CONTROL OF IRON AND MANGANESE AVAILABILITY ON
NITROGEN MINERALIZATION IN SUBTROPICAL PADDY SOILS

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SUMMARY
Nitrogen (N) mineralization from soil organic matter (SOM) provides large proportion of N uptake by rice plants from wetland paddy soils. Hence, the reliable assessment of mineralizable N from SOM is a prerequisite to optimize N fertilizer application and use efficiency, and minimize adverse impact of excess N on environment. The N mineralization in wetland paddy soils is influenced by different biotic and abiotic factors, which are, however, poorly understood. The availability of Fe$^{3+}$ and Mn$^{4+}$ is of interest since they are much more abundant generally in flooded paddy soils than other electron acceptors and may mediate SOM decomposition and NH$_4^+$ production after rapid depletion of O$_2$. Furthermore, Kader et al. (2013) found that N mineralization in paddy soils was significantly correlated with extractable Fe and pH only and not with other general soil properties like soil N content.

Therefore the present MSc thesis was conducted: 1º To investigate the evolution of Fe, Mn, pH and dissolved organic carbon (DOC) in soil solution together with CO$_2$ and CH$_4$ production to gain fundamental insight of SOM decomposition and reductive processes in these soils; 2º To assess if availability of Fe$^{3+}$ and Mn$^{4+}$ limits anaerobic N mineralization in paddy soils of Bangladesh 3º To find out the relative contributions of abiotic release of fixed NH$_4^+$ on mineral N production when compared to biotic anaerobic N mineralization. To achieve these objectives, three controlled laboratory incubation experiments were set up.

A first incubation experiment included soils collected from five fertilizer treatments (Control, N, NP, NPK and N+FYM) of a long-term field experiment with double rice cropping in BAU. All the soils, either untreated (T$_1$), treated with Fe$_2$O$_3$ (T$_2$) or with Mn/Al mixed oxides (T$_3$) were anaerobically incubated in the laboratory. Soil solution pH, dissolved Fe and Mn and DOC were monitored by collecting samples through rhizon soil moisture samplers (RSMS). Produced CH$_4$ and CO$_2$ were measured at a regular time interval. The KCl-extractable NH$_4^+$, microbial biomass carbon (MBC) and pH-KCl at the end of the experiment were also determined.

With an exception in the Fe content of T$_2$ at week 6, generally in all five BAU fertilizer treatments there were no considerable differences in soil solution Fe and Mn concentrations between the T$_1$ and T$_2$ treatments for the entire incubation length. Over all the five soils, in T$_3$, the soil solution Fe contents were lower and Mn contents were higher than in T$_2$. The DOC gradually decreased and contributed 0.4 to 0.6% of total SOC during 1 to 8 weeks after onset of flooding. In all five soils amended with mixed Mn/Al oxides (T$_3$), the DOC concentrations were lower than those in T$_1$ and T$_2$ treatments and a similar trend was seen for the CH$_4$ emission after 6 and 8 weeks. This suggested that the application of amorphous Mn-oxides retarded or delayed CH$_4$ production by limiting the substrate for methanogenic bacteria. After 8 weeks, the content of both KCl-extractable NH$_4^+$ and MBC were not differed significantly among the treatments. The data generated here on KCl-extractable NH$_4^+$
indicated that the limited availability of Mn\textsuperscript{4+} and Fe\textsuperscript{3+} did not influence release of NH\textsubscript{4}\textsuperscript{+} in the studied paddy soils. However, this experiment was conducted in one soil type and we were not able to monitor the actual release of NH\textsubscript{4}\textsuperscript{+} during the incubation due to RSMS set up.

Therefore, a **second incubation experiment** included four farmers’ paddy fields in Northern Bangladesh were conducted. The pH-KCl, dissolved Fe and Mn and KCl-extractable NH\textsubscript{4}\textsuperscript{+} of soils were measured destructively at a regular interval. Per soil the concentrations of dissolved Fe and Mn in control (T\textsubscript{1}) and Fe\textsubscript{2}O\textsubscript{3} (T\textsubscript{2}), and the Mn content in Mn/Al mixed oxides (T\textsubscript{3}) treatments revealed similar results as in BAU soils except few cases. The NH\textsubscript{4}\textsuperscript{+} release patterns were asymptotic and differed significantly among the farmers’ field soils after 10 weeks. However, there were no considerable variations of NH\textsubscript{4}\textsuperscript{+} content among the treatments (T\textsubscript{1}, T\textsubscript{2} and T\textsubscript{3}) within each farmers’ field soil. Based on these results, the hypothesis on the lack of available inorganic electron acceptors affecting anaerobic N mineralization in paddy soils of Bangladesh could be rejected. The rapid increase of exchangeable NH\textsubscript{4}\textsuperscript{+} in farmers’ fields soils during earlier period provoked the question whether this outcomes were entirely due to microbial activity or from the release of fixed-NH\textsubscript{4}\textsuperscript{+}.

That is why, a **third incubation experiment** was conducted to assess the relative contribution of released fixed NH\textsubscript{4}\textsuperscript{-N} and microbial biomass to build up mineral N in farmers’ fields paddy soils. The amount of non-exchangeable NH\textsubscript{4}\textsuperscript{-N} increased over incubation period in all four soils and the contents in week 0 were significantly (p<0.01) lower than after onset of 2 and 4 weeks of flooding. In all farmers’ fields paddy soils, the MBC was already substantial after 2 weeks of flooding which contributed 0.6 to 2.3\% of total SOC. The results here suggested that released mineral N in farmers’ fields paddy soils is mainly derived from the biotic anaerobic N mineralization, not from defixation of NH\textsubscript{4}\textsuperscript{+}.

The overall findings of the study indicated that further research on the availability of inorganic electron acceptors, in relation to anaerobic N mineralization in paddy soils of Bangladesh could not be expanded any more. More research is required on different fractions of fixed NH\textsubscript{4}\textsuperscript{+}, soil microbial and enzyme activities, biochemical composition of SOM like lignin and polyphenol content, to find out the potential predictors of anaerobic N mineralization in subtropical paddy soils.
# Table of Contents

ACKNOWLEDGEMENTS ......................................................................................... iii
SUMMARY ............................................................................................................... iv
Table of Contents .................................................................................................. vi
List of Tables ......................................................................................................... vii
List of Figures ........................................................................................................ ix
List of Abbreviations ............................................................................................ x

CHAPTER 1 INTRODUCTION ................................................................................ 1

CHAPTER 2 LITERATURE REVIEW ................................................................. 4
  2.1 Paddy soils ........................................................................................................ 4
  2.2 Nitrogen and its transformation in flooded paddy soils .............................. 5
  2.3 Nitrogen mineralization and its controlling factors in wetland soils .... 7
  2.4 Alternate electron acceptors, their availability and anaerobic N
      mineralization in wetland soils .................................................................... 8
      2.4.1 N mineralization in relation to available Fe and Mn content in paddy
           soils ........................................................................................................ 12
  2.5 Fixed ammonium and soil N availability to rice plants ........................... 13
  2.6 Microbial biomass C, N and anaerobic N mineralization ......................... 14

CHAPTER 3 MATERIALS AND METHODS ................................................... 15
  3.1 Site description and soils ............................................................................... 15
  3.2 Soil incubation methods ................................................................................ 16
      3.2.1 Experiment I: Changes in pH, dissolved Fe, Mn and OM content in
           relation to fertilizer management in paddy soils ..................................... 17
      3.2.2 Experiment II: Influence of Fe and Mn availability on anaerobic N
           mineralization in paddy soils ................................................................. 19
      3.2.3 Experiment III: Short-term evolution of fixed NH$_4^+$ content and MBC
           in paddy soils ........................................................................................ 20
  3.3 Soil chemical analysis .................................................................................... 21
      3.3.1 pH ........................................................................................................ 21
      3.3.2 Characterization of Fe and Mn in the paddy soils .................................. 21
      3.3.3 Analysis of mineral N .......................................................................... 22
      3.3.4 Analysis of dissolved organic carbon (DOC) ....................................... 23
      3.3.5 Analysis of non-exchangeable NH$_4^+$ .................................................. 23
      3.3.6 Analysis of microbial biomass C ............................................................ 23
  3.4 Analysis of gas samples .................................................................................. 24
  3.5 Data analysis ................................................................................................... 24
List of Tables

Table 1 Selected soil properties of farmers’ fields paddy soils used for incubation experiments………………………………………………………………………………….. 25
Table 2 Properties of selected young floodplain paddy soils from BAU long-term field experiment…………………………………………………………….. 26
Table 3 The pH-KCl (1:2.5) (± standard error) in soils amended with Fe$_2$O$_3$ or Mn/Al mixed oxides and untreated paddy soils after 8 weeks of flooding………………………………………………………………………………….. 29
Table 4 The mean NH$_4^+$ content (± standard error) of farmers’ fields paddy soils amended with Fe$_2$O$_3$ or mixed Mn/Al oxides and unamended soils after 10 weeks of continuous flooding ……………………………………………………………….. 37
Table 5 The mean NH$_4^+$ content (± standard deviation) in initial soils and soils amended with Fe$_2$O$_3$ or Mn/Al mixed oxides and untreated paddy soils after 8 weeks of flooding…………………………………………………………….. 37
### List of Figures

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure 1</td>
<td>Components of flooded soil-water system (<a href="http://www.knowledgebank.irri.org/submergedsoils">http://www.knowledgebank.irri.org/submergedsoils</a>)</td>
<td>5</td>
</tr>
<tr>
<td>Figure 2</td>
<td>Transformation of N in submerged soil (Source: Buresh et al. 2008)</td>
<td>6</td>
</tr>
<tr>
<td>Figure 3</td>
<td>Sequential reduction of oxidants and accumulation of reductants in wetland soils (Reddy and D’Angleo 1994)</td>
<td>10</td>
</tr>
<tr>
<td>Figure 4</td>
<td>Conceptual horizontal and vertical distribution of reduced iron (Fe$^{2+}$) in paddy soils (Becker and Asch, 2005)</td>
<td>11</td>
</tr>
<tr>
<td>Figure 5</td>
<td>Geographical location of the study area in Bangladesh</td>
<td>15</td>
</tr>
<tr>
<td>Figure 6</td>
<td>Extraction of soil pore solution through glass vacuum tube connected with RSMS via syringe needle</td>
<td>18</td>
</tr>
<tr>
<td>Figure 7</td>
<td>Incubation and collection of gas samples through septum from headspace of glass jar to analyze CH$_4$ and CO$_2$</td>
<td>19</td>
</tr>
<tr>
<td>Figure 8</td>
<td>Incubation setup for farmers’ fields paddy soils treated with Fe$_2$O$_3$ or Mn/Al mixed oxides and untreated soils for 10 weeks</td>
<td>20</td>
</tr>
<tr>
<td>Figure 9</td>
<td>Changes in pH-KCl of farmers’ fields paddy soils amended with Fe$_2$O$_3$ or mixed Mn/Al oxides and unamended soils during ten weeks of continuous flooding (vertical bars indicate standard errors of the means)</td>
<td>27</td>
</tr>
<tr>
<td>Figure 10</td>
<td>Changes in soil solution pH of paddy soils treated with Fe$_2$O$_3$ or mixed Mn/Al oxides and untreated soils during eight weeks of continuous flooding (vertical bars indicate the standard errors of the means)</td>
<td>28</td>
</tr>
<tr>
<td>Figure 11</td>
<td>Evolution of Fe content in farmers’ fields paddy soils amended with Fe$_2$O$_3$ or mixed Mn/Al oxides and unamended soils during ten weeks of continuous flooding (vertical bars indicate the standard errors of the means)</td>
<td>30</td>
</tr>
<tr>
<td>Figure 12</td>
<td>Evolution of soil solution Fe content in paddy soils treated with Fe$_2$O$_3$ or mixed Mn/Al oxides and untreated soils during eight weeks of continuous flooding (vertical bars indicate the standard errors of the means)</td>
<td>31</td>
</tr>
<tr>
<td>Figure 13</td>
<td>Evolution of Mn content in farmers’ fields paddy soils amended with Fe$_2$O$_3$ or mixed Mn/Al oxides and unamended soils during ten weeks of continuous flooding (vertical bars indicate the standard errors of the means)</td>
<td>33</td>
</tr>
<tr>
<td>Figure 14</td>
<td>Evolution of soil solution Mn content in paddy soils treated with Fe$_2$O$_3$ or mixed Mn/Al oxides and untreated soils during eight weeks of continuous flooding (vertical bars indicate the standard errors of the means)</td>
<td>34</td>
</tr>
<tr>
<td>Figure 15</td>
<td>Evolution of soil solution DOC content in paddy soils treated with Fe$_2$O$_3$ or mixed Mn/Al oxides and untreated soils during eight weeks of continuous flooding (vertical bars indicate the standard errors of the means)</td>
<td>35</td>
</tr>
</tbody>
</table>
Figure 16  Evolution of NH$_4^+$ content in farmers’ fields paddy soils amended with Fe$_2$O$_3$ or mixed Mn/Al oxides and unamended soils during ten weeks of continuous flooding (vertical bars indicate the standard errors of the means) ……………… 36

Figure 17  Evolution of relative CH$_4$-C (%) over total C form soils treated with Fe$_2$O$_3$ or mixed Mn/Al oxides and untreated soils during eight weeks of flooding (vertical bars indicate the standard errors of the means) ……………………………………… 39

Figure 18  Evolution of exchangeable and non-exchangeable NH$_4^+$ in four farmers’ fields paddy soils during 28 days of flooding (vertical bars indicate the standard errors of the means) ………………………………………………………………………………… 40

Figure 19  Microbial biomass carbon content in farmers’ fields paddy soils during four weeks of submergence (vertical bars indicate the standard errors of the means) ………………………………………………………………………………… 41

Figure 20  Microbial biomass carbon content in paddy soils treated with Fe$_2$O$_3$ or mixed Mn/Al oxides and untreated soils after eight weeks of continuous flooding (vertical bars indicate the standard errors of the means) …………………………… 42
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAU</td>
<td>Bangladesh Agricultural University</td>
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<tr>
<td>SOM</td>
<td>Soil organic matter</td>
</tr>
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<td>SOC</td>
<td>Soil organic carbon</td>
</tr>
<tr>
<td>NH₄⁺</td>
<td>Ammonium</td>
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<tr>
<td>DOC</td>
<td>Dissolved organic carbon</td>
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<tr>
<td>DOM</td>
<td>Dissolved organic matter</td>
</tr>
<tr>
<td>MBC</td>
<td>Microbial biomass carbon</td>
</tr>
<tr>
<td>MBN</td>
<td>Microbial biomass nitrogen</td>
</tr>
<tr>
<td>Eh</td>
<td>Redox potential</td>
</tr>
<tr>
<td>CBD</td>
<td>Citrate bicarbonate dithionite</td>
</tr>
<tr>
<td>RSMS</td>
<td>Rhizon soil moisture sampler</td>
</tr>
<tr>
<td>CO₂</td>
<td>Carbon dioxide</td>
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<tr>
<td>CH₄</td>
<td>Methane</td>
</tr>
<tr>
<td>CEC</td>
<td>Cation exchange capacity</td>
</tr>
<tr>
<td>DO</td>
<td>Dissolve oxygen</td>
</tr>
<tr>
<td>HYV</td>
<td>High yielding variety</td>
</tr>
<tr>
<td>FYM</td>
<td>Farm yard manure</td>
</tr>
<tr>
<td>TOC</td>
<td>Total organic carbon</td>
</tr>
</tbody>
</table>
CHAPTER 1

Introduction

Rice is the staple food for more than half of the world's human population. In 2010, the estimated world paddy production was approximately 700 million tons with more than 90% produced in Asia (FAO 2012). Cultivated rice (*Oryza sativa*) is derived from a semi-aquatic ancestor plant and is highly sensitive to water shortage. Irrigated lowland rice accounts for 50% of the total rice area, which contributes 75% of the global total rice harvest (IRRI 2011). In Asia more than 70% of rice is produced under wetland conditions with high irrigation requirements and a standing water level is maintained on the soil surface during most of the growing season (Bouman and Tuong 2001). Paddy production systems include nowadays one, two or even three rice crops per year where double and triple rice cropping systems comprising about 40% of the global rice supply (Buresh and Haefele 2010). Consequently, intensified irrigated lowland rice systems with improved rice varieties, modern technology and greater fertilizer use contribute in getting higher rice yield (De Datta 1986).

