NITROUS OXIDE EMISSIONS FROM PADDY AND MANGROVE FOREST SOILS AS AFFECTED BY ENVIRONMENTAL CONDITIONS

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ABSTRACT

Denitrification is an important pathway to remove nitrate (NO$_3^-$) from water and soil under anaerobic conditions. Nitrous oxide (N$_2$O), which is an intermediate product of the denitrification process, contributes to global warming and stratospheric ozone depletion. Most of studies have investigated N$_2$O emission in upland soil. Few research works examined N$_2$O emission in wetlands because N$_2$O is easily transformed to N$_2$ under strongly anaerobic conditions. Recently, hotspots for N$_2$O emissions were reported to be in agricultural areas, riparian wetlands, and mangrove forests. Up to now, environmental conditions responsible for N$_2$O emission in wetlands are still uncertain. The thesis aimed at preventing N$_2$O emission into the atmosphere by gaining better understanding of the mechanisms for N$_2$O emission in paddy and mangrove forest soils. We examined numerous environmental factors responsible for N$_2$O emission in wetlands. We found that: 1) addition of NO$_3^-$ significantly enhanced emissions of N$_2$O and N$_2$. Nitrous oxide was the main denitrification product in acidic soil while N$_2$ was the dominant product in neutral and slightly acidic soils; 2) interactive effects of low pH and higher EC promoted N$_2$O emissions; 3) high EC and low pH in a tropical mangrove forest might inhibit the activity of N$_2$O reductase, and therefore greater N$_2$O was produced in mangrove sediment when sediment receives high NO$_3^-$ inputs; 4) incorporation rice husk char and ash into soil reduced N$_2$O emissions, whereas direct return of rice husk to soil enhanced N$_2$O and CO$_2$ emissions; 5) continuous flooding during fallow periods in Ushimado paddy fields would reduce N$_2$O emissions from soil and NO$_3^-$ concentration in groundwater.

Results obtained from a series of laboratory experiments provide significant insight into the effects of soil pH on N$_2$O emission in wetlands. Low pH inhibited the activity and synthesis of N$_2$O reductase, resulting in promoted N$_2$O emission as an intermediate product. We found that N$_2$O emission in wetlands is governed by soil type, NO$_3^-$ concentration, organic C, salinity, soil moisture, and soil pH. Paddy and mangrove forest soils should be paid attention to as a hotspot for N$_2$O emission under certain conditions. This thesis will contribute to our understanding on N$_2$O emission in wetlands to develop useful strategies to mitigate global warming.

KEYWORDS
Acetylene inhibition method, denitrification, flood and drain cycle, flooded soil, nitrous oxide, mangrove forest, rice husk, soil moisture, water irrigation.
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Chapter 1
GENERAL INTRODUCTION

1.1. NITROUS OXIDE

Nitrous oxide (N\textsubscript{2}O) is an important greenhouse gas as it contributes to global warming and the depletion of the stratospheric ozone layer. Nitrous oxide molecules stay in the atmosphere for an average of 114 years and had a global warming potential almost 298 times greater than carbon dioxide (CO\textsubscript{2}). Atmospheric concentrations of N\textsubscript{2}O have been increasing as a result of human activities, disturbing the natural nitrogen (N) cycle. The Intergovernmental Panel on Climate Change (IPPC) reported that globally averaged atmospheric N\textsubscript{2}O was 324.2 ppb (324.0 to 324.4) in 2011 and has increased by 20% since 1750 (Hartmann et al. 2013).

Figure 1.1. Globally averaged N\textsubscript{2}O dry-air mole fractions from Advanced Global Atmospheric Gases Experiment (red) and National Oceanic and Atmospheric Administration, Earth System Research Laboratory, Global Monitoring Division (blue) at monthly resolution (Hartmann et al. 2013).

Nitrous oxide is naturally present in the atmosphere as part of the Earth's nitrogen cycle, and has produced by both natural and anthropogenic sources. Important natural sources include soils under natural vegetation and the oceans which create 62% of total
N$_2$O emissions. Human activities such as agriculture, fossil fuel combustion and industrial processes are responsible for 38% of total N$_2$O emissions (Denman et al. 2007). Since the Industrial Revolution, human sources of N$_2$O emissions have been growing. Activities such as agriculture, fossil fuel combustion and industrial processes are the primary cause of the increased N$_2$O concentrations in the atmosphere. Agricultural soils are recognized as the major source of atmospheric N$_2$O, contributes to 67% of the global anthropogenic emissions (Denman et al. 2007). In the estimation approach, the IPPC divides N$_2$O emissions from agricultural landscape into direct and indirect emissions. Direct N$_2$O emission refers to N$_2$O derived from applied fertilizer and manure N, which is believed to increase by fertilizer use. Indirect N$_2$O emission consists of 3 parts: N$_2$O emission associated with atmospheric N deposition, human waste, and with N lost via surface runoff and leaching. Indirect N$_2$O emissions represent 1/3 of the total agriculture emission, in which the majority (75%) come from riparian zones (riparian wetlands, drainage ditch, stream sediments), where NO$_3^-$ in leachate and NH$_4^+$ in runoff from farm land are microbially convert to N$_2$O and N$_2$ (Zaman et al. 2012).

1.2. MAIN PROCESSES RESPONSIBLE FOR N$_2$O EMISSIONS FROM SOIL

Nitrous oxide is formed in soil microbiological processes of nitrification and denitrification (Pilegaard 2013; Signor and Cerri 2013). Nitrification is the stepwise oxidation of NH$_4^+$ to NO$_2^-$ and to NO$_3^-$ with the use of O$_2$ as the electron acceptor. In this process, the NO$_2^-$ concentration increases as NH$_4^+$ is being oxidized and then decreases as NO$_3^-$ is being formed. The predominant bacteria responsible for the conversion from NH$_4^+$ to NO$_2^-$ are ammonium oxidizing bacteria and nitrite oxidizing bacteria. Denitrification is the stepwise reduction of NO$_3^-$ to NO$_2^-$ and further on to N$_2$ via NO and N$_2$O intermediates and with the use of a carbon source and the electron
donor. The predominant bacterium responsible for the conversion is heterotrophic denitrifying bacteria. Ammonium oxidizing bacteria can under oxygen limiting condition reduce NO$_2^-$ to N$_2$O, but not all the way to free N$_2$. This pathway is called the Nitrifier Denitrification pathway and is believed to be one of the major sources to N$_2$O formation and emission, especially stimulated during energy optimization of the aeration strategies. The main processes and factors for nitrous oxide formation are presented below:

![Figure 1.2. Transformations of mineral nitrogen in soil (Wrage et al. 2001).](image)

1.3. FACTORS AFFECTING N$_2$O EMISSION

The contribution of microbiological processes of nitrification and denitrification to N$_2$O emissions will vary with climate, and soil management and properties. These processes and N$_2$O production are controlled by several soil factors: soil water-filled pore space (WFPS), oxygen availability, pH, mineral nitrogen (NO$_3^-$, NH$_4^+$), available soil organic carbon (SOC), and temperature (Blackmer and Bremner 1978; Weier et al. 1993; Cheng et al. 2004; Chiu et al. 2004; Van den Heuvel et al. 2011; Ha et al. 2015; Maeda et al. 2015).

1.3.1. Soil NO$_3^-$ and NH$_4^+$ contents

Application of N fertilizers (urea ammonium, urine N) supplies mineral N to soil. Both NO$_3^-$ and NH$_4^+$ are critical for the production of N$_2$O. Under aerobic conditions, NH$_4^+$,
which is available for nitrification, can directly regulate N\textsubscript{2}O emission via nitrifier-denitrification (Wrage et al. 2001), while NO\textsubscript{3} as a substrate for denitrification affect N\textsubscript{2}O emission under anaerobic conditions (Cheng et al. 2004).

**Table 1.1. Factors controlling N\textsubscript{2}O emission from soils.**

<table>
<thead>
<tr>
<th>Factors</th>
<th>N\textsubscript{2}O emission</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mineral N (NO\textsubscript{3}, NH\textsubscript{4}+)</td>
<td>Increase with more mineral N</td>
<td>Letey et al. (1980)</td>
</tr>
<tr>
<td>Available C</td>
<td>Increase with more available C</td>
<td>Weier et al. (1993); Huang et al. (2004); Lou et al. (2007)</td>
</tr>
<tr>
<td>Soil moisture (WFPS)</td>
<td>Increased with higher WFPS</td>
<td>Bateman and Baggs (2005); Bahl et al. (2013)</td>
</tr>
<tr>
<td>pH</td>
<td>Greater at lower pH values</td>
<td>Šimek and Cooper (2002); Šimek et al. (2002); Ha et al. (2015)</td>
</tr>
<tr>
<td>Temperature</td>
<td>Increases exponentially with higher temperature</td>
<td>Smith et al. (1998)</td>
</tr>
<tr>
<td>Soil texture</td>
<td>Lower emission in fine-textured soil than in coarse textured soil,</td>
<td>Maag and Vinther (1996)</td>
</tr>
</tbody>
</table>

**1.3.2. Temperature**

Decomposition of soil organic matter and mineralization of N is stimulated by temperature increases (Leirós et al. 1999), resulting in increase N\textsubscript{2}O emissions. The optimum temperature for enzyme kinetics and metabolic turnover rates of nitrifiers and denitrifiers was at approximately 25–27°C (Canion et al. 2014).

**1.3.3. Soil moisture**

Soil moisture regulated O\textsubscript{2} availability to soil microbes and affected nitrification and denitrification (Bahl et al. 2013). Both processes can produce N\textsubscript{2}O, but the production of N\textsubscript{2}O by denitrification process is higher than that produced from the nitrification process. Nitrification is the main process of N\textsubscript{2}O emission at 35-60% WFPS. Denitrification becomes more important at soil water contents greater than 60% WFPS.
due to a decreased oxygen supply (Bateman and Baggs 2005). The optimum soil WFPS for N₂O production is 60–65%. At WFPS values >70–80%, emissions are strongly reduced, since N₂O in the soil profile will be further reduced to N₂, while under high soil nitrate concentrations N₂O can be the end product of denitrification (Conrad 1996).

1.3.4. Soil available organic C concentration

Soil organic C is an important controller of N₂O and N₂ production in soils and sediments. Available C influences nitrification and denitrification reactions, because it can stimulate microbial growth and activity, and also provide the organic carbon needed by soil denitrifiers (Weier et al. 1993; Chiu et al. 2004). Additionally, microbial growth increases the consumption of O₂ and generates anaerobic conditions necessary for denitrification (Millar et al. 2004). Increasing the soil organic C content can increase the N₂O production (Huang et al. 2004; Lou et al. 2007).

1.3.5. Soil pH

Soil pH is an important regulator of nitrification and denitrification rates as well as the ratio of products derived from these processes. Soil pH controls denitrification by mainly affecting the nitrification process. Knowles (1982) affirmed that in soils with pH of 4.0, N₂O is the main product of denitrification. For low pH soils, the emission of N₂O would be greater, because a small amount of this gas would be reduced to N₂ (Ha et al. 2015).

1.3.5. Soil characteristics

Fine–textured soils have more capillary pores within the aggregates than sandy soils, and hold soil water more tightly. As a result, anaerobic conditions may be more easily reached and maintained for longer periods within aggregates in fine–textured than in coarse-textured soils (Weier et al. 1993). Therefore, fine-textured soils with poorly drained conditions are likely to emit more N₂O for a long period than those of coarse
textured soils with well-drained conditions. Maag and Vinther (1996) indicated that the N$_2$ to N$_2$O ratios were lower in the coarse sandy soil than in the sandy loam soil.

1.4. WETLAND IS A HOTSPOT FOR N$_2$O EMISSIONS

Wetland soils have in common the condition of prolonged saturation. This condition favors certain physical, chemical, biological, and morphological tendencies that help to distinguish wetlands soil from their counterparts (Huang et al. 2011). Wetland soils including agricultural fields and bordering riparian buffer zones have received ample attention with respect to denitrification and N$_2$O emissions (Van den Heuvel 2010). Typically N$_2$O emission hotspots are found in agricultural areas (Reddy and Patrick 1986; Buresh and De Datta 1990), riparian wetlands (Hefting et al. 2004; Van den Heuvel 2010), and mangrove forests (Chiu et al. 2004; Fernandes and Loka 2011). It is important to understand the influence that environmental conditions have on both the removal of NO$_3^-$ from receiving waters (a water quality perspective) and the emission of N$_2$O to the atmosphere (a climate change perspective). However, environmental conditions responsible for N$_2$O emission from wetland are still uncertain. More knowledge on the spatial variability of N$_2$O emission will give us additional information on the factors controlling N$_2$O emission and could eventually lead to generating knowledge on potential measures to counteract this emission.

1.5. OBJECTIVES AND ORGANIZATION

The main objective of this research is to find out factors affecting mechanisms of N$_2$O emission in wetlands ecosystems. Understanding of the N$_2$O formation processes in soils and the factors influencing these emissions is fundamental to develop efficient strategies to reduce N$_2$O emissions from wetlands.

The following research questions are addressed:

- Determine the effect of soil type and nitrate concentration on denitrification
products (N₂O and N₂) under flooded condition (Chapter 2);

- Investigate effects of salt concentrations on N₂O emission from paddy soil adjusted with different pH (Chapter 3);

- Investigate emission of N₂O from sediment of a tropical mangrove forest in Central Vietnam-Implications of short-term laboratory experiment (Chapter 4);

- Investigate effects of rice husk addition and moisture content on N₂O and CO₂ emissions from paddy soil in short-term laboratory experiments (Chapter 5);

- Investigate effects of different water irrigation regimes with high nitrate concentration on N₂O emissions from fallow paddy fields (Chapter 6);

To answer these questions, a series of microcosm and pot experiments were set up under controlled condition of 25°C for short-term incubation periods.

![Figure 1.3. General nitrogen cycle in wetlands.](image)

In Chapter 1, N₂O is an important greenhouse gas and its emissions from wetlands were reviewed.

Denitrification is expected to be the main process of nitrogen (N) transformation in areas where soil and sediments contain sufficient organic C under anaerobic conditions and receive water with a high NO₃⁻ concentration. These areas include flooded rice fields riparian wetlands and mangrove forests. In such flooded soils, the main products of denitrification (N₂O or N₂) were dependent on environmental conditions. The factors
affecting denitrification end products (N$_2$O and N$_2$) in flooded soils remain uncertain. **Chapter 2** describes a laboratory microcosms study to investigate the emissions of N$_2$O and N$_2$ from three soils (two paddy soils and a mangrove soil) under flooded conditions with different NO$_3^-$ concentrations.

Long-term fertilizer applications and saline water irrigation lead to the accumulation of salts and changes in soil pH. These changes may in turn affect N$_2$O emissions from soil. The interactive effect of soil pH and salt concentrations on N$_2$O emission has not been studied well. **Chapter 3** showed a study using a paddy rice soil that receives chemical fertilizer to investigate how salt concentrations affected N$_2$O emissions from soil adjusted with different pH under flooded condition.

Regarding geographical distribution, mangroves are found in the Americas, Africa, Asia and Oceania. There are considerable uncertainties about sediment properties and environmental conditions among mangroves, which affect N$_2$O emission. Therefore, the understanding of sediment properties influencing N$_2$O emission is crucial to determine the risk of N$_2$O emission from zones where sediments contain sufficient organic carbon under anaerobic conditions and receive water with high NO$_3^-$ concentration. The Ru Cha mangrove forest has the typical tidal regime and organically rich sediment in the Central coastal areas of Vietnam. Results of this study are showed in **Chapter 4** which may contribute to the knowledge for the future risk assessment of the N$_2$O emission from tidal ecosystems in the coastal areas of Central Vietnam.

