive water supply caused high NO_3^- -N leaching and N_2O emissions, particularly in CL soil, but NH_3 volatilization was minimal (Figure 4.2). Conversely, reduced water supply in 2022 increased NH_3 volatilization while decreasing NO_3^- -N leaching and N_2O emissions for both soil types. These effects were more

pronounced in CL soil due to its greater capacity for retaining water and nutrients (Liu et al. 2007; Muhammad et al. 2022). Regarding maize grain yield, the high NO_3^- -N leaching in 2021 negatively impacted yields, especially in CL soil. In contrast, the high NH_3 volatilization observed in 2022 did not enhance maize grain yields, suggesting that managing water supply is essential for optimizing MFW application to balance N recovery and minimize environmental impacts.

Unobserved Ndfif losses in the 2021 season were likely attributed to NH_3 volatilization and gaps in sampling (Ju et al. 2011; Schissel and Allen 2022). Maris et al. (2021) noted that NH_3 losses during the initial pouring and mixing stages of MFW application can be substantial, particularly when trapping methods are not employed effectively. In 2022, these losses were reduced, ranging from 2% to 14% (data not shown), possibly due to better management of NH_3 volatilization during MFW application and adjustments in water supply.

Overall, SL soil exhibited lower N retention but also lower N losses through leaching and volatilization compared to CL soil. These findings highlight the importance of soil texture-specific strategies when applying MFW, with particular emphasis on managing water supply to mitigate NO_3^- -N leaching in CL soil and NH_3 volatilization in both soil types.

4.4.3. Optimum MFW rate for maize under contrasting soil textures

Except for CL in 2021, no significant differences in maize biomass or yield were observed between 1N-Chem and 1N-MFW (Table 4.4). This suggests that 1N-MFW, containing 70%–80% inorganic N, is effective in stabilizing maize grain yields across seasons, particularly in SL soil. This is consistent with Robles-Aguila et al. (2019), who reported comparable performance for biofertilizers on sandy and loamy soils.

However, increasing MFW rates caused higher N losses, mainly through NH_3 emissions, compared to chemical fertilizers (Figure 4.2). This highlights a critical trade-off between yield and environmental impact, as increasing MFW application may exacerbate environmental pollution. Furthermore, maize grain yield did not significantly differ among MFW treatments for each soil type, underscoring the need to optimize MFW rates to balance yield performance with minimized environmental losses, such as N leaching (Zheng et al. 2019) and reduced fertilization costs (Ziadi et al. 2013).

Using the min-max normalization method (Zheng et al. 2019), I identified the optimum MFW rates for each soil type by evaluating the maximum distance between biomass/yield and N loss lines (Figure 4.3). In 2021, total biomass was used as the primary indicator for determining the optimum MFW rates due to widespread yield losses in CL soil. In 2022, both biomass and yield were considered. For SL soil, the optimum rates ranged from 700 mg N pot⁻¹ in 2022 to 1000 mg N pot⁻¹ in 2021. In 2021, under high irrigation volumes, N losses in SL soil were primarily driven by NO₃⁻-N leaching, while NH₃ volatilization and N₂O emissions were minimal. In 2022, reduced irrigation led to NH₃ volatilization becoming the dominant N loss pathway in SL soil, driven by the elevated pH and NH₄⁺-N content of MFW, factors that were common across both soil textures. These findings emphasize the importance of managing water supply in SL soil to minimize both leaching and volatilization losses. For CL soil, the optimum rates were lower than SL soil, ranging from 600 to 776 mg N pot⁻¹. In 2021, excessive irrigation during the early growth stages caused significant NO₃⁻-N leaching in CL soil, likely due to a combination of high nitrification rates and water flow through preferential pathways. In 2022, NH₃ volatilization became the dominant loss pathway in CL soil, as observed in SL soil, due to the elevated pH and NH₄⁺-N content of MFW. Across both years, N₂O emissions were consistently higher in CL soil compared to SL soil, attributed to the greater water-filled pore space and microbial activity in clay soils, conditions conducive to denitrification. These findings suggest that MFW application rates should be carefully adjusted based on soil texture and water management practices. In SL soil, MFW can be applied at rates equivalent to chemical fertilizer in SL soil, provided that irrigation is carefully managed to mitigate NH₃ volatilization under reduced water supply. In CL soil, MFW application rates should be reduced by approximately 30% compared to SL soil to minimize NO₃⁻-N leaching and gaseous N losses while achieving optimal maize grain yields. This soil-specific approach highlights the importance of tailored fertilizer management strategies to maximize the agronomic benefits of MFW while reducing its environmental footprint.

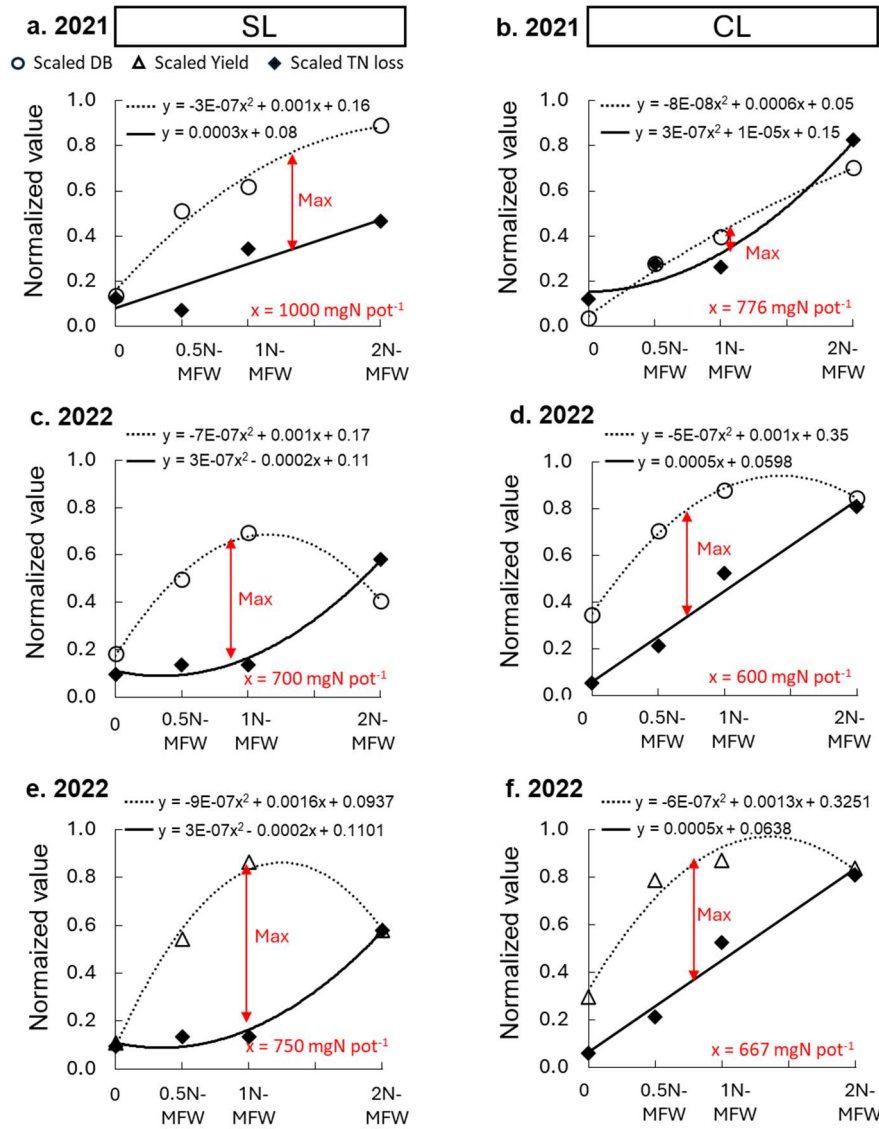


Figure 4.3. Normalization regression of total dry biomass (DB) (a-d), grain yield (e, f), and total N (TN) loss under MFW treatments in contrasting soil textures. Data are presented for sandy loam (SL; a, c, e) and clay loam (CL; b, d, f) across the 2021 (a, b) and 2022 (c–f) seasons. Fertilizer treatments: 0N: no fertilizer; 1N-Chem: 750 mg $\text{NH}_4\text{Cl-N}$; 0.5N-MFW: 375 mg N as MFW; 1N-MFW: 750 mg N as MFW; and 2N as MFW: 1500 mg N as MFW. Data represent mean $\pm$ standard error (SE) from four replications, except for SL-1N-MFW, SL-2N-MFW, and CL-0N treatments in 2022 with three replications. Normalized value was determined by the min-max normalization method to scale features to a $[0, 1]$ range, using the formula $x' = (x - \min(x)) / (\max(x) - \min(x))$, where x is an original value and x' is the normalized value. The maximum distance between the black line (normalized TN loss value) and dot lines (normalized DB/or grain yield value) was presented as the optimum MFW for each soil.

4.5. Conclusion

This study revealed that soil texture and MFW application rates significantly influence maize growth, N dynamics, and environmental impact. Maize growth and yield were generally higher in SL soil than in CL soil, regardless of fertilizer types. While MFW achieved yields and N uptake comparably to chemical fertilizers, its application must be carefully managed to minimize N losses, particularly in CL soil, where NH_3 volatilization and NO_3^- -N leaching were more pronounced at higher MFW rates.

The optimum MFW rates for maize were identified as 700–1000 mg N pot^{-1} for SL soil and 600–776 mg N pot^{-1} for CL soil under controlled water management conditions. These rates highlight the necessity of tailoring the MFW application to soil textures to achieve high crop productivity while reducing environmental losses. Notably, water management emerged as a critical factor influencing N loss pathways: excessive irrigation in 2021 amplified NO_3^- -N leaching, particularly in CL soil, while reduced irrigation in 2022 increased NH_3 volatilization in both soil types. These findings support the potential of MFW as a viable alternative to chemical fertilizers, provided its application is optimized for soil texture and irrigation practices. When applied through tailored nutrient management strategies, MFW can enhance agricultural productivity while mitigating its environmental footprint, contributing to more sustainable farming systems.

CHAPTER V

GENERAL DISCUSSION

5.1. The impact of MFW applications on crop growth and N fate under contrasting soil conditions

My results indicated that soil textures and MFW significantly influenced crop growth (Table 3.3, Table 4.4). Increasing MFW application rates significantly increased crop performance regardless of soil texture. Meanwhile, the impact of soil texture on crop growth was the opposite between sandy loam and clay loam soil, which is dependent on the cultivation conditions. Under submerged conditions, the impacts of clay loam soil on rice growth were significantly higher than those in sandy loam soil, where it was present not only in growth indexes (height and panicle numbers) but also in production index (total biomass and yield) (Table 3.3). In contrast, its impact on maize performance was significantly lower than that of sandy loam soil under aerobic conditions (Table 4.3). The effect of soil texture associated with the MFW rates had a significant impact on rice biomass (Table 3.3) and maize yield (Table 4.4) in the 2022 season, indicating that it is necessary to both soil texture and MFW application rates were vital factors to be managed to improve crop performance.

I found that rising MFW rates in clay loam soil with higher clay content significantly reduced Ndfif loss to the environment, thus improving Ndfif uptake by plants compared to that in sandy loam soil under paddy rice conditions (Chapter III). In contrast, higher clay content and rising MFW rates under upland conditions caused a significant Ndfif loss, leading to lower Ndfif uptake by plants in clay loam soil than in sandy soil (Chapter IV). Despite that, the Ndfif that remained in the soil was significantly higher in clay loam soil than in sandy loam soil, regardless of cultivation conditions or crops (Tables 3.4 and Table 4.5). That indicated the role of clay particles in retaining $\text{NH}_4\text{-N}$ in the soil (Dixon 1991; Lazaratou et al. 2020).

The results indicated that N loss from MFW application was significantly increased relative to increasing MFW rates, which was related to the characteristics of MFW and promoted by soil texture. In addition, the rapid loss of N quickly leads to a lack of N for crop performance. This was indicated by the low yield in control treatments and the lower

rate of MFW application (0.5N-MFW) for rice and maize (Table 3.3; Table 4.3), especially in the maize experiment of 2021.

The $\text{NH}_4\text{-N}$ in MFW reacted with clay particles in the clay loam soil (Dixon 1991), which could be explained by slowly releasing less N leached in clay loam soil rather than in sandy loam soil in the rice experiments (Figure S3.3 and Table 3.4). On the one hand, $\text{NH}_4\text{-N}$ in leachate was found for both soil types in rice pots, while N_2O was negligible (Fig. 3.2 c, d), indicating that the nitrification was inhibited under submerged conditions. By contrast, I observed a high amount of N leached from maize pots under upland conditions, mainly in $\text{NO}_3\text{-N}$, in clay loam soil compared to sandy loam soil (Figure S4.5). Furthermore, a further amount of N_2O was detected in my pot experiments (Figure 2). As I mentioned in Chapters I and IV, the nitrification converted $\text{NH}_4\text{-N}$ to $\text{NO}_3\text{-N}$ rapidly after fertilization in clay soil compared to sandy soil under upland conditions (Aulakh et al. 1996), and that could be improved by high soil pH (Gilmour 1984). Thus, increasing $\text{NH}_4\text{-N}$ from MFW rates combined with the high pH stimulated $\text{NO}_3\text{-N}$ to appear in clay loam soil more than in sandy loam soil. Moreover, Ottaiano et al. (2020) and Meng et al. (2022) indicated that increasing clay contents significantly increased water content retention, directly enhancing nitrification and denitrification. The high amount of $\text{NO}_3\text{-N}$ but less water porosity capacity of clay soil enhanced denitrification, where bacteria produced N_2O by utilizing oxygen from $\text{NO}_3\text{-N}$ (Law et al. 2011).

Ammonia volatilization was strongly influenced by soil type, fertilizer source, pH, soil temperature, and water regime (Liu et al. 2017). It had a higher potential to be emitted from coarser texture, low CEC, and high pH (White and Broadley 2001). However, I could not observe significantly different amounts of NH_3 volatilization on both soil types with chemical fertilizer treatments using NH_4Cl (Figure 3.2; Figure 4.2b; Figure S4.6). Moreover, NH_3 volatilization in both submerged and upland conditions was linear with MFW rates, which was more severe in clay loam soil than in sandy loam soil (Figure 3.1a,b; Figure 4.2b). He et al. (1999) reported that NH_3 volatilization from $(\text{NH}_4)_2\text{SO}_4$ could be rapidly increased by raising soil pH from 6.5 to 8.5, but it was not significantly different between 3.5 and 5.5. Therefore, I assume that the high pH of MFW (7.9-8.8) (Table 2.2), associated with the high pH of clay loam soil (pH 6.4), significantly increased the NH_3 volatilization compared to sandy loam soil (pH 5.4-5.5) (Table 2.1). Therefore,

the pH of MFW needs to be managed while using MFW for crops, especially under upland conditions.

5.2. MFW for crops serves as a fertilizer, but N loss must be managed

My results indicated that chemical fertilizers are the most effective for N use efficiency, providing the highest Ndfif recovery for crops under different cultivation conditions (Table 3.6; Table 4.5). The percentage of N recovered from chemical fertilizer by rice and maize was close to the highest record from the previous observation. Even though I conducted the amount of MFW based on total N, and the high N loss from MFW was more significant than chemical fertilizer, the yield of crops from chemical fertilizer was not significantly higher than MFW application at the same level of N input, except for 2021 in clay loam soil (Table 3.4; Table 4.4). However, the unobserved yield of maize in 1N-MFW from clay loam soil in the 2021 season indicated that if N loss from MFW was severe at an early stage, it could significantly reduce maize yield, even though the biomass in this treatment was not significantly lower than in chemical fertilizer (Table 4.4). Therefore, I assume that MFW can be used as a chemical fertilizer for both types of soil, but it requires well-managed N to ensure yield in soils with high clay content under upland conditions. The optimum amounts of MFW identified for rice and maize on sandy loam soil were 183 mg N pot⁻¹ and 750 mg N pot⁻¹, respectively, which are close to the recommended doses for rice (200 mg N) and maize (750 mg N) in Japan. Meanwhile, clay loam soil should be used with a lower amount, which was 175 mg N pot⁻¹ and 667 mg N pot⁻¹ for rice and maize, respectively.

