

**NITRATE REDUCTION AND METHANE FORMATION AS INFLUENCED BY
IRON-CENTERED INTERMEDIATE REDOX PROCESSES IN RICE SOILS**

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of
requirements for the degree of
Doctor of Philosophy

in

The Department of Oceanography and Coastal Sciences

by

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May 2005

ACKNOWLEDGMENTS

My sincere thanks go to my major professors Dr. William H. Patrick Jr. and Dr. Robert P. Gambrell for their guidance and encouragement in my study. My sincere thanks also go to other committee members Dr. Harry H. Roberts, Ralph J. Portier and Jaye E. Cable, for their time and suggestions, and to Dr. Wayne H. Hudnall and Dr. Lewis A. Gaston, for serving as the representative of the Graduate School Dean in my general and final exams, respectively.

I would like to extend my grateful acknowledgment to Dr. Kewei Yu, Dr. Ron Delaune, Dr. Chuck Lindau, and everyone else from the Wetland Biogeochemistry Institute, Louisiana State University for all kinds of help in my study program. The funding support from the United States Department of Agriculture is appreciated. Finally, I thank my wife Rui and our daughter Yiming, who gave me care and encouragement through the whole period of study.

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ABSTRACT

Rice fields are a major source of the greenhouse gases methane (CH_4) and nitrous oxide (N_2O) and contribute to nitrate (NO_3^-) pollution in waters. Ferric iron (Fe^{3+}) and manganic manganese (Mn^{4+}) are two intermediate alternative electron acceptors (AEAs) capable of regeneration in freshwater soils. In this investigation, the influences of iron-centered intermediate redox processes on NO_3^- reduction and CH_4 formation in rice soils were studied using soil slurries, soil columns, and potted rice.

Reduction of Fe^{3+} -centered intermediate AEAs was mainly mediated by obligate anaerobes relying on fermentation products. Ferric iron reducers are bioelectrochemically active, supporting bioelectricity generation through a fuel cell process from the flooded soil coupled to the reduction of O_2 or NO_3^- in the overlying water. As a major electron accepting process in anaerobic carbon decomposition, Fe^{3+} reduction stimulated N_2O production but had little influence on overall NO_3^- reduction in the homogenized soil slurries under near-neutral pH conditions. In the flooded soil column and pot experiments, intensification of iron-centered intermediate redox processes under amendments of iron and/or manganese oxides changed the fate of NO_3^- in the overlying water, decreasing heterotrophic denitrification and increasing NO_3^- percolation and N_2O emission. Ferric iron reduction competitively suppressed methanogenic activity in the homogenized soil slurries. The diffusion of the stronger oxidants O_2 and NO_3^- controlled temporal and vertical variations of iron-centered intermediate redox processes, which subsequently controlled temporal and vertical variations of methanogenic activity in the flooded soil

columns. In the pot experiment, Fe^{3+} reduction had small effect on CH_4 emission in the early season when CH_4 emission was low but effectively reduced CH_4 emission after midseason drainage intervals through Fe^{3+} regeneration. The roles of iron-centered intermediate redox processes need to be considered in the evaluation and prediction of NO_3^- reduction and CH_4 formation in rice fields.

CHAPTER 1

INTRODUCTION

Biochemical Redox Reactions

Every organism obtains energy from light or inorganic and organic substances through biochemical reduction and oxidation (redox) reactions to synthesize adenosine triphosphate (ATP) for growth and reproduction. All organisms are autotrophs or heterotrophs, according to their pathways of energy harvest. Autotrophs, including phototrophs and chemoautotrophs, obtain energy from redox reactions of inorganic substances with or without need of light. Heterotrophs, including animals and most microorganisms, obtain energy from organic substances. Nevertheless, the boundaries between autotrophs and heterotrophs are not always clear for microorganisms. When organic substances are not available, some heterotrophs become chemoautotrophs harvesting energy from inorganic substances.

Anaerobic Redox Processes

Anaerobic redox processes depend on oxidants other than O_2 , namely alternative electron acceptors (AEAs) as the terminal electron acceptor. Both inorganic and organic substances can be used as an AEA. The common inorganic AEAs include NO_3^- , Mn^{4+} , Fe^{3+} , SO_4^{2-} , and CO_2 . Many other elements such as U^{6+} and Se^{6+} can also be used as AEAs (Lovley et al., 1991; Lovley, 1993; Haveman and Pedersen, 2002). Many quinone-containing organic substances are reduced as AEAs (Fredrickson et al., 1998) for anaerobic respiration. The availability and dynamics of the common inorganic redox

species are more important in redox processes and carbon mineralization in anaerobic environments.

Sequential Reduction of Alternative Electron Acceptors

Although some redox reactions such as Fe^{2+} oxidation with nitrite may happen chemically, redox reactions in anaerobic environments are largely microbially mediated with AEAs being sequentially reduced in the order of NO_3^- , Mn^{4+} , Fe^{3+} , SO_4^{2-} and CO_2 (Patrick and DeLaune, 1972; Froelich et al., 1979; Reddy et al., 1989). The reduction of one AEA precedes the reduction of another AEA for three reasons. First, the reduction of the preceding AEA (i.e., Fe^{3+} before SO_4^{2-}) yields more energy and is preferred by anaerobes. Secondly, energy sources are used so efficiently in the reduction of the preceding AEA that their levels are too low to be able to simultaneously support the reduction of the following AEAs (Lovely and Goodwin, 1998). Thirdly, the preceding AEA in some cases is toxic to microbes capable of using its following AEAs. For example, NO_3^- and its intermediate products NO and N_2O are toxic to methanogens (Chidthaisong and Conrad, 2000). When NO_3^- is present, even a high level of acetate cannot be used for CH_4 production. In general, the closer two AEAs are in the sequence, the less toxic is the preceding AEA to the reduction of the following AEA. For example, SO_4^{2-} is much less toxic than NO_3^- to methanogens (Chidthaisong and Conrad, 2000).

Among these three mechanisms, the thermodynamic principle of energy yield is believed to be most important and is applied as the base for modeling of microbially driven redox processes (VanCappellen and Wang, 1996; Park and Jaffe, 1996; van

Bodegom and Scholten, 2001). In addition, some microbes, including fermenting bacteria, some SO_4^{2-} reducers and CH_4 producers may reduce some metals such as Fe^{3+} but not couple energy yield to support growth (Coleman et al., 1993; Bond and Lovley, 2002). Such redox reactions may not be regulated by the above three mechanisms but a physiological response for de-toxicity.

Overlapping of Redox Processes

While the sequential reduction of AEAs has been found to be largely true in homogenized environments such as soil suspensions, it is largely obscured in natural environments (Gao et al., 2002). Different anaerobic redox processes occur simultaneously on a wide range of temporal and spatial scales. There are a number of reasons for such overlapping. The first one is soil/sediment heterogeneity. Various sizes of particles and other soil components, including organic matter are heterogeneously distributed, providing microsites suitable for various functional microbial groups. The bioavailability and distribution of AEAs are also heterogeneous. Among the common inorganic redox species, NO_3^- , SO_4^{2-} and CO_2 are soluble while Fe^{3+} and Mn^{4+} are largely solid and capable of recycling and redistribution (Frenzel et al., 1999; King and Garey 1999; Ratering and Schnell 2000). Direct contact is required for microbes to transfer electrons to these solid electron acceptors but an exception occurs when alternative mechanisms such as use of electron-shuttling substances are adopted (Straub et al., 2001; Luu and Ramsay, 2003). The presence of aquatic plants and bioturbation of fauna additionally create heterogeneous aerobic/anaerobic interfaces in soils and sediments. A

high excess of electron donors relative to electron acceptors also cause simultaneous occurrence of different redox processes (Roy et al., 1997; Yu and Patrick 2003).

Microbial species responsible for specific redox processes also tend to overlap. There is no clear distinction between NO_3^- reducers, Fe^{3+} reducers, SO_4^{2-} reducers, and CH_4 producers in that many species of microbes that reduce other AEAs are capable of Fe^{3+} reduction. Many NO_3^- reducers are Fe^{3+} reducers. Even some SO_4^{2-} reducers and methanogens can reduce Fe^{3+} (Coleman et al., 1993; Lovley et al., 1993). Due to the prevailing ability of a large number of microbial species to use Fe^{3+} as an electron acceptor, it has been suggested that Fe^{3+} reducers are very important in the origin of life on the earth (Vargas et al., 1998).

Dissimilatory Microbial Reduction of Iron and Manganese

Iron and manganese represent the 4th and 12th most abundant elements, respectively, in the Earth's crust (Horne, 1978). Both of these transition metals are not only essential trace elements for life but also important as intermediate AEAs in anaerobic carbon mineralization. Most iron compounds are particulate and incapable of microbial reduction. Still, due to its abundance, Fe^{3+} is normally the dominant AEA in freshwater soils (Yao et al., 1999). More importantly, Fe^{3+} is capable of regeneration. Rapid iron redox cycling around aerobic/anaerobic interfaces of wetland and aquatic environments further increases the importance of microbial Fe^{3+} reduction in anaerobic carbon decomposition. Even in marine sediments where SO_4^{2-} reduction is normally the dominant pathway for anaerobic carbon mineralization, microbial Fe^{3+} reduction may contribute substantially to

anaerobic carbon decomposition due to intensive iron redox recycling (Aller et al., 1996; VanCappellen and Wang, 1996).

Microbial reduction of Mn^{4+} is normally mediated by microorganisms which are also capable of Fe^{3+} reduction (Lovley et al., 2004). Since manganese is normally less abundant than iron (Horne, 1978) and Mn^{2+} reoxidation is much slower than Fe^{2+} reoxidation at oxic-anoxic interfaces (Stumm and Morgan, 1981), Mn^{4+} reduction is generally less important than Fe^{3+} reduction in anaerobic carbon decomposition. The importance of microbial reduction of other intermediate AEAs such as As^{5+} is even smaller. As such, Fe^{3+} reduction is of central importance among all intermediate redox processes occurring after the depletion of NO_3^- and before the initiation of SO_4^{2-} reduction and CH_4 formation.

A number of factors hamper quantitative approaches to estimation of microbial Fe^{3+} reduction in anaerobic environments. Iron compounds have two oxidation states but have too many structurally different forms differing in solubility and bioavailability.

Dissolution and precipitation of iron compounds are associated with many inorganic and organic substances, such as metals, sulfides and humic substances. Because of these complex interactions, the isotopic approach, a powerful approach to quantification of N, S and C cycling, is not applicable for quantification of microbial Fe^{3+} reduction (Roden and Lovley, 1993). Sampling of iron compounds under field conditions and quantifying the different compounds is also difficult due to their heterogeneous distribution and dynamic transformations. Reducible iron content inferred from direct extraction or

extraction after a period of anaerobic incubation (Lovley and Phillips, 1986; van Bodegom et al., 2003) has been used to assess the potential/scale of microbial Fe^{3+} reduction in anoxic soils and sediments. The influences of soil/sediment properties on the reliability of these extraction approaches deserve further investigations. A quantitative approach to estimation of microbial Fe^{3+} reduction in field conditions under rapid redox cycling is yet to be found.