Identifying the suitable management practices for rice husks products as soil amendments are strongly required to increase soil fertility and mitigate greenhouse gas emissions. Rice husks are a great potential resource to be used as soil amendment in Asian countries. However, effects of rice husk products as soil amendment on greenhouse gas emissions were not well studied. **Chapter 5** described short-term effects
of char, ash and raw materials of rice husks additions on nitrous oxide (N\textsubscript{2}O) and carbon dioxide (CO\textsubscript{2}) emissions from paddy soil at different moisture contents.

In **Chapter 6**, we set up the pot study to simulate a paddy field in Ushimado, Japan where could be hot spots for nitrous oxide (N\textsubscript{2}O) emissions because the nitrate (NO\textsubscript{3}\textsuperscript{−}) concentration in groundwater used for irrigation was as high as 60 mg N L\textsuperscript{−1}. In general, alternating flood and drain cycles are repeated in paddy fields, depending on precipitation and irrigation. High NO\textsubscript{3}\textsuperscript{−} inputs and variable moisture content could promote N\textsubscript{2}O emissions. A study simulated a fallow period in Ushimado paddy fields to reduce the high NO\textsubscript{3}\textsuperscript{−} concentration in groundwater via irrigation management. Effects of different water irrigation regimes with high NO\textsubscript{3}\textsuperscript{−} concentrations on N\textsubscript{2}O emissions were investigated in this chapter.

In **Chapter 7** summarized the main results of chapter 2 to 6. The results from the separate chapter could give the overall explanations for the mechanism of N\textsubscript{2}O emission from studied wetland ecosystems. In addition, base on the finding of these results, we suggested for future works.
Chapter 2

EFFECTS OF SOIL TYPE AND NITRATE CONCENTRATION ON DENITRIFICATION PRODUCTS (N\textsubscript{2}O and N\textsubscript{2}) UNDER FLOODED CONDITIONS IN LABORATORY MICROECOSM

Abstract

Denitrification products (N\textsubscript{2}O and N\textsubscript{2}) were measured in three flooded soils (paddy soil from Vietnam, PV; mangrove soil from Vietnam, MV; paddy soil from Japan, PJ) with different NO\textsubscript{3}\textsuperscript{-} concentrations. Closed incubation experiments were conducted in 100 mL bottles for 7 days at 25°C. Each bottle contained 2 g of air-dried soil and 25 mL solution with NO\textsubscript{3}\textsuperscript{-} concentration (0, 5, or 10 mg N L\textsuperscript{-1}) with or without acetylene (C\textsubscript{2}H\textsubscript{2}). The N\textsubscript{2}O+N\textsubscript{2} emissions were estimated by the C\textsubscript{2}H\textsubscript{2} inhibition method. Results showed that N\textsubscript{2}O+N\textsubscript{2} emissions for 7 days were positively correlated with those of NO\textsubscript{3}\textsuperscript{-} removal from solution with C\textsubscript{2}H\textsubscript{2} ($R^2 = 0.9872$), indicating that most removed NO\textsubscript{3}\textsuperscript{-} was transformed to N\textsubscript{2}O and N\textsubscript{2} by denitrification. In PJ soil, N\textsubscript{2}O and N\textsubscript{2} emissions were increased significantly ($P < 0.05$) by the addition of greater NO\textsubscript{3}\textsuperscript{-} concentrations. However, N\textsubscript{2}O and N\textsubscript{2} emissions from PV and MV soils were increased by addition of 0 to 5 mg N L\textsuperscript{-1}, but not by 5 to 10 mg N L\textsuperscript{-1}. At 10 mg N L\textsuperscript{-1}, N\textsubscript{2} emissions for 7 days were greater in PJ soil (pH 7.0) than in PV (pH 5.8) or MV (pH 4.3) soils while N\textsubscript{2}O emissions were higher in PV and MV soils than in PJ soil. In MV soil, N\textsubscript{2}O was the main product throughout the experiment. In conclusion, NO\textsubscript{3}\textsuperscript{-} concentration and soil pH affected N\textsubscript{2}O and N\textsubscript{2} emissions from three flooded soils.

Keywords: Acetylene inhibition method, denitrification, flooded soil, nitrate removals, nitrous oxide.
2.1. INTRODUCTION

Denitrification is a microbial reaction in terrestrial and aquatic ecosystems in which nitrate (NO$_3^-$) is used as an alternative electron acceptor when oxygen is limited (Philippot et al. 2007). Denitrification is responsible for emission of nitrous oxide (N$_2$O) as an intermediate product. Nitrous oxide is a greenhouse gas and contributes to depletion of the stratospheric ozone layer (Conrad 1996). The Intergovernmental Panel on Climate Change (IPCC) reported that direct emission of N$_2$O from agricultural soils has increased over the last few decades, in parallel with increasing use of fertilizers. In 2005, N$_2$O emission from agriculture soils accounted for 60% of global anthropogenic fluxes (Smith et al. 2007).

Denitrification requires a substrate of NO$_3^-$ as an electron acceptor (Buresh and De Datta 1990; Weier et al. 1993; Cheng et al. 2004), available organic C as an electron donor (Weier et al. 1993; Chiu et al. 2004), the absence of oxygen, which is related to soil moisture contents at > 60% water-filled pore space (WFPS) (Weier et al. 1993), suitable soil temperature, which is optimal at 25°C, and suitable soil pH, which ranges from 3.8 to 8.2 (optimum around pH 7) (Šimek and Cooper 2002).

Denitrification is expected to be the main process of nitrogen (N) transformation in areas where soil and sediments contain sufficient organic C under anaerobic conditions and receive water with a high NO$_3^-$ concentration. These areas include flooded rice fields (Reddy and Patrick 1986; Buresh and De Datta 1990), riparian wetlands (Hefting et al. 2004; Van den Heuvel 2010), and mangrove forests (Chiu et al. 2004; Fernandes and Loka 2011). In such flooded soils, the main products of denitrification (N$_2$O or N$_2$) were dependent on environmental conditions. In flooded rice soils, N$_2$ was mainly produced by denitrification, because N$_2$O was further reduced to N$_2$ under anaerobic conditions (Huang et al. 2007; Iida et al. 2007). In contrast, in Tuvem mangrove
sediments (Goa, India), incomplete denitrification was responsible for up to 43–93% of N₂O production (Fernandes et al. 2010).

To our knowledge, the factors affecting denitrification end products (N₂O and N₂) in flooded soils remain uncertain. We supposed that different physical and chemical properties of soil under the same flooded condition affect denitrification products. Our study was conducted in laboratory microcosms to investigate the emissions of N₂O and N₂ from three soils (two paddy soils and a mangrove soil) under flooded conditions with different NO₃⁻ concentrations (0, 5, or 10 mg N L⁻¹) in standing water. The objective of the study was to identify the controlling factors of N₂O and N₂ emissions from soils of different types under flooded conditions.

2.2. MATERIALS AND METHODS

2.2.1. Soil sampling sites

Soil samples were collected in May 2012 from lowlands, where soils are usually flooded: a paddy field in the Thanh Trung hamlet, Vietnam (PV) (16°31’02’’N, 107°30’10’’E), a mangrove forest located in the Huong Phong commune, Vietnam (MV) (16°33’28’’N, 107°36’35’’E), and a paddy field in Ushimado, Japan (PJ) (34°37’22’’N, 134°07’40’’E). The sampling sites were surrounded by agricultural fields.

The PV, MV and PJ soils were classified according to Food and Agriculture Organization (FAO) as Dystric Fluvisols, Gleyi-Salic Fluvisols and Dystric Gleysols, respectively. These flooded soils are usually under anaerobic conditions and rich in organic matter, which are favorable for denitrification. These areas would be expected to reduce the NO₃⁻ concentration of water entering from the agriculture fields by denitrification.

2.2.2. Soil properties

Soil samples were collected from the 0–20 cm layer, air-dried at room temperature, then
passed through a 2-mm mesh sieve. All soil samples were subjected to measurements of pH and electrical conductivity (EC) at a soil:water ratio of 1:5 after shaking for 1 h at 175 rpm, using a digital pH meter (F-23, Horiba, Japan) and EC meter (DS-14, Horiba, Japan), respectively. Total carbon (TC) and total nitrogen (TN) contents of soil were determined using a CN Corder (MT-700, Yanaco, Japan). Mineral N was extracted with 2 M KCl solution (soil:solution ratio of 1:10). The ammonium (NH$_4^+$-N) and NO$_3^-$-N contents in extracts were measured by spectrophotometry using a continuous flow analyzer (QuAAtro 2-HR, Bltec, Japan). Dissolved organic carbon (DOC) was measured by a total organic carbon (TOC) analyzer (TOC-5000A, Shimadzu, Japan) at a soil:water ratio of 1:10. Cation exchange capacity (CEC) was determined with neutral ammonium acetate (NH$_4$OAc) (Chapman 1965). Soil texture was determined by the pipette method (Sarkar and Haldar 2005). Denitrifying bacteria in soils were estimated by the most probable number (MPN) method with Giltay’s medium (Ishikuri 2011).

Soils properties were described as follows: TN, TC, DOC, and CEC in the two paddy soils (PV and PJ) were higher than in the MV soil (Table 2.1). PJ soil was neutral while PV soil was moderately acid and MV soil was extremely acid. The density of denitrifying bacteria was the highest in PJ soil (2.7×10$^4$ MPN g$^{-1}$), approximately 18 and 23 times higher than in PV and MV soils, respectively. Nitrate was not detected in all soil samples. The NH$_4^+$-N content was highest in the PV soil at 30.1 mg N kg$^{-1}$, followed by those in MV and PJ soils.
<table>
<thead>
<tr>
<th>Parameter</th>
<th>PV</th>
<th>MV</th>
<th>PJ</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH (1:5)</td>
<td>5.8</td>
<td>4.3</td>
<td>7.0</td>
</tr>
<tr>
<td>EC (mS m(^{-1}))</td>
<td>5.9</td>
<td>77.0</td>
<td>7.1</td>
</tr>
<tr>
<td>Particle size distribution (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clay</td>
<td>22</td>
<td>16</td>
<td>7</td>
</tr>
<tr>
<td>Silt</td>
<td>42</td>
<td>25</td>
<td>16</td>
</tr>
<tr>
<td>Sand</td>
<td>36</td>
<td>59</td>
<td>77</td>
</tr>
<tr>
<td>NO(_3^-)-N (mg kg(^{-1}))</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>NH(_4^+)-N (mg kg(^{-1}))</td>
<td>30.1</td>
<td>12.3</td>
<td>1.4</td>
</tr>
<tr>
<td>DOC (mg kg(^{-1}))</td>
<td>102.5</td>
<td>83.8</td>
<td>97.5</td>
</tr>
<tr>
<td>TC (g kg(^{-1}))</td>
<td>10.4</td>
<td>6.3</td>
<td>7.9</td>
</tr>
<tr>
<td>TN (g kg(^{-1}))</td>
<td>1.2</td>
<td>0.6</td>
<td>0.8</td>
</tr>
<tr>
<td>C:N ratio</td>
<td>8.9</td>
<td>9.9</td>
<td>10.4</td>
</tr>
<tr>
<td>CEC (cmol(_e) kg(^{-1}))</td>
<td>6.8</td>
<td>5.6</td>
<td>8.0</td>
</tr>
<tr>
<td>Density of denitrifying bacteria (MPN g(^{-1}))</td>
<td>0.14×10(^4)</td>
<td>0.12×10(^4)</td>
<td>2.7×10(^4)</td>
</tr>
</tbody>
</table>

PV = paddy soil from Vietnam; MV = mangrove soil from Vietnam; PJ = paddy soil from Ushimado, Japan; n.d. = not detected. The density of denitrifying bacteria was measured by the most probable number method.

2.2.3. Incubation experiment

Denitrification is a series of processes that transforms NO\(_3^-\) and NO\(_2^-\) to gaseous forms of NO, N\(_2\)O, or N\(_2\). The acetylene inhibition method was used for estimating denitrification (Zaman et al. 2012). Because C\(_2\)H\(_2\) inhibits the reduction of N\(_2\)O to N\(_2\), we determined the denitrification rates as N\(_2\)O production.

To prepare the C\(_2\)H\(_2\) saturated solutions, C\(_2\)H\(_2\) gas was passed through three Pyrex gas-washing bottles which contained 0.5 M H\(_2\)SO\(_4\), deionized water, and a certain concentration of NO\(_3^-\) solution (0, 5, or 10 mg N L\(^{-1}\)) with a flow rate of 100 mL min\(^{-1}\) for 30 minutes. For three soil samples, closed incubation experiments were conducted in a 100 mL bottle at 25\(^{\circ}\)C for 7 days under flooded conditions. Each bottle contained 2 g
of air-dried soil and 25 mL of NO$_3^-$ solution (0, 5, and 10 mg N L$^{-1}$ in a solution are equivalent to 0, 63.4, and 126.5 mg N kg$^{-1}$ oven-dried soil, respectively) with or without C$_2$H$_2$. All treatments were conducted in triplicate. A 0.5 mL gas sample was collected after 3 h on day 0 and days 1, 3, and 7 and analyzed for N$_2$O using a gas chromatograph (GC-8A, Shimadzu, Japan), equipped with a $^{63}$Ni electron capture detector. Differences in N$_2$O emissions during 7 days incubation with and without C$_2$H$_2$ were considered to be the amount of N$_2$ emission during denitrification. The N$_2$O and N$_2$ emissions were calculated by the equation below on the weight basis of air-dried soil (mg N kg$^{-1}$) (Sun et al. 2012):

$$V_{N2O} = \frac{\rho \times C_\text{g} \times (V_g + V_L \times \alpha) \times 273}{W \times (273 + T)}$$

$$V_{N2} = V_1 - V_2$$

where $V_{N2O}$ is the N$_2$O emission (mg N kg$^{-1}$), $V_1$ and $V_2$ are the N$_2$O emissions with and without C$_2$H$_2$, respectively, $\rho$ is the density of N$_2$O (1.25 kg m$^{-3}$), $C_\text{g}$ is the concentration of N$_2$O in the gas phase (ppmv), $V_g$ is the volume of head space (m$^3$), $V_L$ is the volume of the liquid phase (m$^3$), $\alpha$ is the Bunsen absorption coefficient (0.544 at 25°C), $W$ is the dry soil weight (kg), and $T$ is the temperature at sampling (25°C).

On day 7 of incubation, the butyl septum on the bottle was opened. The solution was taken out to analyze NO$_3^-$ concentration. Nitrate removal was obtained from the difference between NO$_3^-$ concentrations on days 0 and 7.

2.2.4. Statistical analysis

Data was expressed as a mean ± standard deviation (SD). An analysis of variance (ANOVA) was performed to examine the effects of NO$_3^-$ concentrations and soil types on N$_2$O and N$_2$ emissions at a significance level of 0.05. Linear regressions were analyzed to identify the relationship between N$_2$O+N$_2$ emissions and NO$_3^-$ removal.
2.3. RESULTS AND DISCUSSION

2.3.1. Effect of NO$_3^-$ concentrations on N$_2$O and N$_2$ emissions

At 25°C, N$_2$O and N$_2$ emissions in 63.4 and 126.5 mg N kg$^{-1}$ NO$_3^-$ treatments were much greater than those in treatments without NO$_3^-$ (Fig. 2.1). Addition of NO$_3^-$ significantly enhanced denitrification and emissions of N$_2$O and N$_2$ (Weier et al. 1993; Cheng et al. 2004). The effect of NO$_3^-$ concentrations (63.4 and 126.5 mg N kg$^{-1}$) on N$_2$O and N$_2$ emissions were significantly observed only in PJ soil (pH 7), while this effect was not clear in PV and MV soils ($P > 0.05$, Fig. 2.1). In PJ soil, N$_2$O and N$_2$ emissions increased with increasing concentration of NO$_3^-$.