A summary of the effects of MFW and chemical applications under different conditions on crop performance and inorganic N fate is provided in Table 5.1 and Figure 5.1. Generally, chemical fertilizers directly enhance N uptake by plants but also increase the potential for N leaching. In contrast, MFW primarily influences N emissions.

When considering the impact of soil texture, sandy loam soils lead to greater N loss during rice cultivation but result in improved maize performance and N uptake by maize. Conversely, clay loam soils enhance N retention regardless of soil conditions, whether submerged or upland conditions, and increase rice yields. However, clay loam soil also results in significant N loss under upland conditions, especially through leaching and emissions, leading to lower maize yield.

Overall, the application of rising MFW levels can boost yields but also accelerate N loss across both cultivation conditions. It was more efficient for rice in clay soil under submerged conditions, while better for maize in sandy soil under upland conditions. This highlights the need for effective management practices when utilizing MFW inputs in the field.

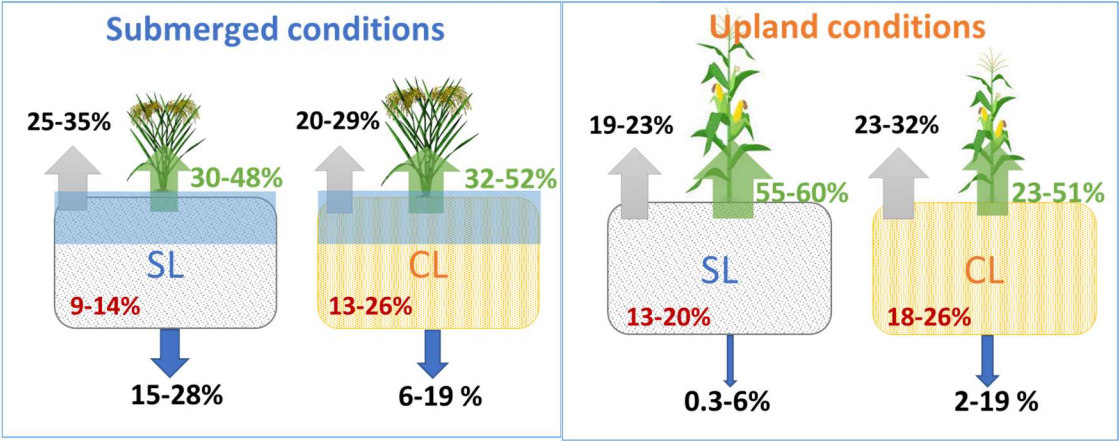


Figure 5.1. Summary of the impact of soil texture and MFW rates on crops and the fate of inorganic N under submerged and upland conditions

Table 5.1 Summary of the results representing crop performance and inorganic N distribution from fertilizer application under contrasting soil texture

Crop type	Fertilizer (mg. pot ⁻¹)	Soil texture	Dry biomass (g.pot ⁻¹)	Grain yield (g.pot ⁻¹)	N derived from fertilizer proportion (%)						Optium MFW (g.pot ⁻¹)
					N Plant uptake	N Remained in soil	N Loss				
							<i>N leached</i>	<i>NH₃</i>	<i>N₂O</i>	<i>Unknown</i>	
Rice	N-NH ₄ Cl (200mg)	Sandy loam	59.7-90.6	6.5-22.9	37.0-50.5	9.3-12.4	20.3-32.7	0.44	-	16.3-21.0	
		Clay loam	85.5-103.1	9.9-27.1	45.0-56.1	16.8-24.0	8.0-18.7	1.3	-	10.6-19.6	
	N-MFW (100 – 400 mg)	Sandy loam	53.8-87.2	5.1-24.4	30.6-48.6	8.8-14.4	15.4-28.3	0.5-0.8	-	25-35	183
		Clay loam	80.2-98.8	7.3-28.9	31.9-51.8	12.5-26.1	6.5-19.1	0.5-1.6	-	20-29	175
Maize	N-NH ₄ Cl (200mg)	Sandy loam	124-137	41.7-49.7	67–68	17–18	1.8–4.9	0–0.3	0.9–2.4	8.6–13	
		Clay loam	114-152	30.9-44.7	53–66	19	3–17	0–0.4	5.8–6.6	11–12	
	N-MFW (100 – 400 mg)	Sandy loam	96-142	24.4-52.4	55–60	13–20	0.3–5.8	8–10	0.6–3.7	11–31	750
		Clay loam	79-151	0- 43.7	23–51	18–26	2–19	14–22	4.6–8.2	2–35	667

5.3. The limitation 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 yield (Table 3.5; Table 4.3), plant Ndfun or DON in leachate (Figure 3.2), 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 for rice in submerged conditions, and the limited time for daily irrigation for maize in aerobic conditions under shallow pots may have obscured natural differences in infiltration between sandy and clay 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 clay soils may have been overestimated, as clay soils generally have lower infiltration rates than sandy soils under field conditions (Gärdenäs et al. 2005). For example, under aerobic conditions (Chapter 4), the contrasting patterns of NO_3^- -N leaching under frequent irrigation in 2021 and NH_3 volatilization under reduced watering in 2022 (Figure 4.2) were observed in CL soil more than in SL soil. 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, understanding how more realistic assessments of soil-specific infiltration and water management practices affect inorganic N distribution is necessary. This aspect warrants further investigation to resolve the uncertainties in the behaviour of unaccounted-for N.

Additionally, because fertilizers were mixed into the soil immediately after application, NH_3 volatilization was likely suppressed under my experimental conditions. In practical field situations where digestate may remain on the soil surface before incorporation, NH_3 emissions

could be higher. Therefore, my measured NH_3 losses may underestimate volatilization under typical field conditions, which likely influenced the observed N dynamics.

Future research should validate the optimum MFW rates identified in this study under field conditions, accounting for the effects of soil depth, climatic variability, and long-term organic matter accumulation. Investigations into the long-term impacts of MFW application on soil health and microbial activity will also be critical. By addressing these challenges, this research can provide the foundation for developing sustainable nutrient management strategies tailored to different soil types and environmental conditions.

CHAPTER VI

CONCLUSION AND PROSPECTIVE STUDY

6.1. Conclusions

The primary conclusion of this research underscores the intrinsic relationship between the effective and environmentally sustainable application of methane fermentation waste fluid (MFW) as a fertilizer and soil texture under contrasting soil water conditions. This relationship necessitates developing differentiated application strategies specifically tailored to accommodate various crops and soil conditions.

The findings strongly support the judicious increase of MFW application rates for paddy rice cultivated under submerged conditions, particularly in clay loam soils. The data on N fate demonstrate that as MFW rates increase, CL soils exhibit a superior capacity for retaining N-MFW and facilitating plant uptake. This starkly contrasts with sandy loam soils, where increasing MFW rates lead to proportionally higher leaching losses, significantly compromising nitrogen use efficiency and increasing environmental risk.

By contrast, in upland conditions, sandy loam soils promote a higher overall recovery of applied N by the soil-plant system, ranging from 73% to 79% total N recovery by maize. This observation underscores the potential for efficient N uptake in sandy loam soils, which have a lower N loss (gas emission and leaching) potential than clay loam soils with rising MFW, even though clay loam soils have high N retention. Consequently, while the application of MFW can be advantageous for maize in sandy loam soils, it is essential to implement strategic timing and application rates to optimize N uptake while minimizing leaching.

The distinct N fate patterns between different soil textures for rice and maize highlight the shortcomings of a generalized approach to applying MFW. To optimize agronomic outcomes and mitigate environmental impacts, it is essential to customize MFW application rates and management techniques according to the specific soil texture and the needs of the target crop. Such a soil-specific methodology is crucial for implementing MFW into agricultural nutrient management strategies.

6.2. Prospective Study

Building upon the insights gained from this study, which was conducted under controlled pot experiment conditions, several avenues for future research are apparent to address the inherent

limitations and expand the applicability of these findings. Future investigations should be upscaled to field-scale experiments across a wider range of environmental conditions and management practices to better reflect real-world agricultural scenarios. Long-term studies are also crucial to understanding the cumulative effects of repeated MFW applications on soil fertility, carbon sequestration, and N cycling dynamics over multiple cropping seasons. Furthermore, exploring the responses of a more diverse set of crop rotations and the interactions of MFW with other organic amendments or cover cropping systems in different soil textures would provide more comprehensive recommendations for sustainable nutrient management. Addressing these areas in future research will be vital for translating the findings from this controlled study into practical, widespread, and environmentally sound agricultural applications of MFW.

REFERENCES

- Abdel-Shafy, H. I., and M. S. M. Mansour. 2018. "Solid Waste Issue: Sources, Composition, Disposal, Recycling, and Valorization." *Egyptian Journal of Petroleum* 27 (4): 1275-1290. <https://doi.org/10.1016/j.ejpe.2018.07.003>.
- Abubaker, J., H. Cederlund, V. Arthurson, and M. Pell. 2013. "Bacterial Community Structure and Microbial Activity in Different Soils Amended with Biogas Residues and Cattle Slurry." *Applied Soil Ecology* 72: 171-180. <https://doi.org/10.1016/j.apsoil.2013.07.002>.
- Abubaker, J., K. Risberg, E. Jönsson, A. S. Dahlin, H. Cederlund, and M. Pell. 2015. "Short-Term Effects of Biogas Digestates and Pig Slurry Application on Soil Microbial Activity." *Applied and Environmental Soil Science, Hindawi*, 2015: 15. <https://doi.org/10.1155/2015/658542>.
- Akhiar, A. 2017. "Characterization of Liquid Fraction of Digestates After Solid-Liquid Separation from Anaerobic Co-Digestion Plants". *PhD Diss.*, Chemical and Process Engineering. Université Montpellier, 2017. English.
- Akiyama, H., I. P. McTaggart, B. C. Ball, and A. Scott. 2004. "N₂O, NO, and NH₃ Emissions from Soil After the Application of Organic Fertilizers, Urea and Water." *Water, Air, and Soil Pollution* 156 (1): 113-129. <https://doi.org/10.1023/B:WATE.0000036800.20599.46>.
- Alburquerque, J. A., C. de la Fuente, M. Campoy, L. Carrasco, I. Nájera, C. Baixauli, F. Caravaca, A. Roldán, J. Cegarra, and M. P. Bernal. 2012. "Agricultural Use of Digestate for Horticultural Crop Production and Improvement of Soil Properties." *The European Journal of Agronomy* 43: 119–128. <https://doi.org/10.1016/j.eja.2012.06.001>.
- Alhaj Hamoud, Y., X. Guo, Z. Wang, H. Shaghaleh, S. Chen, A. Hassan, and A. Bakour. 2019. "Effects of Irrigation Regime and Soil Clay Content and Their Interaction on the Biological Yield, Nitrogen Uptake and Nitrogen-Use Efficiency of Rice Grown in Southern China." *Agricultural Water Management* 213 (March): 934-946. <https://doi.org/10.1016/j.agwat.2018.12.017>.
- Allison, F. E. 1966. "The Fate of Nitrogen Applied to Soils." *Advances in Agronomy* 18: 219-258. [https://doi.org/10.1016/S0065-2113\(08\)60651-3](https://doi.org/10.1016/S0065-2113(08)60651-3).
- Anaba, B. D., M. Yemefack, M. Abossolo-Angue, et al. 2020. "Soil Texture and Watering Impact on Pot Recovery of Soil-Stripped Oil Palm (*Elaeis Guineensis* Jacq.) Seedlings." *Heliyon* 6 (10): e05310. <https://doi.org/10.1016/j.heliyon.2020.e05310>.

- Anas, M., F. Liao, K. K. Verma, M. A. Sarwar, A. Mahmood, Z. L. Chen, Q. Li, X. P. Zeng, Y. Liu, and Y. R. Li. 2020. "Fate of Nitrogen in Agriculture and Environment: Agronomic, Eco-Physiological and Molecular Approaches to Improve Nitrogen Use Efficiency." *Biological Research* 53 (1): 47. <https://doi.org/10.1186/s40659-020-00312-4>.
- APHA, American Water Works Association, and Water Pollution Control Federation. 1989. *Standard Methods for the Examination of Water and Wastewater*. 17th ed. Washington, DC: American Public Health Association. <https://tamug-ir.tdl.org/handle/1969.3/24401>.
- Aulakh, M., K. Singh, B. Singh, and J. Doran. 1996. "Kinetics of Nitrification Under Upland and Flooded Soils of Varying Texture." *Communications in Soil Science and Plant Analysis* 27 (9-10): 2079-2089. <https://doi.org/10.1080/00103629609369689>.
- Baloch, M. S., I. U. Awan, and G. Hassan. 2006. "Growth and Yield of Rice as Affected by Transplanting Dates and Seedlings per Hill Under High Temperature of Dera Ismail Khan, Pakistan." *Journal of Zhejiang University-Science B* 7 (7): 572-579. <https://doi.org/10.1631/jzus.2006.B0572>.
- Barbieri, P. A., H. E. Echeverría, H. R. Sainz Rozas, and F. H. Andrade. 2008. "Nitrogen Use Efficiency in Maize as Affected by Nitrogen Availability and Row Spacing." *Agronomy Journal* 100 (4): 1094–1100. <https://doi.org/10.2134/agronj2006.0057>.
- Baştabak, B., and G. Koçar. 2020. "A Review of the Biogas Digestate in Agricultural Framework." *Journal of Material Cycles and Waste Management* 22 (5): 1318-1327. <https://doi.org/10.1007/s10163-020-01056-9>.
- Battye, R., W. Battye, S. Fudge, and C. Overcash. 1994. "Development and Selection of Ammonia Emission Factors". *Final Report*, Atmospheric Research and Exposure Assessment Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina 27711: 111.
- Bin Rahman, A. N. M. R., and J. Zhang. 2023. "Trends in Rice Research: 2030 and Beyond." *Food and Energy Security* 12 (2): e390. <https://doi.org/10.1002/fes3.390>.
- Boer, H. C. de. 2008. "Co-Digestion of Animal Slurry Can Increase Short-Term Nitrogen Recovery by Crops." *Journal of Environmental Quality* 37 (5): 1968-1973. <https://doi.org/10.2134/jeq2007.0594>.

- Butterbach-Bahl, K., E. M. Baggs, M. Dannenmann, R. Kiese, and S. Zechmeister-Boltenstern. 2013. "Nitrous Oxide Emissions from Soils: How Well Do We Understand the Processes and Their Controls?" *Philosophical Transactions of the Royal Society B: Biological Sciences* 368 (1621): 20130122. <https://doi.org/10.1098/rstb.2013.0122>.
- Cai, Z., G. Xing, X. Yan, H. Xu, H. Tsuruta, K. Yagi, and K. Minami. 1997. "Methane and Nitrous Oxide Emissions from Rice Paddy Fields as Affected by Nitrogen Fertilisers and Water Management." *Plant and Soil* 196 (1): 7-14. <https://doi.org/10.1023/A:1004263405020>.
- Cambouris, A. N., N. Ziadi, I. Perron, K. D. Alotaibi, M. St. Luce, and N. Tremblay. 2016. "Corn Yield Components Response to Nitrogen Fertilizer as a Function of Soil Texture." *Canadian Journal of Soil Science* 96 (4): 386-399. <https://doi.org/10.1139/cjss-2015-0134>.
- Capone, D. G., and J. P. Montoya. 2001. "Nitrogen Fixation and Denitrification." *Marine Microbiology* 30: 505-515. [https://doi.org/10.1016/S0580-9517\(01\)30060-0](https://doi.org/10.1016/S0580-9517(01)30060-0).
- Capra, F., D. Abalos, S. C. Maris, F. Ardenti, M. Lommi, V. Tabaglio, and A. Fiorini. 2023. "Towards Efficient N Cycling in Intensive Maize: Role of Cover Crops and Application Methods of Digestate Liquid Fraction." *GCB Bioenergy* 15 (7): 867-885. <https://doi.org/10.1111/gcbb.13036>.
- Cardoso, E. J. B., R. L. F. Vasconcellos, D. Bini, M. Y. H. Miyauchi, C. A. Santos, P. R. L. Alves, A. M. Paula, A. S. Nakatani, J. M. Pereira, and M. A. Nogueira. 2013. "Soil Health: Looking for Suitable Indicators. What Should Be Considered to Assess the Effects of Use and Management on Soil Health?" *Scientia Agricola* 70 (4): 274-289. <https://doi.org/10.1590/S0103-90162013000400009>.
- Carpenter, S. R. 2005. "Eutrophication of Aquatic Ecosystems: Bistability and Soil Phosphorus." *Biological Sciences. Proceedings of the National Academy of Sciences* 102 (29): 10002-10005. <https://doi.org/10.1073/pnas.0503959102>.
- Cassity - Duffey, K., M. Cabrera, D. Franklin, J. Gaskin, and D. Kissel. 2020. "Effect of Soil Texture on Nitrogen Mineralization from Organic Fertilizers in Four Common Southeastern Soils." *Soil Science Society of America Journal* 84 (2): 534-542. <https://doi.org/10.1002/saj2.20039>.