Rice plants need sufficient nitrogen (N) for both the vegetative and reproductive growth as well as optimum yield. Accordingly, rice grain yield is highly sensitive to an excess or a deficiency of N at these critical growth phases (Ponnamperuma 1977). Paddy rice removes 16 to 17 kg N for the production of one ton rough rice, including straw (Choudhury *et al.* 1997; Ponnamperuma and Deturck 1993; Sahrawat 2000). As such, N is the most limiting nutrient element in wetland intensive rice cultivation. Hence, nitrogenous fertilizer application is essential to meet the crop’s demand and urea is the most widely used N source for lowland rice cultivation. The urea-N use efficiency of lowland rice is, however, very low (30–40% or lower) (Choudhury and Khanif 2001, 2004; Choudhury *et al.* 2002) due to ammonia volatilization, denitrification, leaching, runoff losses etc. At present in a large part of South-East Asia (Bangladesh, Myanmar, India, Laos), the N requirement of wetland rice is dominantly met from the soil N supply since half to two-thirds of total N uptake by rice comes from the soil N pool, even in N fertilized fields (Broadbent 1978; IAEA 1978; Ando *et al.* 1992; Manguiat *et al.* 1994). Therefore, soil organic N mineralization (dominantly ammonium or NH$_4^+$ production) is the key process for N nutrition of lowland rice (Sahrawat and Narteh 2001). Consequently, reliable estimation of mineralizable N is essential to determine the rate and time of N fertilizer application, optimize N use efficiency as well as minimize adverse impacts of excessive N on environment (Watanabe *et al.* 1987; Mikha *et al.* 2006, Sharifi *et al.* 2007). However, N mineralization in wetland soils is influenced by several physicochemical factors including C/N ratio of soil organic matter (SOM), size and activity of the microbial biomass pool, temperature, pH, redox potential (Eh), contents of alternate electron acceptors etc. (White and Reddy 2001). All these factors make estimation of N mineralization complex and little is known on the relative control of these abiotic factors on the N mineralization process in subtropical paddy soils.
Introduction

Different efforts have been taken to predict N mineralization in lowland paddy soils (Watanabe et al. 1987; Nateh and Sahrawat 1997). SOM content has been proposed as an index of available N in submerged paddy soils due to significant correlation of organic C and total N with mineralizable N (Narteh and Sahrawat 1997; Sahrawat 2006). However, other studies reveal poor, insignificant or negative correlation of anaerobic N mineralization with organic C and total N content in wetland rice soils (Kader et al. 2013; Adhikari et al. 1999; Cassman et al. 1996; Sahrawat 2010; Yeasmin 2011). Anaerobic decomposition of crop residues under continuous lowland rice systems promotes accumulation of phenolic-lignin compounds, which covalently bind nitrogenous compounds into recalcitrant forms and decrease soil N supply (Olk et al. 2006). This, for one, may explain why relation between soil N content and anaerobic N mineralization is not straightforward.

Specific soil fractions may show a better relation with soil N mineralization. For instance, dissolved organic matter (DOM) content and composition influence N mineralization in subtropical paddy soils (Li et al. 2010). Microbial biomass C (MBC) and N (MBN) were significantly correlated with the anaerobic N mineralization rate (White and Reddy 2001) which indicate availability of C and potentially mineralizable N in wetland soils (McLatchey and Reddy 1998). More recently, Kader (2012) found significant positive correlations between N mineralization rate and physio-chemical SOM fractions in highly weathered paddy soils, whereas the relationship was insignificant or even negative in young floodplain soils of Bangladesh. Therefore, not only organic matter quality but also other inorganic factors like availability of inorganic alternate electron acceptors appear to control N mineralization in subtropical paddy soils.

In waterlogged paddy soils, microbes utilize reducible species (O₂, NO₃⁻, Mn⁴⁺, Fe³⁺, SO₄²⁻, and CO₂) successively as terminal electron acceptors to mediate SOM decomposition (Ponnamperuma 1972; Gao et al. 2002) and NH₄⁺ production. Likewise, both Mn⁴⁺and Fe³⁺ serve as good electron acceptors for anaerobic respiration and are present in anoxic soils as oxides, hydroxides and as structural parts of the clay minerals (Nealson and Myers 1992; Stucki 1988). Ferric iron is one of the most important oxidants in wetland paddy soils and predominantly supports oxidation of SOM by capturing electrons released from organic compounds (58-79% by Yao et al. 1999; 66-84% by Inubushi et al. 1984). It is well known that both Mn⁴⁺ and Fe³⁺ serve as important electron acceptors in submerged soils and the absence of iron oxides and hydroxides in submerged soils and sediments decline organic matter oxidation and mineralization (Sahrawat 2003). Consequently, Sahrawat and Narteh (2001) postulated that mineralizable N and NH₄⁺ production may be greatly influenced by the relative contents of reducible iron and organic C in flooded paddy soils.
Correspondingly smaller amounts of sodium-pyrophosphate-extractable Fe were linked to lower anaerobic N mineralization rates in young floodplain paddy soils (Kader et al. 2013). In addition, NH$_4^+$ fixation and release in tropical rice soils was also correlated with reducible Fe (Sahrawat 1979; Sahrawat 2004).

Due to complexity of anaerobic N mineralization process and its variable relationship with different soil properties, at present there is still not a valid methodology to accurately predict potentially mineralizable N in paddy soils. Kader (2012) investigated anaerobic N mineralization in relation to the length of the annual inundation period, soil mineralogy, pH, contents of C, N, Fe and Mn and physicochemical SOM fractions in floodplain paddy soils in Bangladesh. He only found significant correlations with pH and Fe contents. The mechanisms involved in OM oxidation and anaerobic N mineralization, depending on alternative electron acceptors in absence of O$_2$, are however, not fully understood (Reddy and De Laune 2008). Thus, this thesis’ main focus is on the availability of alternate electron acceptors and their control on anaerobic N mineralization in paddy soils in Bangladesh. The main underlying research hypothesis is that restricted availability of alternate electron acceptors forms a bottleneck for anaerobic N mineralization.

The specific objectives are:

i) To study the progressive evolution of electron acceptors and SOM decomposition in relation to fertilizer management. To this end, we incubated soil samples from a long-term field experiment and we tracked dissolved OM, dissolved Fe and Mn, pH and emission of CO$_2$ and CH$_4$.

ii) To assess if the content of alternate electron acceptors, particularly Fe$^{3+}$ and Mn$^{4+}$, affects anaerobic N mineralization. We addressed this objective by incubating farmers’ fields soils with addition of synthetic Fe$^{3+}$ oxides and mixed Mn/Al oxides and we traced NH$_4^+$, pH-KCl, dissolved Fe and Mn.

iii) A final secondary objective was to estimate the relative contribution of abiotic release of fixed NH$_4^+$ in the build up of mineral N, next to biotic anaerobic N mineralization in young floodplain paddy soils in Bangladesh.
CHAPTER 2
Literature Review

2.1 Paddy Soils

Paddy fields are flooded parcels of arable land used for growing rice and other semi-aquatic crops. Irrigated rice is concentrated on alluvial floodplains, terraces, inland valleys and deltas in humid and sub-humid, sub-tropics and humid tropics of Asia. About 80% of total rice growing area in Asia is managed as paddy soils, corresponding to 139.62 million ha in 2008 (IRRI 2000). Traditionally rice is transplanted and grown, whilst keeping a submerged environment from crop establishment to prior to harvest due to its sensitivity to water shortage (Gong 1983). After rice production water is usually drained and paddy soils face multiple drying-wetting cycles during in between rice-growing seasons (Nishimura et al. 2004). The management-induced change of oxic and anoxic conditions results in temporal and spatial variations in redox reactions, affecting the dynamics of organic and mineral soil constituents (Cheng et al. 2009).

Intensive lowland rice systems differ markedly from upland crop systems in their physical, chemical and trophic properties, which affect C and N cycling processes. Preferential accumulation of organic matter (OM) has been commonly observed in submerged rice soils compared to aerobic soils due to high input of plant residues combined with lessened and incomplete decomposition of OM (Sahrawat 2003; Kögel-Knabner et al. 2010). Components of the flooded soil-water system are floodwater, oxidized surface soil, reduced soil, rhizosphere-oxidized soil, plow pan and oxidized or reduced subsoil (Dobermann and Witt 2000) (Figure 1). The rice plant has the capacity to transport atmospheric O₂ through its stem to the roots and this O₂ diffuses from the roots to the adjacent soil layer, enabling the rice plants to survive in an anaerobic environment (Reddy and Patrick 1984). The aerobic soil layer supports aerobes and provides a site for O₂ required N transforming reactions whereas the anaerobic layer supports anaerobes and provides site for reactions occurring in absence of O₂ (Reddy 1982). After submergence, O₂ supply greatly declined as the diffusivity of O₂ in water or in water saturated soil is 10,000 times lower than in air (Armstrong 1979). Moreover, O₂ present in water is rapidly used up for aerobic microbial respiration and facultative and obligate anaerobes then dominate (Takeda and Furusaka 1970).
Nitrogen is a major component of many important structural, genetic and metabolic compounds in plant cells, such as chlorophyll, proteins, amino acids, enzymes, DNA, ATP etc. Total N uptake by paddy rice cultivars BR22 and BR25 is 74 and 85 kg ha\(^{-1}\) with grain yield of 4.3 and 5.1 Mg ha\(^{-1}\), respectively (Choudhury et al. 1997). Nitrogen in flooded soils and sediments occurs in inorganic forms but predominantly in organic forms. Inorganic forms are \(\text{NH}_4^+\), \(\text{NH}_3\), \(\text{NO}_3^-\), \(\text{NO}_2^-\) and are taken up by the plants and microbes (Reddy and Patrick 1984). The forms of organic N in paddy soils are amide-N, \(\alpha\)-amino-N, hexosamine-N, unknown-N and nonhydrolyzable-N (Yonebayashi and Hattori 1986). In submerged paddy soils both biotic and abiotic nitrogen transforming reactions such as ammonification, immobilization, nitrification, denitrification, volatilization, nitrogen fixation and leaching occur. Since N transformation reactions in wetland soils are greatly influenced by redox potential, different N transformation processes dominate in different zones (Figure 2).
Ammonification i.e. biological conversion of organic N to $\text{NH}_4^+$ is the dominant N mineralization process (Sahrawat 1983) in anoxic soils where $\text{NH}_4^+$ production during decomposition of OM takes place through hydrolytic deamination of amino acids and peptides, degradation of nucleotides and metabolism of methylamine by methanogenic bacteria (King et al. 1983). $\text{NH}_4^+$ accumulates under anaerobic decomposition of organic N due to lower levels of O$_2$ which limits oxidation of $\text{NH}_4^+$ to NO$_3^-$ (Keeney and Sahrawat 1986). This $\text{NH}_4^+$ can be converted back to organic N (immobilization) after it is taken up by microbes and plants (assimilated). Sources of $\text{NH}_4^+$ in the surface aerobic soil layer are fertilizers, organic N mineralization and release of $\text{NH}_4^+$-fixed in clay mineral interlayers.

In flooded soils, nitrogen transport occurs through ion diffusion ($\text{NH}_4^+$ diffusion from anaerobic soil layers and NO$_3^-$ diffusion from aerobic soil layers), leaching, interflow and surface runoff. Positively charged $\text{NH}_4^+$ ions are adsorbed by negatively charged clay particles whereas negatively charged nitrite and nitrate ions are not usually adsorbed by clay particles (Cho et al. 2010), which results in leaching of nitrate or nitrite from the field. By diffusion to the aerobic zone may either be nitrified to NO$_3^-$ or NH$_3$ may be lost through volatilization. The NO$_3^-$ formed in the aerobic zone can move back to the anaerobic layer via diffusion and can then be subsequently removed through denitrification (Patrick and Reddy 1976). Atmospheric N$_2$ can be fixed into soil and rhizosphere in rice paddy fields through biological nitrogen fixation (Yoshida and Ancajas 1973).

The N availability to plants results from the balance between the processes of SOM mineralization, immobilization by soil microbial biomass, plant uptake, atmospheric deposition and losses through denitrification and leaching (Moretto and Distel 2002). Organic N mineralization is a key process in regulating bioavailability of N, wetland productivity and environmental quality (Reddy and De Laune 2008; White and Reddy 2000).

![Figure 2](image-url) Transformation of N in submerged soil (Source: Buersh et al. 2008)
2.3 Nitrogen mineralization and its controlling factors in wetland soils

Nitrogen mineralization is a biotic decomposition process in which high-molecular weight organic N compounds are sequentially hydrolyzed into simpler inorganic N forms through extra-cellular enzyme activity (Sinsabaugh et al. 1991; Li et al. 2001). The breakdown of SOM is typically slower in submerged soil than in aerobic soil (Villegas-Pangga et al. 2000). Microbial decomposition of OM in submerged soil depends on restricted bacterial microflora which operates at lower energy level and is less efficient than aerobes. This results in incomplete decomposition of carbohydrates and a lower synthesis of microbial cells per unit degraded organic carbon (Reddy and Patrick 1984). Sahrawat (2004) also suggested that OM accumulates with time in water-logged soils due to lack of oxygen and other terminal electron acceptors, like Fe^{3+} and SO_{4}^{2-}, resulting in inefficient OM oxidation at low Eh values. Accordingly, lower gross N mineralization and immobilization in submerged soils often provide a higher net N mineralization than aerobic soils, releasing higher inorganic N (Buresh et al. 2008). Mineralized N contents in wetland rice soils vary between 21 to 166 mg kg^{-1}, during two weeks of anaerobic incubation where NH_{4}^{+}-N ranges from 2 to 7% of the total N (Narteh and Sahrawat 1997). The rate of OM decomposition and N mineralization in wetland soils is largely influenced by different factors such as temperature, soil water regime, redox potential, pH, amount and chemical composition of SOM (lignin, cellulose, protein, C: N ratio), availability of alternate electron acceptors, microbial activity and microbial biomass, CEC, amount and nature of clay, other nutrients availability like P etc. (Inamura et al. 2009; White and Reddy 2001; Sahrawat 2010). The effect of these factors is fairly well understood in well drained soils but little known for flooded soils (Reddy and Patrick 1984). Like most biological processes, N mineralization rate firstly increases with temperature under submerged conditions (Takahashi and Yamamuro 1995). At 112 days incubation, estimated mean N mineralization are 33 mg N kg^{-1} at 25°C and 47 mg N kg^{-1} at 30°C (Manguiat et al. 1996). Clay mineralogy is of importance to abiotic preservation and release of NH_{4}^{+}. After flooding a share of the mineralized NH_{4}^{+} is transferred to non-exchangeable form in the presence of a sufficient amount of expandable 2:1 minerals. This ‘fixed NH_{4}^{+}’ becomes available to the following rice crop with highest release in rhizosphere and the availability decrease with increasing distance from roots (Schneiders and Scherer 1998). The soil redox potential (E_{o}) influences this cycle of fixation and release of NH_{4}^{+} by affecting charge conditions of some expandable clay minerals, which may also alter their cation exchange capacity (CEC).