Denitrification rates depend more on the bacterial population (Philippot and Hallin 2005). The populations of denitrifying bacteria in PV and MV soils were smaller than in PJ soil (Table 2.1). Accordingly, PV and MV soils transformed less NO$_3^-$ to N$_2$O and N$_2$ at higher NO$_3^-$ concentrations than PJ soil (Fig. 2.1, Table 2.2). Nitrate removal from solutions with C$_2$H$_2$ were positively correlated with those of denitrification in three flooded soils ($R^2 = 0.9872$, Fig. 2.2). Most removed NO$_3^-$-N was transformed to N$_2$O and N$_2$ by denitrification within 7 days because the slope of the regression line was nearly equal to 1 (Fig. 2.2).
Figure 2.1. Accumulated N\textsubscript{2}O emissions (mg N kg\textsuperscript{-1}) in a) PV, b) MV, and c) PJ soils and N\textsubscript{2} (mg N kg\textsuperscript{-1}) in d) PV, e) MV, and f) PJ soils during 7 days incubation at 25\textdegree C. Bars indicate standard deviation, \(n=3\).

Note: N\textsubscript{2}O emission (mg N kg\textsuperscript{-1}) was measured in the incubation bottle without C\textsubscript{2}H\textsubscript{2}. N\textsubscript{2} emission (mg N kg\textsuperscript{-1}) was calculated by the differences in N\textsubscript{2}O emissions during 7 days incubation with and without C\textsubscript{2}H\textsubscript{2}. PV, paddy soil from Vietnam; MV, mangrove soil from Vietnam; PJ, paddy soil from Japan.
Table 2.2. Change of NO$_3^-$ (mg N kg$^{-1}$)† after 7 d incubation with acetylene at 25°C.

<table>
<thead>
<tr>
<th>NO$_3^-$ mg N kg$^{-1}$</th>
<th>PV</th>
<th>MV</th>
<th>PJ</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>day 0</td>
<td>day 7 NO$_3^-$ removal</td>
<td>day 0</td>
</tr>
<tr>
<td>0.0</td>
<td>0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>63.4</td>
<td>65.9</td>
<td>7.7 ± 8.1</td>
<td>58.2</td>
</tr>
<tr>
<td>126.5</td>
<td>131.4</td>
<td>78.9 ± 14.4</td>
<td>52.5</td>
</tr>
</tbody>
</table>

Data are the mean ± standard deviation, $n=3$. † NO$_3^-$ concentration (mg N L$^{-1}$) in solutions were converted to NO$_3^-$ (mg N kg$^{-1}$).

Figure 2.2. Relationship between nitrate (NO$_3^-$) removal (x axis) and denitrification (y axis) in MV soil (triangles), PV soil (squares) and PJ soil (circles) on day 7 at 25°C. Bars indicate standard deviation, $n=3$.

2.3.2. Effect of soil pH on $N_2$O and $N_2$ emissions

After 7 days incubation at 25°C with 126.5 mg N kg$^{-1}$, $N_2$O emissions in PV and MV soils (1.8 and 1.6 mg N kg$^{-1}$, respectively) were significantly higher than in PJ soil (0.4 mg N kg$^{-1}$) (Fig. 2.1 a-c). Nitrogen ($N_2$) emission in the 126.5 mg N kg$^{-1}$ treatment was highest in PJ soil (89.2 mg N kg$^{-1}$), followed by PV soil (53.7 mg N kg$^{-1}$). MV soil produced the lowest $N_2$ emissions regardless of NO$_3^-$ concentrations, with a range from 0 to 1.1 mg N kg$^{-1}$ (Fig. 2.1 d-f). Denitrification ($N_2+ N_2O$) was the highest in PJ soil (pH 7.0), followed by PV (pH 5.8) and MV (pH 4.3) soils.

In MV soil, $N_2$O was the main product of denitrification. In contrast, $N_2$ was the main product of denitrification in PJ and PV soils. The net $N_2$O production will be
strongly affected by the rate of further reduction to N$_2$ and therefore the N$_2$O:N$_2$ ratio is an important parameter in determining N$_2$O production originating from denitrification (Van den Heuvel 2010). Results on day 7 showed that the N$_2$O:N$_2$ molar ratio was the highest in acidic MV soil (1.7), followed by PV soil (0.03), and PJ soil (0.005). The N$_2$O:N$_2$ ratio increased with the decrease in soil pH.

Previous studies have highlighted the effects of pH on denitrification and N$_2$O, N$_2$ emissions (Koskinen and Keeney 1982; Šimek and Cooper 2002; Čuhel and Šimek 2011; Van den Heuvel et al. 2011). Denitrification was low at pH 5 or below, and the optimum pH seemed to lie between 7 and 8 (reviewed in Šimek and Cooper 2002)). In this study, soil pH was extremely acid, moderately acid, and neutral for MV, PV, and PJ soils, respectively. Probably due to the neutral pH, denitrification (N$_2$O+N$_2$) in PJ soil was higher than in PV and MV soils. Our study is consistent with previous studies in terms of effect of pH on denitrification. Several laboratory experiments using pH modified treatments and field experiments have confirmed that soil pH affects denitrification end products N$_2$O and N$_2$. Sun et al. (2012) showed that N$_2$O was the major component of gas products of denitrification in forest soils (acid soils), while N$_2$ was the dominant product in grassland soil (close to neutral soil). Koskinen and Keeney (1982) conducted a field experiment, in which silt loam soil was maintained at different pH. They found that at the near-neutral pH of 6.9, N$_2$ was the main product, and a more acidic condition (pH 4.6 and 5.4) favored the emission of N$_2$O. In our study, higher N$_2$O emission by denitrification occurred in MV soil, because a small amount of N$_2$O was transformed to N$_2$. The mechanism of pH controlling denitrification products is explained by the sensitivity of N$_2$O reductase activity to pH. Under acidic pH, the activity and synthesis of N$_2$O reductase is inhibited, resulting in increased N$_2$O accumulation (Šimek and Cooper 2002). Soil pH can be used as a predictive parameter
for denitrification and N$_2$O emission (Čuhel and Šimek 2011).

**2.4. CONCLUSIONS**

We investigated controlling factors of N$_2$O and N$_2$ emissions from three flooded soils of different types and NO$_3^-$ concentrations. Results showed that denitrification (N$_2$O+N$_2$) in PJ soil was NO$_3^-$ limited. In PV and MV soils, denitrification was increased by NO$_3^-$ addition from 0 to 63.4 mg N kg$^{-1}$, but was not affected by that from 63.4 to 126.5 mg N kg$^{-1}$. Denitrification products (N$_2$O and N$_2$) varied among the studied soils. Denitrification (N$_2$O+N$_2$) was higher in the neutral soil (PJ) than that in the moderately acidic soil (PV) and extremely acidic soil (MV). Nitrous oxide was the main product in MV (acidic soil) while N$_2$ was the dominant product in PJ and PV soils. We suggest that NO$_3^-$ concentration and soil pH affected N$_2$O and N$_2$ emissions in three flooded soils.
Chapter 3

EFFECT OF SALT CONCENTRATIONS ON N\textsubscript{2}O EMISSION FROM PADDY SOIL ADJUSTED WITH DIFFERENT pH

Abstract

Long-term fertilizer applications and saline water irrigation lead to the accumulation of salts and changes in soil pH. These changes may in turn affect N\textsubscript{2}O emissions from soil. Effects of salt concentrations on N\textsubscript{2}O emissions from paddy soil adjusted with different pH were examined in a closed incubation experiment in 100 mL glass bottles at 25°C. Each bottle contained 5 g of air-dried soil and 25 mL of solution, which includes K\textsubscript{NO\textsubscript{3}} (10 mg N L\textsuperscript{-1}) and NaCl (0, 0.005, 0.01, 0.05, 0.1 or 0.2 M) for adjusting different electrical conductivity (EC) levels under different pH: acid (HCl 0.005 M), alkali (NaHCO\textsubscript{3} 0.005 M), and neutral (no adjustment). Nitrous oxide emissions increased with increasing of NaCl concentrations in the acidic treatment. Nitrous oxide emissions peaked at 24 h of incubation and fell sharply afterwards due to the further transformation of N\textsubscript{2}O to N\textsubscript{2} by the dentrification process. At 24 h, the highest N\textsubscript{2}O emission was measured in 0.2 M NaCl (7.5 mg N kg\textsuperscript{-1}), followed by in 0.1, 0.05, 0.01, 0.005 and 0 M treatments. In neutral and alkali treatments, there was no significant effect of NaCl concentrations on N\textsubscript{2}O emissions during 72 h incubation (P > 0.05). We conclude that higher NaCl concentrations at acidic pH promote N\textsubscript{2}O emissions.

Keywords: Denitrification, pH effects, nitrous oxide, NaCl concentrations.
3.1. INTRODUCTION

Nitrous oxide is a greenhouse gas and contributes to the depletion of the stratospheric ozone layer (Conrad 1996). The Intergovernmental Panel on Climate Change (IPCC) reported that N\textsubscript{2}O emissions from agricultural soil have increased over the last few decades, in parallel with increasing use of fertilizer (Houghton et al. 1990). In 2005, N\textsubscript{2}O emission from agriculture soil accounted for 60% of global anthropogenic emissions (Smith et al. 2007). Nitrous oxide is mainly produced as a byproduct of nitrification and an intermediate product denitrification processes (Mosier 1998).

Long-term fertilizer application accumulates salts in soil. Sodium chloride (NaCl) is found as a predominant salt in most saline soils. Many researchers reported that salt accumulation affected microbial activities and therefore N transformations in relation to N\textsubscript{2}O production (Menyailo et al. 1998; Inubushi et al. 1999; Laverman et al. 2007; Yuan et al. 2007). However, there still remained conflict results about the effect of salt on N\textsubscript{2}O emission from soil. High salt concentration inhibited N\textsubscript{2}O reductase, resulting in increased N\textsubscript{2}O production (Menyailo et al. 1998; Azam and Müller 2003; Marton et al. 2012). In contrast, N\textsubscript{2}O production was decreased by more salt addition (Cheng et al. 2013) or not significantly affected by different salt concentrations (Inubushi et al. 1999).

The application of chemical fertilizer acidifies the soil, the extent of which depends on the type of fertilizer (Adams 1984; Sumner 1994; Hardy 2008). Sumner (1994) and Hardy (2008) reported that fertilizer salt application often causes the decreases of soil pH over 0.5 units. The pH of soil has been called the master variable, since it affects numerous soil processes and chemical, physical and biological properties (Brady and Weil 1999; Šimek and Cooper 2002). In particular, pH markedly affects nitrification and denitrification processes and thereby N\textsubscript{2}O production (Šimek et al. 2002; Čuhel and

The interactive effect of soil pH and salt concentrations on N$_2$O emission has not been studied well. Our study was undertaken by using a paddy rice soil that receives chemical fertilizer to investigate how salt concentrations affected N$_2$O emissions from soil adjusted with different pH under flooded condition.

3.2. MATERIAL AND METHODS

3.2.1. Sampling site description

Soil was collected from a paddy field in Ushimado, Okayama Prefecture, Japan (34°37′23″N, 134°07′40″E) before rice transplanting in May 2013. Soil samples were taken randomly from 6 points in the field from the 0–20 cm layer. After the collection, soil was well mixed and air-dried at room temperature, then passed through a 2 mm sieve. The soil was characterized as loamy sand (77% sand, 16% silt, and 7% clay), and classified as Dystric Gleysols according to the Food and Agriculture Organization (FAO 1998). The soil properties were described as follows: pH 6.5, electric conductivity (EC) 0.105 dS m$^{-1}$, NO$_3^{-}$ content 25.6 mg N kg$^{-1}$, NH$_4^{+}$ contents 2.5 mg N kg$^{-1}$, total carbon (TC) 11.6 g C kg$^{-1}$, total nitrogen (TN) 1.2 g N kg$^{-1}$. The C:N ratio was a normal ratio in the agriculture soil (C:N 9.7).

3.2.2. Experimental set up

Five grams of air-dried soil were placed in 100 mL glass bottles. Twenty five mL of solution, including KNO$_3$ (10 mg N L$^{-1}$ equivalent to 50 mg N kg$^{-1}$ soil), and NaCl (0 M, 0.005 M, 0.01 M, 0.05 M, 0.1 M, or 0.2 M) with different pH treatments: acid (HCl 0.005 M), or alkali (NaHCO$_3$ 0.005 M), or neutral (no adjustment) was added in the bottle. The bottles were sealed with a butyl septum, and incubated at 25°C for 72 hours. The headspace gas in each glass bottle was taken out 3, 24, 48, and 72 h after incubation. Nitrous oxide concentrations in the collected gas samples were analyzed with a gas
chromatograph (GC-8A, Shimadzu, Japan) equipped with an electron capture detector. Nitrous oxide emissions (ppm,\(_v\)) were converted to a mass unit (mg N kg\(^{-1}\)) using the following equation (Sun \textit{et al.} 2012):

\[
V_{N_2O} = \frac{(\rho \times C_g \times (V_g + V_L \times \alpha) \times 273)}{(W \times (273 + T))}
\]

\(V_{N_2O}\) is the \(N_2\)O emission (mg N kg\(^{-1}\)), \(\rho\) is the density of \(N_2\)O (1.25 kg m\(^{-3}\)) at 25°C and standard pressure, \(C_g\) is the concentration of \(N_2\)O in the gas phase (ppm,\(_v\)), \(V_g\) is the volume of head space (m\(^3\)), \(V_L\) is the volumes of liquid phase (m\(^3\)), \(\alpha\) is the Bunsen absorption coefficient (0.549 at 25°C), and \(W\) is the dry soil weight (kg), and \(T\) is the temperature at determination (25°C).

After gas collection, solutions were taken out for measuring pH, EC and NO\(_3\) concentration. All experiments were performed in triplicate.

\textbf{3.2.3. Statistical analysis}

All results are reported as a mean ± standard deviation. The Tukey HSD test was used to examine the difference in concentrations of \(N_2\)O or NO\(_3\) among different NaCl concentrations and pH treatments. An analysis of variance (ANOVA) was used to examine the effect of pH and salinity on \(N_2\)O emission \((P < 0.05)\). All statistical analyses were performed using the R software (version 2.6.2).

\textbf{3.3. RESULTS AND DISCUSSION}

\textbf{3.3.1. The changes in pH and EC of solutions under different NaCl concentrations and pH treatments}

At the same NaCl concentration, EC did not change during 72 h incubation \((P > 0.05)\). However, EC values increased in the pH adjustments. Electrical conductivity was the highest in the acidic treatment, following by alkali and neutral treatments (Table 3.1).

There was no significant difference in pH among different NaCl concentrations \((P > 0.05)\). The HCl and NaHCO\(_3\) adjustment to soil changed pH values. pH values at 3 h
after incubation were 5.7, 6.5, and 7.5 in acidic, neutral and alkali treatments, respectively. The sharp increase was observed in the acid treatment. The pH solution was acidic (pH 5.7) at the beginning, and shifted to neutral afterwards. There was no signification change in pH under the alkali treatment (Table 3.2).

Table 3.1. Changes in EC (dS m\(^{-1}\)) of solutions with different NaCl concentrations and pH treatments.