- Castellano, M. J., D. B. Lewis, and J. P. Kaye. 2013. "Response of Soil Nitrogen Retention to the Interactive Effects of Soil Texture, Hydrology, and Organic Matter." *Journal of Geophysical Research: Biogeosciences* 118 (1): 280-290.
<https://doi.org/10.1002/jgrg.20015>.
- Cb, O., S. Ho, and A. Go. 2018. "Effect of Liquid Digestate on Agricultural Soil-II: Microbial Population Dynamics." *Applied Microbiology* 4 (1): 1000145.
<https://doi.org/10.4172/2471-9315.1000145>.
- Chalk, P., A. Magalhães, and C. Inácio. 2012. "Towards an Understanding of the Dynamics of Compost N in the Soil-Plant-Atmosphere System Using ^{15}N Tracer." *Plant and Soil* 362 (1-2): 373-388. <https://doi.org/10.1007/s11104-012-1358-5>.
- Chantigny, M. H., P. Rochette, D. A. Angers, D. Massé, and D. Côté. 2004. "Ammonia Volatilization and Selected Soil Characteristics Following Application of Anaerobically Digested Pig Slurry." *Soil Science Society of America Journal* 68 (1): 306–312.
<https://doi.org/10.2136/sssaj2004.3060>.
- Chen, D., L. Jiang, H. Huang, K. Toyota, R. A. Dahlgren, and J. Lu. 2012. "Nitrogen Dynamics of Anaerobically Digested Slurry Used to Fertilize Paddy Fields." *Biology and Fertility of Soils* 49 (6): 647-659. <https://doi.org/10.1007/s00374-012-0752-8>.
- Chen, G., G. Zhao, H. Zhang, Y. Shen, H. Fei, and W. Cheng. 2017. "Biogas Slurry Use as N Fertilizer for Two-Season *Zizania Aquatica* Turcz. in China." *Nutrient Cycling in Agroecosystems* 107 (3): 303-320. <https://doi.org/10.1007/s10705-017-9831-4>.
- Cheng, J., Y. Chen, T. He, R. Liu, M. Yi, and Z. Yang. 2018. "Nitrogen Leaching Losses Following Biogas Slurry Irrigation to Purple Soil of the Three Gorges Reservoir Area." *Environmental Science and Pollution Research* 25 (29): 29096-29103.
<https://doi.org/10.1007/s11356-018-2875-4>.
- Cheng, J., Y. Chen, T. He, R. Liu, M. Yi, and Z. Yang. 2020. "Effects of Biogas Slurry Irrigation DOC/N Ratios on the Fate of Soil Nitrogen and GHG Emissions: A Laboratory Study." *Geoderma* 375 (October): 114458.
<https://doi.org/10.1016/j.geoderma.2020.114458>.
- Coelho, J. J., A. Hennessy, I. Casey, C. R. S. Bragança, T. Woodcock, and N. Kennedy. 2020. "Biofertilisation with Anaerobic Digestates: A Field Study of Effects on Soil Microbial Abundance and Diversity." *Applied Soil Ecology* 147: 103403.
<https://doi.org/10.1016/j.apsoil.2019.103403>.

- Craswell, E., and P. Vlek. 1983. "Fate of Fertilizer Nitrogen Applied to Wetland Rice." *Gaseous Loss of Nitrogen from Plant-Soil Systems* Book chapter: 237-264. https://doi.org/10.1007/978-94-017-1662-8_10.
- de Boer, H. C. 2008. "Co-Digestion of Animal Slurry Can Increase Short-Term Nitrogen Recovery by Crops." *Journal of Environmental Quality* 37 (5): 1968-1973. <https://doi.org/10.2134/jeq2007.0594>.
- de Vries, M. P. C. 1980. "How Reliable are Results of Pot Experiments?" *Communications in Soil Science & Plant Analysis* 11 (9): 895-902. <https://doi.org/10.1080/00103628009367090>.
- de Vries, W. 2021. "Impacts of Nitrogen Emissions on Ecosystems and Human Health: A Mini Review." *Current Opinion in Environmental Science & Health* 21: 100249. <https://doi.org/10.1016/j.coesh.2021.100249>.
- Dekker, L., and C. Ritsema. 1996. "Preferential Flow Paths in a Water Repellent Clay Soil with Grass Cover." *Water Resources Research* 32 (5): 1239-1249. <https://doi.org/10.1029/96wr00267>.
- Dicke, C., J. Andert, C. Ammon, J. Kern, A. Meyer-Aurich, and M. Kaupenjohann. 2015. "Effects of Different Biochars and Digestate on N₂O Fluxes Under Field Conditions." *Science of the Total Environment* 524-525: 310-318. <https://doi.org/10.1016/j.scitotenv.2015.04.005>.
- Ding, W., S. Li, P. He, S. Huang, and D. Sihi. 2019. "Contribution and Fate of Maize Residue-¹⁵N and Urea-¹⁵N as Affected by N Fertilization Regime." *PLOS ONE* 14 (1): 0210176. <https://doi.org/10.1371/journal.pone.0210176>.
- Dixon, J. B. 1991. "Roles of Clays in Soils." *Applied Clay Science* 5 (5-6): 489-503. [https://doi.org/10.1016/0169-1317\(91\)90019-6](https://doi.org/10.1016/0169-1317(91)90019-6).
- Dou, F., J. Soriano, R. E. Tabien, and K. Chen. 2016. "Soil Texture and Cultivar Effects on Rice (*Oryza Sativa*, L.) Grain Yield, Yield Components and Water Productivity in Three Water Regimes." *PLOS ONE* 11 (3): e0150549. <https://doi.org/10.1371/journal.pone.0150549>.
- Du, H., W. Gao, J. Li, S. Shen, F. Wang, L. Fu, and K. Zhang. 2019. "Effects of Digested Biogas Slurry Application Mixed with Irrigation Water on Nitrate Leaching During Wheat-Maize Rotation in the North China Plain." *Agricultural Water Management* 213:882-893. <https://doi.org/10.1016/j.agwat.2018.12.012>.

- Duan, Z., and H. Xiao. 2000. "Effects of Soil Properties on Ammonia Volatilization." *Soil Science and Plant Nutrition* 46 (4): 845-852.
<https://doi.org/10.1080/00380768.2000.10409150>.
- Erenstein, O., M. Jaleta, K. Sonder, K. Mottaleb, and B. M. Prasanna. 2022. "Global Maize Production, Consumption and Trade: Trends and R&D Implications." *Food Security* 14 (5): 1295-1319. <https://doi.org/10.1007/s12571-022-01288-7>.
- Fan, X. H., Y. C. Li, and A. K. Alva. 2011. "Effects of Temperature and Soil Type on Ammonia Volatilization from Slow-Release Nitrogen Fertilizers." *Communications in Soil Science & Plant Analysis* 42 (10): 1111-1122.
<https://doi.org/10.1080/00103624.2011.566957>.
- Fang, J., and Y. Z. Su. 2019. "Effects of Soils and Irrigation Volume on Maize Yield, Irrigation Water Productivity, and Nitrogen Uptake." *Scientific Reports* 9 (1): 7740.
<https://doi.org/10.1038/s41598-019-41447-z>.
- FAO. 1986. "*The International Support Programme for Irrigation Water Management, Land and Water Development Division.*" Irrigation Water Management: Irrigation Water Needs, FAO. <https://www.fao.org/3/S2022E/s2022e00.htm#Contents>.
- FAO. 2023. *World Food and Agriculture – Statistical Yearbook 2023*. Rome.
<https://doi.org/10.4060/cc8166en>.
- Feng, G., Y. Zhang, Y. Chen, Q. Li, F. Chen, Q. Gao, and G. Mi. 2016. "Effects of Nitrogen Application on Root Length and Grain Yield of Rain-Fed Maize Under Different Soil Types." *Agronomy Journal* 108 (4): 1656-1665.
<https://doi.org/10.2134/agronj2015.0367>.
- Ferguson, R. M. W., F. Coulon, and R. Villa. 2016. "Organic Loading Rate: A Promising Microbial Management Tool in Anaerobic Digestion." *Water Research* 100: 348-356.
<https://doi.org/10.1016/j.watres.2016.05.009>.
- Flores, P., I. Castellar, and J. Navarro. 2005. "Nitrate Leaching in Pepper Cultivation with Organic Manure and Supplementary Additions of Mineral Fertilizer." *Communications in Soil Science & Plant Analysis* 36 (19-20): 2889-2898.
<https://doi.org/10.1080/00103620500306072>.
- Frac, M., and K. Ziemiński. 2012. "Methane Fermentation Process for Utilization of Organic Waste." *International Agrophysics*, 26 (3): 317-330. <https://doi.org/10.2478/v10247-012-0045-3>.

- Fuhrmann, I., Y. He, E. Lehndorff, N. Brüggemann, W. Amelung, R. Wassmann, and J. Siemens. 2018. "Nitrogen Fertilizer Fate After Introducing Maize and Upland-Rice into Continuous Paddy Rice Cropping Systems." *Agriculture, Ecosystems & Environment* 258: 162-171. <https://doi.org/10.1016/j.agee.2018.02.021>.
- Gärdenäs, A. I., P. J. Hanson, D. E. Todd, and J. M. Warren. 2005. "Influence of Soil Texture and Precipitation on Soil Carbon and Nitrogen Dynamics in Southern Appalachian Forests." *Forest Ecology & Management* 220 (1-3): 301-314. <https://doi.org/10.1016/j.foreco.2005.08.001>.
- Ge, S., Z. Zhu, Y. Jiang, S. Ge, Z. Zhu, and Y. Jiang. 2018. "Long-Term Impact of Fertilization on Soil pH and Fertility in an Apple Production System." *Journal of Soil Science and Plant Nutrition* 18 (1): 282-293. <https://doi.org/10.4067/S0718-95162018005001002>.
- Geng, Y., G. Cao, L. Wang, and S. Wang. 2019. "Effects of Equal Chemical Fertilizer Substitutions with Organic Manure on Yield, Dry Matter, and Nitrogen Uptake of Spring Maize and Soil Nitrogen Distribution." *PLOS ONE* 14 (7): e0219512. <https://doi.org/10.1371/journal.pone.0219512>.
- Ghoneim, A., H. Ueno, A. Ebid, N. Asagi, and I. Abou El. 2008. "Analysis of Nitrogen Dynamics and Fertilizer Use Efficiency in Rice Using the Nitrogen-15 Isotope Dilution Method Following the Application of Biogas Slurry or Chemical Fertilizer." *International Journal of Soil Science* 3 (1): 11-19. <https://doi.org/10.3923/ijss.2008.11.19>.
- Gilmour, J. T. 1984. "The Effects of Soil Properties on Nitrification and Nitrification Inhibition." *Soil Science Society of America Journal* 48 (6): 1262-1266. <https://doi.org/10.2136/sssaj1984.03615995004800060012x>.
- Govindasamy, P., S. Muthusamy, M. Bagavathiannan, J. Mowrer, P. Jagannadham, A. Maity, H. M. Halli, et al. 2023. "Nitrogen Use Efficiency-A Key to Enhance Crop Productivity Under a Changing Climate." *Frontiers in Plant Science* 14:1121073. <https://doi.org/10.3389/fpls.2023.1121073>.
- Grant, C. A., R. Wu, F. Selles, K. N. Harker, G. W. Clayton, S. Bittman, B. J. Zebarth, and N. Z. Lupwayi. 2012. "Crop Yield and Nitrogen Concentration with Controlled Release Urea and Split Applications of Nitrogen as Compared to Non-Coated Urea Applied at Seeding." *Field Crops Research* 127: 170-180. <https://doi.org/10.1016/j.fcr.2011.11.002>.

- Grave, R. A., R. S. Nicoloso, P. C. Cassol, et al. 2018. "Determining the Effects of Tillage and Nitrogen Sources on Soil N₂O Emission." *Soil and Tillage Research* 175: 1-12.
<https://doi.org/10.1016/j.still.2017.08.011>.
- Groenigen, J. W. van, G. J. Kasper, G. L. Velthof, A. van den Pol-van Dasselaar, and P. J. Kuikman. 2004. "Nitrous Oxide Emissions from Silage Maize Fields under Different Mineral Nitrogen Fertilizer and Slurry Applications." *Plant and Soil* 263 (1): 101-111.
<https://doi.org/10.1023/B:PLSO.0000047729.43185.46>.
- Gu, B., D. Chen, Y. Yang, P. Vitousek, and Y. Zhu. 2021. "Soil-Food-Environment-Health Nexus for Sustainable Development." *Research* 2021: 9804807.
<https://doi.org/10.34133/2021/9804807>.
- Hansen, B., E. S. Kristensen, R. Grant, H. Høgh-Jensen, S. E. Simmelsgaard, and J. E. Olesen. 2000. "Nitrogen Leaching from Conventional versus Organic Farming Systems - a Systems Modelling Approach." *The European Journal of Agronomy* 13 (1): 65-82.
[https://doi.org/10.1016/S1161-0301\(00\)00060-5](https://doi.org/10.1016/S1161-0301(00)00060-5).
- Haraldsen, T. K., U. Andersen, T. Krogstad, and R. Sørheim. 2011. "Liquid Digestate from Anaerobic Treatment of Source-Separated Household Waste as Fertilizer to Barley." *Waste Management & Research* 29 (12): 1271-1276.
<https://doi.org/10.1177/0734242X11411975>.
- Hassink, J., L. A. Bouwman, K. B. Zwart, J. Bloem, and L. Brussaard. 1993. "Relationships Between Soil Texture, Physical Protection of Organic Matter, Soil Biota, and C and N Mineralization in Grassland Soils." *Geoderma* 57 (1): 105-128.
[https://doi.org/10.1016/0016-7061\(93\)90150-J](https://doi.org/10.1016/0016-7061(93)90150-J).
- Hayashi, K., S. Nishimura, and K. Yagi. 2006. "Ammonia Volatilization from the Surface of a Japanese Paddy Field During Rice Cultivation." *Soil Science and Plant Nutrition* 52 (4): 545-555. <https://doi.org/10.1111/j.1747-0765.2006.00053.x>.
- He, Z., A. Kumar, D. Calvert, and D. Banks. 1999. "Ammonia Volatilization from Different Fertilizer Sources and Effects of Temperature and Soil pH." *Soil Science* 164 (10): 750-758. <https://doi.org/10.1097/00010694-199910000-00006>.
- Hernández, M., L. Echarte, A. Della Maggiora, M. Cambareri, P. Barbieri, and D. Cerrudo. 2015. "Maize Water Use Efficiency and Evapotranspiration Response to N Supply Under Contrasting Soil Water Availability." *Field Crops Research* 178: 8-15.
<https://doi.org/10.1016/j.fcr.2015.03.017>.