Ecoenvironmental Aspects of Iron Redox Processes

Iron redox cycling directly or indirectly interacts with many processes including almost all other major redox processes. Due to its close interactions with so many processes, iron redox cycling plays a key role in most anaerobic environments. Iron redox processes are a major factor shaping geochemistry of ancient environments. Formation and distributions of iron compounds, such as pyrite, are important in interpretation of modern and ancient environments (Danielson et al., 1992; Marnette et al., 1993). In modern times, iron redox processes play a number of important ecoenvironmental roles including organic pollutant degradation, metal mobilization/immobilization in subsurface environments and NO_3^- and CH_4 biogeochemistry in wetland soils, etc. (Lovley, 1991; Lovley et al., 1994; Roden and Wetzell, 1996; Yao et al., 1999; Senn et al., 2002). In addition, iron-reducing bacteria, with an ability to use an electrode as the electron acceptor, are involved in bioelectricity generation from anoxic sediments preventing or decreasing the formation of CH_4 (Rabaey et al., 2003) while coupling to the reduction of oxidants including nitrate (Gregory et al., 2004).

Organic Pollutant Degradation and Metal Stability in Subsurface Environments

The redox state of shallow groundwater/subsurface soils is maintained by the relative rates of O_2 and organic carbon inputs, microbial carbon respiration and the availability of the various AEAs. Oxidized iron and manganese minerals provide significant oxidative capacity to buffer groundwater redox quality against the input of organic carbon in the absence of oxygen. However, at point-source pollution sites, e.g. oil spill sites, electron acceptors are normally in short supply, especially after the injection of available carbon to stimulate growth of microorganisms for organic pollutant degradation. The efficiencies of use of O_2 and NO_3^- as electron acceptors are low in this situation due to the difficulties in confining them within the targeted zones. Chelated Fe^{3+} has been used as an AEA to stimulate organic pollutant degradation (Lovley et al., 1994). The Fe^{2+} produced from Fe^{3+} reduction may be collected in a downgradient well for recycling.

Similarly, since redox cycling of many other elements such as U is closely associated with iron redox cycling, Fe^{3+} reduction or regeneration also controls the dissolution/precipitation of these elements (Lovley et al., 1991; Lovley, 1993; Haveman and Pedersen, 2002). An injection of acetate as the electron donor not only stimulates Fe^{3+} reduction but also U(VI) reduction to U(IV), which has a low solubility and hence is less likely to be transported.

Control on Nitrate in Wetlands

Nitrate is a major pollutant in surface and subsurface waters. Natural wetlands normally function as a sink for NO_3^- . Constructed and natural wetlands have been used

for NO_3^- removal from waters (Reilly et al., 2000; Lin et al., 2002; Rutherford and Nguyen, 2004). Both the retention time and the reduction capacity of the functioning zone of a wetland are important in the efficiency of NO_3^- removal. In general, a low redox condition favors NO_3^- removal with less N_2O being formed (Kralova et al., 1992), although an excess of electron donors under highly reducing conditions may favor dissimilatory reduction of NO_3^- to ammonium but not to N_2 (King and Nedwell, 1985). When wetlands (e.g. riparian wetlands and rice fields) are intermittently or seasonally flooded, the redox conditions are variable and so is their ability for NO_3^- removal. Nitrate may not be efficiently removed by the functioning zone but be transported through, causing surface/subsurface water pollution. Such NO_3^- transport has been found in rice fields where high nitrogen fertilization rates are used (Zhu et al., 2000). Groundwater discharge contributes significantly to the NO_3^- load in rivers (Staver and Brinsfield, 1996; Dowling et al., 2004).

Organic carbon sources as electron donors are important in NO_3^- transformation in wetland soils (Vanoostrom and Russell, 1994; Weisner et al., 1994; Ingersoll and Baker, 1998; Hill and Cardaci, 2004). Microbial Fe^{3+} reduction contributes substantially to anaerobic carbon oxidation in freshwater soils and sediments (Roden and Wetzel, 1996; Yao et al., 1999) and should affect the pool of electron donors. Nevertheless, little information is available on the significance of microbial Fe^{3+} reduction in anaerobic carbon oxidation, and the role of Fe^{3+} reduction in NO_3^- transformation in wetland environments. The ability of the functioning zone to transform NO_3^- is of key importance

in determining how much NO_3^- may be transported through the functioning zone. Under carbon-limiting conditions, the availability of Fe^{3+} -centered intermediate AEAs, typically including Mn^{4+} , becomes important. Ferric iron-centered intermediate AEAs may be reduced, increasing the limitation of electron donors at certain depths before NO_3^- is transported there. The reduced iron compounds may promote chemoautotrophic NO_3^- reduction (Murase and Kimura 1997; Benz et al., 1998) but it is not expected to be as efficient as heterotrophic NO_3^- reduction based on thermodynamic principles. As a result, when Fe^{3+} -centered AEAs are abundant, NO_3^- transformation is likely lowered while N_2O as the end product relative to N_2 increases and NO_3^- transport is promoted. Nitrous oxide is an important biogenic greenhouse gas with a global warming potential 310 times that of CO_2 on a molecular basis (IPCC, 2001). Nitrous oxide emission at high rates is observed in treatment wetlands (Freeman et al., 1997; Fey et al., 1999; Mander et al., 2003). Evaluation of loading capacities of treatment wetlands for NO_3^- removal is needed to ensure effective NO_3^- removal while minimizing N_2O emission.

Control on Methane in Wetlands

Methane is a major biogenic greenhouse gas, second only to CO_2 in its contribution to global warming (IPCC, 2001). Wetlands are a major source of CH_4 (IPCC, 2001). It is not reasonable to drain natural wetlands for CH_4 mitigation due to their important economic and ecoenvironmental roles (Patrick, 1994), including their functioning as an active carbon and nitrate sink (Brix et al., 2001; Vitt et al., 2001; Whiting and Chanton, et al., 2001). Artificial wetlands are generally not an effective carbon sink but are an

important CH₄ source (IPCC, 2001). Many approaches have been tested for CH₄ mitigation in rice fields, including selective use of rice cultivars, timing and rate of organic and inorganic fertilizer applications, and, intermittent flooding through water management, etc (Sigren et al., 1997; Yagi et al., 1997; Wang et al., 2000; Wassmann et al. 2000; Krüger et al. 2001). Among these approaches, intermittent flooding is most effective and promising (Yagi et al., 1997).

The availability of reducible iron is an important soil characteristic in determination of CH₄ emission in rice soils (Wang et al., 1993b; Watanabe and Kimura 1999; Yao et al., 1999; Liu et al., 2003). Ferric iron can directly inhibit CH₄ production (van Bodegom et al., 2004). An increase in microbial Fe³⁺ reduction, e.g. through Fe³⁺ amendment, reduces CH₄ emission in continuously flooded rice soils (Jäckel and Schnell, 2000; Furukawa and Inubushi, 2002).

Rapid Fe²⁺ reoxidation is found at the oxygenated soil-water interface and also in the oxidized rhizosphere where it is important in controlling root exudation and CH₄ dynamics (Frenzel et al., 1999; King & Garey 1999; Ratering & Schnell 2000). Ferric iron regeneration during non-flooding periods contributes to CH₄ mitigation upon reflooding (Sigren et al., 1997; Ratering and Conrad, 1998), and hence is important in determination of non-flooding durations required for effective CH₄ mitigation without the risks of reducing rice yield and increasing the emission of N₂O (Henckel and Conrad, 1998; Cai et al., 1999).

In general, the importance of redox cycling of iron in regulation of CH₄ dynamics is

poorly understood. The effect of Fe^{3+} reduction on CH_4 emission from rice paddies is only preliminarily considered in very recent model studies predicting CH_4 emission from rice paddies (Matthews et al., 2000; van Bodegom et al., 2000). Large uncertainties still remain in evaluation of the impact of iron redox cycling on CH_4 emission from rice paddies. Specifically, the roles of reduction and regeneration of Fe^{3+} need to be evaluated in water management based options for CH_4 mitigation in rice fields.

Bioelectricity Generation from Anaerobic Environments for Pollution Control

Large amounts of potential energy are associated with organic matter in anaerobic soils/sediments but are difficult to utilize. When a soil/sediment develops highly reducing conditions, bioelectrochemically active microorganisms such as iron-reducing bacteria can use an electrode (e.g. graphite plate) as the electron acceptor when it is electrically connected to another electrode placed in the oxidized overlying water to support bioelectricity generation (Bond et al., 2002; Reimers et al., 2001). The addition of an external electron shuttling substance is not necessary in this process, providing a possibility to directly harvest power from anaerobic sediments/soils.

The microbially harvested power normally ranges from 10 to 100 mW m^{-2} (Liu et al., 2004) and can be used to drive low-power sensors in marine environments (Bond et al., 2002; Reimers et al., 2001). Difficulties remain in collecting, concentrating, and storage of this energy for practical applications. Nevertheless, since the internal resistance of a microbial fuel cell is relatively high, low bioelectricity yield is not necessarily as important a goal as using this energy for in situ bioelectrochemical pollutant removal.

During bioelectricity generation, waste organic matter (e.g. from wastewater) is bioelectrochemically oxidized by O_2 (Liu et al., 2004) to prevent or lower the formation of CH_4 (Rabaey et al., 2003) and oxidized pollutants such as NO_3^- can be bioelectrochemically reduced when or where O_2 is not available (Gregory et al., 2004). Nitrate as a major pollutant in drinking water can be removed with physico/chemical and biological processes (Kapoor and Viraraghavan, 1997). With the microbial fuel cell approach, organic carbon from wastewaters, soils, and sediments is available as the reductant for NO_3^- removal even with no direct contact with the water. The use of microbially mediated reductants as energy sources by the biobattery approach also makes it different from electrochemical/bioelectrochemical NO_3^- removal by an electric current field (Feleke and Sakakibara, 2001; Zaveri and Flora, 2002; Beschkov et al., 2004).

Research Objectives

Wetlands are a major source of CH_4 and a sink for NO_3^- . Artificial wetlands can also be a source of NO_3^- . Iron redox processes play important roles in NO_3^- and CH_4 biogeochemistry in wetland environments. These roles are poorly quantified due to the heterogeneity of iron redox processes. The temporal and spatial distribution of iron-centered intermediate redox processes, typically including manganese redox processes, not only controls methanogenesis heterogeneity but also affects the fate of NO_3^- in wetland soils.

In this dissertation, soil suspension, soil column, and pot experiments were carried out to investigate the influences of iron-centered intermediate redox processes on NO_3^-

reduction and CH₄ formation in rice soils. The importance of microbial Fe³⁺ reduction in timing and scaling of denitrification and methanogenesis was evaluated in anoxic soil suspensions (Chapter 2). The role of cycling of Fe³⁺-centered AEAs in temporal and vertical variations of N₂O and CH₄ production were evaluated in flooded soil columns (Chapter 3). The effect of a pool of Fe³⁺-centered intermediate AEAs on NO₃⁻ dynamics was evaluated in flooded soil columns at different percolation rates (Chapter 4). Microbial activity responsible for the reduction of Fe³⁺-centered AEAs was estimated using an iron redox processes-dominated intermediately reducing zone with comparison to other redox zones (Chapter 5). The ability of a flooded rice soil as a reductant source to bioelectrochemically remove NO₃⁻ from water was evaluated (Chapter 6). A pot experiment was carried out to study effects of Fe³⁺ reduction and regeneration on NO₃⁻ percolation and emissions of N₂O and CH₄ from a soil-rice system (Chapter 7). Chapter 8 was a summary of the results obtained from the above six experiments.