Narteh and Sahrawat (1997) found that mineralized N shows a significant positive correlation with general soil properties like pH, organic C, total N, clay% and CEC but correlations were
insignificant with extractable iron and the soil C/N ratio. Multiple regression analysis of mineralized 
N with soil characteristics indicated that inclusion of C/N ratio and extractable iron did, however, 
improve the prediction of mineralized N (Narteh and Sahrawat 1997). Kader (2012) on the contrary 
found no significant correlation between the anaerobic nitrogen mineralization rate and soil properties 
like soil N, soil C, soil C: N ratio, contents of NO$_3^-$, SO$_4^{2-}$, soil texture and pH-KCl in floodplain soils. 
On the other hand in terrace soils, correlations between anaerobic N mineralization rate and SOC 
($P<0.01$) and total N ($P<0.01$) content were positive and significant (Kader 2012). In line with the 
observations for these terrace soils, Sahrawat (1983) found that soil organic C is closely related with 
mineralizable N and nitrogen uptake by rice in tropical wetland rice soils.

**Soil organic matter quality** may influence N mineralization. Organic N fractions and chemical 
structure of soil organic matter pools and chemical environment of submerged soil can have a greater 
influence in N mineralization than soil organic C or total N, as reported by Olk et al. (1996). 
Stabilization of applied OM and fertilizer N into humic fractions under waterlogged paddy soils 
influences mineralization of organic matter and release of mineral N (Bird et al. 2003). Accordingly, 
decreased crop N uptake and soil N availability from SOM is associated with accumulation of 
phenolic lignin residues in SOM under continuous irrigated lowland rice production, which might 
chemically bind N (Olk et al. 2007). **Double rice cropping systems**, have an increased period of 
aerobic soil conditions compared to single rice cropping. This prolonged anaerobic period inhibits 
N mineralization and decomposition of OM compared to aerated systems and may result in decreased 
soil N supply (Olk et al. 2007).

N mineralization is largely influenced by the degree of soil reduction and decreases with an increase 
of soil reduction from nitrate reducing conditions to methanogenic conditions (White and Reddy 
2001). Consequently, the changes in the supply of alternate electron acceptors influence the size of 
microbial biomass, enzyme activity, OM decomposition and N mineralization in wetland soils 
(McLatchey and Reddy 1998).

### 2.4 Alternate electron acceptors, their availability and anaerobic N mineralization in 
wetland soils

Generally, paddy soils go through periodic changes of oxic and anoxic conditions. During anaerobic 
OM degradation reaction electron transfer must be balanced between products and reactants. Most 
important redox couples in flooded paddy soils are: O$_2$/H$_2$O, NO$_3^-$/N$_2$, Mn$^{4+}$/Mn$^{2+}$, Fe$^{3+}$/Fe$^{2+}$, SO$_4^{2-}$ 
/H$_2$S, and CO$_2$/CH$_4$ with O$_2$, NO$_3^-$, Fe$^{3+}$, SO$_4^{2-}$, Mn$^{4+}$ and CO$_2$ etc. as main electron-acceptors (Gao et 
al. 2002). Microbes catalyze the breakdown of OM to get nutrients and energy released from
oxidation of OM (Reddy and D’Angleo 1994). The rate of OM decomposition and nutrient release in wetland soils are influenced by chemical composition of organic substrate, availability of electron acceptors, redox potential, temperature, pH etc. (Reddy and D’Angleo 1994; McLatchey and Reddy 1998). Organic matter decomposition and assimilation in submerged soils are carried out by facultative and obligate anaerobes, which operate at much lower energy level than those of aerobes in well drained soils (Ponnamperuma 1972). Oxygen, the first preferred and most efficient electron acceptor as it receives electrons from reductant (OM) more readily than other oxidants (Inglett et al. 2005), becomes undetectable within a day after submergence by the respiration of aerobic soil microbes and plant roots (Ponnamperuma 1972; Becker and Asch 2005; Kögel-Knabner et al. 2010).

With the depletion of O₂, facultative and obligate anaerobes sequentially use of NO₃⁻, Mn⁴⁺, Fe³⁺, SO₄²⁻, dissimilation products of organic matter, CO₂, N₂ and even H⁺ as electron acceptors for their respiration and reduce them to N₂, Mn²⁺, Fe²⁺, H₂S, CH₄, NH₃ and H₂ (Ponnamperuma 1972) (Figure 3). In wetland soils, the order of using these electron acceptors by microbial population usually follows above sequence depending on their electron affinity, energy yield and related enzyme system in bacteria (Mc Latchey and Reddy 1998; Inglett et al. 2005). Moreover, the rate of consuming electron acceptors in anoxic soil systems depends on concentration of electron acceptors, the nature and content of OM and activity of microbial population etc. Higher amounts of easily decomposable OM bear a more intensive demand for (alternate) electron acceptors during microbial energy conservation, resulting in a more complete sequential exhaustion of electron acceptors (Jacq et al. 1986). Consequently, the availability and dominance of any single inorganic electron acceptors may logically influence OM oxidation and NH₄⁺ production in submerged soils (White and Reddy 2001). In submerged soils, redox potential (Eh) usually ranges from +700 to -300mV. This reflects reduction intensity or electron activity and provides an indication of the availability of dominant electron acceptors (Patrick and Reddy 1978; Inglett et al. 2005).

Upon flooding, Eh in the puddle layer drops to a fairly stable range of +200 to -300mV, depending on soil OM content and reducible elements. In the relatively oxidized few mm top soil layer and surface water zone Eh remains +300 to +500mV (Sahrawat 2012; Pnamperuma 1972). Reduction of specific oxidant occurs in a defined range of redox potential (Eh). For instance, near neutral pH, reduction of Mn³⁺,⁴⁺, Fe³⁺ and SO₄²⁻ takes place over a wide range of Eh (+350 to +100 mV) whereas reduction of dissolved O₂ (DO) and NO₃⁻ occurs at high but narrow Eh range (+400 to +350mV) in paddy soils (Gao et al. 2002).
Since presence of $O_2$ hinders $NO_3^-$ reduction, it begins when $O_2$ concentration goes down very low value after submergence. Nitrate reduction in wetland soils undergoes two major pathways, namely dissimilatory nitrate reduction to ammonium ($NO_3^- \rightarrow NO_2^- \rightarrow NH_4^+$) and denitrification ($NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$) by variety of facultative bacteria. In submerged soils nitrate is extremely unstable and bulk of $NO_3^-$ disappears through nitrate respiration within a few days (Ponnampuruma 1972). Gao et al. (2002) revealed that dissolved $O_2$ (DO) and $NO_3^-$ in paddy soils drop from >0.2 to <0.02 mmol L$^{-1}$ and 1.8 to 0.1 mmol L$^{-1}$, respectively, within the first 5 days of flooding. In addition, $O_2$ transport by roots of growing rice plants may provide a minor electron acceptor throughout the growing season (Kumazawa 1984).

In submerged soils reduction of Mn is one of the major transformation processes, which takes place prior to Fe but after $O_2$ and $NO_3^-$ reduction. The concentration of Mn in anoxic soil solution corresponds to a balance between release of Mn$^{2+}$ by reduction and its removal by cation exchange reactions, precipitation and formation of insoluble complexes (Ponnampuruma 1972). Upon flooding, Mn$^{3+}$ concentration in soil solution thus steadily increases with an eventual decreasing trend indicating the use of Mn oxides as electron acceptors during very early rice growing period (Gao et al. 2002; Jahan et al. 2013). The decrease in concentration of Mn$^{2+}$ is also possibly due to precipitation as MnS (Gao et al. 2002). Solubility of Mn oxides is very low in water and only a limited number of bacteria participate in their reduction.
Next to O$_2$, NO$_3^-$ and Mn, reduction of Fe$^{3+}$ iron begins within a few days after submergence at redox potential of $< +180$ to $+150$ mV (Patrick and Reddy 1978) with gradual increase of soil solution Fe$^{2+}$ concentration (Ponnamperuma 1977; Gao et al. 2002; Jahan et al. 2013). The Fe redox systems are dominant to other inorganic electron acceptors for OM oxidation and NH$_4^+$ production in tropical submerged soils (Reddy and De Laune 2008; Sahrawat 2010; Yao et al. 1999; Jackel and Schnell 2000a). Iron in paddy soils remains as Fe$^{3+}$ oxides and oxyhydroxides and in the structure of clay minerals (Stucki 1988). Different iron species dominate in various stratified zones of flooded paddy soils, where solid-phase Fe$^{2+}$ concentrations are lowest in the oxic surface soil and higher in the 4-8 mm soil layer (Ratering and Schnell 2000). Fe$^{3+}$ is maximally present at shallow 2-4 mm depth and in the rhizosphere (Ratering and Schnell 2000). All these result horizontal and vertical differentiation of Fe$^{2+}$ on a small scale in the paddy soil profile (Figure 4).

Although both microbial and chemical Fe reduction take place in wetland soils, chemical Fe$^{3+}$ reduction plays minor role in paddy soils due to small pool of total sulfur (Ratering and Schnell 2000). Accordingly, reduction of Fe$^{3+}$ oxide under anoxic conditions is largely controlled by wide variety of microorganisms and Fe$^{3+}$ reducing bacteria (e.g. Clostridium, Paenibacillus and Bacillus etc.) gain electrons from organic substrates and then transfer the electron to Fe$^{3+}$ and produce Fe$^{2+}$ rather than Fe assimilation into cell components (Yi et al. 2012). Microbial reduction of Fe$^{3+}$
compounds (e.g. amorphous Fe(OH)₃) continues according to following equation until ferric compounds remain available in the system (Jacq et al. 1986).

\[
\text{Fe(OH)}_3 + 3\text{H}^+ + \text{e}^- \xrightarrow{\text{Fe}^{3+}-\text{reductase(s)}} \text{Fe}^{2+} + 3\text{H}_2\text{O}
\]

The reduction of ferric oxides depends on temperature, OM content and degree of crystallinity of Fe³⁺ (hydr-) oxides (Ponnamperuma 1972). Poorly or low crystalline Fe³⁺ oxides are readily reduced by iron reducing microbes (Qu et al. 2004). Accordingly, the intensity of Fe²⁺ production increases with decreasing crystallinity of pedogenic Fe³⁺ oxides and hydroxides and with an increase in easily mineralizable organic substances (Schwertmann 1966). Consequently, amorphous Fe³⁺ oxides are the predominant source of available reducible Fe and on average 35-50% of amorphous Fe³⁺ oxides Fe is reducible (van Bodegom et al. 2003). In total, it has been estimated that iron reduction drives 42% of anaerobic OM decomposition in flooded paddy soils with a low OM content and this can decrease methanogenic OM degradation (Jackel and Schnell, 2000a). The iron reduction rate is also influenced by the concentration of organic reductant (Sparks 1989). In the line with this, (Gao et al. 2002) found that the rate of Fe reduction was faster in straw-incorporated submerged paddy field which was indicated by the presence of higher soluble, reduced Fe²⁺.

Major reduction reactions of soil oxidants generate CO₂ with OM as the electron donor (Yu et al. 2007). In flooded rice soils CO₂ production increases with reduction of inorganic electron acceptors and reaches its maximum value with the depletion of these electron acceptors. After that methanogenesis initiates and reaches its maximum value with subsequent production of CO₂ and CH₄ at a constant rate (Yao et al. 1999). This terminal step of anaerobic OM degradation is the conversion of acetate and H₂/CO₂ to CH₄ by obligate methanogens (Inglett et al. 2005). Methanogenesis can dominantly participate in anaerobic degradation of SOM (73% of C mineralization) in paddy soils (Jackel and Schnell 2000a).

2.4.1 N mineralization in relation to available Fe and Mn content in paddy soils

An earlier study on N mineralization in diverse West African rice soils showed that extractable Fe is not directly correlated to NH₄⁺ production under waterlogged condition but its inclusion in multiple regression analysis improved the prediction of NH₄⁺ production (Narteh and Sahrawat 1997). A recent study on 15 West African soils (Sahrawat and Narteh 2001) report a significant correlation between mineralizable N under anaerobic conditions with organic C (r²=0.79) and EDTA extractable (r²=0.86) or ammonium oxalate extractable Fe (r²=0.75).
Accordingly, soils high in both organic C and reducible Fe were found to be high in mineralizable N whereas soils having low organic C or reducible Fe has relatively lower mineralized N (Sahrawat and Narteh 2003). More recently, Kader et al. (2013) also found that smaller amounts of Na-pyrophosphate-extractable Fe resulted in lower N mineralization rate under anaerobic conditions. Reducible Fe also influences NH$_4^+$ fixation and release in tropical rice soils (Sahrawat 1979) and sorbed NH$_4^+$ in reduced soils and sediments may be released by exchanging with Fe$^{2+}$ (Sahrawat, 1979) (see 2.5).

### 2.5 Fixed ammonium and soil N availability to rice plants

Ammonium fixation is the adsorption or absorption of NH$_4^+$ ions by the mineral or organic soil fraction, which makes them relatively non-exchangeable by standard cation exchange methods (SSSA 1984). The amount of native fixed NH$_4^+$ greatly differs from soil to soil depending on parent material, texture, clay content, composition of clay mineral, moisture content, potassium (K) status and K saturation of the interlayers of 2:1 clay minerals (Nieder et al. 2011). For instance, vermiculite and illite fix NH$_4^+$ to a greater degree than montmorillonite, and kaolinite (1:1) is unable to bind NH$_4^+$ (Nieder et al. 2011). In flooded rice soils a substantial part of NH$_4^+$ formed by mineralization is converted to non-exchangeable form in presence of sufficient amounts of expandable 2:1 clay minerals (Schneiders and Scherer 1998). Furthermore, a higher concentration of exchangeable NH$_4^+$ due to mineralization of organically bound N (Schneiders and Scherer 1998) favors NH$_4^+$ fixation (Zhang and Scherer 1999; Juang et al. 2001). It is also postulated that NH$_4^+$ fixation is also linked to microbial reduction of Fe$^{3+}$ with subsequent dissolution of Fe oxides coating on clay surface at low Eh, promoting NH$_4^+$ diffusion to the interlayers (Scherer and Zhang 1999). Besides these, iron redox reactions influence both NH$_4^+$ fixation and release (Sahrawat 2004). In flooded soils, NH$_4^+$ fixation might be explained by decreasing redox potentials which lead to reduction of structural Fe$^{3+}$, as such increasing net negative charge of certain clay minerals (Stucki et al. 1984). On the other hand, rice plants are able to transport O$_2$ which may oxidize Fe$^{2+}$ to Fe$^{3+}$ and release fixed NH$_4^+$ by rising redox potentials in the rhizosphere (Schneiders and Scherer 1998). In reduced soils and sediments, sorbed NH$_4^+$ is also released by exchanging with Fe$^{2+}$ (Sahrawat 1979). Intermittently flooded Vertisols with rice crop, results higher CEC due to reduction of octahedral Fe$^{3+}$ and reductive dissolution of Fe oxyhydroxides coatings (Favre et al. 2002). Recently fixed NH$_4^+$ is also more available to plants than native fixed NH$_4^+$ and soil microflora play important roles in this defixation process (Nieder et al. 2011). For instance, 100 kg fixed NH$_4^+$-N ha$^{-1}$ releases during rice growing period in a soil dominated by vermiculite (Keerthisinghe et al. 1984). Consequently, NH$_4^+$ fixation and release could play a decisive role in soil N cycle and efficiency of applied N fertilizer which further influence crop N uptake (Steffens and Sparks 1999; Juang et al. 2001) and N nutrition of wetland rice crops.
2.6 Microbial biomass C, N and anaerobic N mineralization