<table>
<thead>
<tr>
<th>NaCl conc.</th>
<th>Acid treatment</th>
<th>Neutral treatment</th>
<th>Alkaline treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Incubation period (hours)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>24</td>
<td>48</td>
</tr>
<tr>
<td>0 M</td>
<td>0.9</td>
<td>0.9</td>
<td>0.8</td>
</tr>
<tr>
<td>0.005 M</td>
<td>1.4</td>
<td>1.4</td>
<td>1.4</td>
</tr>
<tr>
<td>0.01 M</td>
<td>1.9</td>
<td>1.6</td>
<td>1.9</td>
</tr>
<tr>
<td>0.05 M</td>
<td>5.8</td>
<td>5.8</td>
<td>5.7</td>
</tr>
<tr>
<td>0.1 M</td>
<td>10.3</td>
<td>10.4</td>
<td>10.2</td>
</tr>
<tr>
<td>0.2 M</td>
<td>18.6</td>
<td>18.4</td>
<td>18.5</td>
</tr>
</tbody>
</table>

Data are represented as means of triplicate samples.

Table 3.2. Changes in pH of solutions with different NaCl concentrations and pH treatments.

<table>
<thead>
<tr>
<th>NaCl conc.</th>
<th>Acid treatment</th>
<th>Neutral treatment</th>
<th>Alkaline treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Incubation period (hours)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>24</td>
<td>48</td>
</tr>
<tr>
<td>0 M</td>
<td>5.7</td>
<td>6.3</td>
<td>6.6</td>
</tr>
<tr>
<td>0.005 M</td>
<td>5.8</td>
<td>6.3</td>
<td>6.4</td>
</tr>
<tr>
<td>0.01 M</td>
<td>5.5</td>
<td>6.4</td>
<td>6.7</td>
</tr>
<tr>
<td>0.05 M</td>
<td>5.3</td>
<td>6.2</td>
<td>6.9</td>
</tr>
<tr>
<td>0.1 M</td>
<td>5.3</td>
<td>6.2</td>
<td>6.7</td>
</tr>
<tr>
<td>0.2 M</td>
<td>5.3</td>
<td>6.1</td>
<td>6.6</td>
</tr>
</tbody>
</table>

Data are represented as means of triplicate samples.

3.3.2. Effect of salt on NO\(_3\)\(^-\) concentrations under pH treatments

Nitrate concentrations showed similar trends among treatments (Fig. 3.1). At the beginning of the experiment, NO\(_3\)\(^-\) concentration was 75.6 mg N kg\(^{-1}\) (the sum of concentrations in soil and solution) and then decreased with the incubation time. The sharp reduction of NO\(_3\)\(^-\) concentration was recorded from 3 to 24 h after incubation.
Nitrate remained a half by 24 h as compared with an initial concentration and decreased afterwards, the rest was supposed to be removed by denitrification.

No significant difference was detected in NO$_3^-$ concentration among different NaCl treatments during 72 h incubation ($P > 0.05$). The result indicated that salinity did not affect NO$_3^-$ decreases and denitrification process. Similarly, NO$_3^-$ was completely loss by denitrification and not affected by EC at 90% WFPS (Adviento-Borbe et al. 2006). On the other hand, our study is in contrast to other findings (Rysgaard et al. 1999; Seo et al. 2008; Nakano et al. 2010). Rysgaard et al. (1999) showed that denitrification of NO$_3^-$ from the water column decreased with increasing salinity. Salinity may provide a physiological stress on a microbial level, lead to inhibit denitrifying activity. In the acidic treatment, a significantly higher NO$_3^-$ concentration was detected than those in the neutral and alkaline treatments at 24 h ($P < 0.05$, Fig. 3.1). pH regulated denitrification. Acid conditions suppressed the activities and populations of denitrifiers in soil resulting in low NO$_3^-$ removal (Šimek et al. 2002; Čuhel and Šimek 2011; Sun et al. 2012).

![Figure 3.1](image.png)

**Figure 3.1.** Nitrate concentration (mg N kg$^{-1}$) remained in solution with different salt concentrations in a) acidic treatment; b) neutral treatment; c) alkali treatment.
3.3.3. Effect of salt concentrations on $N_2O$ emission from soil with different pH

During 72 h incubation, effects of NaCl concentrations on $N_2O$ emissions were different among pH treatments (Fig. 3.2). The ANOVA two-way test indicated that salinity significantly affected $N_2O$ emission from soil treated acidic pH ($P < 0.05$).

In the acidic treatment, $N_2O$ emissions peaked at 24 h incubation. Nitrous oxide emissions increased with increasing NaCl concentrations ($P < 0.05$). The highest $N_2O$ emission was 7.5 mg N kg$^{-1}$ in 0.2 M NaCl, followed by NaCl 0.1 M, 0.05 M, 0.01 M, 0.005 M, and 0 M, respectively. There was a statistically significant relationship between NaCl concentrations and $N_2O$ emissions (Fig. 3.3). The activity and synthesis of $N_2O$ reductase that transforms $N_2O$ to $N_2$, is inhibited at low pH (Šimek et al. 2002; Marton et al. 2012) and high salts concentrations (Menyailo et al. 1998; Marton et al. 2012; Zhao et al. 2013). Negative correlation between $N_2O$ emission and $NO_3^-$ concentration was observed in Fig. 3.1-3.2, suggesting that $N_2O$ was formed as intermediate product of the denitrification process at 24 h incubation. In the later period, $N_2O$ reductase transformed $N_2O$ to $N_2$. Results showed a sharp decrease of $N_2O$ emission in all salinity treatments afterwards. Interactive effect of low pH and higher salinity under flooded conditions retarded $N_2O$ reduction to $N_2$, resulting in promoting $N_2O$ emissions at 24 h incubation. Similarly, Marton et al. (2012) confirmed that $N_2O$ increased with salinity in low pH soil (pH 5.5), while neutral pH soils were unaffected.

In neutral and alkali treatments, there was no significant effect of NaCl concentrations on $N_2O$ emission during 72 h incubation ($P > 0.05$). The $N_2O$ emission was not detected at 3 h of incubation, then ranged from 0.02 to 0.11 mg N kg$^{-1}$ among salt levels during 24 to 72 h incubation.
Figure 3.2. Nitrous oxide emission (mg N kg\(^{-1}\)) from soil with different salt concentrations in a) acidic treatment; b) neutral treatment; c) alkali treatment. Bars show standard deviation.

Figure 3.3. The relationship between \(\text{N}_2\text{O}\) emission and NaCl concentrations in soil incubated for 24 h in different pH treatments. The increase of symbol size indicated the increase of NaCl concentration.

3.4. CONCLUSION

Greater NaCl concentration promoted \(\text{N}_2\text{O}\) emissions when pH was adjusted to acid under flooded condition. In some regions, paddy soils are changed in pH and EC because of long-term fertilizer application and saline water irrigation. These areas have a great potential of \(\text{N}_2\text{O}\) emissions.
Chapter 4

NITROUS OXIDE EMISSION FROM SEDIMENT OF A TROPICAL MANGROVE FOREST IN CENTRAL VIETNAM-IMPLICATION OF SHORT-TERM LABORATORY EXPERIMENTS

Abstract

This study investigated nitrous oxide (N\textsubscript{2}O) emission and the environmental parameters responsible for its generation in Ru Cha mangrove sediment (Thua Thien Hue, Vietnam). To determine cumulative amounts of N\textsubscript{2}O emission from different sediment layers, closed incubation experiments were conducted at 25\degree C for 7 days in 100 mL glass bottles that contained 5 g of sectioned sediments (0‒5, 5–10 and 10–15 cm depths) and 25 mL of the ambient water. Cumulative amounts of N\textsubscript{2}O emission decreased with sediment depth, which coincided with decreases in the density of denitrifiers, and contents of total organic carbon, total nitrogen and ammonium from upper to lower sediment layers. A similar incubation experiment was conducted using the 0‒5 cm sediment layer to investigate the effect of different nitrate (NO\textsubscript{3}\textsuperscript{-}) concentrations (0, 1, 3 and 5 mg N L\textsuperscript{-1}) on potential N\textsubscript{2}O emission. Nitrous oxide emission increased with NO\textsubscript{3}\textsuperscript{-} addition, indicating that N\textsubscript{2}O emission was limited by NO\textsubscript{3}\textsuperscript{-}. Nitrate was completely removed from the solution in all NO\textsubscript{3}\textsuperscript{-} treatments by denitrification. Nitrous oxide emission accounted for 20\%, 55\% and 100\% of the amounts of NO\textsubscript{3}\textsuperscript{-} removed in 1, 3 and 5 mg N L\textsuperscript{-1} treatments, respectively. Our laboratory experiments suggest that the Ru Cha mangrove forest could be a hotspot of N\textsubscript{2}O emissions when sediment receives high NO\textsubscript{3}\textsuperscript{-} inputs.

Keywords: Anaerobic condition, denitrification, mangrove forest, nitrate limited, N\textsubscript{2}O.
4.1. INTRODUCTION

Mangroves are salt-tolerant evergreen forests found along sheltered coastlines, shallow-water lagoons, estuaries, rivers and deltas in 124 tropical and subtropical countries and areas, covering about 15.2 million hectares in 2005 (FAO 2007). They function as efficient buffer zones by reducing excess nutrients in estuarine systems (Corredor and Morell 1994). Mangrove sediments are rich in organic matter under anaerobic conditions, which is favorable for denitrification (Fernandes et al. 2010). Chiu et al. (2004) reported that as much as 55% of the nitrogen (N) input in mangrove sediment was lost through denitrification. This process reduces the NO$_3^-$ concentration in surface water and therefore controls eutrophication (Marylynn et al. 1992; Fletcher et al. 2000). On the other hand, incomplete denitrification is responsible for emissions of N$_2$O, which is a greenhouse gas and contributes to depletion of the stratospheric ozone layer (Conrad 1996). Nitrous oxide emissions depend on numerous factors such as NO$_3^-$ concentration, oxygen levels, pH, salinity, temperature, organic matter availability and microbial population (Cheng et al. 2004; Van den Heuvel et al. 2011; Ha et al. 2015; Maeda et al. 2015). Regarding geographical distribution, mangroves are found in South China (Chen et al. 2010), Taiwan (Chiu et al. 2004), Puerto Rico (Corredor and Morell 1994), India (Fernandes et al. 2010; Fernandes and Loka 2011), Australia (Kreuzwieser et al. 2003). There are considerable uncertainties about sediment properties and environmental conditions in mangroves that would affect N$_2$O emission. Chen et al. (2010) found that N$_2$O fluxes increased with soil organic carbon, nitrogen, iron concentrations and soil porosity while they were negatively correlated with salinity in mangrove swamps of South China. Pore-water nutrient concentrations, organic carbon availability and denitrifiers were important sediment properties affecting the production of N$_2$O in a mangrove in Goa, India (Fernandes et al. 2010). Corredor and Morell
(1994), Fernandes and Loka (2011) and Kreuzwieser et al. (2003) investigated the effects of NO$_3^-$ on potential denitrification and N$_2$O emission by using a wide range of NO$_3^-$ concentrations. They showed that N$_2$O emissions in a mangrove were NO$_3^-$ limited. Percentage of N$_2$O emission and NO$_3^-$ consumption varied in mangrove forests. Therefore, the target of these studies was to investigate the key parameters that govern N$_2$O emission from mangrove sediments.

The Ru Cha mangrove forest is located in Thua Thien Hue province, Vietnam, functions as an ecological buffer between the mainland and the Tam Giang lagoon. This mangrove forest experiences semi-diurnal tides with an amplitude of 0.4–0.5 m, the lowest in Vietnam (Phan and Hoang 1993). To our knowledge, no study so far has been undertaken to investigate environmental conditions and N transformations in any mangrove forests of Central Vietnam. A microcosm study was conducted in the laboratory to assess the pathway for N$_2$O emission and environmental parameters responsible for its emissions from mangrove sediment. Results of this study may contribute to the knowledge for the assessment of N$_2$O emission from tidal ecosystems in the coastal areas of Central Vietnam.

4.2. MATERIALS AND METHODS

4.2.1. Site description
The Ru Cha mangrove forest (16°33’27”N, 107°36’43”E) is located in Thua Thien Hue province, Vietnam. There are two seasons in this region: a rainy season (from August to January) and a dry season (from February to September). Average annual rainfall is 3598 mm and annual mean temperature is 25°C (Office 2011).

The Ru Cha mangrove forest has an area of ca. 5 ha and is part of tidal ecosystems in coastal areas of Thua Thien Hue province (Fig. 4.1). The Ru Cha mangrove forest is adjacent to rice paddy fields, aquaculture ponds and the Tam Giang lagoon. The
sediment is classified as Gleyi-Salic Fluvisols according to the Food and Agriculture Organization (FAO 1998). The forests are dominated by *Excoecaria agallocha* (4–7 m high), *Aegiceras corniculatum*, *Acanthus ilicifolius* and *Clerodendrum inerme*.

![Site map of the Ru Cha mangrove forest.](image)

**Figure 4.1.** Site map of the Ru Cha mangrove forest.

### 4.2.2. Sample collection

Sediment and surface water samples were collected inside the Ru Cha mangrove forest in June, 2014 (the middle of dry season). Four sediment cores were randomly collected using a sediment core sampler (50 cm long, 7.5 cm inner diameter). Sediment cores were sectioned at every 5 cm depth interval from the surface to 15 cm. Sediments and water samples were kept in the ice-box and transported to the laboratory for initial property analyses.

### 4.2.3. Sample analysis

Surface water was subjected to measurements of pH and electrical conductivity (EC) by using a digital pH meter (F-23, Horiba, Japan) and EC meter (DS-14, Horiba, Japan), respectively. Total nitrogen (TN) and total phosphorus (TP) in water samples were digested by Persulfate method. Water samples were filtered through 0.2 μm pore size, 25 mm diameter mixed cellulose ester membrane filters (Dismic-25AS020AN, Advantec, Japan). Nitrate, ammonium (NH$_4^+$), TN, TP, and chloride (Cl$^-$) were
measured by the colorimetric method using a continuous flow autoanalyzer (QuAAtro 2-HR, Bltec, Japan).

Sediment was air-dried at room temperature, then passed through a 2 mm mesh sieve. Sediment layers were subjected to measurement of pH, EC, water-extractable total organic carbon (TOC), contents of NH$_4^+$, NO$_3^-$, total carbon (TC) and TN, density of denitrifiers and soil texture. Water-extractable TOC, pH and EC values were determined at a sediment:water ratio of 1:10. Water-extractable TOC was measured with a TOC analyzer (TOC-5000A, Shimadzu, Japan). Mineral N was extracted with 2M KCl at a ratio of 1:10. The extracts were analyzed for NO$_3^-$ and NH$_4^+$. Total nitrogen and TC of sediment samples were measured by the dry combustion method with a CN Corder (MT-700, Yanaco, Japan). Soil textures were determined by the pipette method (Sarkar and Haldar 2005). The density of denitrifiers was measured as the most probable number (MPN), using Giltay’ medium (Ishikuri 2011). Initial properties of sediments are listed in Table 4.2.

4.2.4. Experimental set up

To investigate the cumulative amounts of N$_2$O emission from mangroves, laboratory microcosms were set up using the sectioned sediments. Closed incubation experiments were conducted in 100 mL glass bottles. Each bottle contained 5 g of air-dried sediment and 25 mL of the ambient water (surface water collected at sampling site). The bottle was closed with a butyl septum, then incubated at 25°C for 7 days. Nitrous oxide concentration was determined at 3 h on day 0, and days 1, 2, 3, 5 and 7 after starting incubation. A 0.5 mL gas sample was collected by using a syringe and then analyzed with a gas chromatograph (GC-8A, Shimadzu, Japan) equipped with an electron capture detector. Nitrous oxide concentration (ppmv) was converted to a mass unit (mg N kg$^{-1}$) using the following equation (Sun et al. 2012):
\[ V = (\rho \times C \times (V_g + V_l \times \alpha) \times 273) / (W \times (273 + T)) \]

where \( V \) is the cumulative amounts of N\(_2\)O emission from sediment (mg N kg\(^{-1}\)), \( \rho \) is the density of N\(_2\)O (1.25 kg m\(^{-3}\)) at 25°C and standard pressure, \( C \) is the N\(_2\)O concentration (ppmv), \( V_g \) (m\(^3\)) is the head space volume, \( V_l \) is the volume of liquid phase (m\(^3\)), \( \alpha \) is the Bunsen absorption coefficient (0.544 at 25°C), \( W \) (kg) is the dried soil weight, \( T \) is the temperature at determination (25°C).