- Herrmann, A., K. Sieling, B. Wienforth, F. Taube, and H. Kage. 2012. "Short-Term Effects of Biogas Residue Application on Yield Performance and N Balance Parameters of Maize in Different Cropping Systems." *The Journal of Agricultural Science* 151 (4): 449-462. <https://doi.org/10.1017/S0021859612000548>.
- Holub, S. M., and K. Lajtha. 2004. "The Fate and Retention of Organic and Inorganic ¹⁵N-Nitrogen in an Old-Growth Forest Soil in Western Oregon." *Ecosystems* 7 (4): 368-380. <https://doi.org/10.1007/s10021-004-0239-z>.
- Hong, S. G., J. D. Shin, S. I. Kwon, W. K. Park, D. B. Lee, and J. G. Kim. 2010. "Effects of Co-Digestate Application on the Soil Properties, Leachate and Growth Responses of Paddy Rice." *Journal of the Korea Organic Resources Recycling Association* 18 (4): 31-37. <https://doi.org/10.17137/korrae.2010.18.4.31>
- Hou, H., S. Zhou, M. Hosomi, K. Toyota, K. Yosimura, Y. Mutou, T. Nisimura, M. Takayanagi, and T. Motobayashi. 2007. "Ammonia Emissions from Anaerobically-Digested Slurry and Chemical Fertilizer Applied to Flooded Forage Rice." *Water, Air, and Soil Pollution* 183 (1): 37-48. <https://doi.org/10.1007/s11270-007-9353-9>.
- Hu, B., L. Shen, X. Xu, and P. Zheng. 2011. "Anaerobic Ammonium Oxidation (Anammox) in Different Natural Ecosystems." *Biochemical Society Transactions* 39 (6): 1811-1816. <https://doi.org/10.1042/BST20110711>.
- Hu, X., H. Liu, C. Xu, X. Huang, M. Jiang, H. Zhuang, and L. Huang. 2021. "Effect of Digestate and Straw Combined Application on Maintaining Rice Production and Paddy Environment." *International Journal of Environmental Research and Public Health* 18 (11): 5714. <https://doi.org/10.3390/ijerph18115714>.
- Hu, Z., J. W. Lee, K. Chandran, S. Kim, and S. K. Khanal. 2012. "Nitrous Oxide (N₂O) Emission from Aquaculture: A Review." *Environmental Science & Technology* 46 (12): 6470-6480. <https://doi.org/10.1021/es300110x>.
- Ichinose, Y., Y. Ise, T. Kanda, Y. Maejima, T. Yagi, Y. Takahashi, S. Nakagawa, et al. 2023. "Factors Controlling Available Soil Nitrogen in Japanese Paddy Fields." *Soil Science and Plant Nutrition* 69 (5-6): 303-314. <https://doi.org/10.1080/00380768.2023.2231013>.
- Iocoli, G. A., M. C. Zabaloy, G. Pasdevicelli, and M. A. Gómez. 2019. "Use of Biogas Digestates Obtained by Anaerobic Digestion and Co-Digestion as Fertilizers: Characterization, Soil Biological Activity and Growth Dynamic of *Lactuca Sativa* L." *Science of the Total Environment* 647: 11-19. <https://doi.org/10.1016/j.scitotenv.2018.07.444>.

- Ji, X. H., S. X. Zheng, L. H. Shi, and Z. B. Liu. 2011. "Systematic Studies of Nitrogen Loss from Paddy Soils Through Leaching in the Dongting Lake Area of China." *Pedosphere* 21 (6): 753-762. [https://doi.org/10.1016/S1002-0160\(11\)60179-3](https://doi.org/10.1016/S1002-0160(11)60179-3).
- Jiang, C., M. Johkan, M. Hohjo, S. Tsukagoshi, and T. Maruo. 2017. "A Correlation Analysis on Chlorophyll Content and SPAD Value in Tomato Leaves." *Horticulture Research* 71: 37-42. <https://doi.org/10.20776/S18808824-71-P37>.
- Jimenez, J., M. Grigatti, E. Boanini, D. Patureau, and N. Bernet. 2020. "The Impact of Biogas Digestate Typology on Nutrient Recovery for Plant Growth: Accessibility Indicators for First Fertilization Prediction." *Waste Management* 117 (2020): 18-31. <https://doi.org/10.1016/j.wasman.2020.07.052>.
- Jokela, W. E., and G. W. Randall. 1997. "Fate of Fertilizer Nitrogen as Affected by Time and Rate of Application on Corn." *Soil Science Society of America Journal* 61 (6): 1695-1703. <https://doi.org/10.2136/sssaj1997.03615995006100060022x>.
- JORA (Japan Organics Recycling Association). 2023. "Case Studies of Utilization of Digestate as Fertilizer (In Japanese)." https://www.jora.jp/wp-content/uploads/2024/04/baioekihijireisyu_hiryo2023_rev.pdf
- Ju, X., X. Lu, Z. Gao, X. Chen, F. Su, M. Kogge, V. Römheld, P. Christie, and F. Zhang. 2011. "Processes and Factors Controlling N₂O Production in an Intensively Managed Low Carbon Calcareous Soil Under Sub-Humid Monsoon Conditions." *Environmental Pollution* 159 (4): 1007-1016. <https://doi.org/10.1016/j.envpol.2010.10.040>.
- Kanbar, A., M. Toorchi, and H. E. Shashidhar. 2009. "Relationship Between Root and Yield Morphological Characters in Rainfed Low Land Rice (*Oryza Sativa* L.)." *Cereal Research Communications* 37 (2): 261-268. <https://doi.org/10.1556/CRC.37.2009.2.14>.
- Kandel, B. P. 2020. "Spad Value Varies with Age and Leaf of Maize Plant and Its Relationship with Grain Yield." *BMC Research Notes* 13 (1): 475. <https://doi.org/10.1186/s13104-020-05324-7>.
- Kim, S. U., H. H. Lee, S. M. Moon, H. R. Han, and C. O. Hong. 2021. "Nitrous Oxide Emissions and Maize Yield as Influenced by Nitrogen Fertilization and Tillage Operations in Upland Soil." *Applied Biological Chemistry* 64 (1): 18. <https://doi.org/10.1186/s13765-021-00593-7>.
- Law, Y., P. Lant, and Z. Yuan. 2011. "The Effect of pH on N₂O Production Under Aerobic Conditions in a Partial Nitrification System." *Water Research* 45 (18): 5934-5944. <https://doi.org/10.1016/j.watres.2011.08.055>.

- Lazaratou, C. V., D. V. Vayenas, and D. Papoulis. 2020. "The Role of Clays, Clay Minerals and Clay-Based Materials for Nitrate Removal from Water Systems: A Review." *Applied Clay Science* 185: 105377. <https://doi.org/10.1016/j.clay.2019.105377>.
- Li, K., T. Inamura, and M. Umeda. 2003. "Growth and Nitrogen Uptake of Paddy Rice as Influenced by Fermented Manure Liquid and Squeezed Manure Liquid." *Soil Science and Plant Nutrition* 49 (3): 463-467. <https://doi.org/10.1080/00380768.2003.10410033>.
- Li, X., Y. Wang, G. Feng, Z. Xu, F. Meng, and Q. Gao. 2023. "Differential Fertilizer Nitrogen Fates in Maize Cropping System Among Three Soil Textures Based on ^{15}N ." *Field Crops Research* 291:108780. <https://doi.org/10.1016/j.fcr.2022.108780>.
- Liang, G. 2022. "Nitrogen Fertilization Mitigates Global Food Insecurity by Increasing Cereal Yield and Its Stability." *Global Food Security* 34: 100652. <https://doi.org/10.1016/j.gfs.2022.100652>.
- Liu, D., Y. Fang, Y. Tu, and Y. Pan. 2014. "Chemical Method for Nitrogen Isotopic Analysis of Ammonium at Natural Abundance." *Analytical Chemistry* 86 (8): 3787-3792. <https://doi.org/10.1021/ac403756u>.
- Liu, G., Y. Li, and A. K. Alva. 2007. "High Water Regime Can Reduce Ammonia Volatilization from Soils Under Potato Production." *Communications in Soil Science and Plant Analysis* 38 (9-10): 1203-1220. <https://doi.org/10.1080/00103620701328289>.
- Liu, G., Y. Li, K. W. Migliaccio, Y. Ouyang, and A. K. Alva. 2017. "Identification of Factors Most Important for Ammonia Emission from Fertilized Soils for Potato Production Using Principal Component Analysis." *Journal of Sustainable Watershed Science and Management* 1 (1): 21-30. <https://doi.org/10.5147/jsWSM.v1i1.130>.
- Liu, X., H. Wang, J. Zhou, et al. 2016. "Effect of N Fertilization Pattern on Rice Yield, N Use Efficiency and Fertilizer-N Fate in the Yangtze River Basin, China." *PLOS ONE* 11 (11): e0166002. <https://doi.org/10.1371/journal.pone.0166002>.
- Logan, M., and C. Visvanathan. 2019. "Management Strategies for Anaerobic Digestate of Organic Fraction of Municipal Solid Waste: Current Status and Future Prospects." *Waste Management & Research* 37 (1): 27-39. <https://doi.org/10.1177/0734242X18816793>.
- Makádi, M., T. Szegi, A. Tomócsik, V. Orosz, E. Michéli, A. Ferenczy, K. Posta, and B. Biró. 2016. "Impact of Digestate Application on Chemical and Microbiological Properties of Two Different Textured Soils." *Communications in Soil Science and Plant Analysis* 47 (2): 167-178. <https://doi.org/10.1080/00103624.2015.1109652>.

- Malone, T. C., and A. Newton. 2020. "The Globalization of Cultural Eutrophication in the Coastal Ocean: Causes and Consequences." *Frontiers in Marine Science* 7 (670): 1-30. <https://doi.org/10.3389/fmars.2020.00670>.
- Maris, S. C., F. Capra, F. Ardeni, R. Boselli, D. Pochintesta, G. M. Beone, V. Tabaglio, and A. Fiorini. 2021. "The Interaction Between Types of Cover Crop Residue and Digestate Application Methods Affects Ammonia Volatilization During Maize Cropping Season." *Journal of Environmental Quality* 50 (2): 504-512. <https://doi.org/10.1002/jeq2.20205>.
- Mei, K., Z. Wang, H. Huang, C. Zhang, X. Shang, R. A. Dahlgren, M. Zhang, and F. Xia. 2018. "Stimulation of N₂O Emission by Conservation Tillage Management in Agricultural Lands: A Meta-Analysis." *Soil and Tillage Research* 182: 86-93. <https://doi.org/10.1016/j.still.2018.05.006>.
- Meng, F., K. Hu, P. Feng, G. Feng, and Q. Gao. 2022. "Simulating the Effects of Different Textural Soils and N Management on Maize Yield, N Fates, and Water and N Use Efficiencies in Northeast China." *Plants* 11 (23): 3338. <https://doi.org/10.3390/plants11233338>.
- Minamikawa, K., N. Sakai, and K. Yagi. 2006. "Methane Emission from Paddy Fields and Its Mitigation Options on a Field Scale." *Microbes and Environments* 21 (3): 135-147. <https://doi.org/10.1264/jsme2.21.135>.
- Möller, K. 2015. "Effects of Anaerobic Digestion on Soil Carbon and Nitrogen Turnover, N Emissions, and Soil Biological Activity. A Review." *Agronomy for Sustainable Development* 35 (3): 1021-1041. <https://doi.org/10.1007/s13593-015-0284-3>
- Möller, K., and T. Müller. 2012. "Effects of Anaerobic Digestion on Digestate Nutrient Availability and Crop Growth: A Review." *Engineering in Life Sciences* 12 (3): 242-257. <https://doi.org/10.1002/elsc.201100085>.
- Monostori, I., T. Árendás, B. Hoffman, K. Gierczik, F. Szira, and A. Vágújfalvi. 2016. "Relationship Between SPAD Value and Grain Yield Can Be Affected by Cultivar, Environment, and Soil Nitrogen Content in Wheat." *Euphytica* 211:103-112. <https://doi.org/10.1007/s10681-016-1741-z>.
- Muhammad, I., J. Z. Lv, L. Yang, S. Ahmad, S. Farooq, M. Zeeshan, and X. B. Zhou. 2022. "Low Irrigation Water Minimizes the Nitrate Nitrogen Losses Without Compromising the Soil Fertility, Enzymatic Activities and Maize Growth." *BMC Plant Biology* 22 (1): 159. <https://doi.org/10.1186/s12870-022-03548-2>.

- Murphy, R., M. Saunders, K. G. Richards, D. Król, A. W. Gebremichael, J. Rambaud, N. Cowan, and G. Lanigan. 2022. “Nitrous Oxide Emission Factors from an Intensively Grazed Temperate Grassland: A Comparison of Cumulative Emissions Determined by Eddy Covariance and Static Chamber Methods.” *Agriculture, Ecosystems & Environment* 324: 107725. <https://doi.org/10.1016/j.agee.2021.107725>.
- Nakamura, M., Y. Yuyama, M. Yamaoka, and N. Shimizu. 2014. “Global Warming Impacts of the Process to Utilize Digested Slurry from Methane Fermentation as a Fertilizer: Case Study of the Yamada Biomass Plant.” *Paddy and Water Environment* 12 (2): 295-299. <https://doi.org/10.1007/s10333-013-0375-1>.
- Nelson, G. C., M. W. Rosegrant, A. Palazzo, I. Gray, C. Ingersoll, R. Robertson, S. Tokgoz, et al. 2010. “Food Security, Farming, and Climate Change to 2050: Scenarios, Results, Policy Options.” In *Research Reports*, edited by Gerald C. Nelson, 155. Washington, DC: International Food Policy Research Institute (IFPRI). <https://doi.org/10.2499/9780896291867>.
- Nguyen, B. T., B. T. Phan, T. X. Nguyen, V. N. Nguyen, T. V. Tran, and Q. V. Bach. 2020. “Contrastive Nutrient Leaching from Two Differently Textured Paddy Soils as Influenced by Biochar Addition.” *Journal of Soils and Sediments* 20 (1): 297–307. <https://doi.org/10.1007/s11368-019-02366-8>.
- Nicholson, F., A. Bhogal, L. Cardenas, D. Chadwick, T. Misselbrook, A. Rollett, M. Taylor, R. Thorman, and J. Williams. 2017. “Nitrogen Losses to the Environment Following Food-Based Digestate and Compost Applications to Agricultural Land.” *Environmental Pollution* 228: 504–516. <https://doi.org/10.1016/j.envpol.2017.05.023>.
- Nieder, R., and D. K. Benbi. 2022. “Reactive Nitrogen Compounds and Their Influence on Human Health: An Overview.” *Reviews on Environmental Health* 37 (2): 229-246. <https://doi.org/10.1515/reveh-2021-0021>.
- Nieder, R., D. K. Benbi, and H. W. Scherer. 2011. “Fixation and Defixation of Ammonium in Soils: A Review.” *Biology and Fertility of Soils* 47 (1): 1-14. <https://doi.org/10.1007/s00374-010-0506-4>.
- Nishida, M., H. Sumida, and N. Kato. 2008. “Fate of Nitrogen Derived from ¹⁵N-Labeled Cattle Manure Compost Applied to a Paddy Field in the Cool Climate Region of Japan.” *Soil Science and Plant Nutrition* 54 (3): 459-466. <https://doi.org/10.1111/j.1747-0765.2008.00255.x>.