Microbial biomass is the living component of soil and essential regulator of ecosystem functions, nutrient cycling, soil fertility, SOM turnover and global C changes (Katsaliourou 2006). Consequently, soil microbial biomass has potential to use as sensitive indicator of OM dynamics due to their rapid and detectable changes with altering management practices in soil (Powlson and Jenkinson 1981). In paddy soils as well microbial biomass has important roles in nutrient pool, nutrient turnover and early indicator of soil/crop management (Yoshikawa and Inubushi 1995, Shibahara and Inubushi 1997). Moreover, microbial biomass acts as both labile sink and source of nutrients (Inubushi et al. 1997a, b). In wetland soils mineralization of organic N is accomplished by wide variety of heterotrophic microorganisms (White and Reddy, 2001) where changes in pH and microbial population together with lower Eh influence OM mineralization (McLatchey and Reddy 1998). Accordingly, MBC and MBN representing C and N mineralization rate which gradually decrease in soils turning into more reduced conditions possibly due to lowering microbial populations (McLatchey and Reddy 1998). Moreover, MBC, inorganic N, dissolved organic carbon (DOC), total C and total N are significantly correlated to potential rates of O₂ consumption, denitrification, SO₄²⁻ reduction and methanogenesis in wetland soils (D'Angelo and Reddy 1999). The inorganic N and MBC also illustrate best correlation with heterotrophic activity in wetland soils (D'Angelo and Reddy 1999). However, organic N mineralization rates disclose significant positive correlation with MBC, MBN and extractable NH₄⁺ under aerobic, NO₃⁻ reducing, SO₄²⁻ reducing and methanogenic conditions (White and Reddy 2001). Similarly, potentially mineralizable N (PMN) reveals significant correlation with net N mineralization rate (NMR), MBC, MBN and NH₄⁺-N indicating that the fraction of PMN mediated by soil microbial biomass serves as important source of mineralizable N in wetland paddy soils (Zhang et al. 2009).
CHAPTER 3

Materials and Methods

3.1 Site description and soils

A primary goal of this MSc thesis was to find out the link between availability of alternative electron acceptors (Fe and Mn) and anaerobic N mineralization in Bangladesh. To this end, a set of representative paddy soils were collected from the study area and controlled laboratory incubation experiments were conducted. The study area covers the District of Mymensingh in the North (24°43’ N, 90°25’ E) of Bangladesh, dominated by Boro-Fallow-T. Aman rice cropping systems. This region has a subtropical monsoon climate, characterized by wide seasonal variations in rainfall, high temperatures and humidity. There are three distinct seasons: a hot, humid summer from March to June; a cool, rainy monsoon season from June to October; and a cool, dry winter from October to March. The annual mean temperature and rainfall of the study area are 25°C and 2550 mm, respectively (Rahman 2012).

Figure 5 Geographical location of the study area in Bangladesh
Soil samples were firstly collected from a long-term field experiment at the Bangladesh Agricultural University (BAU) in August 2012 before transplanting T. Aman rice. The experiment was initiated in 1978 at the experimental field of the Department of Soil Science, BAU, Mymensingh on Old Brahmaputra Floodplain Alluvium. The farm belongs to the Sonatola Series under the general soil type of non-calcareous dark grey Floodplain Alluvium with Aeric Haplaquepts (USDA soil taxonomy) and Eutic Gleysol (FAO-UNESCO) soil unit. Initially the soil was silt loam in texture (19, 63 and 18% sand, silt and clay, respectively) with pH of 6.8, a total N content of 0.06% and an OM content of 1.17%. The bulk density of surface depth layer (0-20cm) varies from 1 to 1.43 g cm⁻³. A broadcast Aus - T. Aman - Pulse cropping pattern was followed until 1982 with local rice varieties. After that the cropping pattern was modified to Boro- Fallow - T. Aman Rice with the inclusion of high yielding (HYV) rice varieties.

For this thesis research five treatment combinations were sampled (Control, N, NP, NPK and N+FYM). These are:

1. Unfertilized control
2. N : (100 kg N ha⁻¹)
3. NP : (100 kg N ha⁻¹ + 20 P kg ha⁻¹)
4. NPK : (100 kg N ha⁻¹ + 20 P kg ha⁻¹ + 19 kg K ha⁻¹)
5. N+FYM: (100 kg N ha⁻¹ + 5 Mg FYM ha⁻¹)

Nitrogen, P and K were applied in each crop as urea, triple super phosphate and muriate of potash respectively. All chemical fertilizers and one-third of the urea were incorporated to soil during final land preparation in each crop. Another two splits of urea were applied as top-dressing at 35 and 50 days after transplanting (DAT). The experiment was laid out in a randomized block design with three replications and the unit plot size was 12m x 6 m.

On the basis of data derived by Kader (2012), in addition four farmers’ fields (Sonatala-1, Sonatala-2, Noaddah, and Melandooho) with varying soil type and properties were selected for another incubation experiments for this M Sc. thesis research. All the samples were collected from 15 spots per plot at 0-15cm depth by auger (2.5cm diameter) and were mixed thoroughly into one composite sample for each treatment. After that the soils were broken, air dried, ground and sieved through a 2mm mesh sieve.

3.2 Soil incubation methods

Three separate controlled laboratory incubation experiments were set up. Two with four farmers’ fields soils (3.2.2 and 3.2.3) and another with 15 (5 treatments x 3 field replicates) soils of the BAU long-term field experiment (3.2.1). In first two incubation experiments the soils were either
1° untreated, 2° amended with Fe$_2$O$_3$ (MW: 159.69g; 99% assay) powder or 3° amended with mixed Mn/Al-oxides. The application rate of Fe$_2$O$_3$ and mixed oxides of Mn/Al in experiment-I and II were made primarily on the basis of Fe/Al and Mn/Al ratio in paddy soils of Bangladesh, which ranges from 2-9 and 0.1 to 1 respectively (Kader 2012). Ammonium oxalate extracts all amorphous iron oxides (predominant source of reducible Fe) whereas 0.5M HCl extraction represented all reducible Fe after prolonged incubation (Van Bodegom et al. 2003). Consequently, the content of ammonium oxalate and 0.5M HCl extractable Fe were also considered when selecting the Fe$_2$O$_3$ doses for experiment-I and experiment-II. Mixed oxides of Mn/Al (Mn/Al molar ratio: 0.3) were synthesized by raising the pH to 7.54 by addition of 0.2M NaOH to a continuously stirred solution of 0.06M MnO$_2$ and 0.2M Al$_2$(SO$_4$)$_3$ (modified from Schwertmann and Cornell 1991). In this way, a precipitate is formed, which was filtered through Whatman no. 4 filter paper, rinsed four times with distilled water, air-dried, ground and sieved with a 300μm mesh sieve. The Mn and Al content of the synthetic mixture was determined by dissolving a fixed mass in 2% HNO$_3$ followed by analysis by Inductively Coupled Plasma-Atomic Emission Spectroscopy ICP–AES; on an iCAP 6300 series (Thermo Scientific). In a third incubation experiment only untreated farmers’ fields soils were used.

3.2.1 Experiment I: Changes in pH, dissolved Fe, Mn and OM content in relation to fertilizer management in paddy soils

The first incubation experiment was set up with 5 treatments x 3 field replicates of the long-term BAU field experiment. The aim was to study the evolution of Fe and Mn in the soil solution in detail together with pH to achieve insight in the course of the time evolution of the redox-condition in both unamended and either Fe or Mn/Al amended soil. In addition, concentrations of dissolved organic carbon (DOC) and the evolution of CO$_2$ and CH$_4$ were measured to track SOM decomposition.

The practical design deviated from the second experiment in that soil was not sampled destructively but instead the soil solution was sampled regularly with rhizon soil moisture samplers (RSMS) (Produced by: Rhizosphere Research Products; Article no. 19.21.05). Each RSMS was outfitted with 5cm porous and 2.5mm OD part, stainless steel strengthener wire, a 12 cm PE/PVC tube and male luer lock which used for collecting soil pore solution to analyze macro elements (except metals) and OM in undisturbed soils (Figure 6). 112g dry soil was filled in plastic recipients (10cm height and 5.1cm inner diameter) for anaerobic incubation of 8 weeks. Adjusted bulk density and soil height were 1.1 Mg m$^{-3}$ and 5cm, respectively. Three treatments, viz. T$_1$: Control, T$_2$: Fe$_2$O$_3$ at a rate of 2g kg$^{-1}$ soil and T$_3$: Mn/Al at a rate of 3.04g kg$^{-1}$ soil were included. Thus, 45 tubes were prepared for each of the 15 soils (5 field treatments x 3 field replicates x 3 lab treatments) and oversaturated with deionized water to bring standing water level of 2cm. Next, RSMS were inserted cautiously into each
soil column. The soil was incubated at 20°C temperature and a standing water level of 2cm was maintained. Each week soil pore water (9ml each) was extracted by connecting a 10 ml glass vacuum tube with syringe needle connected to each RSMS. Collected samples were stored in freezer for the analysis of Fe, Mn, pH and dissolve organic carbon (DOC). Beside these, 210 gas samples (5 field treatments x 2 field replicates x 3 lab treatments x 7 sampling time) were collected throughout the incubation. For collecting gas, each sample was incubated in individual glass jar for 2 hours at 20°C temperature and 1ml gas sample was withdrawn from the head space of jar through a septum (Figure 7). At the end of the experiment, the soil was homogenized and weighed separately to determine NH$_4^+$, MBC, pH-KCl and gravimetric soil moisture content.

Figure 6 Extraction of soil pore solution through glass vacuum tube connected with RSMS via syringe needle
3.2.2 Experiment II: Influence of Fe and Mn availability on anaerobic N mineralization in paddy soils

In a second incubation experiment the aim was to test the hypothesis that a limited availability of alternative electron acceptors would limit anaerobic N mineralization in young floodplain paddy soils. To test this hypothesis the time evolution of a series of relevant soil variables were compared in unamended and Fe or Mn/Al amended soil. We followed concentrations of KCl-extractable NH$_4^+$, pH-KCl and water soluble Fe and Mn in the Sonatala-1, Sonatala-2, Noaddah and Melandoho soils for 10 weeks. All soils were incubated anaerobically in small plastic tubes (6.8cm height and 3.4cm inner diameter) for 10 weeks. The amount of dry soil per tube was 36g for Sonatala-1 and Sonatala-2 soils and 45g for the Noaddah and Melandoho soils. Dry soil amount was calculated on the basis of the actual bulk density in the field and a 4cm soil height. Bulk density was adjusted to 1 Mg m$^{-3}$ for Sonatala-1 and Sonatala-2 soils and 1.2 Mg m$^{-3}$ for the Noaddah and Melandoho soils by slight compaction. Based on ammonium-oxalate extractable Fe, Al and Mn content of these soils by Kader (2012) three treatment combinations such as T$_1$: Control, T$_2$: Fe$_2$O$_3$ at a rate of 7.2g Kg$^{-1}$ soil and T$_3$: Mn/Al (0.08 ratio) mixture at a rate of 1g kg$^{-1}$ soil were tested. Over all four soils in total 144 tubes were prepared (4 soils x 3 treatments x 2 replicates x 6 sampling time). After that all tubes were kept in incubation room and deionized water was added to achieve a standing water level of about 2cm (Figure 8).
Materials and Methods

Throughout the incubation the water level was checked regularly and water was added if required. Per treatment two tubes were sampled destructively at 7, 14, 28, 42, 56 and 70 days after the start of the incubation. The soil in each tube was mixed properly by hand shaking and the soil paste was transferred into an 85ml Nalgene centrifuge tube and centrifuged at 8000 rpm for 3 minutes. Thereafter, the supernatant was filtered through a Whatman (GF/C) glass microfibre filter fitted in a vacuum holder. The filtrate was stored in the freezer until analysis of water soluble Fe and Mn. The soil residue was again homogenized and 15g moist soil was extracted with 1M KCl at a 1:5 extraction ratio to determine NH$_4^+$ content. Another 15 g soil was taken to measure pH-KCl (at an approximate 1:2.5 extraction ratio). The gravimetric moisture content of each soil sample was determined by oven drying at 105°C for 24 hours.

![Figure 8](image_url) Incubation setup for farmers’ fields paddy soils treated with Fe$_2$O$_3$ or Mn/Al mixed oxides and untreated soils for 10 weeks

3.2.3 Experiment III: Short-term evolution of fixed NH$_4^+$ content and MBC in paddy soils

Third incubation experiment was conducted with untreated farmers’ fields soils to find out the involvement of abiotic defixation of NH$_4^+$ and biotic anaerobic N mineralization on mineral N release. To achieve this fixed NH$_4^+$ and MBC were assessed in unamended and anaerobically incubated soils. As a whole 24 (4 soils x 3 lab replicates x 2 sampling time) small plastic tubes (6.8cm x 3.4cm) were prepared and incubated in same way as described in experiment-II (section 3.2.2) for 4 weeks. Twelve soil samples (4 soils x 1 treatment x 3 replicates) were taken out destructively in every sampling time of 2 and 4 weeks after start of incubation. The soil in each tube was mixed properly by hand shaking.
and weighted separately to determine MBC and gravimetric soil moisture content. Lastly remaining soils of all tubes were dried in oven at 20ºC for 72 hours, ground and sieved through 50μm mesh sieve to analyze fixed NH₄⁺.

3.3 Soil chemical analysis

3.3.1 pH

The pH-KCl was measured with a glass electrode in a soil suspension in 1M KCl (soil: extractant ratio 1:2.5). The pH of soil pore solution was measured directly with glass electrode.

3.3.2 Characterization of Fe and Mn in the paddy soils

Before incubation contents of Fe, Al and Mn of air dried soils were determined by various extraction methods. The filtrates were stored in the freezer for later analysis of Fe and Mn. All the Fe and Mn content (soil pore solution of experiment-I, in supernatant of experiment-II and in initial soil extracted by different methods) were analyzed by ICP–AES (on an iCAP 6300, Thermo Scientific).

Extraction by ammonium oxalate

Fe and Al from amorphous minerals and ferrihydrite were extracted by ammonium oxalate (Blakemore et al. 1987). One gram air-dried soil was weighed into an 85ml nalgene centrifuge tube and 50 ml of 0.2 M ammonium oxalate buffer (adjusted pH 3.0) was added. The centrifuge tubes were shaken in a shaker for 4 hours under dark condition. The tubes were then left for 1 hour to settle and were centrifuged at 1500 rpm for 10 minutes. After centrifugation the supernatants were filtered through Whatman (GF/C) glass microfibre filters, fitted in a vacuum holder.