CHAPTER 2

EFFECT OF MICROBIAL IRON REDUCTION ON DENITRIFICATION AND METHANOGENESIS IN ANOXIC SOIL SLURRIES

Introduction

Anaerobic carbon oxidation occurs after the depletion of oxygen in the porewater in flooded soils, coupled to sequential reduction of a series of electron acceptors, mainly NO_3^- , Mn^{4+} , Fe^{3+} , SO_4^{2-} and finally CO_2 (Patrick and DeLaune, 1972; Reddy et al., 1989). In freshwater soils, the presence of the element S is normally low while Fe is an abundant element. While most iron compounds are particulate and largely incapable of being reduced by microbial respiration, due to the large amount and variety of forms of iron present, microbial Fe^{3+} reduction is an electron accepting process dominant or second only to methanogenesis in anaerobic carbon decomposition in most freshwater soils (Yao et al., 1999).

A number of factors affect microbial Fe^{3+} reduction in soil. Acidic conditions increase solubility of iron compounds and may increase microbial Fe^{3+} reduction if such pH conditions are physiologically acceptable. The presence of quinone-containing substances such as humic substances may facilitate microbial reduction of particulate Fe^{3+} (Straub et al., 2001; Luu and Ramsay, 2003). More importantly, Fe^{3+} is capable of regeneration. Iron redox cycling occurs so intensively around aerobic/anaerobic interfaces in wetland and aquatic environments that microbial Fe^{3+} reduction even contributes substantially to anaerobic carbon mineralization in marine sediments (Aller et al., 1996; VanCappellen and

Wang, 1996). Because of its importance in anaerobic carbon decomposition, microbial Fe^{3+} reduction plays many important ecoenvironmental roles in organic pollutant degradation, toxic metal mobilization and immobilization in soils and sediments, NO_3^- dynamics, and CH_4 biogeochemistry in wetland and aquatic environments (Lovley, 1991; Lovley et al., 1994; Roden and Wetzel, 1996; Yao et al., 1999; Senn et al., 2002).

In wetland soils, microbial Fe^{3+} reduction as an intermediate redox process is an important factor determining the duration of intermediate reducing conditions between the depletion of nitrate and initiation of methanogenesis, a condition where N_2O and CH_4 fluxes are low (Yu and Patrick, 2003). As such, Fe^{3+} reduction is an important factor affecting CH_4 emissions from rice fields (Wang et al., 1993b; Watanabe and Kimura 1999; 1999; Liu et al., 2003). Rice fields are a major biogenic source of CH_4 (IPCC, 2001), which is second only to CO_2 in its contribution to global warming.

Ferric iron reduction may directly and indirectly affect methanogenesis and denitrification in anoxic conditions. In homogenized soil slurries, Fe^{3+} reduction suppresses methanogenesis (Achthich et al., 1995) but may con occur with denitrification especially when available carbon is abundant. While Fe^{3+} reduction con occurring with denitrification may be difficult to detect due to rapid chemical and/or biological reoxidation of Fe^{2+} that is produced (Finneran et al., 2002), this process may promote N_2O formation (Brons et al., 1991) and even affect overall denitrification. In field conditions, due to soil heterogeneity, microbial Fe^{3+} reduction may con occur with denitrification and methanogenesis on various spatial scales and more intensively affect them through

redistribution and regeneration of Fe^{3+} in the soil (Benz et al., 1998; Nielsen et al., 1998; Frenzel et al., 1999; Rysgaard et al., 2001).

In this chapter, we selected two rice soils differing in pH and iron content to study the effects of microbial Fe^{3+} reduction on denitrification and methanogenesis in homogenized soil slurries. Studies on the effects of microbial Fe^{3+} reduction on denitrification and methanogenesis under more natural soil conditions were reported in other chapters.

Materials and Methods

Soils

Two rice soils were used in the experiments. One is a Crowley silt loam soil (fine, smectitic, hyperthermic Typic Albaqualfs) from Crowley, Louisiana (LA soil) and the other is a Beaumont clay soil (fine, montmorillonitic, thermic Entic Pelluderts) from Beaumont, Texas (TX soil). The soil samples were collected from the top layer (0-20 cm), air-dried, stored at room temperature (20 °C), and sieved (<1-mm) for the slurry experiment. Soil characteristics of interest were analyzed and shown in Table 2.1.

Table 2.1 Selected characteristics of the soils.

Soil	pH	OM (%)	Total N (%)	P	K	Extractable Fe †	Extractable Mn †	Extractable S ‡
						mg kg ⁻¹		
LA	7.3	1.67	0.07	42	291	68.2	19.5	10.7
TX	5.1	2.54	0.11	68	104	115.6	35.1	38.4

† Extracted with DTPA (diethylene triamine pentaacetic acid) solution to remove soluble and labile solid phases (Lindsay and Norvell, 1978).

‡ Extracted with ammonium acetate and acetic acid solution to remove S that was presumed to be mostly SO_4^{2-} (Bardsley and Lancaster, 1960).

Slurry Experiment

The microcosm technique of Patrick et al. (1973) was used in the slurry experiment with some modification (Yu and Patrick, 2003). The soil slurry was prepared by adding 400 g air-dried soil and 1600 ml deionized water into a 2300-ml Erlenmeyer flask, with or without ferrihydrite amendment at $40 \mu\text{mol Fe}^{3+} \text{ g}^{-1}$. Ferrihydrite was prepared (Jäckel and Schnell, 2000) and resuspended in deionized water at pH 7. The soil slurry was aerobically preincubated under constant stirring at $25 \text{ }^\circ\text{C}$ for 4 days. Then 4 g of ground rice straw was added as an energy source and potassium nitrate was added at $8 \mu\text{mol N g}^{-1}$ into the soil slurry. The flask was capped with a rubber stopper in which a septum was installed for gas sampling, and a gas inlet and outlet were installed for headspace purging. The headspace was purged with pure N_2 for 20 min to start the anaerobic incubation. Pure N_2 was bubbled through the suspension at a rate of 15 ml m^{-1} to maintain anaerobic conditions during the incubation. Instead of using treatment replication, an approach of frequent sampling and measurement was used to ensure the duplicability and accuracy of experiment with this homogenized microcosm containing a large soil mass (Yu and Patrick, 2003).

At hours 0, 12, 24, 48, 72, 120 and 172 after the start of the anaerobic incubation, 10 ml of soil slurry was anaerobically taken from the flask, centrifuged, filtered ($<0.45 \mu\text{m}$) and stabilized 10 ml of NO_3^- buffer (APHA, 1995) for NO_3^- determination with an ion meter coupled to a combination NO_3^- selective electrode (detection limit $7 \times 10^{-6} \text{ M}$). Ten ml of deionized water was added after the sampling of soil slurry to compensate for the volume change of soil slurry. The removal of soil was considered in the calculation of the

rates of NO_3^- reduction and production of N_2O , CO_2 , and CH_4 on a mass basis. Redox potential and gas production were normally daily measured until CH_4 production from the microcosm became less variable, and pH was measured on selected dates. The redox potential was measured before gas sampling and was reported as the mean reading (Eh) of Pt electrodes (n=2) coupled to a saturated calomel reference electrode after correction to the standard H_2 electrode by adding 245 mV to the instrument reading. The microcosm was purged with N_2 at a higher flow rate (at least double than normal) for two hours before gas sampling. Then gas samples were taken three times from the headspace of the microcosm at 0, 20, and 40 minutes after stop purging and analyzed for N_2O , CH_4 , and CO_2 concentrations (see below). Ten ml of soil slurry (n=2) was taken at the end of anaerobic incubation for measurement of the content of microbially reducible iron, which was extracted by 0.5 M HCl (Lovley and Phillips, 1986) and determined by ICP.

Analysis of Gas Samples

Gas samples were taken and analyzed with a Tometrics 9001 gas chromatograph (GC) using an electron capture detector (ECD) for determining N_2O and a flame ionization detector (FID) for determining concentrations of CH_4 and CO_2 after calibration with certified standards. A methanizer catalyst option was installed after the GC column to convert CO_2 to CH_4 for FID detection.

Calculation and Statistical Analysis

Gas production rate was calculated by linear regression of the three analyses in 40 minutes (Yu and Patrick, 2003). The dissolution of N_2O (5.07×10^{-4} , mole ratio), CO_2

(0.707), and CH₄ (0.028) (Handbook of Chemistry and Physics, 1991) in the liquid phase was considered in their production rate calculation. The cumulative production of N₂O or CH₄ in the slurry was the sum of the production rate on each sampling date multiplied by the time interval, which was calculated from the midpoints of neighboring sampling dates. The cumulative production of CO₂ was not calculated due to the difficulty in considering the effect of pH on CO₂ solubility in the microcosm.

Statistical analysis was conducted with SAS (SAS Institute Inc., 1999-2001). The curves of NO₃⁻ reduction and N₂O production in the soil slurries versus time or Eh were linearly or exponentially fitted. The statistical level of significance was chosen at $\alpha = 0.05$ for all statistical analysis.

Results

Temporal Change of Redox Potential and pH

The Eh decrease was more rapid in the Louisiana soil slurries than in the Texas soil slurries (Fig. 2.1). Ferrihydrite amendment had little effect on Eh decrease in the Louisiana soil, but apparently delayed the Eh decrease in the Texas soil between days 5 and 18. The pH increase during the anaerobic incubation was less than 0.6 pH unit in the Louisiana soil, but close to 2 units in the Texas soil (Fig. 2.1). Ferrihydrite amendment slightly increased the pH in the Louisiana soil after day 10, but apparently delayed the pH rise in the Texas soil between days 5 and 20.