- Nishida, M., K. Tsuchiya, and S. Yamamuro. 2004. "Fate of N and Relative Efficiency of ^{15}N -Labeled Organic Materials Applied to Transplanted Rice in Northern Kyushu Region of Japan." *Soil Science and Plant Nutrition* 50 (2): 225-232.
<https://doi.org/10.1080/00380768.2004.10408471>.
- Nishikawa, T., K. Li, and T. Inamura. 2014. "Nitrogen Uptake by the Rice Plant and Changes in the Soil Chemical Properties in the Paddy Rice Field During Yearly Application of Anaerobically-Digested Manure for Seven Years." *Plant Production Science* 17 (3): 237-244. <https://doi.org/10.1626/ppp.17.237>.
- Nissen, T., and M. M. Wander. 2003. "Management and Soil - Quality Effects on Fertilizer - Use Efficiency and Leaching." *Soil Science Society of America Journal* 67 (5): 1524-1532. <https://doi.org/10.2136/sssaj2003.1524>.
- Nkoa, R. 2014. "Agricultural Benefits and Environmental Risks of Soil Fertilization with Anaerobic Digestates: A Review." *Agronomy for Sustainable Development* 34 (2): 473-492. <https://doi.org/10.1007/s13593-013-0196-z>.
- O'Brien, B. J., D. A. Neher, and E. D. Roy. 2020. "Nutrient and Pathogen Suppression Properties of Anaerobic Digestates from Dairy Manure and Food Waste Feedstocks." *Waste and Biomass Valorization* 11 (12): 6565–6573.
<https://doi.org/10.1007/s12649-019-00906-4>.
- Odlare, M., V. Arthurson, M. Pell, K. Svensson, E. Nehrenheim, and J. Abubaker. 2011. "Land Application of Organic Waste - Effects on the Soil Ecosystem." *Applied Energy* 88 (6): 2210-2218. <https://doi.org/10.1016/j.apenergy.2010.12.043>.
- Oelmann, Y., Y. Kreutziger, R. Bol, and W. Wilcke. 2007. "Nitrate Leaching in Soil: Tracing the NO_3^- Sources with the Help of Stable N and O Isotopes." *Soil Biology and Biochemistry* 39 (12): 3024-3033. <https://doi.org/10.1016/j.soilbio.2007.05.036>.
- Oritate, F. 2016. "Use of Digested Slurry from Livestock Manure in Paddy Fields and Its Environmental Effect in Southeast Asia." PhD Diss., Renewable Energy and Resources unit, Regional Resources Engineering, Division: Kyoto University.
<https://doi.org/10.14989/doctor.r13024>.
- Ottaiano, L., I. Di Mola, P. Di Tommasi, M. Mori, V. Magliulo, and L. Vitale. 2020. "Effects of Irrigation on N_2O Emissions in a Maize Crop Grown on Different Soil Types in Two Contrasting Seasons." *Agriculture* 10 (12): 623.
<https://doi.org/10.3390/agriculture10120623>.

- Palacio, S., R. Milla, J. Albuixech, C. Pérez-Rontomé, J. J. Camarero, M. Maestro, and G. Montserrat-Martí. 2008. "Seasonal Variability of Dry Matter Content and Its Relationship with Shoot Growth and Nonstructural Carbohydrates." *The New Phytologist* 180 (1): 133-142. <https://doi.org/10.1111/j.1469-8137.2008.02569.x>.
- Pandey, A., S. M. Eldridge, A. Weatherley, et al. 2023. "High Fertilizer Nitrogen Input Increases Nitrogen Mining in Sandy Paddy Soils." *Nutrient Cycling in Agroecosystems* 125 (1): 77-88. <https://doi.org/10.1007/s10705-022-10257-7>.
- Panuccio, M. R., F. Romeo, C. Mallamaci, and A. Muscolo. 2021. "Digestate Application on Two Different Soils: Agricultural Benefit and Risk." *Waste and Biomass Valorization* 12 (8): 4341-4353. <https://doi.org/10.1007/s12649-020-01318-5>.
- Partey, S., K. Saito, R. Preziosi, and G. Robson. 2015. "Biochar Use in a Legume-Rice Rotation System: Effects on Soil Fertility and Crop Performance." *Archives of Agronomy and Soil Science* 62 (2): 199-215. <https://doi.org/10.1080/03650340.2015.1040399>.
- Pastorelli, R., A. Lagomarsino, N. Vignozzi, G. Valboa, R. Papini, A. Fabiani, S. Simoncini, S. Mocali, and R. Piccolo. 2013. "Anaerobic Digestate from Biogas Production as a Resource for Improving Soil Fertility: Effects on Crop Yield and Soil Properties." *Geophysical Research Abstracts* 15: EGU2013-7664. <https://ui.adsabs.harvard.edu/abs/2013EGUGA.15.7664P>.
- Paz-Ferreiro, J., M. A. Marinho, C. A. de Abreu, and E. Vidal-Vázquez. 2018. "Soil Texture Effects on Multifractal Behaviour of Nitrogen Adsorption and Desorption Isotherms." *Biosystems Engineering, Computational Tools to Support Soil Management Decisions*, 168 (April): 121-132. <https://doi.org/10.1016/j.biosystemseng.2018.01.009>.
- Przygocka-Cyna, K., and W. Grzebisz. 2018. "Biogas Digestate - Benefits and Risks for Soil Fertility and Crop Quality - An Evaluation of Grain Maize Response." *Open Chemistry* 16 (1): 258-271. <https://doi.org/10.1515/chem-2018-0027>.
- Quan, Z., X. Zhang, E. A. Davidson, F. Zhu, S. Li, X. Zhao, X. Chen, et al. 2021. "Fates and Use Efficiency of Nitrogen Fertilizer in Maize Cropping Systems and Their Responses to Technologies and Management Practices: A Global Analysis on Field ¹⁵N Tracer Studies." *Earth's Future* 9 (5): e2020EF001514. <https://doi.org/10.1029/2020EF001514>.
- Ran, Y., X. Bai, Y. Long, and P. Ai. 2022. "Yield and Quality of Rice Under the Effects of Digestate Application." *Agriculture* 12 (4): 514. <https://doi.org/10.3390/agriculture12040514>.

- Reis, A. R., J. L. Favarin, E. Malavolta, J. L. Júnior, and M. F. Moraes. 2009. "Photosynthesis, Chlorophylls, and SPAD Readings in Coffee Leaves in Relation to Nitrogen Supply." *Communications in Soil Science and Plant Analysis* 40 (9-10): 1512-1528. <https://doi.org/10.1080/00103620902820373>.
- Rigby, H., and S. R. Smith. 2013. "Nitrogen Availability and Indirect Measurements of Greenhouse Gas Emissions from Aerobic and Anaerobic Biowaste Digestates Applied to Agricultural Soils." *Waste Management* 33 (12): 2641-2652. <https://doi.org/10.1016/j.wasman.2013.08.005>.
- Rimski-Korsakov, H., G. Rubio, and R. S. V. Lavado. 2009. "Effect of Water Stress in Maize Crop Production and Nitrogen Fertilizer Fate." *Journal of Plant Nutrition* 32 (4): 565-578. <https://doi.org/10.1080/01904160802714961>.
- Risberg, K., H. Cederlund, M. Pell, V. Arthurson, and A. Schnürer. 2017. "Comparative Characterization of Digestate Versus Pig Slurry and Cow Manure - Chemical Composition and Effects on Soil Microbial Activity." *Waste Management* 61: 529-538. <https://doi.org/10.1016/j.wasman.2016.12.016>.
- Ritter, W. F., and A. E. M. Chirnside. 1987. "Influence of Agricultural Practices on Nitrates in the Water Table Aquifer." *Biological Wastes* 19 (3): 165-178. [https://doi.org/10.1016/0269-7483\(87\)90050-4](https://doi.org/10.1016/0269-7483(87)90050-4).
- Riva, C., V. Orzi, M. Carozzi, M. Acutis, G. Boccasile, S. Lonati, F. Tambone, G. D'Imporzano, and F. Adani. 2016. "Short-Term Experiments in Using Digestate Products as Substitutes for Mineral (N) Fertilizer: Agronomic Performance, Odours, and Ammonia Emission Impacts." *Science of the Total Environment* 547: 206-214. <https://doi.org/10.1016/j.scitotenv.2015.12.156>.
- Robles-Aguilar, A. A., V. M. Temperton, and N. D. Jablonowski. 2019. "Maize Silage Digestate Application Affecting Germination and Early Growth of Maize Modulated by Soil Type." *Agronomy* 9 (8): 473. <https://doi.org/10.3390/agronomy9080473>.
- Rochette, P., D. A. Angers, M. H. Chantigny, and N. Bertrand. 2008. "Nitrous Oxide Emissions Respond Differently to No-Till in a Loam and a Heavy Clay Soil." *Soil Science Society of America Journal* 72 (5): 1363-1369. <https://doi.org/10.2136/sssaj2007.0371>.

- Rochette, P., D. A. Angers, M. H. Chantigny, M. O. Gasser, J. D. MacDonald, D. E. Pelster, and N. Bertrand. 2013. "NH₃ Volatilization, Soil Concentration and Soil pH Following Subsurface Banding of Urea at Increasing Rates." *Canadian Journal of Soil Science* 93 (2): 261-268. <https://doi.org/10.4141/cjss2012-095>.
- Ronga, D., L. Setti, C. Salvarani, et al. 2019. "Effects of Solid and Liquid Digestate for Hydroponic Baby Leaf Lettuce (*Lactuca Sativa* L.) Cultivation." *Scientia Horticulturae* 244: 172-181. <https://doi.org/10.1016/j.scienta.2018.09.037>.
- Saldivar, S. O. S., and E. Perez-Carrillo. 2016. *Maize in Encyclopedia of Food and Health*, edited by Benjamin Caballero, Paul M. Finglas, and Fidel Toldrá, 601–609. Oxford: Academic Press. <https://doi.org/10.1016/B978-0-12-384947-2.00436-0>.
- Sasada, Y., K. Win, R. Nonaka, A. Win, K. Toyota, T. Motobayashi, M. Hosomi, D. Chen, and J. Lu. 2011. "Methane and N₂O Emissions, Nitrate Concentrations of Drainage Water, and Zinc and Copper Uptake by Rice Fertilized with Anaerobically Digested Cattle or Pig Slurry." *Biology and Fertility of Soils* 47 (8): 949-956. <https://doi.org/10.1007/s00374-011-0601-1>.
- Sawada, K., and K. Toyota. 2015. "Effects of the Application of Digestates from Wet and Dry Anaerobic Fermentation to Japanese Paddy and Upland Soils on Short-Term Nitrification." *Microbes and Environments* 30 (1): 37-43. <https://doi.org/10.1264/jsme2.ME14080>.
- Scarlat, N., J. F. Dallemand, and F. Fahl. 2018. "Biogas: Developments and Perspectives in Europe." *Renewable Energy* 129: 457-472. <https://doi.org/10.1016/j.renene.2018.03.006>.
- Schissel, C., and D. T. Allen. 2022. "Impact of the High-Emission Event Duration and Sampling Frequency on the Uncertainty in Emission Estimates." *Environmental Science & Technology Letters* 9 (12): 1063–1067. <https://doi.org/10.1021/acs.estlett.2c00731>.
- Seadi, T. A., and C. Lukehurst. 2012. "Quality Management of Digestate from Biogas Plants Used as Fertiliser." In *IEA Bioenergy, Task 37, Energy from Biogas*, Vol. 40. IEA Bioenergy.
- Shan, J., P. Yang, X. Shang, M. Rahman, and X. Yan. 2018. "Anaerobic Ammonium Oxidation and Denitrification in a Paddy Soil as Affected by Temperature, pH, Organic Carbon, and Substrates." *Biology and Fertility of Soils* 54 (3): 341-348. <https://doi.org/10.1007/s00374-018-1263-z>.

- Sharifi, M., S. Baker, L. Hojabri, and M. Hajiaghaei-Kamrani. 2019. "Short-Term Nitrogen Dynamics in a Soil Amended with Anaerobic Digestate." *Canadian Journal of Soil Science* 99 (2): 173-181. <https://doi.org/10.1139/cjss-2018-0060>.
- Sharma, B., B. Vaish, Monika, U. K. Singh, P. Singh, and R. P. Singh. 2019. "Recycling of Organic Wastes in Agriculture: An Environmental Perspective." *International Journal of Environmental Research* 13 (2): 409-429. <https://doi.org/10.1007/s41742-019-00175-y>.
- Shen, L., P. Zheng, and S. Ma. 2016. "Nitrogen Loss Through Anaerobic Ammonium Oxidation in Agricultural Drainage Ditches." *Biology and Fertility of Soils* 52 (2): 127-136. <https://doi.org/10.1007/s00374-015-1058-4>.
- Shibata, M., S. Sugihara, A. D. Mvondo-Ze, S. Araki, and S. Funakawa. 2017. "Nitrogen Flux Patterns Through Oxisols and Ultisols in Tropical Forests of Cameroon, Central Africa." *Soil Science and Plant Nutrition* 63 (3): 306-317. <https://doi.org/10.1080/00380768.2017.1341285>.
- Shiferaw, B., B. M. Prasanna, J. Hellin, and M. Bänziger. 2011. "Crops That Feed the World 6. Past Successes and Future Challenges to the Role Played by Maize in Global Food Security." *Food Security* 3 (3): 307-327. <https://doi.org/10.1007/s12571-011-0140-5>.
- Sigman, D. M., K. L. Casciotti, M. Andreani, C. Barford, M. Galanter, and J. K. Böhlke. 2001. "A Bacterial Method for the Nitrogen Isotopic Analysis of Nitrate in Seawater and Freshwater." *Analytical Chemistry* 73 (17): 4145-4153. <https://doi.org/10.1021/ac010088e>.
- Sigurnjak, I., C. Vaneeckhaute, E. Michels, B. Ryckaert, G. Ghekiere, F. M. G. Tack, and E. Meers. 2017. "Fertilizer Performance of Liquid Fraction of Digestate as Synthetic Nitrogen Substitute in Silage Maize Cultivation for Three Consecutive Years." *Science of the Total Environment* 599-600: 1885-1894. <https://doi.org/10.1016/j.scitotenv.2017.05.120>.
- Sikora, L. J., and N. K. Enkiri. 1999. "Growth of Tall Fescue in Compost/Fertilizer Blends." *Soil Science* 164 (1): 62-69. <https://doi.org/10.1097/00010694-199901000-00009>.
- Sileshi, G. W., F. K. Akinnifesi, L. K. Debusho, T. Beedy, O. C. Ajayi, and S. Mong'omba. 2010. "Variation in Maize Yield Gaps with Plant Nutrient Inputs, Soil Type, and Climate Across Sub-Saharan Africa." *Field Crops Research* 116 (1-2): 1-13. <https://doi.org/10.1016/j.fcr.2009.11.014>.

- Šimon, T., E. Kunzová, and M. Friedlová. 2016. "The Effect of Digestate, Cattle Slurry and Mineral Fertilization on the Winter Wheat Yield and Soil Quality Parameters." *Plant, Soil and Environment* 61 (No. 11): 522-527. <https://doi.org/10.17221/530/2015-PSE>.
- Singh B., and E. Craswell. 2021. "Fertilizers and Nitrate Pollution of Surface and Ground Water: An Increasingly Pervasive Global Problem." *SN Applied Sciences* 3 (4): 518. <https://doi.org/10.1007/s42452-021-04521-8>.
- Singla, A., and K. Inubushi. 2014. "Effect of Biogas Digested Liquid on CH₄ and N₂O Flux in Paddy Ecosystem." *Journal of Integrative Agriculture* 13 (3): 635-640. [https://doi.org/10.1016/S2095-3119\(13\)60721-2](https://doi.org/10.1016/S2095-3119(13)60721-2).
- Smith, V. H., G. D. Tilman, and J. C. Nekola. 1999. "Eutrophication: Impacts of Excess Nutrient Inputs on Freshwater, Marine, and Terrestrial Ecosystems." *Environmental Pollution* 100 (1-3): 179-196. [https://doi.org/10.1016/S0269-7491\(99\)00091-3](https://doi.org/10.1016/S0269-7491(99)00091-3).
- Snapp, S., T. B. Sapkota, J. Chamberlin, C. M. Cox, S. Gameda, M. L. Jat, P. Marenja, et al. 2023. "Spatially Differentiated Nitrogen Supply is Key in a Global Food-Fertilizer Price Crisis." *Nature Sustainability* 6 (10): 1268-1278. <https://doi.org/10.1038/s41893-023-01166-w>.
- Sogbedji, J. M., H. M. van Es, C. L. Yang, L. D. Geohring, and F. R. Magdoff. 2000. "Nitrate Leaching and Nitrogen Budget as Affected by Maize Nitrogen Rate and Soil Type." *Journal of Environmental Quality* 29 (6): 1813-1820. <https://doi.org/10.2134/jeq2000.00472425002900060011x>.
- Soinne, H., R. Keskinen, M. Rätty, et al. 2020. "Soil Organic Carbon and Clay Content as Deciding Factors for Net Nitrogen Mineralization and Cereal Yields in Boreal Mineral Soils." *European Journal of Soil Science* 72(4): 1497-1512. <https://doi.org/10.1111/ejss.13003>.
- Sørensen, P., E. S. Jensen, and N. E. Nielsen. 1994. "The Fate of ¹⁵N-Labelled Organic Nitrogen in Sheep Manure Applied to Soils of Different Texture under Field Conditions." *Plant and Soil* 162 (1): 39-47. <https://doi.org/10.1007/BF01416088>.
- St. Luce, M., J. K. Whalen, N. Ziadi, B. J. Zebarth, and M. H. Chantigny. 2014. "Labile Organic Nitrogen Transformations in Clay and Sandy-Loam Soils Amended with ¹⁵N-Labelled Faba Bean and Wheat Residues." *Soil Biology and Biochemistry* 68: 208-218. <https://doi.org/10.1016/j.soilbio.2013.09.033>.