Extraction by Sodium Pyrophosphate

Pyrophosphate usually extracts Fe and Al from organic complexes and to a small extent from short-range order minerals (Mc Keague 1966). In this method by Blakemore et al. (1987), 1g air-dried soil was weighed into a nalgene centrifuge tube and 50 ml of 0.1M sodium pyrophosphate (Na₄P₂O₇.10H₂O) (adjusted pH 10 by NaOH or HCl) was added. The centrifuge tubes were shaken in a shaker for 4 hours, left for 1 hour to settle and centrifuged at 1500 rpm for 10 minutes. After that the supernatants were filtered through a Whatman (GF/C) glass microfibre filter fitted in a vacuum holder and the filtrates were stored in freezer for later analysis.
Materials and Methods

Extraction by Citrate Bicarbonate-Dithionite (CBD)
Dithionite-citrate extracts Fe from both amorphous and crystalline iron oxides, except for siderite and iron silicates (Canfield 1989). The method described by Blakemore et al. (1987) was followed. One g air-dried soil was weighed into a nalgene centrifuge tube and 45 ml of 0.3M C₆H₅Na₃O₄.2H₂O (sodium citrate) and 5ml of 1M NaHCO₃ (sodium bicarbonate) were added. The tubes were then placed in a hot water bath at 80°C. While the sample reached the desired temperature, 1g of Na₂S₂O₄ (sodium dithionite) powder was added, stirred constantly for 1 minute and intermittently every 5 minutes for 15 minutes. One gram more Na₂S₂O₄ powder was added and stirred occasionally every 5 minutes for 10 minutes. The samples were then taken out from the water bath, allowed to cool and centrifuged at 1500 rpm for 10 minutes. After that the supernatants were filtered through Whatman (GF/C) glass microfibre filter fitted in a vacuum holder and the filtrates were stored in freezer for later analysis.

Extraction by 0.5M HCl
Soils were extracted by 0.5M HCl, as it has been forward as a method to quantify reducible Fe with no oxidized forms (Loveley and Phillips 1986). One g air-dried soil was taken into a plastic centrifuge tube and 50 ml 0.5 M HCl was added. The centrifuge tubes were shaken in a shaker for 4 hours, left for 1 hour to settle and centrifuged at 1500 rpm for 10 minutes. After that the supernatant was filtered through a Whatman (GF/C) glass microfibre filter fitted in a vacuum holder and the filtrates were stored in freezer for later analysis.

Water soluble Fe and Mn extraction
Water extracts only water soluble Fe which is fully and directly available for reduction (Gotoh and Patrick 1974). One g air-dried soil was weighed into a plastic centrifuge tube and 50 ml distilled water was added. The centrifuge tubes were shaken in a shaker for 4 hours, left to settle for 1 hour and centrifuged at 1500 rpm for 10 minutes. Later, the supernatant was filtered through a Whatman (GF/C) glass microfibre filter fitted in a vacuum holder and the filtrates were stored in freezer for later analysis of Fe and Mn.

3.3.3 Analysis of mineral N
An amount of 15g homogenized moist soil, equivalent to about 10g dry soil was weighed in a 250ml Erlenmeyer and 50ml 1M KCl was added. The samples were shaken for 2 hours in a shaker and the soil slurries were filtered through Ø150mm filter paper. Finally the samples were stored in freezer for later analysis of NH₄⁺ by continuous flow auto analyzer (Scalar, The Netherlands).
Materials and Methods

3.3.4 Analysis of dissolve organic carbon (DOC)

The dissolved organic carbon content in the pore solution of experiment-I were determined by means of a total organic carbon (TOC) analyzer.

3.3.5 Analysis of non-exchangeable NH$_4^+$

To analyze non-exchangeable NH$_4^+$, method described by Silva and Bremner (1966) was followed. Firstly a 0.5g soil sample was weighed in a 200ml Erlenmeyer flask and treated with 20ml of alkaline potassium hypobromite (KOBr-KOH) solution to eliminate exchangeable NH$_4^+$ and organic N compounds. After that, the flask was swirled to mix the soil and KOBr solution. The Erlenmeyers were covered by watch glasses and left to stand for 2 hours. Then, 60ml deionized water was added and the Erlenmeyers were heated on an electric hotplate until the mixture boiled vigorously for 5 minutes. Afterwards, the mixture was allowed to cool and settle down overnight. The supernatant was decanted and discarded. The centrifugation pellet was transferred into 85 ml nalgene centrifuge tubes by rinsing the Erlenmeyer with 0.5 M KCl. After closing with polyethylene caps the centrifuge tubes were shaken manually and they were centrifuged at 7000 rpm for 5 minutes. Then, the clear supernatant was decanted and this washing process was repeated once more. Subsequently, 20 ml of 5 M HF-1M HCl solution was added to the centrifuge tubes and they were shaken for 24 hours on a mechanical shaker. The final step of the analysis consisted of steam distillation of released ‘fixed-NH$_4^+$’ and determination of the released NH$_3$ by titration. First, 15 ml 10M KOH was added to a 250 ml Kjeldahl distillation tube with 60 ml marking. After that, the content of the centrifuge tube was transferred to the distillation flask by rinsing with deionized water until the liquid level in the flask reached 60 ml. To avoid contact of the HF-HCl solution with the glass tube, a long-stemmed polyethylene filter funnel was placed in the neck of the Kjeldahl tubes in such a way that the end of funnel reached below the surface of KOH solution. Then the solution was instantly steam distilled and liberated NH$_3$ was collected into boric acid solution. Finally, the NH$_4^+$ content in boric acid-indicator solution was determined by titrating with 0.002 M HCl.

3.3.6 Analysis of microbial biomass C

To measure soil microbial biomass C the chloroform-fumigation-extraction method according to Voroney et al. (1993) was used. Firstly, 15g water-saturated soil equivalent to ~ 7.5g dry soil was weighed into a 50ml glass beaker for fumigation and another 15g was weighed into a 250ml Erlenmeyer for the non-fumigated control. Non-fumigated control soils were extracted immediately with 30 ml 0.5M K$_2$SO$_4$ solution (1: 4 w/v ratio) by shaking for 1 hour and filtration through a
Whatman no. 6 filter paper. For fumigation, the beakers with soils were placed into a desiccator held in fume cupboard with moistened tissue paper at the bottom of the desiccator. Then, a 50ml beaker containing 30 ml ethanol-free CHCl₃ and some boiling chips was placed in the middle of the desiccator. The desiccator was evacuated using a membrane vacuum pump until the CHCl₃ boiled vigorously for 2 minutes after which it was closed and a connecting valve was detached from the pump. The desiccator was covered with black plastic sheet and left inside the fume cupboard at room temperature for 24 hours. After 24 hours, CHCl₃ and moistened tissue paper were removed and the desiccator was attached once more to the vacuum pump to expel out remaining CHCl₃ vapors. Evacuation and subsequent opening of the desiccator was repeated at least 5-6 times until the smell of CHCl₃ became undetectable. The soil samples were then transferred to 250ml Erlenmeyers by rinsing with 30 ml of 0.5 M K₂SO₄ solution, shaken for 1 hour and filtered through a Whatman no. 6 filter paper. All the filtrates were kept in the freezer for later analysis of their C content in TOC analyzer.

The difference between extractable C in fumigated and non-fumigated soils represents the chloroform-labile C pool (EC), which is proportional to microbial biomass C (C). This relationship is expressed as: \( C = \frac{EC}{k_{EC}} \) where \( k_{EC} \) is soil-specific but usually estimated as 0.45 (Beck et al. 1997).

3.4 Analysis of gas samples

Methane and CO₂ concentrations of collected gas samples in extainers were analyzed with a gas chromatograph (GC) (Interscience: Thermo Electron Corporation: Trace GC Ultra /Tuned Trace GC: 20046429) equipped with a flame ionization detector (FID) for CH₄ and a thermal conductivity detector (TCD) for CO₂. For every sample, 1ml gas was injected manually through inlet via a 1ml syringe. Concentrations of CH₄ and CO₂ were calculated using a linear regression equation between peak area of a series of corresponding certified gas standards. Methane and CO₂ concentrations in samples were calculated by subtracting the respective concentration in background air. The production rate (\( \mu g \ g^{-1} \ dry \ soil \ h^{-1} \)) of CH₄ and CO₂ from anaerobically incubated soils was calculated by multiplying the concentration of each gas sample with their corresponding density and volume of headspace and by dividing by the amount of dry soil per tube per unit time. Finally, the percentage of CH₄-C to total C (CH₄-C + CO₂-C) emitted from each soil sample was also calculated.

3.5 Data analysis

All data were processed using SPSS. One-way and two-way ANOVA were used to detect for significant differences in the conspicuous outcomes of relevant soil variables. Such as: KCl-extractable NH₄⁺, dissolved Fe and Mn, CH₄ production rate, pH-KCl, MBC and non exchangeable NH₄⁺ at or after specific week of anaerobic incubation.
CHAPTER 4

Results

4.1 Initial soil characteristics

Characteristics of air dried farmers’ fields soils relevant to this study before anaerobic incubation are presented in Table 1. Both Fe and Mn extracted by different methods varied widely among the four soils included in this thesis research. Beside this, the soils had wide ranges in total soil organic carbon (SOC), total N and pH-KCl. Out of all four soils Noaddah was a typical in terms its high contents of Citrate-Bicarbonate-Dithionite(CBD), Na-pyrophosphate and ammonium-oxalate extractable Fe. Sonatala-1 was specific soil due to higher total SOC, N and Mn contents.

Table 1 Selected soil properties of farmers’ fields paddy soils used for incubation experiments

<table>
<thead>
<tr>
<th>Soils</th>
<th>CBD extractable Fe and Mn (g kg⁻¹)</th>
<th>0.5N HCl extractable Fe and Mn (g kg⁻¹)</th>
<th>Na-pyrophosphate extractable Fe and Mn (g kg⁻¹)</th>
<th>Ammonium oxalate extractable Fe and Mn (g kg⁻¹)</th>
<th>pH-KCl</th>
<th>Total SOC (g kg⁻¹)a</th>
<th>Total N (g kg⁻¹)a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sonatala-1</td>
<td>12.16 0.28</td>
<td>7.52 0.28</td>
<td>1.04 0.14</td>
<td>5.41 0.19</td>
<td>4.88</td>
<td>21.4</td>
<td>2.1</td>
</tr>
<tr>
<td>Sonatala-2</td>
<td>9.85 0.16</td>
<td>7.67 0.19</td>
<td>0.94 0.08</td>
<td>4.92 0.12</td>
<td>4.74</td>
<td>16.3</td>
<td>1.7</td>
</tr>
<tr>
<td>Melandoho</td>
<td>8.2 0.07</td>
<td>3.28 0.06</td>
<td>1.24 0.01</td>
<td>3.25 0.05</td>
<td>3.73</td>
<td>8.2</td>
<td>0.9</td>
</tr>
<tr>
<td>Noaddah</td>
<td>14.18 0.09</td>
<td>3.78 0.06</td>
<td>2.84 0.04</td>
<td>6.17 0.07</td>
<td>3.77</td>
<td>9.2</td>
<td>1.0</td>
</tr>
</tbody>
</table>

aData taken from Kader (2012)

Selected properties of air dried soils collected from the fertilizer treatments of the BAU long-term field experiment are given in Table 2. Among the treatments, Fe and Mn extracted by Na-pyrophosphate and ammonium-oxalate showed very small variations. The pH-KCl, contents of total SOC and total N were statistically similar in NP, NPK and N+FYM treatments (Kader 2012).
Table 2 Properties of selected young floodplain paddy soils from BAU long-term field experiment

<table>
<thead>
<tr>
<th>Treatments</th>
<th>CBD extractable Fe and Mn (g kg⁻¹)</th>
<th>Na pyrophosphate extractable Fe and Mn (g kg⁻¹)</th>
<th>0.5M HCl extractable Fe and Mn (g kg⁻¹)</th>
<th>pH-KCl</th>
<th>SOC (g kg⁻¹)</th>
<th>Total N (g kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fe</td>
<td>Mn</td>
<td>Fe</td>
<td>Mn</td>
<td>Fe</td>
<td>Mn</td>
</tr>
<tr>
<td>Control</td>
<td>11</td>
<td>0.2</td>
<td>0.7</td>
<td>0.1</td>
<td>7</td>
<td>0.2</td>
</tr>
<tr>
<td>N</td>
<td>11</td>
<td>0.2</td>
<td>0.6</td>
<td>0.1</td>
<td>6</td>
<td>0.2</td>
</tr>
<tr>
<td>NP</td>
<td>11</td>
<td>0.2</td>
<td>0.8</td>
<td>0.1</td>
<td>7</td>
<td>0.2</td>
</tr>
<tr>
<td>NPK</td>
<td>11</td>
<td>0.2</td>
<td>0.8</td>
<td>0.1</td>
<td>6</td>
<td>0.2</td>
</tr>
<tr>
<td>N+FYM</td>
<td>11</td>
<td>0.2</td>
<td>0.7</td>
<td>0.1</td>
<td>7</td>
<td>0.2</td>
</tr>
<tr>
<td>ANOVA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: Data adapted from Kader (2012). ** denotes significantly differences at the 1% level of significance based on ANOVA; NS indicates not significantly different. ** Treatments indicated by different lower case letters are statistically different (P ≤0.05) from each other according to Duncan’s Multiple Range Post Hoc Test.

4.2 Changes in the soil properties during flooding of paddy soils

4.2.1 pH

Farmers’ fields
Generally the pH-KCl among all four farmers’ fields soils and pH in soil solution among the five soils from the BAU long-term field experiment varied over time. Variations among the treatments (T₁, T₂ and T₃) within a soil were always very small (Figure 9 and 10). The pH-KCl increased after submergence in all four soils with or without addition of Fe₂O₃ or the Mn/Al-oxides mixture (Figure 9). Both Sonatala-1 and Sonatala-2 soils illustrated only slight increases in pH-KCl within the period between 2 to 10 weeks of inundation. The pH-KCl of the Melandoho and Noaddah soils varied from 4.22 to 5.55 and 4.52 to 6.31, respectively, with considerable increasing trend during 2 to 10 weeks of flooding.
Results

Figure 9 Changes in pH-KCl of farmers’ fields paddy soils amended with Fe₂O₃ or mixed Mn/Al oxides and unamended soils during ten weeks of continuous flooding (vertical bars indicate standard errors of the means).

BAU long-term fertilizer application experiment

The pH in Rhizon soil moisture sampler (RSMS) collected soil solution of both Fe₂O₃ or the Mn/Al-oxides amended and unamended treatments increased sharply between 1 to 8 weeks of anaerobic incubation (Figure 10). Soil solution pH ranged between 6.68 (NP: T₁) to 6.89 (N+FYM: T₃) and 7.08 (NPK: T₁) to 7.25 (N+FYM: T₂) after first and 8th weeks of submergence respectively.
Results

Figure 10 Changes in soil solution pH of paddy soils treated with Fe$_2$O$_3$ or mixed Mn/Al oxides and untreated soils during eight weeks of continuous flooding (vertical bars indicate the standard errors of the means)
Results

The mean pH-KCl after 8 weeks of flooding of the five soils from the BAU long-term field experiment are given in Table 3. In general the pH-KCl in Mn/Al mixed oxides treated soils were significantly greater than those in T1 and T2 treatments. On the other hand, the pH-KCl in N+FYM was also significantly higher than other four field treatments (Control, N, NP and NPK) (level of significance not shown here).

Table 3 The pH-KCl (1:2.5) (± standard error) in soils amended with Fe$_2$O$_3$ or Mn/Al mixed oxides and untreated paddy soils after 8 weeks of flooding

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Control</th>
<th>N</th>
<th>NP</th>
<th>NPK</th>
<th>N+FYM</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>6.54±0.01a</td>
<td>6.58±0.02a</td>
<td>6.58±0.02a</td>
<td>6.57±0.03a</td>
<td>6.63±0.04a</td>
</tr>
<tr>
<td>T2</td>
<td>6.57±0.04a</td>
<td>6.56±0.03a</td>
<td>6.53±0.04a</td>
<td>6.54±0.03a</td>
<td>6.65±0.02a</td>
</tr>
<tr>
<td>T3</td>
<td>6.61±0.03b</td>
<td>6.62±0.03b</td>
<td>6.63±0.03b</td>
<td>6.66±0.01b</td>
<td>6.70±0.04b</td>
</tr>
</tbody>
</table>

* denotes significantly differences at the 5% level of significance based on ANOVA; * Treatments indicated by different lower case letters are statistically different (P≤0.05) from each other according to Duncan’s Multiple Range Post Hoc Test.