According to the water quality (Table 6.1) and Le (2008), NO\(_3^−\) concentration in Tam Giang lagoon were from 0.05 to 0.57 mg N L\(^{-1}\). Therefore, we selected a maximum NO\(_3^−\) concentration of 5 mg N L\(^{-1}\) (10 times more than in the field) to investigate the effects of NO\(_3^−\) on potential N\(_2\)O emission. A similar incubation experiment was prepared using the 0–5 cm sediment layer. Five grams of air-dried sediment and 25 mL of a solution with 0.3 M NaCl and NO\(_3^−\) concentrations (0, 1, 3 and 5 mg N L\(^{-1}\) are equivalent to 0, 5.4, 16.1 and 26.4 mg N kg\(^{-1}\) oven-dried sediment, respectively) were incubated in 100 mL glass bottle at 25°C for 7 days. Nitrous oxide concentration was measured in the same manner with the above experiment.

On day 7 of incubation, the butyl septum on the bottle was opened. The solution was taken out to determine NO\(_3^−\) and NH\(_4^+\) concentrations. Nitrate removal was calculated from the difference between NO\(_3^−\) concentration on days 0 and 7. All treatments were conducted in triplicate.

4.2.5. Statistical analysis

All statistical analyses were performed using R software version 2.6.2. The Tukey HSD test was used to examine the significant difference (\( P < 0.05 \)) in N\(_2\)O concentration, and sediment properties (pH, EC, NH\(_4^+\), TOC, TN, and TC contents). Pearson correlation coefficients were calculated to determine the relationships between initial sediment
properties and cumulative amounts of N\textsubscript{2}O emission on day 7.

4.3. RESULTS

4.3.1. Water quality in the Ru Cha mangrove forest

The EC of mangrove water was 37 dS m\textsuperscript{-1}, almost as high as that in Tam Giang lagoon in a dry season (42.5 dS m\textsuperscript{-1}, Cao et al. (2009)). Nitrate was not detected in the surface water (Table 1). Ammonium concentration was lower than the Vietnamese standard for surface water quality (1 mg L\textsuperscript{-1}, TCVN 5942-95). However, high concentrations of TN (3.4 ± 0.3 mg L\textsuperscript{-1}) and TP (0.3 ± 0.1 mg L\textsuperscript{-1}) were recorded in mangrove water (compared with Japanese standard for lake/pond category V: TN 1 mg L\textsuperscript{-1} and TP 0.1 mg L\textsuperscript{-1}, c.f. no Vietnamese standards).

Table 4.1. Surface water quality in the Ru Cha mangrove forest

<table>
<thead>
<tr>
<th>pH</th>
<th>EC</th>
<th>NH\textsubscript{4}\textsuperscript{+}</th>
<th>NO\textsubscript{3}\textsuperscript{-}</th>
<th>TN</th>
<th>TP</th>
<th>Cl\textsuperscript{-}</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.4 ± 0.0</td>
<td>36.9 ± 0.9</td>
<td>0.4 ± 0.0</td>
<td>n.d.</td>
<td>3.4 ± 0.3</td>
<td>0.3 ± 0.1</td>
<td>13916 ± 19</td>
</tr>
</tbody>
</table>

n.d. indicates not detected.

4.3.2. Physical and chemical properties in the Ru Cha mangrove sediments

Textures of Ru Cha mangrove sediments were classified as silt loam (Table 4.2). Mangrove sediments were acidic with pH ranging from 3.8 to 5.4. Nitrate was not detected in all sediment layers. Total organic carbon, TN and NH\textsubscript{4}\textsuperscript{+} contents, and the density of denitrifiers decreased with sediment depths. No significant difference in TC was detected among different sediment layers (\(P > 0.05\)). The upper layer (0–5 cm) contained higher TN than in deeper layers.
Table 4.2. Physical and chemical properties of the Ru Cha mangrove sediments

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Sediment layers (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0–5</td>
</tr>
<tr>
<td>pH (1:10)</td>
<td>3.8 ± 0.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>EC (dS m&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>5.3 ± 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Particle size distribution (%)</td>
<td></td>
</tr>
<tr>
<td>Clay</td>
<td>8</td>
</tr>
<tr>
<td>Silt</td>
<td>70</td>
</tr>
<tr>
<td>Sand</td>
<td>22</td>
</tr>
<tr>
<td>Total organic carbon (mg kg&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>146.7 ± 2.6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>NH&lt;sub&gt;4&lt;/sub&gt;&lt;sup&gt;+&lt;/sup&gt;-N (mg kg&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>31.7 ± 0.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>NO&lt;sub&gt;3&lt;/sub&gt;-N (mg kg&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>n.d.</td>
</tr>
<tr>
<td>TC (g kg&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>24.9 ± 1.7&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>TN (g kg&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>2.1 ± 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>C:N ratio</td>
<td>13.5</td>
</tr>
<tr>
<td>Density of denitrifiers (MPN g&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>0.49×10&lt;sup&gt;5&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Different superscript letters (a, b and c) indicate significant differences in means at $P < 0.05$ among sediment layers. n.d. means not detected.

4.3.3. Cumulative amounts of N<sub>2</sub>O emission and changes of mineral N in different sediment layers

Cumulative amounts of N<sub>2</sub>O emission were significantly different among sediment depths ($P < 0.05$, Fig. 6.2). At 3 h on day 0 and on day 1, cumulative N<sub>2</sub>O emissions were less than 0.01 mg N kg<sup>-1</sup>. In the latter period of incubation, cumulative N<sub>2</sub>O emission was the highest in the 0–5 cm layer and decreased with sediment depths ($P < 0.05$). Nitrate was not detected in the ambient water on days 0 and 7. More NH<sub>4</sub><sup>+</sup> was released to the water in the upper layer of sediment on both days 0 and 7 ($P < 0.05$, Table 4.3).
0–5 cm, 5–10 cm and 10–15 cm. Bars indicate standard deviations, (n=3). Means with different letters among sediment layers are significantly different at $P < 0.05$ by using Tukey’s multiple comparison test.

### Table 4.3. Inorganic N concentration in the ambient water after 7 day incubation

<table>
<thead>
<tr>
<th>Sediment layers cm</th>
<th>NH$_4^+$ mg N kg$^{-1}$</th>
<th>NO$_3^-$ mg N kg$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>day 0</td>
<td>day 7</td>
</tr>
<tr>
<td>0–5</td>
<td>27.8</td>
<td>32.8 ± 1.7</td>
</tr>
<tr>
<td>5–10</td>
<td>12.7</td>
<td>18.9 ± 1.9</td>
</tr>
<tr>
<td>10–15</td>
<td>11.7</td>
<td>15.6 ± 1.2</td>
</tr>
</tbody>
</table>

n.d. indicates not detected.

### 4.3.4. Cumulative amounts of N$_2$O emission from the 0–5 cm layer sediment at different NO$_3^-$ concentrations

In the treatment without NO$_3^-$ addition, results of cumulative N$_2$O emission were similar to those in the ambient water treatment (0.036 mg N kg$^{-1}$, Fig. 4.3 a). The higher the NO$_3^-$ added to solution, the more N$_2$O emitted from the 0–5 cm layer (Fig. 4.3). Nitrate was completely removed from the solution at the end of incubation in all NO$_3^-$ treatments by denitrification (Table 4.4). Nitrous oxide emissions accounted for 20%,
55% and 100% of the amount of NO$_3^-$ removed in 5.4, 16.1 and 26.4 mg N kg$^{-1}$ treatments, respectively. The amount of N$_2$O emission was nearly equal to the NO$_3^-$ removal in the 26.4 mg N kg$^{-1}$ treatment. Nitrous oxide was the major end product of denitrification when sediment received higher NO$_3^-$ input.

Figure 4.3. Cumulative amounts of N$_2$O emission from the 0–5 cm sediment layer under different NO$_3^-$ treatments. Bar indicate standard deviation, ($n=3$).
### Table 4.4. Inorganic N concentration in NO$_3^-$ added treatments after 7 days incubation.

<table>
<thead>
<tr>
<th>NO$_3^-$ treatments</th>
<th>NH$_4^+$ mg N kg$^{-1}$</th>
<th>NO$_3^-$ mg N kg$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>day 0</td>
<td>day 7</td>
</tr>
<tr>
<td>0 mg N kg$^{-1}$</td>
<td>21.0</td>
<td>31.8 ± 0.7</td>
</tr>
<tr>
<td>5.4 mg N kg$^{-1}$</td>
<td>23.4</td>
<td>35.4 ± 0.8</td>
</tr>
<tr>
<td>16.1 mg N kg$^{-1}$</td>
<td>24.6</td>
<td>34.4 ± 1.9</td>
</tr>
<tr>
<td>26.4 mg N kg$^{-1}$</td>
<td>23.1</td>
<td>29.1 ± 1.9</td>
</tr>
</tbody>
</table>

n.d. indicates not detected.

### 4.4. DISCUSSION

#### 4.4.1. Physical and chemical characteristic of the Ru Cha mangrove sediment

The Ru Cha mangrove forest experiences semi-diurnal tides with low tidal amplitude, meaning that salty water remains in mangrove sediments for a long time (Phan and Hoang 1993). Thus, sediments in the Ru Cha mangrove were more saline (EC was 5.3 dS m$^{-1}$ at a sediment:water ratio of 1:10 for the 0–5 cm layer) than in a mangrove forest of South China (Chen et al. 2010) (EC was 1.67-4.7 dS m$^{-1}$ at a sediment:water ratio of 1:5). The Ru Cha mangrove sediment was extremely acidic, in which sediment pH lower than those in other mangroves (in China (Chen et al. 2010): 5.1-6.6; in India(Fernandes et al. 2010): 5.8-6.0). Mangroves sediments in Ru Cha contain a high content of pyrite (FeS$_2$) (Tran et al. 2005). When the pyrite is exposed to the air, it is oxidized and produces sulfuric acid (H$_2$SO$_4$). Thus pyrite containing soils are extremely acidic (pH < 4) (Dent 1986). In addition, the release of organic acids through the decomposition of mangroves litter acidifies the sediment (Reef et al. 2010) and the litter supplies high contents of N and C in sediment (Donato et al. 2011). Sediments in the Ru Cha mangroves were saline, extremely acidic, frequently waterlogged and rich in
organic matter, which affected the N₂O emission through denitrification.

4.4.2. Environmental factors affecting cumulative amounts of N₂O emission from different sediment layers

Cumulative amounts of N₂O emissions decreased with sediment depth (Fig. 4.2). Sediments showed a decline in the density of denitrifiers, TOC and TN and NH₄⁺ contents with sediment depth, which would lead to a decrease in denitrification activities and N₂O emissions in deeper layers (Fig. 4.2, Table 4.2). Similar findings were reported by previous studies (Koch et al. 1992; Chiu et al. 2004; Fernandes and Loka 2011). The higher the density of denitrifiers in the sediment, the higher the N₂O produced. Microorganisms play an important role in N₂O emission (Fernandes et al. 2010), and their activities are governed by biological, chemical and physical factors in the soil (Knowles 1982; Cheng et al. 2004). The relationships of cumulative amounts of N₂O emission on day 7 with physical chemical parameters in the Ru Cha mangrove forest are shown in Table 4.5. Our results indicate that N₂O emission increased with increasing soil TOC, EC, NH₄⁺, TN and TC content but was negatively correlated to soil pH.

Cumulative amounts of N₂O emission were low during the 7 d incubation. This is probably due to non-detectable NO₃⁻ during the incubation. More NH₄⁺ was released to the water in the upper layer of sediment. Nitrogen mineralization and the pairing of NH₄⁺ ions with anions in saline water (Gardner et al. 1991) resulted in the increase of NH₄⁺ release. We suppose that the NO₃⁻ that was formed by nitrification was immediately reduced to N₂O or N₂. This is again supported by the low concentration of N₂O emission during the 7 d incubation.
Table 4.5. Pearson correlation coefficient values between initial soil properties and cumulative amounts of N$_2$O emission on day 7.

<table>
<thead>
<tr>
<th>Soil properties</th>
<th>N$_2$O</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>-0.91**</td>
</tr>
<tr>
<td>EC</td>
<td>0.82**</td>
</tr>
<tr>
<td>TOC</td>
<td>0.96**</td>
</tr>
<tr>
<td>NH$_4^+$-N</td>
<td>0.99**</td>
</tr>
<tr>
<td>TC</td>
<td>0.89**</td>
</tr>
<tr>
<td>TN</td>
<td>0.99**</td>
</tr>
</tbody>
</table>

** indicates significant $r$ value at $P < 0.01$.

4.4.3. Nitrate affected denitrification and N$_2$O emission from the Ru Cha mangroves sediment

Previous studies were conducted in mangrove forests and tidal salt marshes with a wide range of NO$_3^-$ concentrations (0.014–15.0 mg N L$^{-1}$) to investigate the influence of NO$_3^-$ on potential N$_2$O emission (Koch et al. 1992; Corredor and Morell 1994; Magalhães et al. 2005). Based on the study of Le (2008), we suppose that a NO$_3^-$ concentration higher than 5 mg N L$^{-1}$ is unrealistic in Ru Cha mangrove forest environment. Therefore, we selected NO$_3^-$ concentrations (0, 1, 3 and 5 mg N L$^{-1}$ are equivalent to 0, 5.4, 16.1 and 26.4 mg N kg$^{-1}$ oven-dried sediment, respectively) to measure the potential N$_2$O emission from 0-5 cm sediment layer. Nitrate was completely removed from the solution at the end of incubation (Table 4.4), suggesting that mangrove sediments could reduce NO$_3^-$ through the denitrification. However, in term of greenhouse gas emission, N$_2$O increased with the increase of NO$_3^-$ additions (Fig. 4.3), indicating that NO$_3^-$ was a limiting factor for N$_2$O emission. Our result is in agreement with previous studies (King and Nedwell 1987; Muñoz-Hincapié et al. 2002; Fernandes and Loka 2011). King and Nedwell (1987) showed that the bacterial
communities responsible for N$_2$O emission were adapted to changes in NO$_3^-$ concentrations. Greater NO$_3^-$ exhibited faster rates of NO$_3^-$ reduction and increased N$_2$O emission (Muñoz-Hincapié et al. 2002; Fernandes and Loka 2011). In our study, the percentages of N$_2$O emission to NO$_3^-$ removal were 20%, 55% and 100% in 5.4, 16.1 and 26.4 mg N kg$^{-1}$ treatments, respectively. Focht et al. (1979) and Senbayram et al. (2012) found that the activity and synthesis of N$_2$O reductase (responsible for the transformation N$_2$O to N$_2$) were inhibited by high NO$_3^-$ concentration, leading to greater N$_2$O emission from soil. In addition, N$_2$O reductase is inhibited by low pH (Knowles 1982) or high salinity (Menyailo et al. 1998; Marton et al. 2012), which resulted in a greater production of N$_2$O through denitrification. The Ru Cha mangrove sediments were extremely acidic. In the dry season, salty water remains in mangroves for a long time at high concentration. Therefore, potential N$_2$O emission from the Ru Cha mangrove sediment was promoted when receiving high NO$_3^-$ inputs.