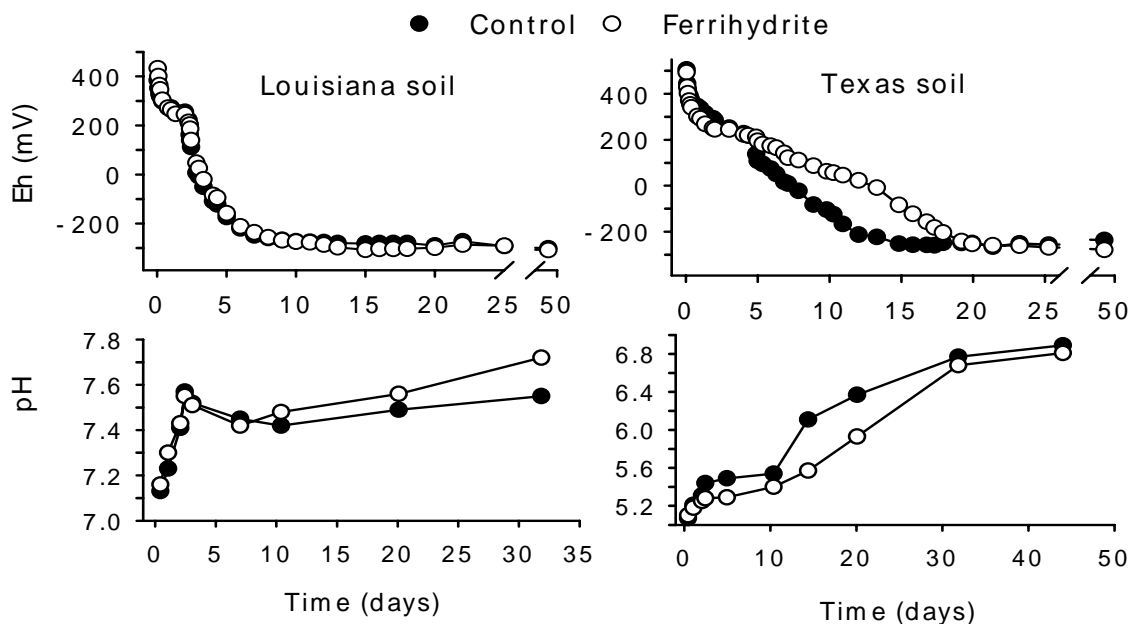


Fig. 2.1 Temporal changes in Eh and pH in the anoxic soil slurries.

Nitrate Reduction and Nitrous Oxide Production

Nitrate reduction was rapid in the Louisiana soil at a linear rate of $4.01 \mu\text{mol N g}^{-1} \text{d}^{-1}$ ($p < 0.001$) for 48 h and was little affected by ferrihydrite amendment (Fig. 2.2).

Ferrihydrite amendment in the Texas soil lowered the NO_3^- reduction rate by 37% from 1.56 to $0.98 \mu\text{mol g}^{-1} \text{d}^{-1}$, and prolonged the depletion time by 48% from 117 to 172 h.

Nitrous oxide production linearly decreased with time in the Louisiana soil ($p < 0.05$), but exponentially decreased in the Texas soil ($p < 0.01$) (Fig. 2.2). Ferrihydrite amendment dramatically increased N_2O production at beginning of the incubation in both soils.

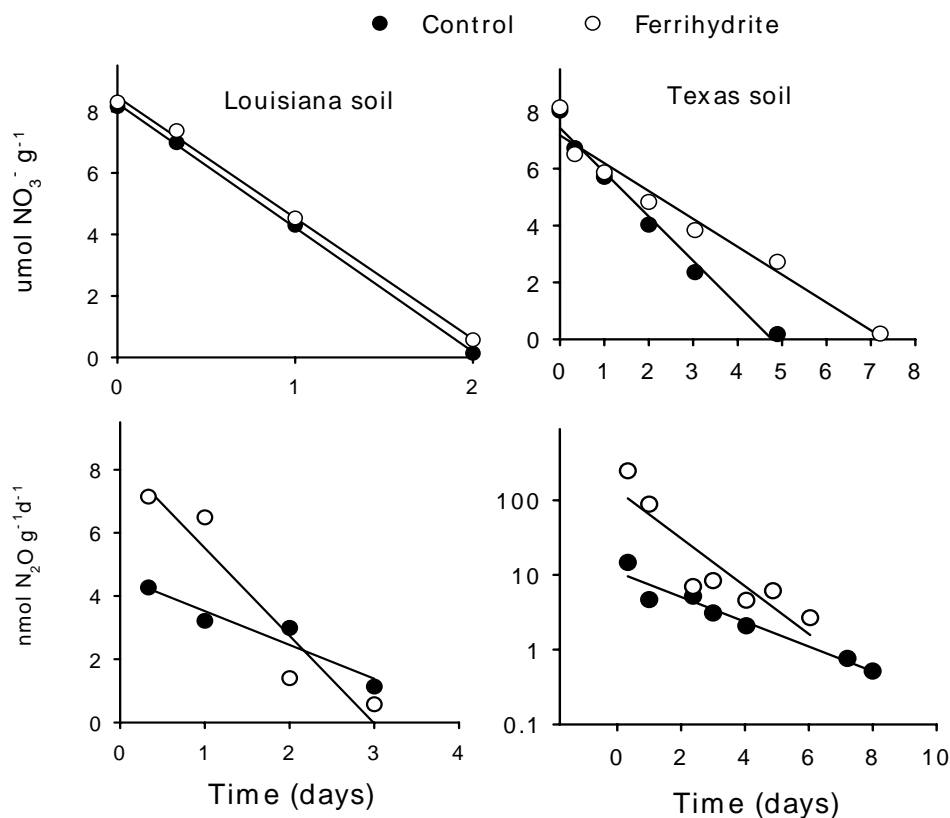


Fig. 2.2 NO_3^- reduction and N_2O production in the soil slurries.

Methane Production

Methanogenesis was more rapidly initiated in the Louisiana soil than in the Texas soil (Fig. 2.3). Ferrihydrite amendment delayed the initiation of significant CH_4 production ($>10 \text{ nmol g}^{-1} \text{ d}^{-1}$) from day 5 to 6 in the Louisiana soil, and from day 13 to 20 in the Texas soil. Ferrihydrite amendment lowered the peak of CH_4 production by 15% in the Louisiana soil, but increased production by 27% in the Texas soil. The reductions in cumulative CH_4 production by ferrihydrite amendment were 23% in the Louisiana soil and 92% in the Texas soil when the CH_4 production peaks in the control treatments were observed (on days 23 and 27, respectively) (Table 2.2). The overall CH_4 reductions were 11% in the Louisiana soil and 13% in the Texas soil during the 46-day incubation.

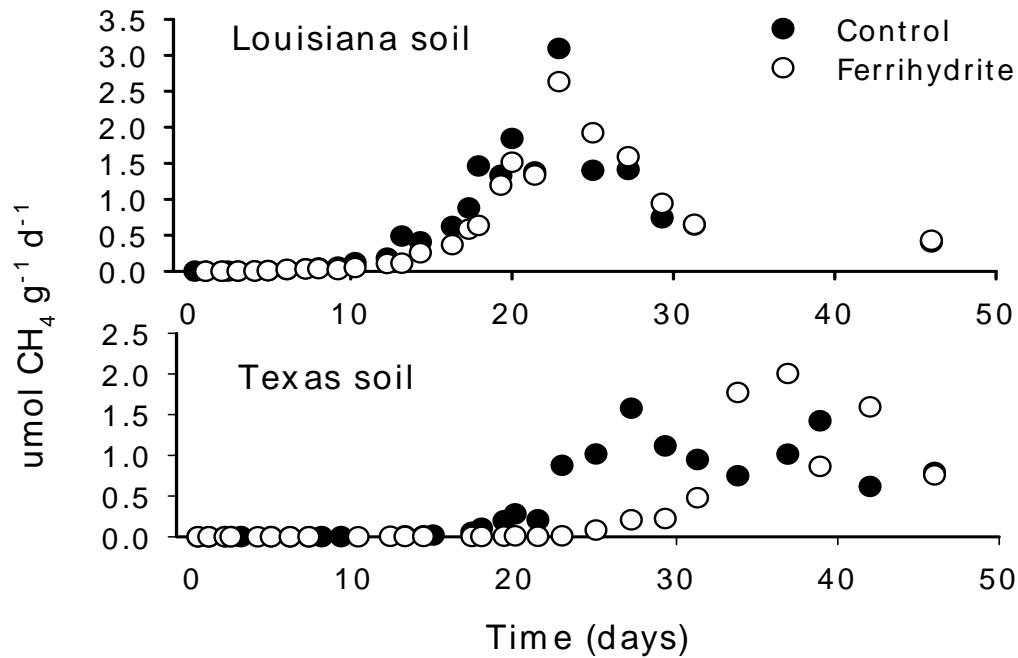


Fig. 2.3 CH₄ production in the soil slurries.

Table 2.2 Effects of ferrihydrite amendment on ferric iron reduction, nitrate reduction, and production of N₂O and CH₄ in the anaerobically incubated soil slurries.

Soil	Fe ³⁺ amended ($\mu\text{mol g}^{-1}$)	Fe ³⁺ reduced ($\mu\text{mol g}^{-1}$)	Nitrate reduction ($\mu\text{mol g}^{-1} \text{d}^{-1}$)	N ₂ O production ($\text{nmol g}^{-1} \text{d}^{-1}$)	CH ₄ formation 1 ($\mu\text{mol g}^{-1} \text{d}^{-1}$) †	CH ₄ formation 2 ($\mu\text{mol g}^{-1} \text{d}^{-1}$) ‡
LA	0	63.3	4.01	2.8	0.66	0.85
LA	40	97.0	3.86	4.4	0.51	0.76
TX	0	196.8	1.56	2.5	0.29	0.54
TX	40	214.6	0.98	39.0	0.024	0.47

† When the corresponding control treatment reached the maximum methane production rate (on days 23 and 27, respectively).

‡ The whole incubation period (day 46).

Carbon Dioxide Production

There was a decrease and then an increase of CO₂ production in the Louisiana soil slurries in the first 10 days, and in the Texas soil slurries in the first 20 days (Fig. 2.4). Thereafter, CO₂ production in all soil slurries was less variable or in a trend of decreasing at low rates along the time course. Ferrihydrite amendment decreased CO₂ production in the Louisiana soil in the first 5 days and in the Texas soil in the first 10 days.

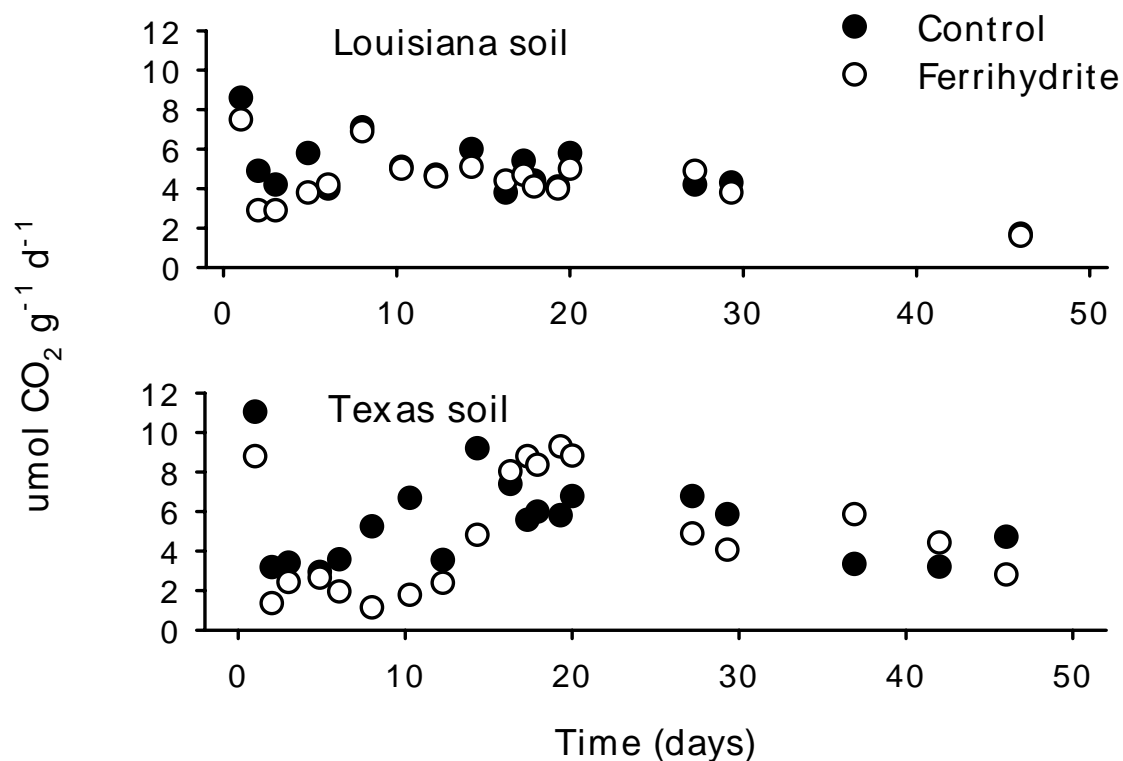


Fig. 2.4 CO₂ production in the soil slurries.