4.2.2 Water soluble Fe and Mn

Farmers’ fields

In contrast to pH, the dynamics of water soluble Fe varied widely among the four farmers’ fields paddy soils (Figure 11). Per soil the variations of water soluble Fe content between control (T1) and Fe$_2$O$_3$ (T2) treatments were limited. The concentration of water soluble Fe (mg kg$^{-1}$) in Sonatala-1, Sonatala-2, Melandoho and Noaddah soils ranged from 0 to 14, 1 to 8, 0 to 16 and 0 to 37 mg kg$^{-1}$ respectively during 10 weeks period of anaerobic incubation. In both Sonatala-1 and Sonatala-2 soils, the water soluble Fe content increased linearly, peaked at week 4(in T1 and T2) while for T3 this maximum was already reached at week 2. After reaching a peak soil solution Fe concentration decreased gradually in the subsequent weeks. Accordingly, dissolved Fe concentration in the Noaddah soil also increased linearly until 6 weeks of flooding and leveled off in the following weeks. For the Melandoho soil in contrast, the concentration of Fe only started to increase linearly after 4 weeks and continued to rise in the succeeding weeks of anaerobic incubation.
Results

Figure 11 Evolution of Fe content in farmers’ fields paddy soils amended with Fe$_2$O$_3$ or mixed Mn/Al oxides and unamended soils during ten weeks of continuous flooding (vertical bars indicate the standard errors of the means).

**BAU long-term fertilizer application experiment**

Generally, in case of all 5 soils (control, N, NP, NPK and N+FYM treatments) the evolution of soil solution Fe with time was similar when the soils were maintained as untreated (T$_1$) control (Figure 12). In all cases Fe in soil solution increased steeply at two weeks of flooding within a range of 12 (N+FYM: T$_1$) to 16 (NP: T$_1$) mg L$^{-1}$ and peaked at 4$^{th}$ week of submergence (14 to 17mg L$^{-1}$). Elevated Fe concentrations (13 to 16 mg L$^{-1}$) continued until the 5$^{th}$ week of inundation and shortly lowered to 4 to 5 mg L$^{-1}$ after 6 weeks. For all the five soils the soil solution Fe concentration in the Fe$_2$O$_3$ treated (T$_2$) soils were significantly higher (p<0.05) than the soils not treated with Fe$_2$O$_3$ (T$_1$ and T$_3$) after 6 weeks of anaerobic incubation. Over all five field experimental treatments, soil solution Fe ranged from 3 to 9 mg L$^{-1}$ in the case of addition of Fe$_2$O$_3$ (T$_2$) in week1 and increased gradually to peak at week 4 (14 to18 mg L$^{-1}$). The Fe concentration in T$_2$ slightly decreased after 5 and 6 weeks and dropped off after 8 weeks of flooding. In the case of soil amendment with mixed Mn/Al oxides
Results

(T3) the Fe concentration (mg L⁻¹) followed a similar course as in the T2 treatment but in general the concentrations on average were about 18 to 32% lower.

Figure 12 Evolution of soil solution Fe content in paddy soils treated with Fe₂O₃ or mixed Mn/Al oxides and untreated soils during eight weeks of continuous flooding (vertical bars indicate the standard errors of the means)
Farmers’ fields

In all farmers’ fields soils, the dissolved Mn content were generally higher in the soils amended with a mixture of Mn/Al-oxides (T3) compared to soil without such addition (T1 and T2 treatments) (Figure 13). The evolution of water soluble Mn with time varied strongly among the four soils. In the Sonatala-1 soil, the water soluble Mn content went up sharply from 1 mg kg⁻¹ in week-0 to 9-13 mg kg⁻¹ in week-2 and declined rapidly to a stable range of 4-5 mg kg⁻¹ after 6 weeks of flooding. Accordingly, a similar evolution was seen for the Sonatala-2 soil but the maximum concentration at 2-4 weeks of flooding was limited to 5 to 7 mg kg⁻¹. In case of both Sonatala-1 and Sonatala-2 soils, the mean Mn contents of the Mn/Al mixed oxides amended soils (T3) were significantly (p<0.05) higher than unamended (T1 and T2) soils after 2 weeks. In contrast, the dissolved Mn content of Melandoho soil stayed at the initial level (2 mg kg⁻¹) in the T1 and T2 treatments whereas in the T3 treatment the concentration increased steadily from 2 to 8 mg kg⁻¹ over the 10 weeks incubation period. Likewise, for the Noaddah soil, water extractable Mn went up gradually until after 8 weeks of flooding for all treatments. In the T1 and T2 treatments, the concentration of Mn started to increase only after the 2nd week of flooding and the Mn concentrations were about double in the case of soil amendment with the mixture of Mn/Al-oxides (T3). Like Sonatala-1 and Sonatala-2, the mean Mn contents in T3 of Melandoho and Noaddah soils were significantly (p<0.05) greater than that in T1 and T2 after 10 weeks of flooding.
Results

Figure 13  Evolution of Mn content in farmers’ fields paddy soils amended with Fe$_2$O$_3$ or mixed Mn/Al oxides and unamended soils during ten weeks of continuous flooding (vertical bars indicate the standard errors of the means)

BAU long-term fertilizer application experiment

Over all five field experimental treatments the evolution of soil solution Mn content over time was similar for the T$_1$ and T$_2$ treatments. The evolutions differed remarkably in the T$_3$ treatment compared to the T$_1$ and T$_2$ treatments (Figure 14). Soil solution Mn was much higher in the soil amended with the mixture of Mn/Al-oxides. These results resemble the outcome for the four farmers’ fields soils. In general the concentration of soil solution Mn always went up gradually from its lowest value of 0 at week-0 to a maximum at 2 weeks followed by a slow decrease to a stable value of 3 mg L$^{-1}$ after 6 and subsequent weeks of submergence. In all five soils, the differences in soil solution Mn content between the Mn/Al mixed oxides treated (T$_3$) and untreated (T$_1$ and T$_2$) soils were significant (p<0.01) after 2 weeks of incubation. In case of the T$_3$ treatments the increase of Mn was much stronger and the content remained at more or less same level until week-4 in contrast to the soils not amended with the mixture of Mn/Al-oxides.
Results

Figure 14 Evolution of soil solution Mn content in paddy soils treated with Fe₂O₃ or mixed Mn/Al oxides and untreated soils during eight weeks of continuous flooding (vertical bars indicate the standard errors of the means).

4.2.3 Dissolved Organic Carbon (DOC)

BAU long-term fertilizer application experiment

Generally, the dynamics of DOC contents in soil solution of all five soils (viz. control, N, NP, NPK and N+FYM) over time were similar, irrespective of Fe or Mn-application treatment (Figure 15). In all cases the concentration of soil solution DOC was higher at the start and then decreased gradually during anaerobic incubation. The soil solution DOC concentration ranged between 76 (Control: T₃)
Results
to 145 (N+FYM: T₁) mg C L⁻¹ and 32 (N: T₃) to 56 (N+FYM: T₂) mg C L⁻¹ after 1 and 8 weeks of submergence, respectively. In all five soils amended with mixed Mn/Al oxides (T₃), the DOC concentrations were 16 to 29% and 26 to 32% lower than those in T₁ and T₂ treatments, respectively during the entire 8-weeks period. The proportion of DOC relative to the total soil organic carbon varied within a range of 0.39 to 0.59% during 1 to 8 weeks after the onset of flooding.

Figure 15 Evolution of soil solution DOC content in paddy soils treated with Fe₂O₃ or mixed Mn/Al oxides and untreated soils during eight weeks of continuous flooding (vertical bars indicate the standard errors of the means)
### Results

#### 4.2.4 Evolution of exchangeable ammonium ($\text{NH}_4^+$) concentration

**Farmers’ fields**

The changes in the KCl-extracted $\text{NH}_4^+$ content of the four farmers’ fields soils during entire 10-weeks of anaerobic incubation are presented in Figure 16. The dynamics of ammonium content over time among the four soils varied widely but within each soil there were no considerable variations among the treatments ($T_1$, $T_2$ and $T_3$). The mean $\text{NH}_4^+$ contents at the beginning of incubation were also very different among the four soils within a range from 5 to 39 mg kg$^{-1}$ soil. In all treatments of both Sonatala-1 and Sonatala-2 soils $\text{NH}_4^+$ contents went up linearly from first sampling event to week 4 and reached a maximum. After that, the $\text{NH}_4^+$ concentration was maintained at a same level in the subsequent weeks. Unlike in the Sonatala-1 and Sonatala-2 soils, at Melandooho and Noaddah soil the concentration of $\text{NH}_4^+$ (mg kg$^{-1}$) more or less remained at its initial value the first 2 weeks, and only then started to go up linearly and continued to increase until 10 weeks.

![Figure 16](image_url)

**Figure 16** Evolution of $\text{NH}_4^+$ content in farmers’ fields paddy soils amended with $\text{Fe}_2\text{O}_3$ or mixed Mn/Al oxides and unamended soils during ten weeks of continuous flooding (vertical bars indicate the standard errors of the means)
The mean $\text{NH}_4^+$ contents of farmers’ paddy fields soils after 10 weeks of continuous flooding are shown in Table 4. In case of all soils, except Melandoho, no significant differences in the $\text{NH}_4^+$ contents were observed among the treatments (T1, T2 and T3). Only in case of the Mn/Al mixed oxides treated soil (T3) of Melandoho, the $\text{NH}_4^+$ content was significantly lower than that in the T1 and T2 treatments.

Table 4 The mean $\text{NH}_4^+$ content (± standard error) of farmers’ fields paddy soils amended with Fe$_2$O$_3$ or mixed Mn/Al oxides and unamended soils after 10 weeks of continuous flooding

<table>
<thead>
<tr>
<th>Treatments</th>
<th>KCl-extractable $\text{NH}_4^+$ (mg kg$^{-1}$ soil)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sonatala-1</td>
</tr>
<tr>
<td>T1</td>
<td>38.23 ± 0.94</td>
</tr>
<tr>
<td>T2</td>
<td>35.34 ± 1.79</td>
</tr>
<tr>
<td>T3</td>
<td>35.34 ± 1.90</td>
</tr>
</tbody>
</table>

ANOVA NS NS * NS

* denotes significantly differences at the 5% level of significance based on one way ANOVA; NS indicates not significantly different. * Treatments indicated by different lower case letters are statistically different ($P \leq 0.05$) from each other according to Duncan’s Multiple Range Post Hoc Test

BAU long-term fertilizer application experiment

The mean $\text{NH}_4^+$ content before incubation and after 8 weeks of flooding of the five soils from the BAU long-term field experiment are given in Table 5. The initial $\text{NH}_4^+$ content in N+FYM was significantly lower than other four field treatments (Control, N, NP and NPK). The $\text{NH}_4^+$ contents increased after 8 weeks to 22.82 to 30.42 mg kg$^{-1}$ without any significant differences among the treated and untreated soils.

Table 5 The mean $\text{NH}_4^+$ content (± standard deviation) in initial soils and soils amended with Fe$_2$O$_3$ or Mn/Al mixed oxides and untreated paddy soils after 8 weeks of flooding

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Control</th>
<th>N</th>
<th>NP</th>
<th>NPK</th>
<th>N+FYM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week-0</td>
<td>5.88 ± 0.95b</td>
<td>5.78 ± 0.42b</td>
<td>5.85 ± 1.18b</td>
<td>5.66 ± 0.85b</td>
<td>4.55 ± 0.35a</td>
</tr>
<tr>
<td>Week-8</td>
<td>25.42 ± 6.27</td>
<td>29.50 ± 4.98</td>
<td>23.48 ± 2.28</td>
<td>28.71 ± 7.59</td>
<td>22.96 ± 5.23</td>
</tr>
</tbody>
</table>

ANOVA NS NS NS NS NS

* denotes significantly differences at the 5% level of significance based on ANOVA; NS indicates not significantly different. * Treatments indicated by different lower case letters are statistically different ($P \leq 0.05$) from each other according to Duncan’s Multiple Range Post Hoc Test
4.2.5 Carbon dioxide and methane production

**BAU long-term fertilizer application experiment**

We measured soil emissions of CH$_4$ and CO$_2$ at regular time intervals. Indicative to the progress of the soil redox potential is the onset of methanogenesis, rather than amount of CH$_4$ emitted. In line the relative fraction of C emitted through CH$_4$ production to the total C-emission (CH$_4$-C+CO$_2$-C) was plotted against time for each of the five paddy soils (Figure 17). The percent of emitted CH$_4$-C to total C generally ranged between 0 to 1 and 3 to 97 after 1 and 8 weeks of submergence. Generally the percentage of produced CH$_4$-C to total C were negligible until 5 weeks, and then started to increase rapidly to reach a maximum rate after 6 to 8 weeks of flooding. Overall the CH$_4$ emitted (μg g$^{-1}$ dry soil h$^{-1}$) from the Mn/Al mixed oxides treated soils (T$_3$) were significantly (p<0.05) lower compared to the T$_1$ and T$_2$ treatments after 6 and 8 weeks of submergence (data not shown). Exceptions were T$_3$ for the NP, N+FYM and to a lesser extent also the NPK treatment, where CH$_4$ emissions were lower or even absent. A similar observation could be made for T$_1$ of the control field treatment. Irrespective of the treated and untreated soils, CO$_2$ produced from the beginning of incubation was within a range 0.3 to 1.4 μg g$^{-1}$ dry soil h$^{-1}$ after 1 week, and then decreased gradually to almost stable value after 6 weeks of flooding (data not shown).
Figure 17 Evolution of relative CH₄-C (%) over total C from soils treated with Fe₂O₃ or mixed Mn/Al oxides and untreated soils during eight weeks of flooding (vertical bars indicate the standard errors of the means).

4.2.6 Exchangeable versus non-exchangeable NH₄⁺

Farmers’ fields
The evolution of exchangeable and fixed NH₄⁺ with time in farmers’ fields soils during 28 days of submergence are presented in Figure 12. The non-exchangeable NH₄⁺ content was approximately...
Results

one fifth of the total soil N content in Sonatala-1 and Sonatala-2 during 28 days of submergence. In case of Melandoho and Noaddah soils the fixed NH₄⁺ content were one third and one fourth of total soil N respectively. After the onset of submergence, exchangeable NH₄⁺ content gradually increased whereas non-exchangeable NH₄⁺ exposed a sharp increase in all four paddy soils during 4 weeks of inundation. However, the amount of non-exchangeable NH₄⁺ differed significantly (p<0.01) among the four soils and was relatively higher in Sonatala-1 (281-435 mg kg⁻¹) followed by the Sonatala-2 (201-319 mg kg⁻¹), then Melandoho (239-289 mg kg⁻¹) and lastly Noaddah (179-250 mg kg⁻¹) soil. In all four soils also the mean non-exchangeable NH₄⁺ contents in week 0 were significantly (p<0.01) lower compared to 2 and 4 weeks of flooding. On the other hand, exchangeable NH₄⁺ content in Noaddah (39-71 mg kg⁻¹) was clearly higher than that in the Melandoho (23-38 mg kg⁻¹), Sonatala-1 (7-40 mg kg⁻¹) and Sonatala-2 (5-30 mg kg⁻¹) soils for the full duration of the 4 weeks incubation experiment.