4.5. CONCLUSIONS

Our results demonstrated that N$_2$O emission decreased with sediment depth. Environmental parameters of EC, TOC, TN, and NH$_4^+$ content, and density of denitrifiers were positively correlated to N$_2$O emission from mangrove sediments. Nitrous oxide emission increased significantly with higher NO$_3^-$ concentrations. High EC and low pH in the Ru Cha mangrove forest might inhibit the activity of N$_2$O reductase, and therefore greater N$_2$O was produced in mangrove sediment. Our microcosm experiments suggest that the Ru Cha mangrove forests could be a hotspot for N$_2$O emission when sediment receives high NO$_3^-$ input.
Chapter 5

NITROUS OXIDE AND CARBON DIOXIDE EMISSIONS FROM PADDY SOIL TREATED WITH RICE HUSK PRODUCTS AT DIFFERENT SOIL MOISTURE CONTENTS IN A SHORT-TERM EXPERIMENT

Abstract: A short-term study was conducted to investigate the effects of additions of rice husk products on nitrous oxide (N₂O) and carbon dioxide (CO₂) emissions from paddy soil at different soil moisture contents. Soil amended with raw rice husk (RR, 2% on a dry weight basis), rice husk char (RC, 2%), rice husk ash (RA, 2%) or no amendment (CT) was incubated at 40%, 60%, or 80% water-holding capacity (WHC) in a 100 mL bottle for 120 h. Results showed that N₂O emission was lower at 40% WHC compared with other WHC treatments. At 60% and 80% WHC, N₂O emissions peaked at 24 h of incubation in most treatments. The highest emission of N₂O was recorded in the RR addition at 80% WHC. Additions of RC and RA decreased cumulative N₂O emissions from soil at 24 h (by 24% at 60% WHC and 51% at 80% WHC for RC, and 90% at 60% WHC and 80% WHC for RA) compared with the CT treatment. Regardless of the soil moisture content, RR enhanced cumulative CO₂ emission for 120 h, whereas no significant difference was detected among RC, RA and CT treatments (P > 0.05). Our short-term results indicated that application of rice husk char or ash to soil reduced N₂O emission, whereas direct incorporation of rice husk into soil enhanced CO₂ and N₂O emissions.

Keywords: Denitrification, greenhouse gas emission, nitrification, rice husk, water-holding capacity.
5.1. INTRODUCTION

Nitrous oxide and CO$_2$ are so-called greenhouse gases, which contribute to global warming (Ussiri and Lal 2013). Nitrous oxide is of great importance because it can stand in the atmosphere for more than 114 years and has a global warming potential 298 times higher than CO$_2$ (Smith et al. 2007). The report of the Intergovernmental Panel on Climate Change (IPCC) documented that agricultural soils are an important source of greenhouse gas emissions. In 2005, N$_2$O and CO$_2$ emissions from agricultural soils accounted for 60% and 10–12% of global anthropogenic emissions, respectively (Smith et al. 2007). Nitrous oxide is mainly produced as a byproduct of nitrification and an intermediate product of denitrification (Mosier 1998). Carbon dioxide is generated from soil through biological oxidation of organic matter, decomposition of crop residues (Schlesinger and Andrews 2000; Lou et al. 2007).

As a practical manner to improve the soil fertility, incorporation of local crop residues into soil has been worldwide applied. Traditionally, farmers returned back crop residues to their fields in the forms of raw residues, char or ash. Addition of crop residues can significantly improve soil properties by increasing soil moisture and providing readily available organic C and N, resulting in an increase in crop yields (Velthof et al. 2002; Krishna et al. 2004). However, the decomposition of crop residues stimulates N$_2$O and CO$_2$ emissions from the soil (Huang et al. 2004; Lou et al. 2007). Char and ash, products of burning crop residues, can also improve soil chemical and physical properties (Wang et al. 2012; Atanu and Rattan 2013). Wang et al. (2012) showed that addition of rice husk char to soil increases soil aeration and soil pH, and promotes microbial immobilization of available N in soil for 60 days, resulting in a decrease of N$_2$O emission. The effect of ash and charcoal made from municipal
biowaste, on N$_2$O emission was reported by Yanai et al. (2007). Application of the ash did not suppress N$_2$O emission, while the char addition decreased N$_2$O emission from soil rewetted at 73% water-filled pore space (WFPS). Nitrous oxide and CO$_2$ emissions were evaluated from soil treated with different types of crop residues (Aulakh et al. 1991; Velthof et al. 2002; Huang et al. 2004; Lou et al. 2007) or their products char or ash (Yanai et al. 2007). Soil moisture governed the decomposition of crop residues added to the soil, which in turn affected greenhouse gas emissions (Abro et al. 2011). Identifying suitable management practices for addition of crop residues and moisture content are strongly required to increase soil fertility and crop yields while mitigating greenhouse gas emissions from agricultural soil.

Rice husks are a great potential resource to be used as a soil amendment in Asian countries (Maclean et al. 2002). However, the effects of rice husk products as soil amendments on greenhouse gas emissions are not well studied. In addition, most studies of crop residues additions to soil were conducted by using long-term experiments (Huang et al. 2004; Lou et al. 2007; Wang et al. 2012). Short-term effects were investigated by few research works. Microbial activities responsible for N and C mineralization are stimulated shortly after organic matter application to soil (Cheng et al. 2006) and larger missions of CO$_2$ and N$_2$O due to organic amendments often occur within 48 h (Yanai et al. 2007; Jones et al. 2011). Accordingly, by using short-term incubation, we investigated the effects of additions of char, ash and raw material from rice husk and moisture contents on N$_2$O and CO$_2$ emissions from paddy soil.

5.2. MATERIALS AND METHODS

5.2.1. Soil sample and rice husk products

Soil samples were collected from the 0–20 cm layer at a paddy field in Ushimado,
Okayama Prefecture, Japan (34°37’23”N, 134°07’40”E) before rice transplanting in May 2013. After collection, soil samples were well mixed and air-dried at room temperature, then passed through a 2-mm sieve. The soil was characterized as loamy sand (77% sand, 16% silt, and 7% clay), and classified as Dystric Gleysols (FAO 1998). Rice husk products (raw rice husk, char, ash) were used as soil amendments. Rice husk ash and char were produced via high temperature pyrolysis (500°C) with and without oxygen, respectively. Rice husk products were milled to pass through a 0.84-mm sieve and mixed with soil for incubation. Soil and rice husks samples were subjected to measurements of pH and electrical conductivity (EC) at a 1:10 ratio of air-dried materials to deionized water followed by shaking for 1 hour at 175 rpm, using a digital pH meter (F-23, Horiba, Japan) and EC meter (DS-14, Horiba, Japan), respectively. Total nitrogen (TN) and total carbon (TC) were measured by the dry combustion method with a C/N Corder (MT-700, Yanaco, Japan). Mineral N in air-dried materials was extracted with 2M KCl solution in ratio of 1:10. The extracts were analyzed for NO$_3^-$ and NH$_4^+$ by the colorimetric method using a continuous flow analyzer (QuAAtro 2-HR, Bltec, Japan). Initial properties of soil and rice husk products are listed in Table 5.1.
Table 5.1. Physical and chemical properties of soil and rice husk products used in the study.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Soil</th>
<th>Rice husk</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Raw</td>
</tr>
<tr>
<td>pH (1:10)</td>
<td>7.5 ± 0.1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.9 ± 0.0&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>EC (dS m&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>0.1 ± 0.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.4 ± 0.1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>NH&lt;sub&gt;4&lt;/sub&gt;&lt;sup&gt;+&lt;/sup&gt;-N (mg kg&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>3.0 ± 1.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>16.9 ± 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>NO&lt;sub&gt;3&lt;/sub&gt;&lt;sup&gt;-&lt;/sup&gt;-N (mg kg&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>20.9 ± 0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.5 ± 0.0&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>TC (g kg&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>10.4 ± 0.5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>365.8 ± 8.8&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>TN (g kg&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>0.9 ± 0.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.2 ± 0.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>C:N ratio</td>
<td>12</td>
<td>114</td>
</tr>
</tbody>
</table>

Data are means ± standard deviation. n.d. indicates not detected. Different letters in each row denote significant differences ($P < 0.05$, $n=3$) according to Tukey’s HSD test.

5.2.2. Experimental setup

Laboratory microcosms were set up in 100-mL glass bottles capped with a butyl rubber septum at 25°C for 120 h. Each bottle contained 5 g of air-dried soil and 0.1 g (2% on a dry weight basis) of raw rice husk (RR), rice husk char (RC), rice husk ash (RA) or no amendment (CT). The percentage of amendments to soil was determined based on the common application rate of 24 t ha<sup>-1</sup> (Harter et al. 2014). The soil-amendments mixture was conditioned at 70 mg NO<sub>3</sub><sup>-</sup>-N kg soil<sup>-1</sup> and at 40%, 60%, or 80% WHC. All treatments were performed in triplicate.

5.2.3. Gas sampling and analysis

Nitrous oxide and CO<sub>2</sub> concentrations were determined after 3, 24, 72 and 120 h incubation. Twenty-five mL of a gas sample was taken using a syringe and stored in a vacuum vial (-80 kPa). Nitrous oxide and CO<sub>2</sub> concentrations in the collected gas samples were analyzed with a gas chromatograph (GC-8A, Shimadzu, Japan) equipped with an electron capture detector (ECD) and a thermal conductivity detector (TCD),
respectively. Nitrous oxide and CO₂ concentrations (ppm v) were converted to a mass unit (mg kg⁻¹) using the following equation:

\[ V_i = (\rho \times C \times V_g \times 273) / [W \times (273 + T)] \]  

(1)

where \( V_i \) is the cumulative N₂O or CO₂ emission at sampling times (mg kg⁻¹), \( \rho \) is the density of N₂O (1.25 kg m⁻³) or CO₂ (0.5357 kg m⁻³) at 0°C, \( C \) is the N₂O or CO₂ concentration (ppm v), \( V_g \) (m³) is the head space volume, \( W \) (kg) is the dry soil weight, \( T \) is the temperature at determination (25ºC).

5.2.4. Soil amendment analysis

After gas collection, soil amendments were extracted with 2M KCl solution at a ratio of 1:10 for analyzing NO₃⁻ and NH₄⁺ contents. pH, EC, and total organic carbon were measured in the extracts of the amended soils with deionized water (1:10). Water-extractable total organic carbon (TOC) was measured by using a TOC analyzer (TOC-5000A, Shimadzu, Japan).

5.2.5. Statistical analysis

The Tukey HSD test was used to examine the significant difference (\( P < 0.05 \)) in N₂O and CO₂ emissions, NO₃⁻, NH₄⁺, and TOC concentrations. A two-way analysis of variance (ANOVA) was used to determine whether rice husk addition and moisture content affect N₂O and CO₂ emissions. Statistical analyses were performed using R software version 2.6.2.

5.3. RESULTS

5.3.1. Differences in soil properties due to additions of rice husk products

Due to the high pH of rice husk char and ash, incorporation of RC or RA increased the soil pH (Table 4.2). The water-extractable TOC in RR was significantly higher than in RA, RC and CT (\( P < 0.05 \)).
Table 5.2. Changes in selected soil properties due to additions of rice husk products

<table>
<thead>
<tr>
<th>WHC (%)</th>
<th>Properties</th>
<th>CT</th>
<th>RR</th>
<th>RC</th>
<th>RA</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>pH</td>
<td>7.5 ± 0.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.5 ± 0.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.6 ± 0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.9 ± 0.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>TOC (mg kg&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>39.3 ± 1.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>73.6 ± 3.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>40.9 ± 1.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>45.1 ± 4.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>60</td>
<td>pH</td>
<td>7.4 ± 0.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.6 ± 0.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.6 ± 0.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.9 ± 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>TOC (mg kg&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>39.3 ± 0.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>54.3 ± 5.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>39.2 ± 3.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>50.2 ± 11.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>80</td>
<td>pH</td>
<td>7.1 ± 0.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.4 ± 0.1&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>7.5 ± 0.2&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>7.9 ± 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>TOC (mg kg&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>30.1 ± 5.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>55.1 ± 16.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>29.8 ± 0.9&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>32.3 ± 4.8&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Data are means ± standard deviation. Different letters in each row denote significant differences (P < 0.05, n=3) according to Tukey’s HSD test.

5.3.2. Effects of addition of rice husk products and moisture content on N<sub>2</sub>O and CO<sub>2</sub> emissions

Nitrous oxide emissions were sensitive to soil moisture. The N<sub>2</sub>O emission was lower at 40% WHC compared with other WHC treatments (Fig. 5.1 a-c). At 40% WHC, there was no significant difference in N<sub>2</sub>O emission among rice husk addition treatments (P > 0.05). The N<sub>2</sub>O emission was nearly 0 mg N kg<sup>-1</sup> at the first 24 h, then increased to about 0.1–0.2 mg N kg<sup>-1</sup> towards the end of incubation. At 60% WHC, N<sub>2</sub>O generally peaked at 24 h incubation, then decreased in RC, RA, and CT while the N<sub>2</sub>O emission in RR increased towards 72 h. The highest cumulative N<sub>2</sub>O emission occurred in RR treatment at 80% WHC (20.3 mg N kg<sup>-1</sup>), which was 5.1, 10.5, and 50.1 times greater than CT, RC and RA, respectively (P < 0.05) at 24 h. Additions of RC and RA reduced N<sub>2</sub>O emission from soil at 24 h (by 24% at 60% WHC, 51% at 80% WHC for RC and 90% at 60% and 80% WHC for RA) compared with the CT (P < 0.05). Carbon dioxide emissions were not affected by soil moisture contents (Fig. 5.1 d-f). The trends were similar in all WHC treatments. Cumulative CO<sub>2</sub> emission increased towards 120 h of
incubation. Incorporation of RR promoted CO$_2$ emission, whereas no significant
difference was detected among RC, RA and CT treatments ($P > 0.05$, Fig. 5.1).
Cumulative CO$_2$ emission in RR was approximately twice those in other treatments.

![Graphs showing cumulative N$_2$O and CO$_2$ emission at different WHC levels.]

**Figure 5.1.** Cumulative N$_2$O emission (mg N kg soil$^{-1}$) at (a) 40% WHC; (b) 60%
WHC; (c) 80% WHC and cumulative CO$_2$ emission (mg C kg soil$^{-1}$) at (d) 40%
WHC; (e) 60% WHC; (f) 80% WHC.

5.3.3. \(\text{NH}_4^+\) and \(\text{NO}_3^-\) contents

Mineralization was enhanced by additions of rice husk to soil. The \(\text{NH}_4^+\) content increased in the first 72 h and decreased thereafter (Fig. 5.2).

Different trends were observed in \(\text{NO}_3^-\) at different soil moisture contents (Fig. 5.2). At 40\% WHC, \(\text{NO}_3^-\) content increased with rice husk additions during 120 h incubation due to mineralization and the subsequent nitrification process. The content of \(\text{NO}_3^-\) increased from 70 mg N kg\(^{-1}\) at the beginning of the experiment to 75, 86, and 90 mg N kg\(^{-1}\) at the end of incubation in RR, RA and RC treatments, respectively. The CT treatment showed no change in \(\text{NO}_3^-\) content during 120 h of incubation. On the other hand, a rapid drop in \(\text{NO}_3^-\) content was observed at 24 h with 60\% and 80\% WHC. The \(\text{NO}_3^-\) content sharply decreased in soil with RR addition at 80\% WHC to nearly 0 mg N kg\(^{-1}\). The \(\text{NO}_3^-\) content increased again in the later period of the incubation.
Figure 5.2. Soil NH$_4^+$ and NO$_3^-$ contents (mg N kg$^{-1}$) under different soil WHC.