- Stanley, H. O., C. B. Ogbonna, G. O. Abu, and C. J. Ugboma. 2019. "Effect of Liquid Digestate on Agricultural Soil - I: Growth Dynamics of Zea Mays Plant." *Journal of Experimental Agriculture International* 31(5): 1-9.
<https://doi.org/10.9734/jeai/2019/v31i530083>.
- Stepanauskas, R., E. Davidsson, and L. Leonardson. 1996. "Nitrogen Transformations in Wetland Soil Cores Measured by ^{15}N Isotope Pairing and Dilution at Four Infiltration Rates." *Applied & Environmental Microbiology* 62 (7): 2345-2351.
<https://doi.org/10.1128/aem.62.7.2345-2351.1996>.
- Stevenson, F. C., F. L. Walley, and C. van Kessel. 1998. "Direct vs. Indirect Nitrogen-15 Approaches to Estimate Nitrogen Contributions from Crop Residues." *Soil Science Society of America Journal* 62 (5): 1327-1334.
<https://doi.org/10.2136/sssaj1998.03615995006200050026x>.
- Subbarao, G. V., K. Nakahara, M. P. Hurtado, et al. 2009. "Evidence for Biological Nitrification Inhibition in Brachiaria Pastures." *Proceedings of the National Academy of Sciences* 106 (41): 17302-17307. <https://doi.org/10.1073/pnas.0903694106>.
- Takakai, F., H. Seiya, Y. Harakawa, K. Hatakeyama, K. Yasuda, T. Sato, K. Kimura, and Y. Kaneta. 2018. "Fate of Fertilizer-Derived N Applied to Enhance Rice Straw Decomposition in a Paddy Field During the Fallow Season Under Cool Temperature Conditions." *Agriculture* 8 (4): 50. <https://doi.org/10.3390/agriculture8040050>.
- Tambone, F., B. Scaglia, G. D'Imporzano, A. Schievano, V. Orzi, S. Salati, and F. Adani. 2010. "Assessing Amendment and Fertilizing Properties of Digestates from Anaerobic Digestion Through a Comparative Study with Digested Sludge and Compost." *Chemosphere* 81 (5): 577-583.
<https://doi.org/10.1016/j.chemosphere.2010.08.034>.
- Tampio, E., T. Salo, and J. Rintala. 2015. "Agronomic Characteristics of Five Different Urban Waste Digestates." *Journal of Environmental Management* 169: 293-302.
<https://doi.org/10.1016/j.jenvman.2016.01.001>.
- Tanaka, T. S. T., Y. Nitta, K. Kido, T. Nishikawa, T. Matoh, and T. Inamura. 2017. "Effect of the Long-Term Application of Anaerobically Digested Residual Slurry on Methane Emissions in a Rice Paddy Field." *Soil Science and Plant Nutrition* 63 (3): 300-305.
<https://doi.org/10.1080/00380768.2017.1322918>.

- Ueno, H., and S. Yamamuro. 2001. "Fate of Nitrogen Derived from ^{15}N -Labeled Plant Residues and Composts in Rice-Planted Paddy Soil." *Soil Science and Plant Nutrition* 47 (4): 747-754. <https://doi.org/10.1080/00380768.2001.10408439>.
- van Groenigen, J. W., G. J. Kasper, G. L. Velthof, A. van den Pol-van Dasselaar, and P. J. Kuikman. 2004. "Nitrous Oxide Emissions from Silage Maize Fields Under Different Mineral Nitrogen Fertilizer and Slurry Applications." *Plant and Soil* 263 (1): 101-111. <https://doi.org/10.1023/B:PLSO.0000047729.43185.46>.
- Verdi, L., P. J. Kuikman, S. Orlandini, M. Mancini, M. Napoli, and A. Dalla Marta. 2019. "Does the Use of Digestate to Replace Mineral Fertilizers Have Less Emissions of N_2O and NH_3 ?" *Agricultural and Forest Meteorology* 269-270: 112-118. <https://doi.org/10.1016/j.agrformet.2019.02.004>.
- Voća, N., T. Krička, T. Ćosić, V. Rupiće, Ž. Jukić, and S. Kalambura. 2005. "Digested Residue as a Fertilizer After the Mesophilic Process of Anaerobic Digestion." *Soil and Environment* 51 (6): 262-266. <https://doi.org/10.17221/3584-PSE>.
- Vries, M. P. C. de. 1980. "How Reliable Are Results of Pot Experiments?" *Communications in Soil Science and Plant Analysis* 11 (9): 895-902. <https://doi.org/10.1080/00103628009367090>.
- Wang, P., C. Wang, X. Wang, Y. Wu, Y. Zhang, Y. Sun, Y. Shi, and G. Mi. 2023. "Increasing Nitrogen Absorption and Assimilation Ability Under Mixed NO_3^- and NH_4^+ Supply is a Driver to Promote Growth of Maize Seedlings." *Journal of Integrative Agriculture* 22 (6): 1896-1908. <https://doi.org/10.1016/j.jia.2023.04.037>.
- Wang, Y., S. Chikamatsu, T. Gegen, K. Sawada, K. Toyota, S. Riya, and M. Hosomi. 2019. "Application of Biogas Digestate with Rice Straw Mitigates Nitrate Leaching Potential and Suppresses Root-Knot Nematode (*Meloidogyne Incognita*)." *Agronomy* 9 (5): 227. <https://doi.org/10.3390/agronomy9050227>.
- Wang, Z. H., X. J. Liu, X. T. Ju, F. S. Zhang, and S. S. Malhi. 2004. "Ammonia Volatilization Loss from Surface-Broadcast Urea: Comparison of Vented- and Closed-Chamber Methods and Loss in Winter Wheat-Summer Maize Rotation in North China Plain." *Communications in Soil Science and Plant Analysis* 35 (19-20): 2917-2939. <https://doi.org/10.1081/CSS-200036499>.

- Watanabe, S., K. Nakamura, C. S. Ryu, M. Iida, and S. Kawashima. 2012. "Effects of Different Application Timings of Methane Fermentation Digested Liquid to Paddy Plots on Soil Nitrogen and Rice Yield." *Soil Science and Plant Nutrition* 58 (2): 224-237. <https://doi.org/10.1080/00380768.2012.661949>.
- White, P. J., and M. R. Broadley. 2001. "Chloride in Soils and Its Uptake and Movement within the Plant: A Review." *Annals of Botany* 88 (6): 967-988. <https://doi.org/10.1006/anbo.2001.1540>.
- Win, A. T., K. Toyota, K. T. Win, T. Motobayashi, T. Ookawa, T. Hirasawa, D. Chen, and J. Lu. 2014. "Effect of Biogas Slurry Application on CH₄ and N₂O Emissions, Cu and Zn Uptakes by Whole Crop Rice in a Paddy Field in Japan." *Soil Science and Plant Nutrition* 60 (3): 411-422. <https://doi.org/10.1080/00380768.2014.899886>.
- Win, K. T., R. Nonaka, K. Toyota, T. Motobayashi, and M. Hosomi. 2010. "Effects of Option Mitigating Ammonia Volatilization on CH₄ and N₂O Emissions from a Paddy Field Fertilized with Anaerobically Digested Cattle Slurry." *Biology and Fertility of Soils* 46 (6): 589-595. <https://doi.org/10.1007/s00374-010-0465-9>.
- Wolf, U., R. Fuß, F. Höppner, and H. Flessa. 2014. "Contribution of N₂O and NH₃ to Total Greenhouse Gas Emission from Fertilization: Results from a Sandy Soil Fertilized with Nitrate and Biogas Digestate with and without Nitrification Inhibitor." *Nutrient Cycling in Agroecosystems* 100 (1): 121-134. <https://doi.org/10.1007/s10705-014-9631-z>.
- Wulf, S., M. Maeting, and J. Clemens. 2002. "Application Technique and Slurry Co-Fermentation Effects on Ammonia, Nitrous Oxide, and Methane Emissions After Spreading." *Journal of Environmental Quality* 31 (6): 1795-1801. <https://doi.org/10.2134/jeq2002.1795>.
- Xiong, D., J. Chen, T. Yu, W. Gao, X. Ling, Y. Li, S. Peng, and J. Huang. 2015. "SPAD-Based Leaf Nitrogen Estimation is Impacted by Environmental Factors and Crop Leaf Characteristics." *Scientific Reports* 5 (1): 13389. <https://doi.org/10.1038/srep13389>.
- Xu, J., S. Peng, S. Yang, and W. Wang. 2012. "Ammonia Volatilization Losses from a Rice Paddy with Different Irrigation and Nitrogen Managements." *Agricultural Water Management* 104: 184-192. <https://doi.org/10.1016/j.agwat.2011.12.013>.
- Yagi, T., S. Toh, S. Otsuka, T. Matsumoto, O. Sakai, T. Nakatsuji, M. Onodera, and T. Saigusa. 2018. "Proper Amount of Basal Nitrogen Application by Banding to Maize (*Zea Mays* L.) in Hokkaido." *日本土壌肥料学雑誌* 89:384-389. https://doi.org/10.20710/dojo.89.5_384.

- Yang, X., H. Li, S. Nie, J. Su, B. Weng, G. Zhu, H. Yao, J. A. Gilbert, and Y. Zhu. 2015. "Potential Contribution of Anammox to Nitrogen Loss from Paddy Soils in Southern China." *Applied & Environmental Microbiology* 81 (3): 938-947. <https://doi.org/10.1128/aem.02664-14>.
- Ye, Q. B., H. C. Zhang, H. Y. Wei, Y. Zhang, B. Wang, K. Xia, Z. Y. Huo, Q. Dai, and K. Xu. 2007. "Effects of Nitrogen Fertilizer on Nitrogen Use Efficiency and Yield of Rice Under Different Soil Conditions." *Frontiers of Agriculture in China* 1 (1): 30-36. <https://doi.org/10.1007/s11703-007-0005-z>.
- Yu, X., Y. Xu, H. Zhu, B. Shutes, B. Yan, X. Chen, and C. Rui. 2019. "Tracking the Fate of Fertilizer Nitrogen in a Paddy Rice Field Using Isotope Technology." *Polish Journal of Environmental Studies* 29 (1): 419-428. <https://doi.org/10.15244/pjoes/103026>.
- Zhao, X., Y. X. Xie, Z. Q. Xiong, X. Q. Yan, G. X. Xing, and Z. L. Zhu. 2009. "Nitrogen Fate and Environmental Consequence in Paddy Soil Under Rice-Wheat Rotation in the Taihu Lake Region, China." *Plant and Soil* 319 (1): 225-234. <https://doi.org/10.1007/s11104-008-9865-0>.
- Zheng, J., Y. Qu, M. M. Kilasara, W. N. Mmari, and S. Funakawa. 2019. "Nitrate Leaching from the Critical Root Zone of Maize in Two Tropical Highlands of Tanzania: Effects of Fertilizer-Nitrogen Rate and Straw Incorporation." *Soil and Tillage Research* 194:104295. <https://doi.org/10.1016/j.still.2019.104295>.
- Zhou, J. B., J. G. Xi, Z. J. Chen, and S. X. Li. 2006. "Leaching and Transformation of Nitrogen Fertilizers in Soil After Application of N with Irrigation: A Soil Column Method1 1Project Supported by the National Natural Science Foundation of China (Nos. 30230230 and 30370288) and the National Key Laboratory for Soil Erosion and Dryland Farming on the Loess Plateau (No. 10501-116)." *Pedosphere* 16 (2): 245-252. [https://doi.org/10.1016/S1002-0160\(06\)60050-7](https://doi.org/10.1016/S1002-0160(06)60050-7).
- Zhou, M., and K. Butterbach-Bahl. 2014. "Assessment of Nitrate Leaching Loss on a Yield-Scaled Basis from Maize and Wheat Cropping Systems." *Plant and Soil* 374 (1): 977-991. <https://doi.org/10.1007/s11104-013-1876-9>.
- Zhou, S., S. Sugawara, S. Riya, M. Sagehashi, K. Toyota, A. Terada, and M. Hosomi. 2011. "Effect of Infiltration Rate on Nitrogen Dynamics in Paddy Soil After High-Load Nitrogen Application Containing ^{15}N Tracer." *Ecological Engineering* 37 (5): 685-692. <https://doi.org/10.1016/j.ecoleng.2010.04.032>.

- Zhou, S., Y. Sakiyama, S. Riya, X. Song, A. Terada, and M. Hosomi. 2012. "Assessing Nitrification and Denitrification in a Paddy Soil with Different Water Dynamics and Applied Liquid Cattle Waste Using the ^{15}N Isotopic Technique." *Science of the Total Environment* 430: 93-100. <https://doi.org/10.1016/j.scitotenv.2012.04.056>.
- Ziadi, N., A. N. Cambouris, J. Nyiraneza, and M. C. Nolin. 2013. "Across a Landscape, Soil Texture Controls the Optimum Rate of N Fertilizer for Maize Production." *Field Crops Research* 148:78-85. <https://doi.org/10.1016/j.fcr.2013.03.023>.
- Zilio, M., A. Pigoli, B. Rizzi, G. Geromel, E. Meers, O. Schoumans, A. Giordano, and F. Adani. 2021. "Measuring Ammonia and Odour Emissions During Full Field Digestate Use in Agriculture." *Science of the Total Environment* 782:146882. <https://doi.org/10.1016/j.scitotenv.2021.146882>.