Figure 18 Evolution of exchangeable and non-exchangeable NH₄⁺ in four farmers’ fields paddy soils during 28 days of flooding (vertical bars indicate the standard errors of the means)
4.2.7 Microbial biomass carbon (MBC)

Farmers’ fields

In general the soil microbial biomass carbon of farmers’ fields paddy soils contributed 0.6 to 2.3% of total soil carbon after 2 weeks of flooding. Over all four farmers’ fields soils MBC strongly differed (Figure 13) and ranged from 46 to 287 mg kg$^{-1}$ soil. The MBC contents after 4 weeks of submergence were slightly higher than those after 2 weeks, except in the Noaddah soil. In case of all four soils the analysis of variance revealed significant (p<0.05) differences in MBC content between 2 and 4 weeks of flooding.

![Figure 19](image)

**Figure 19** Microbial biomass carbon content in farmers’ fields paddy soils during four weeks of submergence (vertical bars indicate the standard errors of the means)

BAU long-term fertilizer application experiment

The soil MBC contents in all five soils ranged from 179 to 227 mg kg$^{-1}$ (Figure 20) irrespective of the Fe$_2$O$_3$ or Mn/Al mixed oxides treated and untreated soils. No significant differences in soil MBC content among the soils and between the amended and unamended treatments could be detected after 8 weeks of anaerobic incubation. At the end of the experiment the soil MBC generally comprised 1.2 to 1.5% of the total soil carbon irrespective of the treated and untreated soils.
**Figure 20** Microbial biomass carbon content in paddy soils treated with Fe\textsubscript{2}O\textsubscript{3} or mixed Mn/Al oxides and untreated soils after eight weeks of continuous flooding (vertical bars indicate the standard errors of the means)
CHAPTER 5

Discussion

In water-logged conditions production of exchangeable ammonium is the central process for N nutrition of rice soils. Anaerobic N mineralization in flooded paddy soils is influenced by different soil physicochemical properties. The release of ammonium in lowland rice soils in relation to general soil properties like SOC, pH, C/N, total N, CEC, Fe, clay content, availability of alternative electron acceptors etc. were studied by a number of researchers in the past. More recently, Kader et al. (2013) only found significant correlations between N mineralization and pH and Fe contents, while no relationships existed with other key soils properties (soil mineralogy, contents of C and N and physicochemical SOM fractions) in floodplain paddy soils of Bangladesh. On the basis of Kader et al. (2013) study we conducted laboratory incubation experiments to study Fe and Mn availability in detail and their control on NH₄⁺ production in paddy soils of Bangladesh.

5.1 Detailed follow-up of soil solution Fe and Mn and DOM in soil collected from field-experimental plots

5.1.1 Evolution of Fe, Mn and pH in soil solution

Iron reduction is one of the dominant processes in wetland paddy soils required to support mineralization of SOM (Van Bodegom et al. 2003; Qu et al. 2004). In all five soils from different fertilizer treatments at the BAU field experiment, increased soil solution Fe concentrations of T₁ and T₃ treatments sustained from week-2 to week-5 of the anaerobic incubations whereas in T₂ this was until week-6. The reduction of different Fe³⁺(hydr-oxide) through enzymatic and abiotic means logically explains the increase in the soil solution Fe content (Ponnamperuma 1977; Zhang et al. 2012). Alongside, the pH-KCl and the pH in soil solution increased by 1.0 and 0.2 to 0.5 units respectively after 8 weeks of submergence, irrespective of the Fe- or Mn-oxide application treatment of the five paddy soils. This overall increasing trend of soil pH to neutrality can be logically ascribed from the reductive consumption of protons (H⁺) during major reduction processes like denitrification, reduction of Mn, Fe and SO₄²⁻ etc. (Ponnamperuma 1972; Sahrawat 2005; Yu et al. 2007; Sahrawat 2012). The following decrease in Fe content after 5-6 weeks of incubation was probably due to precipitation of iron as Fe₃O₄.nH₂O or Fe₃(OH)₆ and FeS.nH₂O, brought about by increasing soil solution pH and declining P CO₂ (Ponnamperuma et al. 1967) and reduction of SO₄. Zhang et al. (2012) found that at high soil pH value, iron oxidizes more easily, and therefore lowered the solubility of Fe²⁺. The higher concentration of soil solution Fe at week 6 since the onset of flooding in the Fe₂O₃ amended (T₂) soils compared to in the control (T₁) soils indicated that iron-oxide addition prolonged the supply of Fe³⁺ as alternative electron acceptor. Relatively lower concentrations of soil solution Fe in T₃ than T₁ and T₂, on the other hand, are most likely due to a
preferential reduction of the added Mn oxides, which limited the need of Fe\(^{3+}\) reduction for mineralization to proceed, limiting build up of high soil solution Fe\(^{2+}\) concentrations (Ponnamperuma 1972; Munch and Ottow 1980).

Next to Fe, Mn is another important electron acceptors with multiple valences in flooded paddy soils. Like Fe oxides, the reduction of Mn oxides is also influenced by the content of SOM, surface chemical reactions, Eh etc. (Sparks 1989). Amendment of Fe\(^{3+}\) did not affect the evolution of Mn\(^{2+}\) concentrations in soil solution at all. Increasing concentrations of water soluble Mn content after submergence of paddy soils primarily results from reduction of Mn\(^{4+}\) oxides and a decrease Mn concentration usually due to removal of Mn\(^{2+}\) by precipitation, formation of insoluble organic complexes and cation exchange reactions (Ponnamperuma 1972). Hence it appears that supply of Fe did not result in lowered Mn reduction, which would be logical since Mn is preferentially utilized as alternative electron acceptor compared to Fe. In accordance with this, Nealson and Myers (1992) even found that the addition of Fe\(^{3+}\) to the cultures growing on Mn\(^{4+}\) can increase the rate of Mn reduction. Adding Fe\(^{3+}\) apparently resulted in the production of Fe\(^{2+}\) which provided a good reductant of Mn\(^{4+}\) to form Mn\(^{2+}\) \(\text{MnO}_2 + 2 \text{Fe}^{2+} + 4\text{H}_2\text{O} \rightarrow \text{Mn}^{2+} + 2\text{Fe(OH)}_3 + 2\text{H}^+\) (Myers and Nealson. 1988; Lovley 1991).

The evolution of soil solution Mn content did not differ noticeably among the five fertilizer treatments soils (viz. control, N, NP, NPK and N+FYM). Hence, Mn reduction and evolution of the soil redox potential appeared to occur at the same rate irrespective of 34 years of distinct fertilization management. On the contrary in any given soil, differences among the T\(_1\) and T\(_2\) treatments on the one hand and the T\(_3\) treatment on the other were conspicuous. The higher soil solution Mn contents in the T\(_3\) compared to those in T\(_1\) and T\(_2\) treatments at any given time is likely due to supplemental reduction of added Mn oxides in T\(_3\). Thus, unlike amendment with Fe\(^{3+}\), soil application of reducible-Mn did affect the sequence of soil redox reactions, irrespective of fertilizer treatment. In all three treatments, the concentration of soil pore water Mn went up to its highest value after two weeks of submergence and then fell down. Similar changes of Mn content in soil extracts of rice growing soils was also attained by Jahan et al. (2013). The declined concentration of soil solution Mn content is possibly due to formation of insoluble organic complexes or precipitation as MnCO\(_3\) (Ponnamperuma 1972). Consequently, the results in all five soils proposed that Mn oxides served as important electron acceptors during 1 to 5 weeks after the onset of flooding. This period of Mn reduction partly overlaps with the Fe reduction. In accordance, Gao et al. (2002) found the overlapping of Mn and Fe as electron acceptors in wetland paddy soils. The reduction of Mn and Fe in combination with increased pH is linked with the release of dissolved organic carbon (DOC), an active electron donor, in anaerobically incubated wetland soils (Grybos et al. 2009). For this reason we also tracked DOC in
soil solution to come to a more complete insight of the effects of Fe- or Mn- application on microbial activity in the studied paddy soils. Next, the relative production of CH₄ instead of CO₂ as a result of microbial activity is indicative of the evolution of redox potential and was further investigated for the established paddy soil incubation experiment (5.1.2).

5.1.2 Dissolved organic carbon (DOC)

Dissolved organic carbon (DOC) is one of the important fractions in active soil carbon pool and plays an important role in the biogeochemical cycling of C, N, P and S (Kalbitz et al. 2000; Zhan et al. 2011). The concentration or flux of DOC in soils depend on its net release from SOM or desorption from solid phase and removal through adsorption or decomposition (Kalbitz et al. 2000). In our study, the concentration of DOC in all five treated and untreated paddy soils was highest at the start of the incubations (after 1 to 3 weeks of flooding). This contrasts with commonly observed gradual release of DOC in the soil solution following the onset of flooding, caused by several mechanisms. Grybos et al. (2009) stated that anaerobic incubation of wetland soils releases 2.5% of total SOC as DOC, which is mainly associated with rising pH from 5.5 to 7.4 together with reduction of soil Fe and Mn. At higher pH, net positive surface charge of minerals decreases and organic molecules become more electronegative due to deprotonation. In another study by Hanke et al. (2013) also revealed that anoxic conditions increased DOC concentrations in paddy soils incubated for 12 weeks under alternating redox conditions.

While these processes could explain initial relatively high DOC concentrations, during our study surprisingly, the DOC concentration decreased gradually after week 2. Consistent with this Liu et al. (2013) also reported a highly significant negative correlation between water-soluble C content and NaOAc/HCl-extractable Fe²⁺ in paddy soils. The decreased concentration of DOC may be accredited by the formation of complexes between functional groups (carboxyl, hydroxyl, and so on) of DOC and metal ions such as Fe²⁺(Stumm and Sulzberger 1992; Kraemer 2004), which are gradually released into the soil solution following submergence. In accordance with this, Nierop et al. (2002) stated that precipitation of DOM by Fe²⁺ under anaerobic conditions removed 75% of DOC from solution at an Fe²⁺/C quotient of 1.

Next to the general decreasing trend of DOC with time, in all five soils, the DOC concentrations were relatively lower in Mn/Al mixed oxides treated paddy soils (T₃) than in the T₁ and T₂ treated soils. This may be linked with the higher soil solution Mn²⁺ concentrations in T₃ than in the other two treatments, with a consequent enhanced formation of Mn²⁺-DOC complexes. In support of this hypothesized pathway, Kalbitz et al. (2000) also stated that chemical reactions between anionic functional groups of organic molecules and cations (such as Al³⁺, Fe³⁺, Ca²⁺, Mg²⁺, Cu²⁺, Mn²⁺, Pb²⁺)
etc.) decline the solubility of OM, either by decreasing surface charge density, by altering the structural conformation of adsorbed species, by flocculation or by cation bridging.

Alongside DOC, microbial biomass carbon (MBC) is another important active SOC pool, despite its small proportion relative to total SOC (Zhan et al. 2011). The MBC represents the quantity of microbes present in the soils and because it responds quickly to changes in soil management it is often used as a soil quality indicator (Lutzow et al. 2007; Shah et al. 2010). For all the five soils used in this study, the MBC after 8 weeks of anaerobic incubation contributed 1.2 to 1.5% of the total SOC pool (in line with e.g. Zhan et al. 2011), without any significant differences between the Fe₂O₃ or Mn/Al mixed oxides treated and untreated soils. Consequently, in line with observations for the evolution of DOC, Fe, and Mn we conclude that availability of Mn or Fe only had at best a modest control on microbial activity in the studied paddy soils. In addition, MBC was not significantly different among the five BAU fertilizer treatments (control, N, NP, NPK and N+FYM). In contrast to this, Zhang et al. (2009) reported that soil MBC and MBN were greater in a NPK + OM treatment compared to no fertilizer, N, NP and NPK treatments in paddy field soils. Also, both DOC content and MBC were significantly and positively correlated with the emission of CH₄ in flooded subtropical paddy soils (Zhan et al. 2011). The relatively small differences in SOC content of the different BAU field experimental treatments probably explains why differences in MBC were smaller in the present study.

In the earlier stage of the incubations, CO₂ emissions logically resulted from microbial oxidation of organic carbon with electron donation to various inorganic electron acceptors. Decreased CO₂ emissions after several weeks of flooding probably result from increased reduction of CO₂ to CH₄ and precipitation of FeCO₃ (Yao et al. 1999). After CO₂, CH₄ is the most important greenhouse gas since it contributes to approximately 20% of the anthropogenic global warming effect (Cheng et al. 2007). Paddy soils are important contributors of atmospheric methane production (Qu et al. 2003) and account for about 15–20% of total methane emitted to the atmosphere (Xu et al. 2007). Methane is a dominant end-product of anaerobic microbial activity and is produced after nearly entire reduction of other electron acceptors (NO₃⁻, Mn⁴⁺, Fe³⁺ and SO₄²⁻) other than CO₂ (Laanbroek 2010; Cheng et al. 2007). The CH₄ production in most BAU soils started after about 5 weeks of continuous flooding. This outcome was possibly related with the completion of Fe³⁺ and Mn⁴⁺ reduction, as indicated by the declined concentrations of Fe and Mn in soil solution after 5 weeks of incubation. The contribution of hematite powder to soil Fe-reduction of amended soils (T₂) seems to have been negligible as was also apparent from unaffected onset and rate of CH₄ production. In accordance, Qu et al. (2004) also found that added iron oxides with higher crystallinity (like goethite, hematite) were not reduced or reduced to but a small extent in paddy soils and resulted in no or only
Discussion

minor suppression of CH₄ production. In addition, significantly lower CH₄ emission in Mn/Al mixed oxides treated soils (T₃) compared to the T₁ and T₂ treatments were found after 6 and 8 weeks. This provided an indication that lessened or delayed CH₄ production could be accomplished by the application of Mn oxides. A lower CH₄ production in T₃ may have been linked either with the competitive consumption of H₂ produced from OM decomposition by the bacteria using Mn⁴⁺ as electron acceptors or with anaerobic oxidation of CH₄ by Mn⁴⁺ oxidant (Yao et al. 1999; Yu et al. 2007). Beside these effects, the lower DOC concentrations in the Mn/Al mixed oxides treated soils perhaps also limited the substrates for methanogenic bacteria. In agreement with this, Zhan et al. (2011) also reported that higher DOC concentrations resulted higher CH₄ production in wetland paddy soils.

From above discussions, it is seen that all the investigated soil properties (pH, Fe, Mn, DOC, MBC, CO₂ and CH₄ emission) are more or less interrelated. They are also directly or indirectly linked with SOM decomposition and may hence be expected to influence anaerobic N mineralization in wetland paddy soils. In the line with this, Narteh and Sahrawat (1997) reported that mineralized N was significantly correlated with soil properties like pH, SOC, total N, clay and CEC but not with extractable Fe and C/N ratio of rice soils. While N mineralization in paddy soils of Bangladesh correlated only with pH and Fe contents (Kader et al. 2013).

The elevated progress of all these soil parameters over time was logically accompanied by the NH₄⁺ production in all soils, regardless of Fe or Mn treatment. The KCl extractable NH₄⁺ content in all five soils were determined at the end of this experiment. The NH₄⁺ content in BAU soils increased by 17 to 25 mg kg⁻¹ after 8 weeks but the differences among the treated and untreated soils were not significant. Hematite (Fe₂O₃) powder addition (T₂) yielded a slight increase of Fe and Mn/Al mixed oxides addition did result in increased soil solution concentrations of Mn (T₃). Therefore, the hypothesis of increasing NH₄⁺ production based on more availability of inorganic alternate acceptors by the addition of Fe₂O₃ or Mn/Al mixed oxides was rejected.