5.4. DISCUSSION

Additions of char, ash and raw materials from rice husk to soil supplied available organic C and increased soil pH, which in turn affected N$_2$O and CO$_2$ emissions. The highest N$_2$O emission was detected at 80% WHC in the RR treatment. Addition of RR promoted N$_2$O and CO$_2$ emissions while those of RC and RA reduced N$_2$O emission.

5.4.1. Effect of soil moisture content on N$_2$O and CO$_2$ emissions

Soil moisture regulates oxygen availability to soil microbes and affects nitrification and denitrification (Bahl et al. 2013), which are closely related to N$_2$O emission from soil. Nitrification was the main process in the production of N$_2$O at 35–60% water-filled pore space (WFPS), while denitrification was the major contributor of N$_2$O emission above 70% WFPS (Bateman and Baggs 2005).
In the present study, \( \text{N}_2 \text{O} \) emission as a byproduct of nitrification was low (0.2 mg N kg\(^{-1}\)) at 40% WHC. At 60–80% WHC, a negative correlation between \( \text{N}_2 \text{O} \) emission and \( \text{NO}_3^- \) reduction was observed at 24 h of incubation, indicated that denitrification was the main biological process for \( \text{N}_2 \text{O} \) emission from soil. Nitrous oxide emission peaked at 24 h and decreased afterwards. Because the activity and synthesis of \( \text{N}_2 \text{O} \) reductase were probably inhibited in the early stage of incubation, more \( \text{N}_2 \text{O} \) was produced as an intermediate product of denitrification at higher moisture content. Similarly, Yanai et al. (2007) reported that \( \text{N}_2 \text{O} \) emission increased with the increase in the soil moisture content at 80% and 91% WHC, whereas no significant \( \text{N}_2 \text{O} \) emission was detected at 70% WHC. Patiño-Zúñiga et al. (2009) demonstrated that the \( \text{N}_2 \text{O} \) emission from soil was more than six times larger at 80% and 100% WHC compared to soil at 40% and 60% WHC. The higher soil moisture content created more anaerobic conditions that stimulated \( \text{N}_2 \text{O} \) emission.

No significant difference was detected in CO\(_2\) emission at different soil moisture contents (\( P > 0.05 \)). Microbial activities for C decomposition were probably kept constant within the range of 40–80% WHC. A similar result was reported by Kruse et al. (2004). They conducted an incubation study of cotton leaf residues under different moisture conditions, in which CO\(_2\) emissions occurred at the same level regardless of soil moisture.

5.4.2. Effect of rice husk additions on \( \text{N}_2\text{O} \) and CO\(_2\) emissions

At 80% WHC, RR incorporation into soil promoted \( \text{N}_2\text{O} \) emission, which was significantly higher than in other treatments (\( P < 0.05 \)). The addition of RR provided organic C as an electron donor for denitrification. At 80% WHC, soil pores are almost completely filled with water, leading to low O\(_2\) concentration. In addition,
decomposition of RR reduced O₂ availability at microsites (Millar et al. 2004). Incorporation of RR into soil stimulated denitrification and N₂O emission under anaerobic conditions. At higher soil moisture content, N₂O emission peaked at 24 h as an intermediate product of denitrification (Fig. 5.1 c). Similarly, N₂O emission was increased by amendment of rice residues during the early stage of incubation (Lou et al. 2007). Regardless of soil moisture contents, the addition of RR doubled cumulative CO₂ emission compared with other treatments (P < 0.05), probably resulting from rapid mineralization of the fresh biomass (Schlesinger and Andrews 2000; Lou et al. 2007). The highest CO₂ emission in RR was correlated with its highest water-extractable TOC content (Table 5.2). Our results on the effect of the raw rice husk addition on greenhouse gas emissions was in agreement with previous studies (Huang et al. 2004; Lou et al. 2007; Abro et al. 2011). Lou et al. (2007) conducted an incubation experiment with rice straw and roots at 60% WHC. They found that CO₂ and N₂O emissions were increased by the addition of rice residues.

The incorporation of RC and RA reduced cumulative N₂O emission from soil by 24% at 60% WHC and 51% at 80% WHC for RC, and 90% at 60% WHC and 80% WHC for RA at 24 h. This depression of N₂O emission was probably because RC and RA additions increased soil pH (Table 5.2). Soil pH affects whether the end products of denitrification is N₂O or N₂ (Šimek and Cooper 2002). In soil with higher pH values, the activity and synthesis of N₂O reductase were enhanced, and promoted the conversion of N₂O to N₂ under anaerobic conditions. The greater the pH effects due to addition of rice husk char and ash, the more the percentage of cumulative N₂O emissions from soil were suppressed. Similarly, Li et al. (2013) and Wang et al. (2012) indicated that addition of biochar to soil reduced N₂O emission.
5.5. CONCLUSIONS

We investigated the short-term effects of rice husk products as a soil amendment with different moisture content on greenhouse gas emissions. The results showed that N$_2$O emission was enhanced by higher soil moisture content, but that CO$_2$ emission was not affected. Short-term laboratory experiments at 80% WHC indicated that RR addition promoted N$_2$O emissions. Regardless of soil moisture contents, CO$_2$ emission in RR addition was twice those in other treatments. This study is a preliminary step to evaluate the effects of rice husk product additions on N$_2$O and CO$_2$ emissions. We did not consider effects of aging rice husk materials on the greenhouse gases in the fields. Spokas (2013) warned that weathered biochar does not reduce N$_2$O emission, whereas it enhances CO$_2$ emissions as compared with fresh biochar or no addition. Further studies on long-term effects of rice husk products should be undertaken in the future.
Chapter 6

NITROUS OXIDE EMISSIONS FROM FALLOW PADDY FIELDS UNDER DIFFERENT WATER REGIMES WITH HIGH NITRATE CONCENTRATION

Abstract: Paddy fields in an area of Ushimado, in Okayama, Japan, could be hot spots for nitrous oxide (N₂O) emissions because nitrate (NO₃⁻) concentration in groundwater used for irrigation was as high as 60 mg N L⁻¹. In general, alternating drain and flood cycles are repeated in paddy fields, depending on precipitation and irrigation. High NO₃⁻ inputs and variable moisture content could promote N₂O emissions. This study simulated a fallow period in Ushimado paddy fields to reduce the high NO₃⁻ concentration in groundwater via irrigation management. Effects of different water irrigation regimes with high NO₃⁻ concentrations on N₂O emissions were investigated. We set up six experimental pots with three water regimes with continuous flooding, 5-day flood and 5-day drainage cycles, and 15-day flood and 15-day drainage cycles. Our results suggest that continuous flooding during fallow periods in paddy fields would reduce N₂O emissions from soil and NO₃⁻ concentration in irrigation water.

Keywords: Flood and drain cycle; nitrous oxide emission; paddy soil; water irrigation regimes; fallow periods.
6.1. INTRODUCTION

Rice fields account for over 9% of the global cropland (Maclean et al. 2002), which include both upland and lowland rice production fields. “Paddy soil” denotes soil in irrigated and rain-fed lowland rice production systems with a prolonged period of submergence (Roland and Stephan 2010). Paddy soil experiences both aerobic and anaerobic conditions during the course of a year (Maclean et al. 2002; Kyuma 2004). The transition cycle from drained to flooded is often gradual, depending on variations in precipitation or irrigation (Maclean et al. 2002). Nitrification and denitrification processes are sensitive to the oxygen concentration in soil, which is affected by soil water status (Davidson 1992; Huang et al. 2012). Both biological processes are responsible for the emission of N₂O, which is a greenhouse gas and contributes to depletion of the stratospheric ozone layer (Huang et al. 2012).

Effects of water irrigation regimes on N₂O emissions from paddy soils have been documented by previous studies. Alternating flood and drain cycles by irrigation regimes increase the risk of N₂O emission from rice fields, particularly nitrogen-rich fields. Several researchers reported that N mineralization, denitrification, and N₂O emissions were stimulated after rewetting (Smith and Parsons 1985; Xing et al. 2002; Ruser et al. 2006; Beare et al. 2009; Jørgensen and Elberling 2012; Guo et al. 2013). In contrast, other researchers showed that peaks of N₂O emission were observed after the flood water was drained (Cai et al. 2001; Towprayoon et al. 2005; Huang et al. 2007; Hou et al. 2012; Liu et al. 2012). Drying of paddy soil creates soil cracking (Huang et al. 2005). Cracks enhance the exchange of O₂ in soil, which affects nitrification and denitrification processes. In the earlier phase of water drainage, nitrification accelerates to produce N₂O as well as more NO₃⁻ is transformed to N₂O by denitrification (Huang et al. 2005). Besides that, Cai et al. (2001) and Xing et al. (2002) showed N₂O emissions
from paddy soils were negligible under continuous flood conditions. However, greater NO$_3^-$ loading (42 mg NO$_3^-$-N L$^{-1}$) was inputted to soil under flooded conditions, more N$_2$O was emitted due to the reductive condition was not enough developed (Kasuya 2008). Therefore, investigating N$_2$O emission from paddy soil receiving irrigation water with high NO$_3^-$ concentration is strongly required.

Paddy fields in Ushimado, Japan, may be hot spots for N$_2$O emissions due to the high NO$_3^-$ concentration (60 mg N L$^{-1}$) in the groundwater used for irrigation. In a year, paddy fields are used for rice cultivation from May to September. Then, the paddy fields lay fallow for 6–7 months. In this study, we simulated the fallow period of Ushimado paddy fields to reducing the high NO$_3^-$ concentration in irrigation water by denitrification via water regime management. We supposed that different water irrigation regimes with high NO$_3^-$ concentration would affect N$_2$O emissions from the paddy field. Our aims were to determine i) which phase of the flood and drain cycles stimulate N$_2$O emissions and ii) which water regimes reduce the amount of N$_2$O emissions. Our results would help farmers choose a suitable water irrigation regime in their paddy fields during fallow periods for reducing N$_2$O emission from soil as well as NO$_3^-$ concentration in irrigation water.

6.2. MATERIALS AND METHODS

6.2.1. Study area

Paddy field in Ushimado (34°37’23”N, 134°07’40”E), which is located in the southeast part of Okayama Prefecture, Japan, is maintained under flooded conditions during most of the rice growing period. Two weeks before harvesting, paddy fields are drained and kept dry until the next cropping. The groundwater used for irrigation contained a high NO$_3^-$ concentration (60 mg N L$^{-1}$). Soil is classified as Dystric Gleysols according to Food and Agriculture Organization (FAO 1998).
6.2.2. Soil properties

Soil samples were collected before rice transplantation in May 2013. Soil samples were taken randomly from the top 0–20 cm layer at six points in the field and mixed well, then air-dried at room temperature and sieved through a 2 mm. Soil sample was subjected to measurements of pH and electrical conductivity (EC) at soil:water ratio of 1:5 after shaking for 1 hour at 175 rpm, using a digital pH meter (F-23, Horiba, Japan) and EC meter (DS-14, Horiba, Japan), respectively. Total carbon (TC) and total nitrogen (TN) contents of soil were determined using a CN Corder (MT-700, Yanaco, Japan). Mineral N was extracted with 2 M KCl solution (soil:solution ratio of 1:10). The ammonium (NH$_4^+$-N) and NO$_3^-$-N contents in extracts were measured by colorimetric method using a continuous flow analyzer (QuAAtro 2-HR, Bttec, Japan). Soil texture was determined by the International pipette method (Sarkar and Haldar 2005). Physico-chemical properties of Ushimado paddy soil were described in Table 5.1.

Table 6.1. Physico-chemical properties of Ushimado paddy soil

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Ushimado paddy soil</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH (1:5)</td>
<td>7.4</td>
</tr>
<tr>
<td>EC (dS m$^{-1}$)</td>
<td>0.1</td>
</tr>
<tr>
<td>Particle size distribution (%)</td>
<td></td>
</tr>
<tr>
<td>Clay</td>
<td>7</td>
</tr>
<tr>
<td>Silt</td>
<td>16</td>
</tr>
<tr>
<td>Sand</td>
<td>77</td>
</tr>
<tr>
<td>NO$_3^-$-N (mg kg$^{-1}$)</td>
<td>25.6</td>
</tr>
<tr>
<td>NH$_4^+$-N (mg kg$^{-1}$)</td>
<td>2.5</td>
</tr>
<tr>
<td>TC (g kg$^{-1}$)</td>
<td>11.6</td>
</tr>
<tr>
<td>TN (g kg$^{-1}$)</td>
<td>1.2</td>
</tr>
<tr>
<td>C:N ratio</td>
<td>9.7</td>
</tr>
</tbody>
</table>
6.2.3. Experimental set-up

Wagner pots (15.8 cm diameter × 19.4 cm height) were open at the top and had a drainage hole on the bottom sealed with a silicon plug. Pots were filled with 2 kg of washed cobblestones, a sheet of nylon cloth to prevent clogging of soil, and 2 kg of air-dried soil, and then 2 L water with 60 mg NO$_3^-$-N L$^{-1}$ was added to achieve about 5 cm water above the soil surface. The NO$_3^-$ concentration of the flooded water was kept constant by replacing water continuously using a peristaltic pump. The hydraulic retention time of water in the experimental pot was 1.6 days. We set up six experimental pots with three water regimes in duplicate for 60 days with continuous flooding (CF), 5-day flood and 5-day drain cycles (FD5), and 15-day flood and 15-day drain cycles (FD15). The pots were kept at 25°C. Water was drained by opening the stopper of a tube inserted into the silicon plug. A platinum electrode was inserted at 5 cm depth. Soil redox potential (Eh) values were measured every day for two months.

6.2.4. Sampling and measurements

Gas measurement

Gas samples were collected every 5 days for two months. In addition, under FD5 and FD15 regimes, gas samples were collected immediately, 4 h, and 1 d after soil drainage or flooding. A closed PVC chamber (8.6 cm diameter × 20 cm height) was used for determination of N$_2$O fluxes from soil. In order to keep the system closed during sample collection, the bottom edge of the chamber was inserted to a depth of 2 cm in soil. Gas samples were taken at 0, 15, 30, and 45 min after placing chambers in the soil. Gas samples (25 mL) were taken with a syringe and stored in a 20 mL vacuum vial. Nitrous oxide gas was measured with a gas chromatograph (GC-8A, Shimadzu, Japan) equipped with an electron capture detector. Nitrous oxide emissions from soil were calculated by the following equation (Peng et al. 2011):
\[ F = \rho \times h \times \frac{273}{273+T} \times \frac{dc}{dt} \]

where \( F \) is the \( \text{N}_2\text{O} \) emission flux (\( \mu \text{g} \text{ m}^{-2} \text{ h}^{-1} \)), \( \rho \) is the \( \text{N}_2\text{O} \) density at 0°C, \( h \) is the height of the chamber above the water surface (m), \( \frac{dc}{dt} \) is determined from the linear regression of a set of four data points obtained during the 45-min sampling period (\( \mu \text{g} \text{ m}^{-3} \text{ h}^{-1} \)), and \( T \) is the air temperature inside the chamber at sampling (°C).

**Water measurement**

Concentrations of \( \text{NH}_4^+ \)-N and \( \text{NO}_3^- \)-N in leached water were measured by colorimetric methods using a continuous flow analyzer (QuAAtro 2–HR, Bltec, Japan).

**6.2.5. Statistical analysis**

The Tukey HSD test was used to examine differences in \( \text{N}_2\text{O} \), \( \text{NO}_3^- \), \( \text{NH}_4^+ \), or Eh among different water regimes. Linear regression analysis was used to evaluate the effect of soil Eh on \( \text{N}_2\text{O} \) emission. All statistical analyses were performed using R software (version 2.6.2).