Nitrous Oxide and Methane Production within Different Redox Ranges

Significant N₂O production (> 2 nmol g⁻¹ d⁻¹) was found in the weakly reducing range (>200 mV), with the rate decreasing linearly with a decrease of Eh in the Louisiana

soil slurries ($p < 0.05$), and decreasing exponentially in the Texas soil slurries (Fig. 2.5) ($p < 0.01$). Significant CH_4 production ($> 10 \text{ nmol g}^{-1} \text{ d}^{-1}$) was found in the highly reducing range ($< -200 \text{ mV}$), with the rate increasing exponentially with a decrease of Eh in all soil slurries ($p < 0.01$).

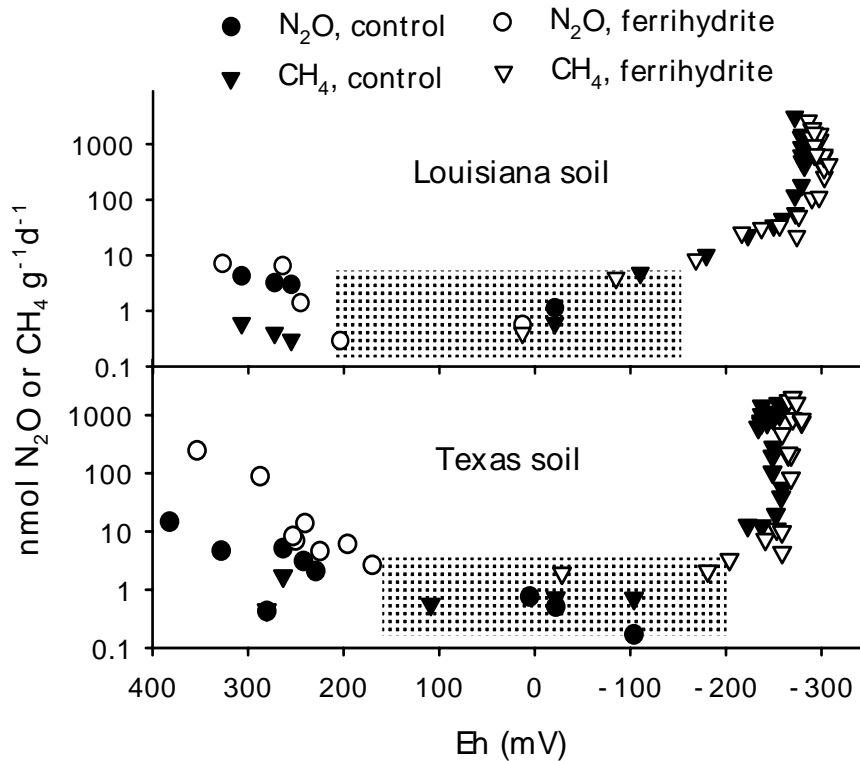


Fig. 2.5 N_2O and CH_4 production in different Eh ranges in the soil slurries. In the shaded Eh ranges N_2O and CH_4 are produced at low rates.

The specific Eh range within which N_2O and CH_4 were both produced at low rates (< 2 and $10 \text{ nmol g}^{-1} \text{ d}^{-1}$, respectively) was 200 to -150 mV in the Louisiana soil, and 150 to -200 mV in the Texas soil. Iron amendment had little effect within this Eh range, but increased the production of N_2O in the weakly reducing range, and reduced the production of CH_4 in the highly reducing range of both soils.

Ferric Iron Reduction

The content of indigenous reducible iron was lower but ferrihydrite amendment induced more Fe^{3+} reduction in the Louisiana soil than in the Texas soil (Table 2.2). Without considering the contribution of microbial reduction of Mn^{4+} , Fe^{3+} reduction as an electron accepting process was second only to methanogenesis in the Louisiana soil but dominant in the Texas soil during the 46-day incubation (Table 2.3).

Table 2.3 Electron transfer in major redox processes of the soil slurries.

Soil	Ferrihydrite amendment ($\mu\text{mol g}^{-1}$)	Electron transfer ($\mu\text{mol g}^{-1}$)			
		NO_3^- reduction †	Fe^{3+} reduction ‡	SO_4^{2-} reduction §	CH_4 production ¶
LA	0	40.0	63.3	2.7	155.7
	40	40.0	97.0	2.7	140.4
TX	0	40.0	196.8	9.6	98.7
	40	40.0	214.6	9.6	86.6

† Assuming background NO_3^- content and NO_3^- transformation other than denitrification to N_2 were negligible.

‡ One electron was required for the reduction of Fe^{3+} to Fe^{2+} .

§ Assuming extractable S was in SO_4^{2-} form and reduced to sulfide.

¶ Assuming organic carbon at oxidation state zero was the only electron donor converted to either CO_2 or CH_4 (Yao et al., 1999).

Discussion

Three major redox processes, denitrification, Fe^{3+} reduction and methanogenesis, in the two anaerobically incubated soils were measured. Sulfate reduction was not quantified but was presumably less important as a redox process than Fe^{3+} reduction (Table 2.3). Microbial Mn^{4+} reduction was also expected to be of minor importance compared to Fe^{3+} reduction (Yao et al., 1999), especially considering the relatively low

content of extractable Mn in each soil. Microbial Fe^{3+} reduction as a major alternative electron accepting process, together with soil pH, was found to be important in the succession of redox processes and timing and scaling of N_2O , CH_4 , and CO_2 production in both soils.

A higher content of indigenous reducible Fe^{3+} , but less favorable pH conditions for anaerobes were probably the two major factors resulting in the slower development of the full range of redox processes in the Texas soil compared to the Louisiana soil, as indicated by the differences in Eh, NO_3^- reduction, and N_2O , CH_4 , and CO_2 production. Acidic conditions retard denitrification activity, especially N_2O reduction (Burford and Bremner, 1975) and not are favorable to methanogenesis (Wang et al., 1993a). The pH values in the Texas soil slurries were not more than 6.1 when denitrification and substantial CH_4 production were initiated; the pH varied much less in a range more favorable to denitrifiers and methanogens in the Louisiana soil slurries. As a result, lower NO_3^- reduction with more N_2O production and later initiation of methanogenesis at lower rates occurred in the Texas soil compared to the Louisiana soil.

Nevertheless, besides the less favorable pH conditions, a higher level of reducible iron also contributed to the lower NO_3^- reduction with higher N_2O production in the Texas soil. This effect was evidenced by ferrihydrite amendment in each soil. Denitrification did not completely inhibit the reduction of Fe^{3+} to Fe^{2+} , especially in the presence of sufficient carbon source (Achnich et al., 1995). The formed Fe^{2+} may rapidly react with nitrite, an intermediate product of denitrification, to promote formation of NO

and N_2O (Brons et al., 1991). Thus, besides soil pH, cycling of Fe between oxidized and reduced states in the presence of NO_3^- also affected gaseous N formation, including N_2O . Ferrihydrite amendment promoted Fe^{2+} formation, resulting in higher N_2O production in both soils. This effect was augmented by a low soil pH to further decrease the overall NO_3^- reduction in the Texas soil.

Similarly, besides the less favorable pH conditions, higher levels of reducible Fe^{3+} also contributed to the later initiation of methanogenesis in the Texas soil slurries compared to the Louisiana soil slurries as evidenced by the suppression of methanogenesis in each soil upon ferrihydrite amendment. In the Louisiana soil, pH, Eh and timing of methanogenesis was slightly affected by ferrihydrite amendment, but the rate of CH_4 production was consistently reduced until day 23. Despite the rice straw amendment, increasing the availability of AEAs relative to available carbon sources by ferrihydrite amendment appeared to be important in reducing CH_4 production in the Louisiana soil. In contrast, ferrihydrite amendment in the Texas soil apparently delayed NO_3^- reduction and change of pH and Eh, resulting in a greater delay of methanogenesis in the Texas soil. Nevertheless, ferrihydrite amendment in the Texas soil changed the soil pH from 6.6 to 6.8 when methanogenesis peaked, a significant favorable pH change for methanogens (Wang et al., 1993a), resulting in the higher CH_4 peak.

The temporal changes in CO_2 production in the period during and soon after denitrification were mainly attributed to the need of initiation of fermenting activity for providing electron donors for the redox processes other than denitrification. Fermenting

bacteria were less competitive than denitrifiers (Dassonville et al., 2004). Nevertheless, according to the rate of CO₂ produced to that of NO₃⁻ reduced, fermenting activity was not completely suppressed by denitrification activity but was weakened to some extent by ferrihydrite amendment as evidenced by generally lowered CO₂ production during the denitrifying stage.

Despite the observed effects of Fe³⁺ reduction on the timing and rates of denitrification and methanogenesis, the interactions of Fe³⁺ reduction with denitrification and methanogenesis appeared to be underestimated in the homogenized soil slurries. Iron amendment could extend the duration of an intermediate Eh but did not change the specific Eh range (e.g., +150 to -150 mV) within which N₂O and CH₄ are produced at low rates (Yu and Patrick, 2003). This Eh range was largely occupied by iron-centered intermediate redox processes, implying that overlapping of Fe³⁺ reduction with denitrification and methanogenesis was small in the unidirectional succession of redox processes in the homogenized soil slurries. However, such overlapping in heterogeneous field conditions is observed through repeated recycling of Fe³⁺ (Frenzel et al., 1999; King and Garey, 1999; Ratering and Schnell, 2000). The effects of recycling of Fe³⁺-centered AEAs on NO₃⁻ reduction and methanogenesis in rice soils will be investigated under more realistic conditions in later chapters.

Conclusions

Microbial Fe³⁺ reduction as a major alternative electron accepting process affected denitrification and methanogenesis in anoxic soil slurries. An increase in reducible Fe³⁺

content by ferrihydrite amendment changed environmental conditions, specifically pH, Eh and AEA availability, to stimulate N₂O production and suppress CH₄ production. The increase in N₂O flux by ferrihydrite amendment was significant but temporary while the suppression of CH₄ production was extended for considerable periods. Ferrihydrite amendment had little effect on the Eh range within which both N₂O and CH₄ were produced at low rates.