The BAU field experiment covered just one soil type and we only measured exchangeable NH₄⁺ content at the end of the incubations. To confirm the correct rejection of this thesis’ primary research hypothesis we therefore conducted another experiment with four farmers’ field paddy soils. We again assessed the effect of artificial alteration of the availability of Fe³⁺ and Mn⁴⁺ on anaerobic N mineralization. In this second experiment the soil samples were analyzed destructively for KCl extractable NH₄⁺, pH-KCl and water soluble Fe and Mn at a regular time interval (5.2).
5.2 Effect of Fe and Mn availability on mineral N-release in farmers’ field soils

In this study logically, we tracked the evolution of total water soluble Fe and Mn content in the course of flooding period and the differences of their concentrations among the farmers’ field paddy soils.

Generally, as in the first BAU experiment, water soluble Fe initially increased linearly, peaked and then slowed down in all farmers’ field soils, except for Melandoho where the concentration kept increasing until 10 weeks of flooding. The reductive dissolution of Fe$^{3+}$ (hydr-) oxides (e.g. Fe$_2$O$_3$ + 1/2CH$_2$O + 4H$^+$ = 2Fe$^{2+}$ + 5/2H$_2$O + 1/2CO$_2$) logically drove the increased concentration of water soluble Fe (Ponnamperuma 1977; Sahrawat 2005; Zhang et al. 2012). As expected, the pH-KCl also increased in all soils between 2 and 10 weeks of flooding. The rapid increase of pH-KCl in Sonatala-1 and Sonatala-2 is probably involved with their higher OM and reducible Fe content (Table 1 and Figure 9). While the slow pH-KCl increased in Melandoho and Noaddah soils may be attained by their lower amount of OM and reducible Fe content (Ponnamperuma 1972). The ranges in water soluble Fe content were soil specific and relatively higher in the Noaddah compared to the Sonatala-2 soil.

In general, the magnitude of Fe$^{3+}$ reduction logically varies from soil to soil depending particularly on the redox changes, SOM (electron donors) content, forms and degree of crystallinity of Fe (hydr-) oxides, pH etc. (Ponnamperuma 1972; Qu et al. 2004; Zhang et al. 2012). Fe is generally more soluble in soils with lower pH while the higher amounts of Fe$^{2+}$ may be derived in more acidic soils under same redox condition (Zhang et al. 2012). The critical value for the reduction and dissolution of Fe is 100 mV at pH 7 (Kogel-Knabner et al. 2010) and this value also varies from one soil to another.

Beside these, the lower degree of crystallinity of Fe oxides generally result in a higher extent of pedogenic Fe$^{3+}$ reduction (Ponnamperuma 1972; Qu et al. 2004). The higher water soluble Fe content in Noaddah soil after 6 weeks of flooding followed its greater ammonium oxalate extractable labile iron oxides content than other three soils. Reduced Fe has been found to be correlated with ammonium-oxalate extractable Fe, meaning that labile iron oxides are probably the most important source of reduced Fe (Van Bodgom et al. 2003). Consequently, the higher contents of labile Fe oxides (Table 1) in Sonatala-1 probably explain its higher dissolved Fe content than in the Sonatala-2 soil.

There were only small or no differences in the contents of water soluble Fe between untreated and Fe$_2$O$_3$ treated soils. This would mean that either: 1° availability of reducible Fe was already sufficient
Discussion
to cover transfer of electrons donated by microbial activity; or 2° the supplied Fe₂O₃ (hematite powder) was not adequately available for reduction due to its physical conformation. In line with the second explanation, Qu et al. (2004) proposed that the addition of ferric oxides like goethite, hematite etc. had no effect on Fe²⁺ contents in paddy soil slurries compared to unamended controls, indicating no microbially mediated reduction of the added oxides. Indeed, Van Bodegom et al. (2003) also found that crystalline iron oxides were no substantial contributors of reducible Fe, in contrast to non-crystalline pedogenic Fe. We cannot exclude that the experimental set-up was flawed since the added Fe₂O₃ does not correspond well to main reducible. Nevertheless we did attempt to synthesize amorphous oxides of Fe and mixed oxides of Fe/Al (4:1 molar ratio) in the laboratory from Fe₂(SO₄)₃ and Al₂(SO₄)₃ according to the method modified from Schwertmann and Cornell (1991). The synthesis of Fe or Fe/Al mixed oxides didn’t succeed and most likely magnetite was the end product, as is most often the case.

Consequently, the experimental setup (application of Fe₂O₃ as a powder) may have been inappropriate to fully reject the present thesis’s primary hypothesis. On the other hand, like BAU soils, the addition of amorphous mixed Mn/Al oxides increased the concentration of dissolved Mn in treated soils (T₃) due to their more easy and preferential reduction. In consistent with this, Lovley (1991) also reported that near neutral pH of 6 to 8, the Mn⁴⁺ is more reactive with organic compounds than Fe³⁺ in submerged soils. An example of the nearly absent effect of Fe-addition on Fe-concentrations was the elevated and higher water soluble Fe content in T₂ compared to T₁ and T₃ treatments in the Noaddah soil after 6 and subsequent weeks of incubation. This result indicates reductive dissolution of added Fe₂O₃.

Togethers with Fe³⁺, Mn⁴⁺ also serves as electron acceptor for anaerobic respiration with Eh near nitrate reduction and well above sulfate reduction (Nealson and Myers 1992). In general, the higher concentration of water soluble Mn content in T₃ than T₁ and T₂ treatments most likely resulted from the enhanced reduction of added Mn oxides in all paddy soils used in this study. The concentration of dissolved Mn at any given treatment and time in Sonatala-1 soil was higher than that in the Sonatala-2 perhaps due its greater SOM and Mn content compared to Sonatala-2 soil. Lower concentrations of dissolved Mn in T₁ and T₂ of Melandoho and Noaddah soils than those in Sonatala-1 could also be explained by their lower pH, SOM and Mn content compared to Sonatala-1 soil. In addition to this, the gradual increase of water soluble Mn content in both Melandoho and Noaddah soils treated with Mn/Al mixed oxides (T₃) peaked only after 8 weeks of submergence. This result corresponds to the previous review work by Ponnamperuma (1972) where he mentioned that acidic soils with low OM and high Mn content gave high and broad peak but at very late. Hence in the soils with low Mn and OM content added Mn most likely served as electron acceptor for a longer period.
Discussion

Ammonium release patterns during anaerobic incubation were asymptotic for all four farmers’ field paddy soils. Generally, the total amount of NH$_4^+$ released ranged from 25 to 54 mg kg$^{-1}$ during 10 weeks of anaerobic incubation. This range was analogous with that attained by Narteh and Sahrawat (1999), who reported NH$_4^+$ released after 15 weeks of anaerobic incubation between 3 to 74 mg L$^{-1}$. Among the four soils, the maximum amount of NH$_4^+$ was produced in the Noaddah soil followed by Melandoho soil. This difference might be related to their differences in amount of Na-pyrophosphate extractable Fe content, which is indicative for the content of reducible Fe, required for the proceeding of microbial activity under anaerobic conditions. Accordingly, Kader et al. (2013) also found that anaerobic N mineralization correlated positively with Na-pyrophosphate-extractable Fe and negatively with pH.

For all the soils from farmers’ fields and BAU long term field experiment there were insignificant differences in NH$_4^+$ content among the treated (T$_2$ and T$_3$) and untreated (T$_1$) soils after 10 and 8 weeks of anaerobic incubation. Addition of Fe$_2$O$_3$ and Mn/Al mixed oxides apparently did not promote production of NH$_4^+$ in both paddy soils. Consequently, this leads us to reject the main hypothesis that lack of available reducible Fe or Mn would form a bottleneck for anaerobic microbial activity and N mineralization in these Bangladeshi paddy soils.

Therefore, based on the dynamics of available Fe and Mn content and produced NH$_4^+$ over time in all treated and untreated farmers’ field paddy soils we could decide that: 1° the idea that low availability of reducible Fe limited N mineralization could not fully be rejected since the applied Fe may have been physically unavailable; 2° On the other hand, better reducible applied amorphous Mn-oxides also did not have any effect on N mineralization. From this it is in fact possible to conclude that a lack of available alternative electron acceptors did not limit anaerobic N mineralization.

It does appear that to some extent Mn application prolonged microbial activity, but still this had no influence on the final release of mineral N. The relative fast and substantial increase of exchangeable NH$_4^+$ in Sonatola-1, Sonatola-2 and Noaddah soils was striking. This prompted the question whether this fast initial mineral N build-up was entirely due to microbial action or possibly at least partly the result of fixed- NH$_4^+$ release. If release of fixed NH$_4^+$ is substantial, then it would be logical that availability of reducible Fe and Mn only had a minor control on the mineral N buildup in the studied soils. Consequently, a third short-term incubation experiment was conducted with four farmers’ field paddy soils to estimate the relative contribution of abiotic release of fixed NH$_4^+$ versus biotic anaerobic N mineralization in the mineral N (5.3).
5.3 Follow-up of exchangeable and non-exchangeable NH$_4^+$ in farmers’ fields

In all four farmers’ field paddy soils both exchangeable and non-exchangeable (i.e. fixed) NH$_4^+$ gradually increased during the 4-week incubation. Apparently, flooding favored NH$_4^+$ fixation, rather than net release of fixed NH$_4^+$. Accordingly, increasing quantity of non-exchangeable NH$_4^+$ in rice soils after flooding was previously also related with decreasing redox potential by Schneiders and Scherer (1996) and Schneiders and Scherer (1998). Moreover, microbial reduction of Fe$^{3+}$ with subsequent dissolution of Fe-oxides coatings on clay surfaces at low Eh enhanced diffusion of NH$_4^+$ ions into the interlayer (Scherer and Zhang 1999).

In all four farmers’ field soils the amount of fixed NH$_4^+$ were substantial after 2 and 4 weeks of flooding due to the presence of vermiculite (2:1 expandable clay mineral) (Kader 2012). In accordance with this, Bajwa (1987) and Nieder et al. (2011) reported that vermiculitic clays strongly fixed both NH$_4^+$ and K in wetland rice soils. The buildup of exchangeable NH$_4^+$ after two weeks of flooding therefore probably derived primarily from SOM decomposition. The MBC data matched this reasoning. MBC was substantial already after 2 weeks of flooding in all four farmers’ field paddy soils, with but a small additional increase between week 2 and 4 since the onset of submergence and even a decrease in the Noaddah soil. These results on fixed NH$_4^+$ and MBC indicated that there was no considerable abiotic release of fixed NH$_4^+$ rather the fixation of NH$_4^+$ was actually increasing with the period of flooding. Therefore, the mineral N was released in the studied soil mainly from the biotic anaerobic N mineralization via SOM decomposition, which was not controlled by availability of reducible Fe.
CHAPTER 6

Conclusions

Our study with Fe₂O₃ or Mn/Al mixed oxides treated and untreated soils mostly verified the consequences of available electron acceptors, particularly Fe³⁺ and Mn⁴⁺ on anaerobic N mineralization and SOM decomposition in young floodplain paddy soils of Bangladesh (BAU long-term field experiment soils and farmers’ field paddy soils).

The evolution of soil solution pH, Fe and Mn, DOC over period of flooding among the five BAU fertilizer treatments field soils were almost similar and followed expectable trends. The soil solution Fe and Mn concentrations were not differed remarkably between untreated and Fe₂O₃ treated soil. From this it could preliminary be concluded that Fe-addition didn’t influence Fe reduction. We cannot exclude that this result is an artifact because the added hematite powder may have been poorly available for reduction. In the Mn/Al mixed oxides treated soils, the contents of Fe were slightly lower and the Mn were considerably greater than those in the soils without such amendment. Relatively lower DOC concentration in the mixed Mn/Al oxides amended soils than the soils not treated with these oxides may be linked with the higher soil solution Mn²⁺, and forming more Mn²⁺-DOC complexes. Like DOC, the CH₄ emission from Mn/Al mixed oxides treated BAU soils were also significantly lower compared to unamended and Fe₂O₃ amended soils. These lower DOC and methane emission in Mn/Al mixed oxides treated soils indicated that the added Mn oxides probably limited the substrate for methanogenic bacteria and then delayed or decreased CH₄ emission. This does point to additional microbially driven reduction of the applied amorphous Mn for the investigated soils.

At the end KCl extractable-NH₄⁺ and MBC contents between the treated and untreated BAU soils did not differ significantly. These results primarily rejected the hypothesis of increasing NH₄⁺ production based on the adequate availability of inorganic alternate acceptors by the addition of Fe₂O₃ or Mn/Al mixed oxides. However, based on this result for a single site and with no follow-up of the evolution of NH₄⁺ with time, it is not possible to draw a general conclusion for paddy soils in Bangladesh.

The evolution pattern and content of pH-KCl, KCl-extractable NH₄⁺, dissolved Fe and Mn varied widely among the four farmers’ fields paddy soils since they were different in their OM (electron donor) as well as reducible Fe and Mn (electron acceptors) contents. Without few exceptions, in every farmers’ field soil generally the water soluble Fe and Mn contents in unamended and Fe₂O₃ treated soils, and the Mn content in Mn/Al mixed oxides treated soils were resemble to those of the BAU soils. This indicated better reduction of the added amorphous Mn/Al mixed oxides and inadequate reduction of applied Fe₂O₃ powder. Within a farmer’s field paddy soil also the KCl-extractable NH₄⁺
Conclusion

contents between the Fe$_2$O$_3$ or Mn/Al mixed oxides treated and untreated soils were not differed considerably during the entire incubation period. This result finally rejected the main hypothesis by confirming that the restricted availability of alternative electron acceptors like Mn$^{4+}$ and Fe$^{3+}$ didn’t influence anaerobic N mineralization in the studied paddy soils.

Lastly, the amount of non-exchangeable NH$_4^+$ differed significantly among the four farmers’ field soils since they were different in clay mineralogy. In each soil, also the contents of fixed NH$_4^+$ at the start of incubation were significantly lower than those after 2 and 4 weeks of flooding. These results pointed out that there was no substantial abiotic release of fixed NH$_4^+$ with increasing period of flooding. Alongside, the MBC in these soils were considerable after 2 weeks of flooding and did not evolve any further until week 4. This suggested that, microbial activity and biomass was already established in the first 2 weeks, even preceding the buildup of exchangeable NH$_4^+$. Hence the subsequent release of mineral N in farmers’ field paddy soils after 2 weeks may well have been the outcome of biotic anaerobic N mineralization of SOM.

From the whole outcome of the study, it could be said that N mineralization in the studied subtropical paddy soils is a complex process and not influenced greatly by the availability of abiotic electron acceptors. Therefore, we see no reason to expand the research on the availability of alternate electron acceptors, particularly Fe$^{3+}$ and Mn$^{4+}$ in relation to anaerobic N mineralization in floodplain paddy soils of Bangladesh. Further research will be needed to look for the potential predictors and to improve the estimation of anaerobic N mineralization in the subtropical paddy soils. The extensive research on the biotic indicators like, soil microbial activity and biomass, amidohydrolase activities in relation to the period of flooding will also provide an insight of SOM decomposition and N mineralization in these soils. More research will be required as well to develop a better estimate of different fractions of fixed NH$_4^+$ and their role on mineral N in flooded paddy soils. In addition, the biochemical composition of SOM particularly the lignin and polyphenol content relevant to N mineralization in lowland paddy soils could also be studied.
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