APPENDIX 1: SUPPLEMENTAL DATA

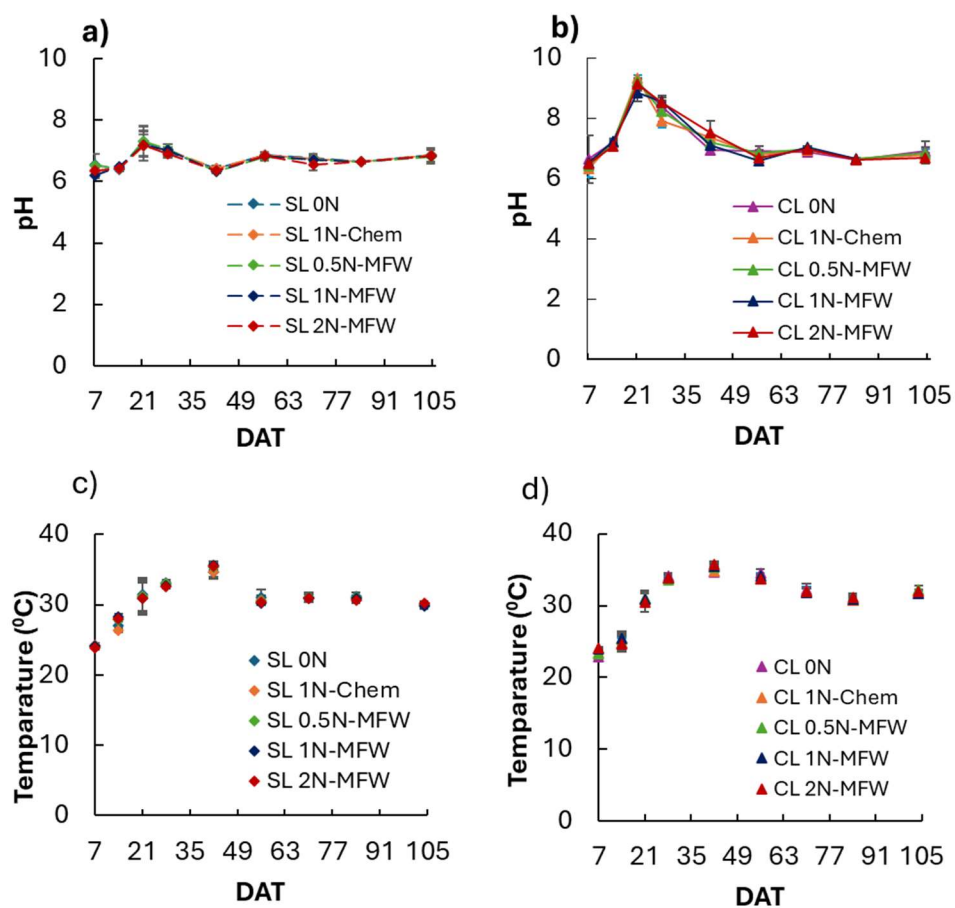


Figure S3.1. Floodwater pH (a, b) and temperature (c, d) under different treatments in 2022 for SL (a, c) and CL (b, d) soils. Error bars represent standard error ($n = 4$), except for SL-0N and CL-2N-MFW treatments in 2022 ($n = 3$). All measurements were conducted between 10:00 and 16:00 on gas collection days. DAT: Days after transplanting.

Table S3.1 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	Authors
						<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		Ghoneim et al. 2008
MFW				50.5	13.2	-	-	-	36.3	N balance	Chen et al. 2012
Urea	Clay loam	1 m ²	Split	36.6	-	2.3	7.5	0.2	53.0		
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			% of N input	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	-	-		Xinqiang et al. 2014
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	-	-		
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		

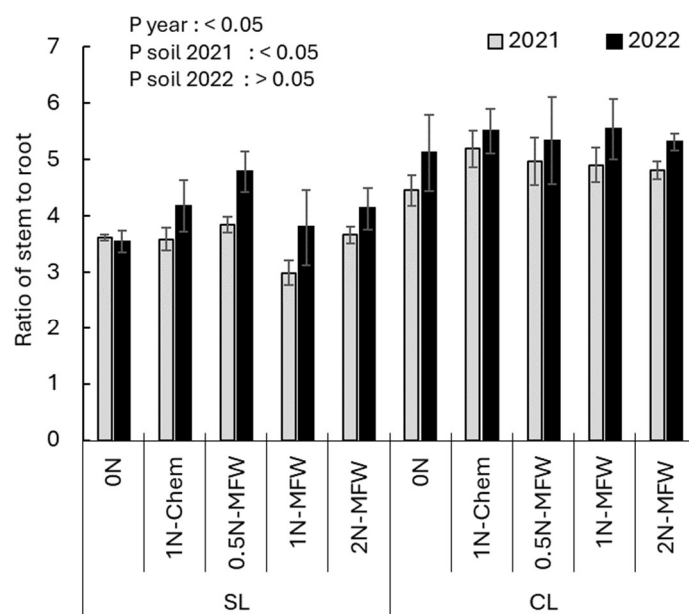


Figure S3.2. Shoot-to-root ratio of rice at harvest in SL and CL soils during 2021 and 2022. Error bars represent standard error ($n = 4$), except for SL-0N and CL-2N-MFW treatments in 2022 ($n = 3$). P-values indicate significant differences between year (2021 vs. 2022) and soil textures (SL vs. CL).

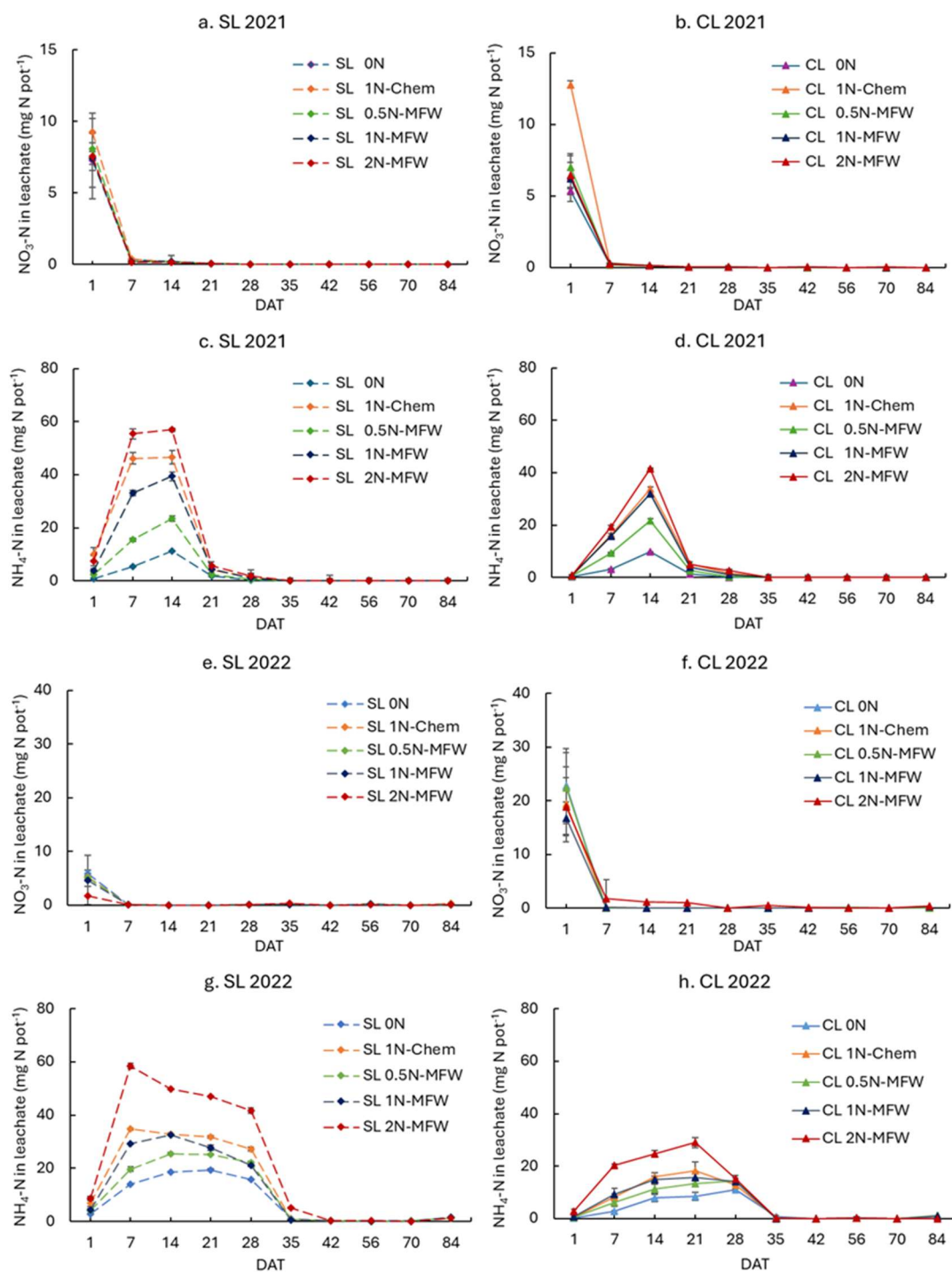


Figure S3.3. Inorganic N (NO₃-N and NH₄-N) content in daily leachate for SL and CL soils in 2021 (a–d) and 2022 (e–h). Error bars represent standard error ($n = 4$), except for SL-0N and CL-2N-MFW treatments in 2022 ($n = 3$). DAT: Days after transplanting.

Table S4.1 Summary of N fate under different fertilizer treatments in maize cultivation.

Author	Chemical rates	MFW rates	Soil type	Scale	Method	Plant uptake	Remained	Loss				Note
	(kg N ha ⁻¹)	N leaching (%)						NH ₃	N ₂ O	Un-detected		
Quan et al. (2021)	100–300		Unknown	field	Unknown	42 ± 14	31 ± 14		28 ± 19			Meta-analysis of ¹⁵ N
Wang et al. (2016)	225		Cumulic Haplustolls	field	Split	27–41	46–53				12–24	¹⁵ N urea
Fuhrmann et al. (2018)	60		Hydragric Anthrosol	field	Split	22	33	34	0–6	1.5		¹⁵ N urea; Maize-paddy rice mix
Rimski-Korsakov et al. (2012)	70–250		O'Higgins Series Typic Argiudoll	field	Split	48–60	10–21	6–11	15–24		0–5	¹⁵ N urea
Yang et al. (2011)	30–90		Sandy loamy	field	Basal	26–31	55–60 ⁽¹⁾				14–24	¹⁵ N urea;
	60–180	Topdressing			41–51	45–47 ⁽¹⁾		20–33	(1): at 0–150cm			
This study	150	75–300	Sandy loam	pot	Basal	67–68	17–18	1.8–4.9	0–0.3*	0.9–2.4	8.6–13	Based
	150		Basal		53–66	19	3–17	0–0.4*	5.8–6.6	11–12	on	
			Basal		55–60	13–20	0.3–5.8	8–10*	0.6–3.7	11–31	¹⁵ NH ₄ C;	
			Basal		23–51	18–26	2–19	14–22*	4.6–8.2	2–35	*, only in the 2022 season	

Table S4.2 Person correlation coefficients between agronomic indicators and MFW application rates over two growing years

Soil	Year	Height	SPAD	Grain yield	Total biomass	Aboveground biomass
SL	2021	0.56*	0.92**	0.72**	0.89**	0.89**
	2022	-0.27	0.78**	0.56*	0.23	0.27
CL	2021	0.77**	0.93**	0.97*	0.86**	0.86**
	2022	0.38	0.21	0.52	0.60*	0.67**

** . Correlation is significant at the 0.01 level.

*. Correlation is significant at the 0.05 level.

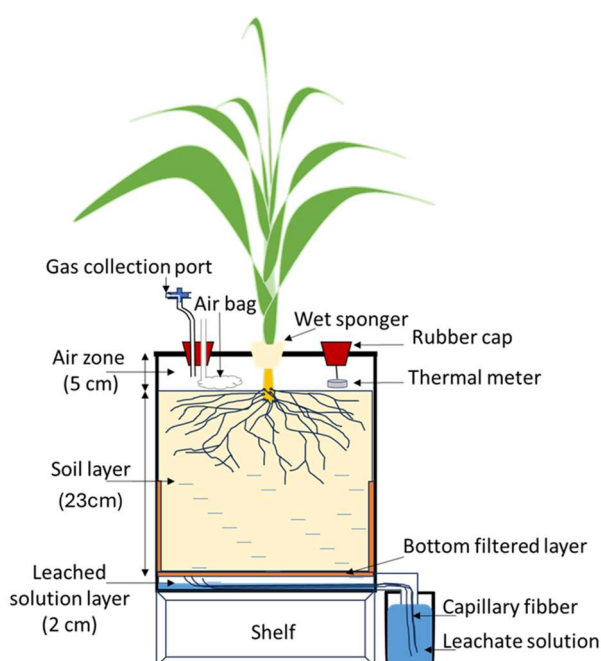


Figure S4.1. Schematic diagram of the experimental pot system and modified chamber for N₂O gas collection during maize cultivation. 1:2000 Wagner pots were used, each containing a soil layer overlaid on a geotextile (filter) layer fixed 2 cm above the pot bottom to allow water percolation. A capillary fiber connected the lower part of the pot to an external collection bottle, enabling passive leachate collection without disturbing the soil. This setup ensured consistent drainage conditions and minimized waterlogging across treatments. The chamber cap was fixed to the pot with tape and positioned near the maize stem with a wet sponge for sealing. A gas-collecting port with a rubber cap was installed on the cap for sample collection during measurement.

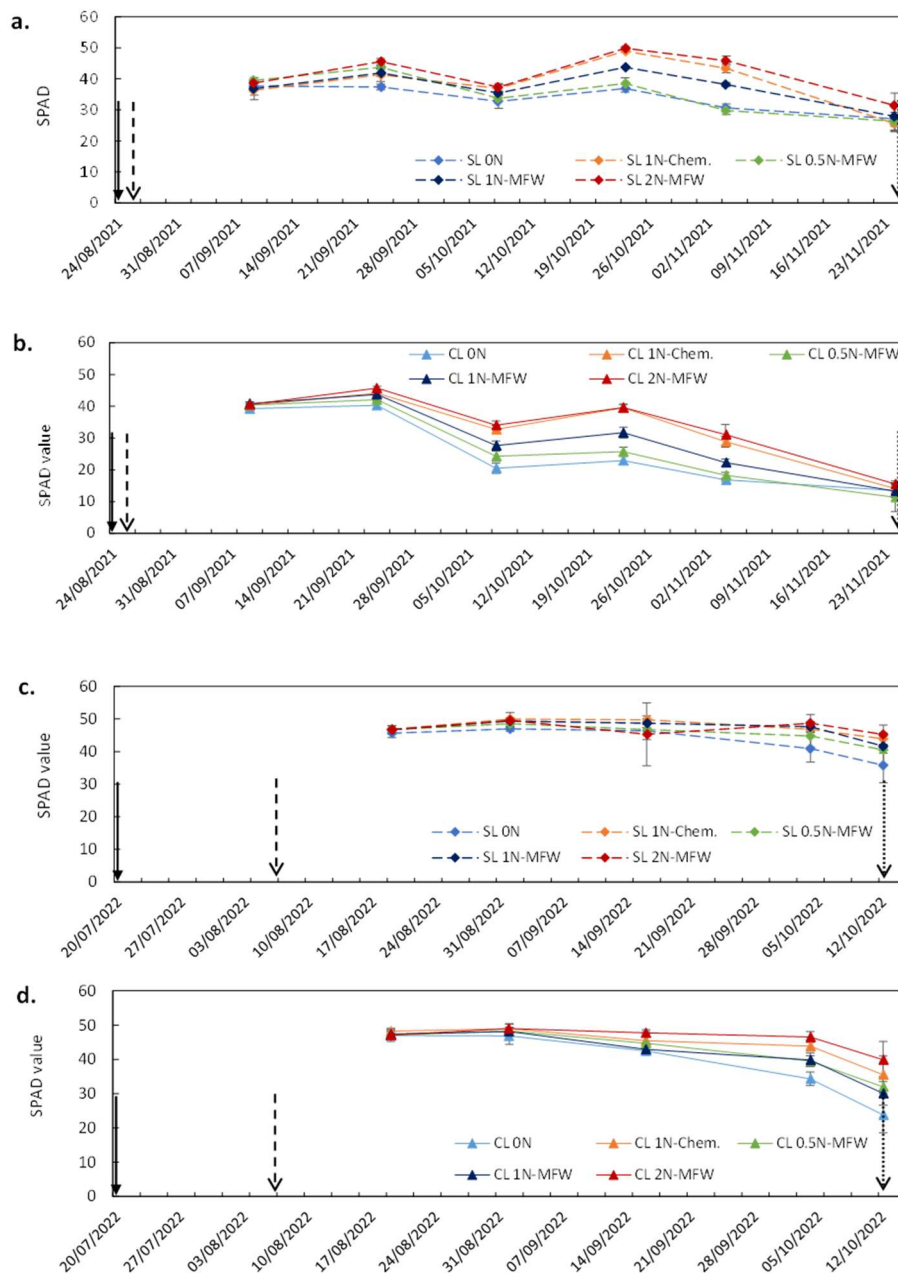


Figure S4.2. SPAD values of maize under different fertilizer treatments and contrasting soil textures (sandy loam - SL; clay loam – CL) across two growing seasons (2021 and 2022). (a, c) SL soil and (b, d) CL soil in 2021 (a, b) and 2022 (c, d). Fertilizer treatments: 0N: no fertilizer; 1N-Chem: 750 mg $\text{NH}_4\text{Cl-N}$; 0.5N-MFW: 375 mg N as MFW; 1N-MFW: 750 mg N as MFW; and 2N as MFW: 1500 mg N as MFW. Data represent mean $\pm$ standard error (SE) from four replications, except for CL-1N-Chem in 2021 and SL-1N-MFW, SL-2N-MFW, and CL-0N treatments in 2022, with three replications. Solid line arrows indicate fertilization dates; dotted line arrows indicate sowing (2021) or transplanting (2022) dates; dashed arrows indicate harvest dates.