CHAPTER 3

TEMPORAL AND VERTICAL VARIATIONS OF NITROUS OXIDE AND METHANE PRODUCTION AFFECTED BY IRON-CENTERED INTERMEDIATE REDOX PROCESSES IN A FLOODED SOIL

Introduction

Most soils undergo a change from oxic to anoxic conditions upon flooding. Oxygen is depleted soon except at and above the aerobic/anaerobic interface while the reduction of NO_3^- , Mn^{4+} , Fe^{3+} , SO_4^{2-} , and CO_2 occurs sequentially (Peters and Conrad, 1996) in the anaerobic layer. The sequential redox processes under field conditions normally overlap and interact to some extent, resulting in heterogeneous redox conditions in wetland soils (Gao et al., 2002).

Iron and manganese redox processes are two major intermediate redox processes in soils and sediments, and in general, iron redox chemistry is of central importance considering the abundance and the ability of redox cycling of iron relative to manganese (Horne, 1978; Stumm and Morgan, 1981). Iron-centered intermediate redox processes closely interact with many important processes such as NO_3^- reduction, metal mobilization/immobilization and methanogenesis in wetland and aquatic environments (Ratering and Conrad, 1998; Ratering and Schnell, 2001; Weber et al., 2001; Senn and Hemond, 2002). Nevertheless, in general these interactions have not been well established under field conditions due to the heterogeneous occurrence of cycling of Fe^{3+} -centered AEA's. In laboratory slurry studies, the succession of redox processes is normally unidirectional, from the oxic condition to the weakly reducing through

intermediately reducing to highly reducing conditions, and the multidirectional interactions are likely underestimated or obscured.

While cycling of Fe^{3+} -centered AEAs normally occurs around aerobic/anaerobic interfaces of wetland and aquatic environments, largely mediated by O_2 diffusion (Frenzel et al., 1999; Rysgaard et al., 2001), this thin redox zone may be expanded under some conditions. For example, low levels of soil organic carbon, the abundant presence of one or more types of AEAs (e.g. NO_3^- and Fe^{3+}), and low temperatures may all promote the expansion of iron redox cycling-centered intermediate redox zones in subsurface and surface anaerobic environments (Ludvigsen et al., 1998).

Specifically, since NO_3^- as an oxidant is soluble and readily reducible, NO_3^- diffusion is able to modify the redox profile to a greater extent than O_2 diffusion through stimulating heterotrophic denitrification and recycling of AEAs such as Mn^{4+} and Fe^{3+} (Klüber and Conrad, 1998). Consequently, iron-centered intermediate redox processes may be vertically expanded and temporally extended in the soil and their interactions with other processes may be studied.

Methanogenesis and denitrification are major redox processes in freshwater soils and sediments. Cycling of Fe^{3+} -centered intermediate AEAs around aerobic/anaerobic interfaces, together with organic carbon inputs from aquatic plants, largely controls temporal and spatial variations of methanogenesis in these environments. The influence of Fe^{3+} -centered AEAs is important to CH_4 flux in rice paddies and should be useful in estimating and predicting CH_4 flux (Matthews et al., 2000; van Bodegom et al., 2000).

Cycling of Fe^{3+} affects N_2O formation from NO_3^- reduction even in soil slurries (Chapter 2). Such interaction may be more intensive under field conditions. In this study we used NO_3^- diffusion to create temporal and vertical variations in availability of electron donors and recycling of Fe^{3+} -centered intermediate AEAs in a rice soil. The importance of cycling of Fe^{3+} -centered intermediate AEAs in temporal and vertical variations of N_2O and CH_4 production was evaluated.

Materials and Methods`

Soil Column Incubation

A rice soil, taken from a rice field at the Crowley Rice Experimental Station, Crowley, Louisiana, was used in the experiment. For the soil properties, see Table 2.1 in Chapter 2.

Plastic syringes (Monoject, 140 ml with catheter tip) were used for soil column incubations. The inner diameter and length of the syringe (not including the tip) were 3.7 and 15 cm, respectively. The syringe was loaded with 135 g soil (dry weight) and gently shaken to form a 10-cm soil column. Deionized water was added to the syringe to saturate the soil column. The soil column was continuously flooded with a 2-cm layer of overlying water. The syringe was incubated at 22°C in the dark. On day 17 of the incubation when the redox potential (Eh) profile in the soil column was relatively stable, the overlying water of the soil column was amended with 0 or 10.5 mg NO_3^- -N as potassium nitrate (equivalent to 0 or 35 mM NO_3^-). The unamended and amended soil columns were continuously incubated as long as 120 days, and Eh and production of N_2O ,

CH₄, and CO₂ with depth were measured at different times during the incubation.

Redox Profile before and after Nitrate Diffusion

Five soil columns were used for the measurement of the Eh profile before and after the NO₃⁻ amendment on day 17 until day 120 on various frequencies (daily to weekly). Platinum electrodes were installed along the sidewall of the syringe before incubation, corresponding to depths of 0.1, 0.5, 1.5, 2.5, 4, and 6 cm in the soil column. The electrodes were tested with quinhydrone-saturated standard pH solutions (Patrick et al., 1996). A calomel reference electrode was used during Eh measurement using a small tube containing a salt agar bridge immersed in the overlying water. The Eh was reported after correction against the standard H₂ electrode by adding 245 mV to the instrument reading.

Production of Nitrous Oxide, Methane and Carbon Dioxide

On days 15, 30, and 60 (and also on day 120 for the NO₃⁻-amended soil column) of the incubation, one set (n=3) of the control or NO₃⁻-amended soil columns was sectioned for measurement of the production of N₂O, CH₄, and CO₂ in the soil column. The top part of the syringe above the soil was cut off in a glove bag under a N₂ atmosphere. The overlying water was discarded and the soil layers of 0-1, 1-2, 3-4, or 6-7 cm was extruded by pushing up the syringe plunger into a 125-ml flask containing 50 ml deionized water, and an anoxic soil slurry was maintained with a magnetic stirrer under a N₂ atmosphere. Five ml of the soil slurry was transferred with a plastic syringe into a test tube (16 x125 mm) to which had been added 1 ml of either a 0 or 12 mM acetate solution, and the tube was capped with a rubber stopper under a N₂ atmosphere. One set of the tubes with no

acetate addition was treated with an injection of 2 ml H₂ per tube after taking 2 ml gas from the tube headspace. All the tubes were incubated at 22 °C under shaking (120 oscillations per min). Gas samples for the tubes without substrate addition were taken after a 2-h of incubation for determination of N₂O concentration. Gas samples from all tubes were taken after 11-12 h incubation for determination of CH₄ and CO₂ concentrations with a Tremetrics 9001 gas chromatograph (GC). The N₂O, CH₄, and CO₂ production rates were expressed as mass cumulated in the headspace per unit weight of soil within a unit time. The N₂O and CO₂ production rates were calculated with consideration of their dissolution in the soil slurry (see Chapter 2).

Data Analysis

Statistical analysis was conducted with SAS (SAS Institute Inc., 1999-2001). Student t test was used for comparing production of CH₄ and CO₂ the soil column between treatments, depths or dates. The statistical level of significance was chosen at $\alpha = 0.05$ for all statistical analysis.

Results

Redox Profile before and after Nitrate Diffusion

The soil column developed highly reducing conditions (i.e., < -200 mV) within a few days after flooding (Fig. 3.1). On day 10, even the Eh at the 0.1-cm depth reached around -200 mV. Influenced by NO₃⁻ diffusion on day 17, the Eh in the 0.5-4 cm layer quickly increased to and was maintained around 0 mV until day 35. Thereafter, the Eh above the 1.5-cm depth continued to rise to and remained in the weakly reducing range (i.e., 200 to

350 mV) until day 50 when the Eh at the 1.5-cm depth started to drift slowly to below -200 mV on day 120. The Eh at the 2.5- and 4-cm depths remained stable for a few days, and then decreased similarly to below -200 mV on day 60. Nitrate diffusion had little effect on the Eh at the 6.0-cm depth. Overall, the intermediately reducing zone (i.e., -100 to +150 mV) was rapidly expanded soon after NO_3^- diffusion, and clearly visible between days 20 and 50.

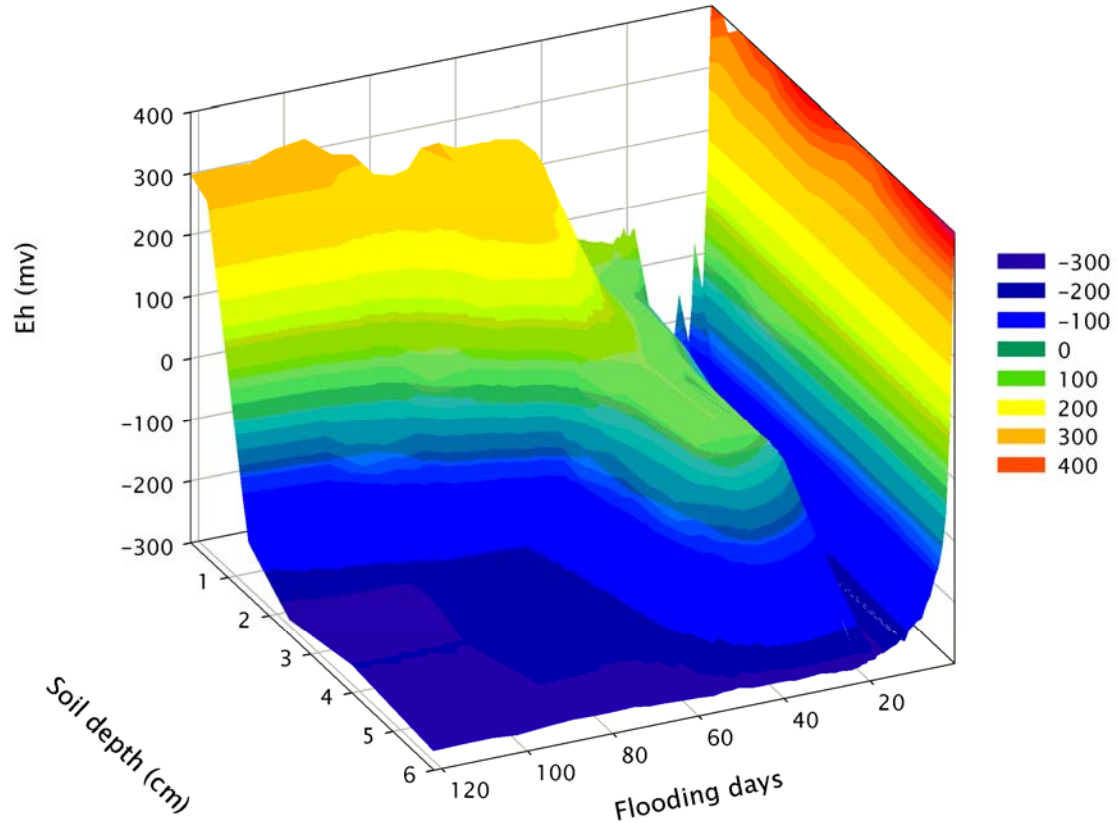


Fig.3.1 Eh profile (n=5) in the flooded soil before and after NO_3^- addition. Nitrate was amended as potassium nitrate at 35 mM to the overlying water (2 cm deep) on day 17 after flooding.