**6.3. RESULTS**

**6.3.1. Soil redox potential in three water regimes during 60-day experiment**

The same soil redox potential trends were observed in the first flooding phase (0–5 days) of CF, FD5, and FD15 water regimes (Fig. 6.1). Soil Eh fell to less than -200 mV on day 3 of the flooding period, and it then approached a steady value (-245 mV) under the CF water regime. Under FD5 and FD15 water regimes, soil Eh values increased from -245 to 0 mV one day after water drainage, then decreased till reflooding. Soil Eh values remained negative even during drained periods, probably because the soil was compressed and oxygen was not supplied to the soil at 5 cm depth.
Figure 6.1. Eh values at 5 cm depth of soil under different water regimes. Downward arrows indicate dates when water drainage was started. CF, FD5 and FD15 mean continuous flooding, 5-day flood and 5-day drainage cycles, and 15-day flood and 15-day drainage cycles, respectively.

6.3.2. Nitrous oxide emissions from paddy soil in three water regimes

Different trends in N₂O emissions were observed in the three water regimes (Fig. 6.2). Nitrous oxide emissions from paddy soil were less than 100 µg N m⁻² h⁻¹ under the CF water regime. Under FD5 and FD15 water regimes, N₂O emissions peaked after drainage of flood water, then declined and remained low until the next drainage event. The highest peak of N₂O emissions (579 µg N m⁻² h⁻¹ for FD15 and 464 µg N m⁻² h⁻¹ for FD5) occurred at the same time on day 16 of incubation. There was a significant difference in N₂O emissions between flooded and drained periods under FD15 and FD5 water regimes (P < 0.05). The highest cumulative N₂O emission for 60 days was seen under the FD15 water regime (113.4 mg N m⁻²), followed by FD5 (105.2 mg N m⁻²), and CF (66.4 mg N m⁻²) water regimes.
Figure 6.2. Nitrous oxide emissions under three water regimes a) continuous flooding b) 5-day flood and 5-day drainage cycles, and c) 15-day flood and 15-day drainage cycles. Letters F and D mean flood and drainage periods, respectively. Downward arrows indicate dates when water drainage was started.
6.3.3. \( \text{NH}_4^+ \)-N and \( \text{NO}_3^- \)-N in leached water

The leached water was collected after drainage of the flooding water in FD5 and FD15 water regimes. Nitrate concentrations in leached water under the FD15 water regime were lower than those under the FD5 water regime (Fig. 5.3). Results indicated that extending the flooding period would decrease the \( \text{NO}_3^- \)-N concentration in leached water.

![Figure 6.3. Nitrogen concentration in leached water. Numbers indicate dates when water was drained.](image)

Figure 6.4. Relationship between soil redox potential (Eh) and \( \text{N}_2\text{O} \) emissions from paddy soil under different water regimes

6.4. DISCUSSION

Paddy fields in Ushimado are irrigated using groundwater with \( \text{NO}_3^- \) concentrations as high as 60 mg N L\(^{-1}\). High \( \text{NO}_3^- \) concentration has been identified as a cause of
eutrophication in surface waters (McClelland and Valiela 1998). The experiment simulated a fallow period in Ushimado paddy fields to reduce the high \( \text{NO}_3^- \) concentration in irrigation water via water regime management. The \( \text{N}_2\text{O} \) emissions from fallow field under different water regimes were investigated.

6.4.1. Effect of water regimes on \( \text{N}_2\text{O} \) emissions

Nitrous oxide emissions depended on water irrigation regime management in the fallow paddy field. Nitrous oxide emission was negligible under continuous flooding irrigation water regime of high \( \text{NO}_3^- \) concentration, while soil Eh was reductive (-245 mV). Our study was inconsistent with study by Kasuya (2008). The continuous flooding regime creates a strongly anaerobic condition, most of the \( \text{N}_2\text{O} \) formed as an intermediate product of denitrification was further reduced to \( \text{N}_2 \) (Huang et al. 2007). In addition, the flooding water hampers \( \text{N}_2\text{O} \) transfer to the air from the soil. Therefore, \( \text{N}_2\text{O} \) formation and emissions were restricted under flooded conditions (Cai et al. 2001; Xing et al. 2002; Liu et al. 2012).

Alternating periods from flooding to drainage promoted \( \text{N}_2\text{O} \) emissions from soil. Similar results were found in previous studies (Cai et al. 2001; Huang et al. 2007; Hou et al. 2012; Liu et al. 2012). Peaks of \( \text{N}_2\text{O} \) emissions were observed one day after soil drainage. In the early stage of soil drying, the soil water content was favorable for \( \text{N}_2\text{O} \) production from nitrification and denitrification. Nitrification is stimulated as the soil water content increases, and more \( \text{N}_2\text{O} \) is formed by nitrification. When the soil anoxic condition is not stronger than in the flooded condition, more \( \text{N}_2\text{O} \) were was produced by incomplete denitrification (Huang et al. 2005). Some studies showed the maximal rate of \( \text{N}_2\text{O} \) emission at 45–75% water-filled pore space (WFPS) (Hansen et al. 1993).

6.4.2. Factors affecting \( \text{N}_2\text{O} \) emissions

Soil redox potential is an important factor affecting \( \text{N}_2\text{O} \) emissions from paddy soil
under different water regimes. The results demonstrated that N$_2$O emissions were greater under the alternating flooding drainage cycles with Eh ranging -250 mV to 0 mV than under the continuous flooding condition with low redox potentials. Nitrous oxide emission peaks were observed after drainage of the flooding water, corresponding to soil Eh, which had slightly reductive values (Eh: -100 mV to 0, Fig. 5.4). The results are in agreement with those of a previous study (Reddy and Delaune 2008). Nitrous oxide emissions from soil in reductive zones were promoted as byproduct of nitrification and an intermediate product of denitrification processes.

Nitrate provides the substrates for denitrifier activities (Wang et al. 2013). When the NO$_3^-$ concentration in irrigation water as high as 60 mg N L$^{-1}$ supplied to the paddy increase the risk of N$_2$O emission from incomplete denitrification process.

6.5. CONCLUSIONS

This study investigated the effects of different water regimes on N$_2$O emissions from fallowed paddy soil that received irrigation from groundwater with a high NO$_3^-$ concentration. Alternating drainage of flooded cycles in paddy soil receiving high NO$_3^-$ inputs created favorable conditions for high N$_2$O emission. Our main finding was that maintaining the continuously flooded condition in the fallow period reduced both N$_2$O emissions from soil and NO$_3^-$ concentration in irrigation water.
SUMMARY AND CONCLUSIONS

Wetlands including agricultural fields and mangrove forest have received ample attention with respect to denitrification and N$_2$O emissions.

Paddy field in Ushimado (34°37’23”N, 134°07’40”E) located in the southeast part of Okayama Prefecture, Japan. In a year, paddy fields are used for rice cultivation from May to September, then, the paddy fields lay fallow for 6–7 months. Alternating flood and drain cycles by irrigation or precipitation increase the risk of N$_2$O emission from rice fields, particularly nitrogen-rich fields. Ushimado paddy fields may be hot spots for N$_2$O emissions due to the high NO$_3^-$ concentration (60 mg N L$^{-1}$) in the groundwater used for irrigation.

The Ru Cha mangrove forest located in Thua Thien Hue province, Vietnam, has been functioning as an ecological buffer between mainland and the Tam Giang lagoon. This mangrove forest experiences semi-diurnal tides with the amplitude of 0.4–0.5 m, the lowest in Vietnam. Due to the presence of ebb tides, salty water remains in estuaries for a long time, particularly in dry seasons. Mangroves sediments in Ru Cha contain a high content of pyrite (FeS$_2$). When the pyrite is exposed to the air, it is oxidized and produces sulfuric acid (H$_2$SO$_4$). Thus pyrite containing soils are extremely acidic. In addition, the release of organic acids through the decomposition of mangroves litter acidifies the sediment and the litter supplies high contents of N and C in sediment. These conditions in Ru Cha mangroves sediment might affect the N$_2$O emission through denitrification.

Our study aimed at finding the influencing factors on the N$_2$O emissions from wetland ecosystems. Understanding the processes of N$_2$O formation is fundamental to
reduce $N_2O$ emissions from wetlands.

For this purpose, we investigated influencing factors on $N_2O$ emission in this thesis: (i) soil type and $NO_3^-$ concentration, (ii) pH and salinity, (iii) residue amendment, (iv) water irrigation regimes management, (v) high organic matter and saturated sediments in tropical mangrove forest.

The effect of soil type, $NO_3^-$ concentration, pH, salinity, residue amendment, mangrove sediments were studied under controlled conditions in 100 mL bottle at 25°C and saturated condition for short-term period. The effect of water irrigation regime was studied in simulated pot experiment for 2 months. We attempt to find out the mechanism regulate $N_2O$ emission from agricultural and mangrove soils under varied influencing environmental and soil factors.

**Nitrous oxide was the main product of denitrification in acidic soil**

In chapter 2, denitrification products ($N_2O$ and $N_2$) were measured in three flooded soils (paddy soil from Vietnam, PV; mangrove soil from Vietnam, MV; paddy soil from Japan, PJ) with different $NO_3^-$ concentrations. Denitrification rates depend more on the bacterial population in soil which is regulated by soil pH. Our result showed that denitrification ($N_2O+N_2$) was higher in the neutral soil (PJ) than that in the moderately acidic soil (PV) and extremely acidic soil (MV). Nitrous oxide was the main product in MV soil while $N_2$ was dominant in PJ and PV soils. The mechanism of pH-controlled denitrification is explained by the sensitivities of $N_2O$ reductase activity to pH. Under acidic pH, the activity and synthesis of $N_2O$ reductase is inhibited, resulting in increased $N_2O$ accumulation. Soil pH regulated denitrification rate and denitrification products. Addition of $NO_3^-$ significantly enhanced denitrification and emissions of $N_2O$ and $N_2$. From results of this study, we suggest that $NO_3^-$ concentration and soil pH affected $N_2O$ and $N_2$ emissions in flooded soils.
Combination of low pH and high EC of irrigated water have a great potential of N\textsubscript{2}O emissions

The application of chemical fertilizer acidifies the soil and accumulates salts in soil. In chapter 3, effects of salt concentrations on N\textsubscript{2}O emissions from paddy soil adjusted with different pH were examined in a closed incubation experiment in 100 mL glass bottles at 25°C. Six NaCl concentrations and three pH levels were monitored in a microcosm study. Greater NaCl concentration promoted N\textsubscript{2}O emissions at low pH (acid treatment). In neutral and alkali treatments, there was no significant effect of NaCl concentrations on N\textsubscript{2}O emissions ($P > 0.05$). The activity and synthesis of N\textsubscript{2}O reductase that transforms N\textsubscript{2}O to N\textsubscript{2} was probably inhibited at low pH and high salts concentrations. Interactive effects of low pH and higher EC promoted N\textsubscript{2}O emissions.

Tropical mangrove forests could be a hotspot for N\textsubscript{2}O emission when sediment receives high N input

The study in chapter 6 investigated nitrous oxide (N\textsubscript{2}O) production and environmental parameters responsible for its generation in the Ru Cha mangrove sediment (Thua Thien Hue, Vietnam). Our results highlighted that N\textsubscript{2}O emission decreased with sediment depth. Environmental parameters of EC, TOC, TN, and NH\textsubscript{4}\textsuperscript{+} content, and density of denitrifiers were positively correlated to N\textsub Hitch N\textsubscript{2}O emission from mangrove sediments. Nitrous oxide emission increased with NO\textsubscript{3}\textsuperscript{−} additions, indicating that N\textsubscript{2}O emission was NO\textsubscript{3}\textsuperscript{−} limited. Nitrate was completely removed from the solution in all NO\textsubscript{3}\textsuperscript{−} treatments by denitrification. Nitrous oxide emission accounted for 20%, 55% and 100% of the amounts of NO\textsubscript{3}\textsuperscript{−} removed in 1, 3 and 5 mg N L\textsuperscript{−1} treatments, respectively. High EC and low pH in the Ru Cha mangrove forest might inhibit the activity of N\textsubscript{2}O reductase, and therefore greater N\textsubscript{2}O was produced in mangrove sediment. Our microcosm
experiments suggest that the Ru Cha mangrove forests could be a hotspot for N$_2$O emission when sediment receives high N input.

**Incorporation of rice husk products (char and ash) as soil amendment mitigated N$_2$O and CO$_2$ emissions from paddy soil**

Short-term effects of additions of char, ash and raw materials made from rice husks on N$_2$O and CO$_2$ emissions from paddy soil at different water holding capacity (WHC) (40%, 60%, or 80%). Nitrous oxide emission was enhanced by higher soil moisture content but CO$_2$ emission was not affected. Short-term laboratory experiments at 80% WHC indicated that raw rice husk (RR) addition promoted N$_2$O emissions. Regardless of soil moisture contents, CO$_2$ emission in RR addition was 2 times greater than those in other treatments. Decomposition of RR consumed oxygen and provided organic C and N available for microbial activities that stimulated N$_2$O and CO$_2$ emissions. Incorporation of rice husk products (char and ash) increased soil pH which in turn reduced N$_2$O emission from soil compared with control. Our study from the short-term experiment pointed out that addition char and ash could increase soil properties and mitigate greenhouse gas emissions.

**Continuous flooding during fallow periods in paddy fields would reduce N$_2$O emissions from soil and NO$_3^-$ concentration in groundwater**

The study in chapter 6 conducted in a pot experiment simulated a fallow period in Ushimado paddy fields to reduce the high NO$_3^-$ concentration in groundwater via irrigation management. The objective in this study was to determine the effects of different water irrigation regimes with high NO$_3^-$ concentrations on N$_2$O emissions. Continuously flooded condition in the fallow period reduced both N$_2$O emissions from soil and NO$_3^-$ concentration in groundwater. Because the continuous flooding regime
creates a strongly anaerobic condition, most of the N\textsubscript{2}O formed as an intermediate product of denitrification was further reduced to N\textsubscript{2}. In addition, the flood water hampers N\textsubscript{2}O transfer to the air from the soil. In contrast, alternating periods from flooding to drainage favored for N\textsubscript{2}O production from nitrification and denitrification.

**Suggestions from the results**

Results obtained from a series of laboratory experiments provide significant insight into the effects of soil pH on N\textsubscript{2}O emission in wetlands. Low pH inhibited the activity and synthesis of N\textsubscript{2}O reductase, resulting in promoted N\textsubscript{2}O emission as an intermediate product. We found that affecting factors such as soil type, NO\textsubscript{3}\textsuperscript{-} concentration, organic C, salinity, soil moisture, and soil pH governed N\textsubscript{2}O emission in wetlands. Paddy and mangrove forest soils should be paid attention as a hotspot for N\textsubscript{2}O emission under certain conditions. As mentioned above, this thesis will contribute to our understanding of factors affecting N\textsubscript{2}O emission in wetlands to develop useful strategies to mitigate global warming. For management options, increasing average pH would probably lead to a decrease in N\textsubscript{2}O emissions of acidic soil. Decreased nitrogen loadings into paddy and mangrove forest will result in decrease denitrification and N\textsubscript{2}O emissions. Increasing the water retention in paddy field will enhance the buffering function of the system, in which most of NO\textsubscript{3}\textsuperscript{-} rich groundwater entering the paddy will then be removed with minor N\textsubscript{2}O emissions.

It should be pointed out that the factors observed in this study were only obtained in the laboratory experiments. Future studies are needed to investigate environmental factors affecting greenhouse gas emissions at field scales in prolonged periods.
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