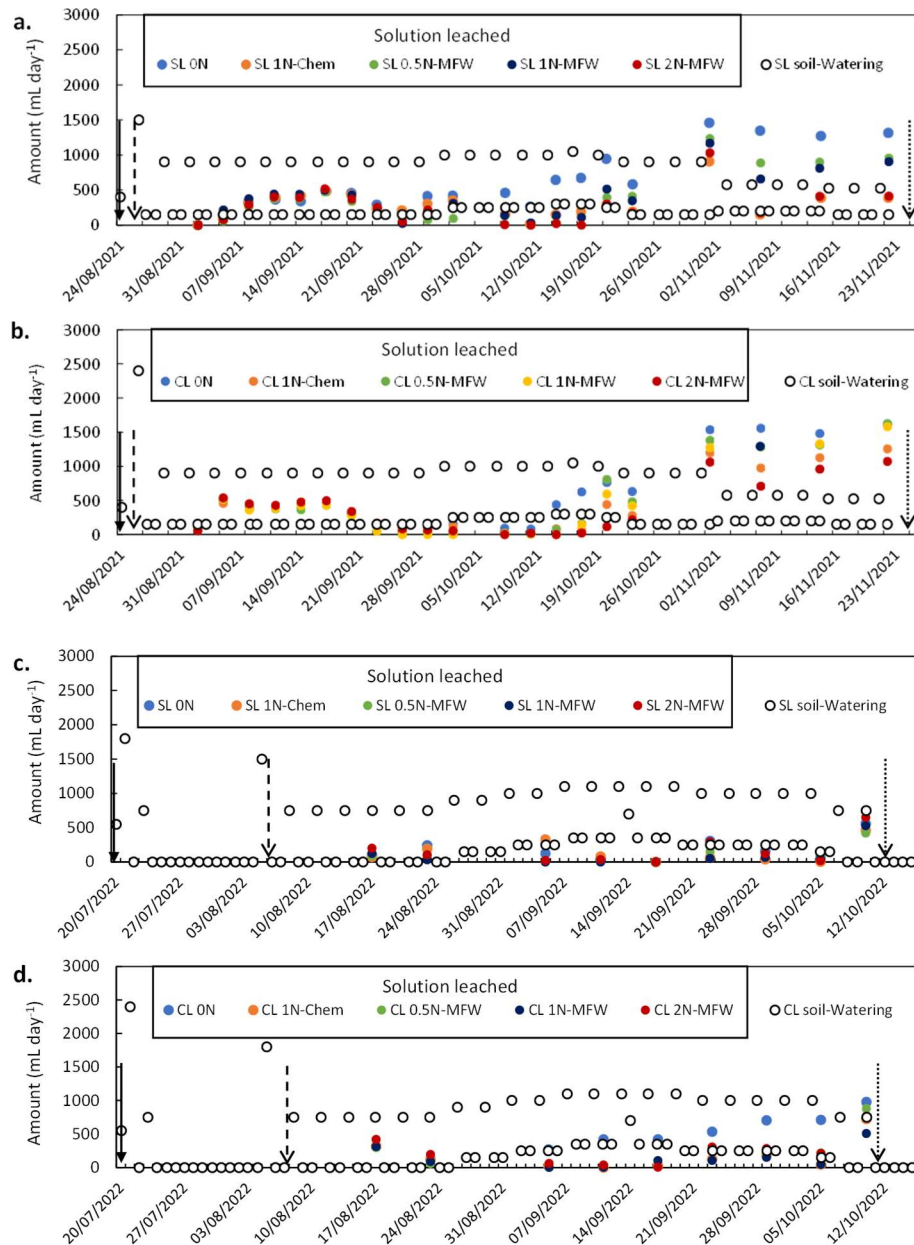


Figure S4.3. Daily water supply and leachate volume under different fertilizer treatments and contrasting soil textures across two growing seasons (2021 and 2022). (a, c) SL soil and (b, d) CL soil in 2021 (a, b) and 2022 (c, d). Fertilizer treatments: 0N: no fertilizer; 1N-Chem: 750 mg $\text{NH}_4\text{Cl-N}$; 0.5N-MFW: 375 mg N as MFW; 1N-MFW: 750 mg N as MFW; and 2N as MFW: 1500 mg N as MFW. Data represent mean $\pm$ standard error (SE) from four replications, except for CL-1N-Chem in 2021 and SL-1N-MFW, SL-2N-MFW, and CL-0N treatments in 2022, with three replications. Solid line arrows indicate fertilization dates; dotted line arrows indicate sowing (2021) or transplanting (2022) dates; dashed arrows indicate harvest dates.

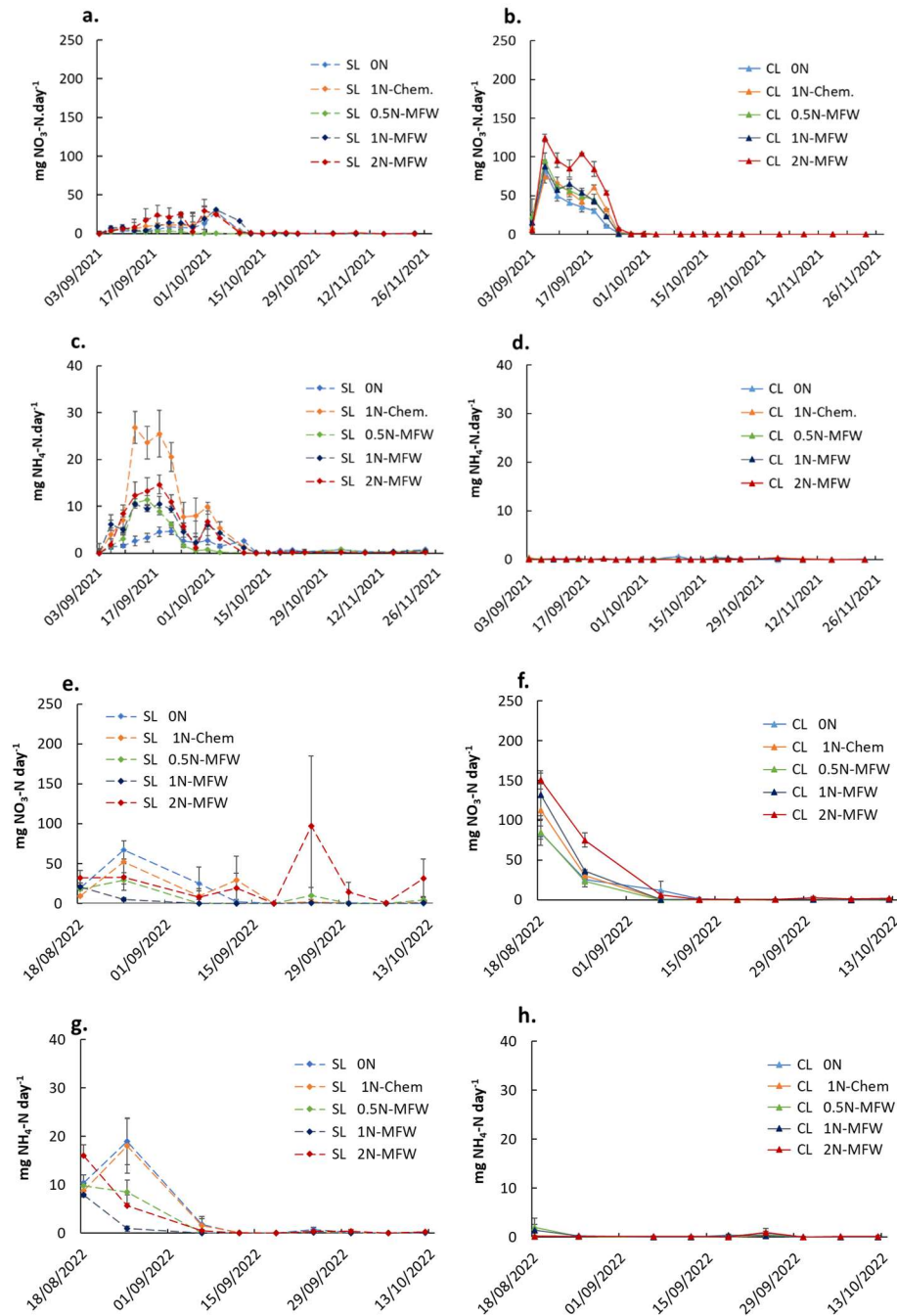


Figure S4.4. Fraction of N leached ($\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$) under different fertilizer treatments and contrasting soil textures during two growing seasons (2021 and 2022). (a, c, e, g) SL soil and (b, d, f, h) CL soil in 2021 (a, b, c, d) and 2022 (e, f, g, h). Fertilizer treatments: 0N: no fertilizer; 1N-Chem: 750 mg $\text{NH}_4\text{Cl-N}$; 0.5N-MFW: 375 mg N as MFW; 1N-MFW: 750 mg N as MFW; and 2N as MFW: 1500 mg N as MFW. Data represent mean $\pm$ SE from four replications, except for CL-1N-Chem in 2021 and SL-1N-MFW, SL-2N-MFW, and CL-0N in 2022, which have three replications.

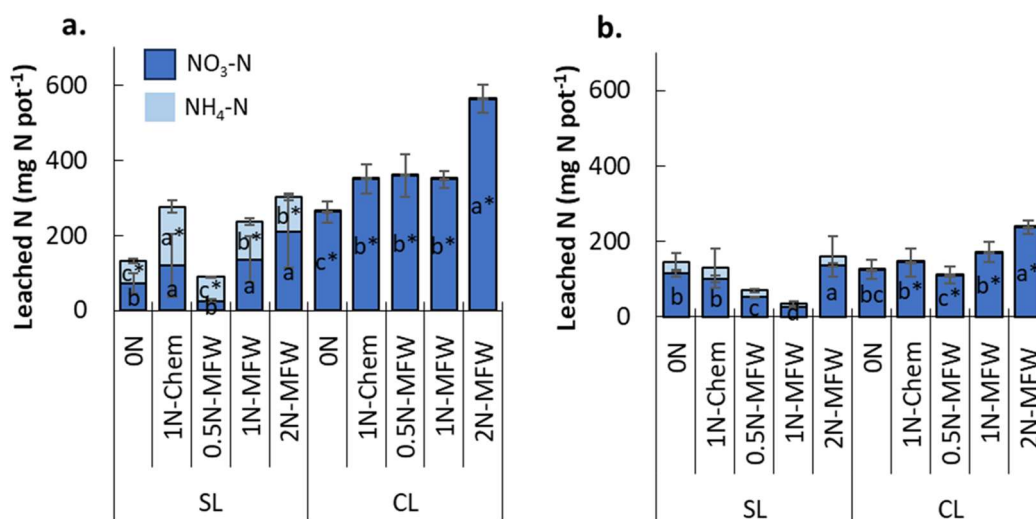


Figure S4.5. Cumulative inorganic N leached under different fertilizer treatments and soil textures during two growing seasons (2021 and 2022). (a) 2021 and (b) 2022. Fertilizer treatments: 0N: no fertilizer; 1N-Chem: 750 mg $\text{NH}_4\text{Cl-N}$; 0.5N-MFW: 375 mg N as MFW; 1N-MFW: 750 mg N as MFW; and 2N as MFW: 1500 mg N as MFW. Data represent mean $\pm$ SE from four replications, except for CL-1N-Chem in 2021 and SL-1N-MFW, SL-2N-MFW, and CL-0N in 2022, which have three replications.

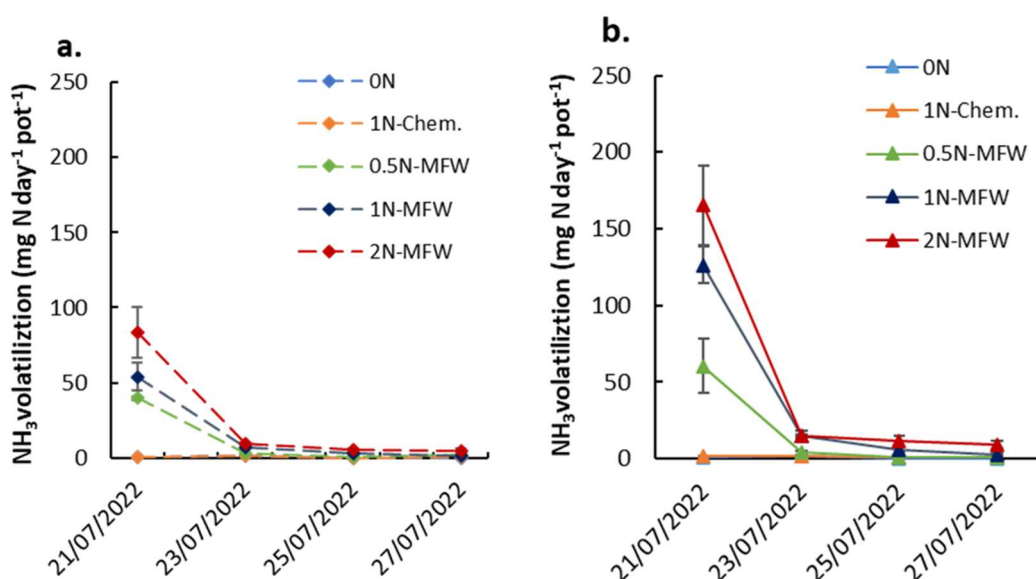


Figure S4.6. Ammonia volatilization under different fertilizer treatments and contrasting soil textures during the first week of the 2022 season. (a) SL soil and (b) CL soil. Fertilizer treatments: 0N: no fertilizer; 1N-Chem: 750 mg $\text{NH}_4\text{Cl-N}$; 0.5N-MFW: 375 mg N as MFW; 1N-MFW: 750 mg N as MFW; and 2N as MFW: 1500 mg N as MFW. Data represent mean $\pm$ SE from four replications, except for CL-1N-Chem in 2021 and SL-1N-MFW, SL-2N-MFW, and CL-0N in 2022, which have three replications.

APPENDIX 2: PHOTOS



Picture 1: The system of rice experiment



Picture 2: The system of the maize experiment



Picture 3: The modification of gas collection for maize in the first season



Picture 4: The growing stage of maize and rice in the second season



Picture 5: The rice plants in treatments of the first season



Picture 6: The rice plants in treatments of the second season



Picture 7: The maize plants in treatments of the second season

PUBLICATIONS

Chapter III

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

Article DOI: <https://doi.org/10.1080/00380768.2025.2508979>.

Author(s): Bui Ngoc Tan, Makoto Shibata, Keisuke Matsubara, Keitaro Fukushima, Toru Matoh, Shinya Funakawa

Journal Title: Soil Science and Plant Nutrition

Published online: 27 May 2025.

Chapter IV

Article Title: Impact of methane fermentation waste fluid application rates on maize yield and fate of N with special reference to soil textures.

Article DOI: <https://doi.org/10.1080/00380768.2025.2521507>.

Author(s): Bui Ngoc Tan, Makoto Shibata, Keisuke Matsubara, Keitaro Fukushima, Han Lyu, Toru Matoh, Shinya Funakawa

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