Production of Nitrous Oxide, Methane and Carbon Dioxide

Nitrous oxide was either not detectable or produced at rates less than $0.1 \text{ nmol g}^{-1} \text{ d}^{-1}$ in the control soil column on each date (Fig. 3.2). Nitrous oxide production rates higher than $1 \text{ nmol g}^{-1} \text{ d}^{-1}$ were found in the 0-1, 1-2, and 3-4 layers of the amended soil column on day 30, but were significantly lowered to around $0.1 \text{ nmol g}^{-1} \text{ d}^{-1}$ on Day 60.

Methane production in the control soil was low on day 15 but high on days 30 and 60 except in the 0-1 cm layer (Fig. 3.3). Methane production in each layer on day 30 was significantly higher than the corresponding layer on days 15 and 60 ($p < 0.05$). Under NO_3^- diffusion, CH_4 production in the 6-7 cm layer was only observable on day 30 but was comparably high as found in the same layer of the control soil on day 60. Methane production was negligible or not found in the other layers of the NO_3^- -diffusing soil on days 30 and 60, but became observable in the 1-2 cm layer, and relatively high in the 3-4 cm layer on day 120.

Hydrogen-induced CH_4 production in the control soil was low on day 15 but significant except in the 0-1 cm layer on day 30. On day 60, H_2 -induced CH_4 production was small in the 3-4 cm and 6-7 cm layers, while increased CH_4 production with the acetate addition was significant relative to the control in all layers. Acetate addition also stimulated more CH_4 production than by H_2 addition when considerable CH_4 production was found in the NO_3^- -diffusing soil, such as in the 6-7 cm layer on day 60, and in the 3-4 and 6-7 cm layers on day 120.

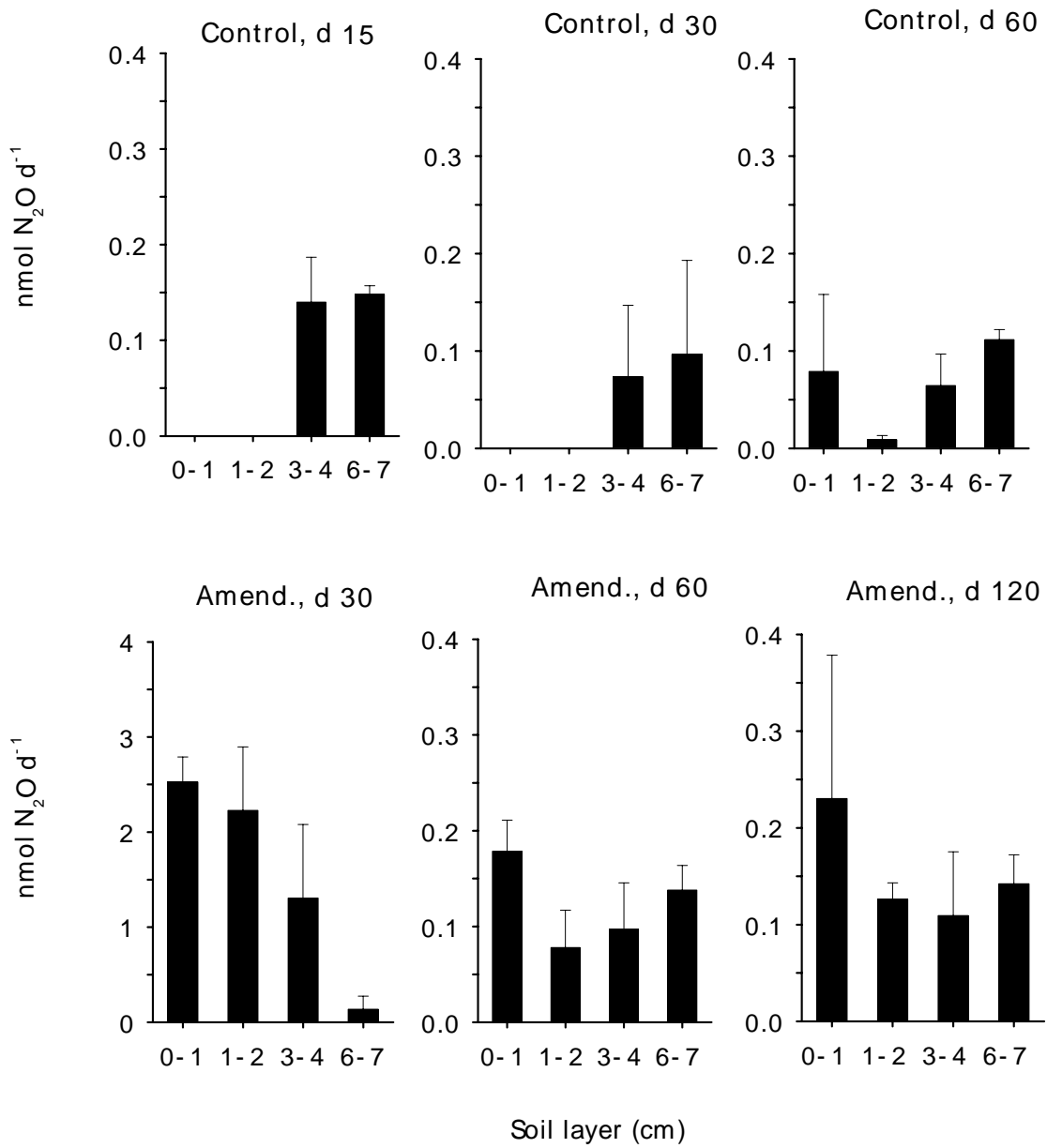


Fig. 3.2 Nitrous oxide production (mean \pm SE, n=3) in the control and NO₃⁻-diffusing soil columns. Nitrate was amended at 35 mM to the overlying water (2 cm deep) on day 17 after flooding.

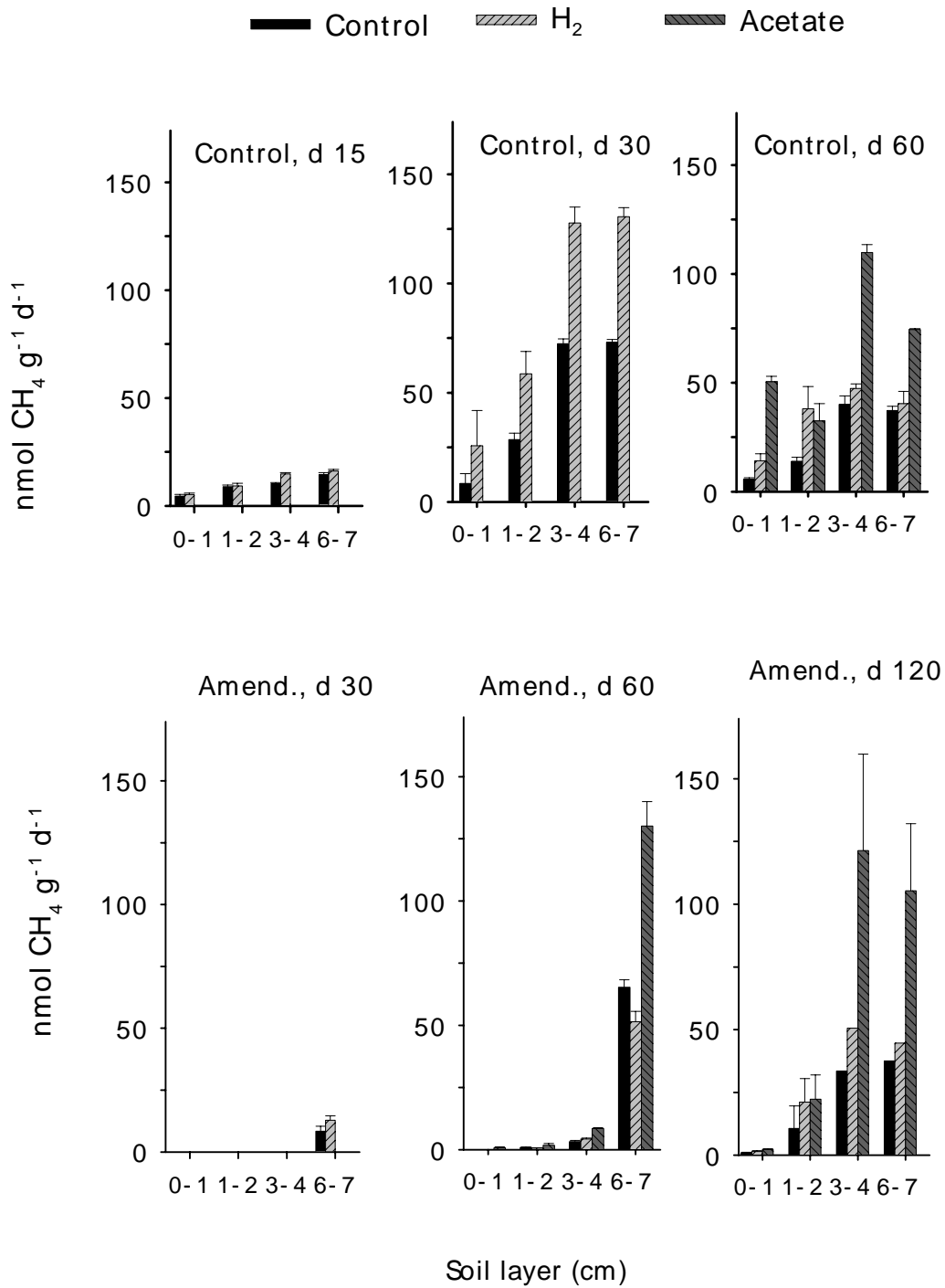


Fig. 3.3 Methane production (mean \pm SE, n=3) in the control and NO₃⁻-diffusing soil columns. Nitrate was amended at 35 mM to the overlying water (2 cm deep) on day 17 after flooding.

Carbon dioxide production in the two top layers was generally lower than in the two deep layers in the control soil on days 15 and 30, but became similar on day 60 (Fig. 3.4). Carbon dioxide production in the NO_3^- -diffusing soil was generally lower than in the control soil to the 7-cm depth on day 30 and to the 4-cm depth on day 60. In most cases, CO_2 production was little affected by H_2 addition, but was increased to some extent by acetate addition in both soil columns.

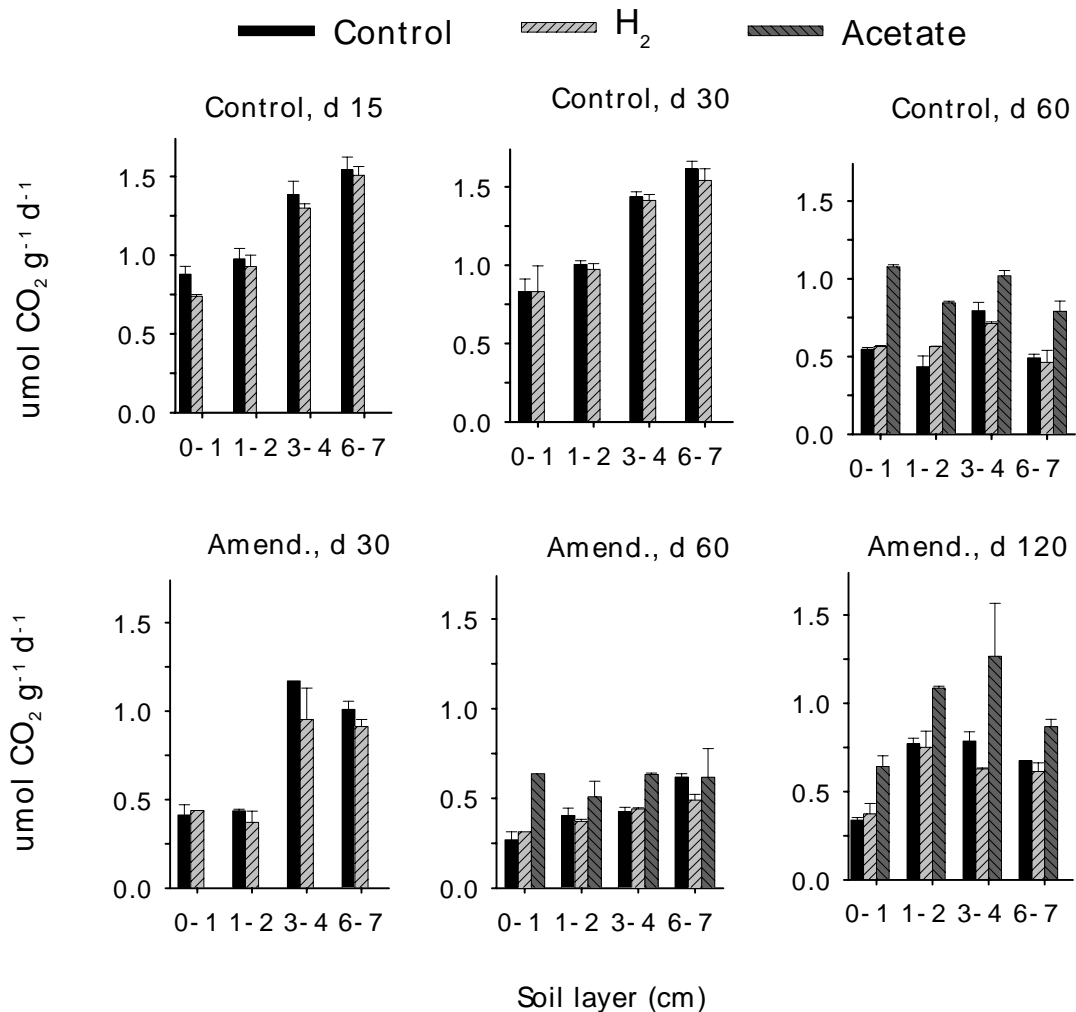


Fig. 3.4 Carbon dioxide production (mean \pm SE, n=3) in the control and NO_3^- -diffusing soil columns. Nitrate was amended at 35 mM to the overlying water (2 cm deep) on day 17 after flooding.

Nitrous Oxide and Methane Production in Different Redox Ranges

Relatively high rates of N_2O production at Eh around 0 mV (Fig. 3.5) were found in the amended soil column on day 30 (Fig. 3.2), while high rates of CH_4 production were only found at Eh less than -200 mV in the control and amended soil columns.

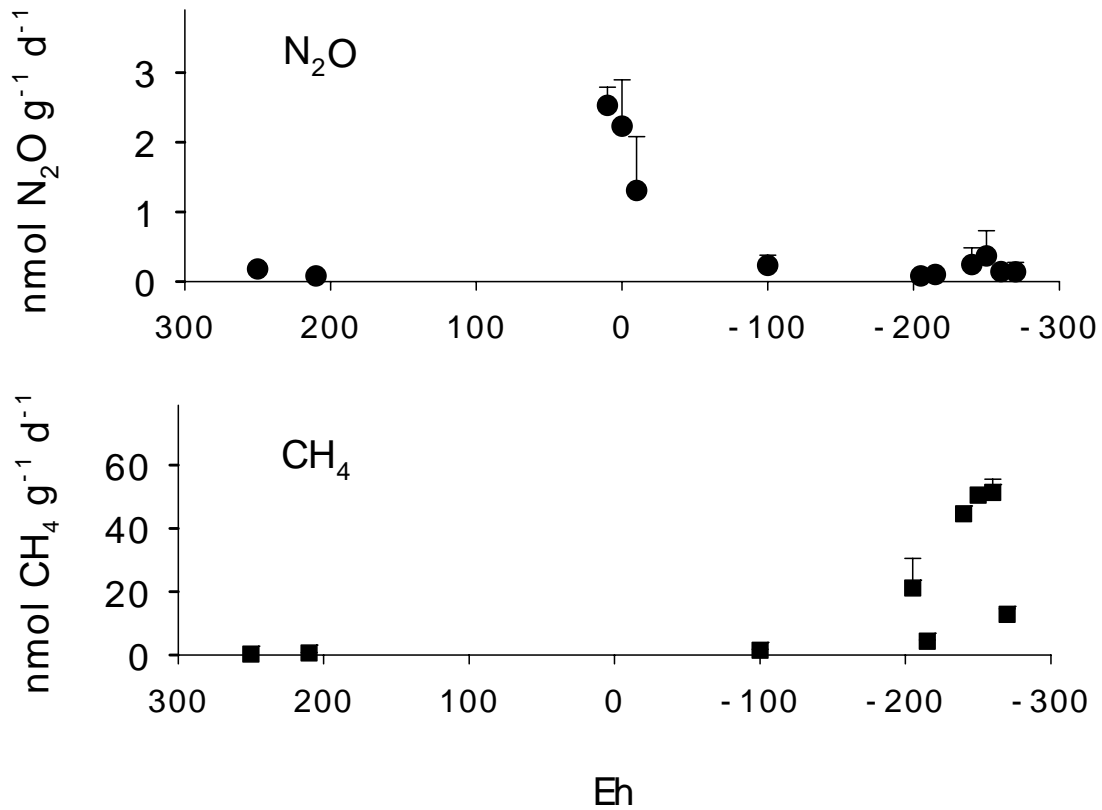


Fig. 3.5 N_2O and CH_4 production in different Eh ranges of the control and NO_3^- diffusing soil columns.

Discussion

Redox Conditions

The Eh profile before NO_3^- diffusion began on day 17 indicated the control soil quickly developed highly reducing conditions. Thereafter, the Eh profile for the control

soil was not separately measured. The aerobic/anaerobic interface of the control soil may move deeper under O₂ diffusion with time (Phillips et al., 1978), but would be above the 0.5-cm depth according to the Eh profile after NO₃⁻ diffusion and within the 0-1 cm layer since methanogenic activity was occurring in this layer on day 60. The soil layer below the 1-cm depth largely remained under highly reducing conditions through day 60.

Nitrate diffusion since day 17 temporally extended and spatially expanded the intermediate reducing zone in the soil column. Nitrate diffusion would first stimulate organic carbon consumption through heterotrophic denitrification and consequently lower carbon availability for all heterotrophic anaerobes in the soil column. This was evidenced by CO₂ production in the NO₃⁻-diffusing soil being generally lower than in the control soil to the 7-cm depth on day 30 and to the 4-cm depth on day 60.

Under increasing carbon limitation, NO₃⁻ diffused deeper to stimulate cycling of Fe³⁺-centered AEAs in the soil column. The Eh profile showed that O₂ could not reach below the 0.5-cm depth. The rapid Eh increase above the 4-cm depth after NO₃⁻ diffusion was primarily due to the regeneration of Fe³⁺-centered AEAs coupling to NO₃⁻ reduction (Klüber and Conrad, 1998). The regenerated AEAs may be microbially reduced again but would be repeatedly recycled under the continuing influence of NO₃⁻ diffusion, greatly expanding and extending the intermediately reducing zone in the NO₃⁻-diffusing soil. When such influence was weak or absent, the net reduction of Fe³⁺-centered AEAs occurred, as indicated by the restart of Eh decrease at the 2.5- and 4.0-cm depths since day 40 and at the 1.5-cm depth since day 55.

Nitrous Oxide Production

Due to the rapid depletion of NO_3^- in the soil soon after flooding, N_2O production was either undetectable or negligible in the control soil. Nitrate diffusion dramatically induced N_2O production at Eh around 0 mV above the 4-cm depth on day 30. High N_2O production around 0 mV was also observed under Eh-controlled slurries (Kralova et al., 1992). The Eh increase above the 4-cm depth during this period indicated Fe^{3+} -centered AEAs were under intensive regeneration. Such high N_2O production was not only ascribed to intensified denitrification, but also due to the effect on N_2O production of Fe^{2+} reoxidation (Chapter 2). While such intensive interaction of Fe^{2+} with NO_3^- reduction could not easily occur in soil slurries where redox processes were largely sequentially initiated (Chapter 2), it can be found in NO_3^- -polluted aquatic environments and even in the rhizosphere. Such interaction not only affect the fate of NO_3^- including N_2O production, but may also affect the stability of heavy metals associated with the consequently changed iron-centered intermediate redox processes (Senn et al., 2002).

Methanogenesis

While the soil layer below the 1-cm depth largely remained under highly reducing conditions through day 60 in the control soil, cycling of Fe^{3+} -centered AEAs, occurring around the anaerobic/anaerobic interface and largely mediated by O_2 diffusion (Frenzel et al., 1999; Rysgaard et al., 2001), was effective in lowering electron donor availability for anaerobic activity, especially methanogenic activity above the 2-cm depth.

The importance of electron donor availability in controlling anaerobic microbial activity was clearly revealed by substrate-induced CH₄ and CO₂ production at different times. In the early period (on day 15), H₂ addition did not significantly increase CH₄ production, mainly because methanogenic activity was weak while more competitive redox processes such as Fe³⁺ reduction may still continue. During the mid- and late-periods when methanogenic activity was sustained, the addition of substrate, especially acetate, significantly stimulated CH₄ formation, even below the 3-cm depth where strong methanogenesis was found. Thus, substrate availability would largely control the vertical and temporal variations of methanogenesis in these periods. Meanwhile, the addition of hydrogen or acetate may also stimulate other anaerobic microbial activity besides methanogenesis. Only H₂ induced microbial activity was not directly reflected by changes in CO₂ production. Acetate-stimulated CO₂ production, e.g. in the 0-1 and 1-2 cm layer on day 60, was not only derived from CO₂ produced in methanogenesis but also from anaerobic oxidation of acetate by other anaerobes such as Fe³⁺ reducers (Chidthaisong and Conrad, 2000).

Methanogenic activity in the NO₃⁻-diffusing soil was greatly lowered, not only due to increased carbon limitation and NO₃⁻ inhibition, but also due to the suppressing effect of regeneration of Fe³⁺-centered AEAs. While the regenerated AEAs under NO₃⁻ diffusion, such as Fe³⁺ as an inhibitor on methanogens, were not as strong as NO₃⁻ and its intermediate products NO and N₂O (Chidthaisong and Conrad, 2000), they were strong enough to suppress methanogenic activity under carbon-limiting conditions (Achthich et

al., 1995). The 0-1 cm layer remained under weakly reducing conditions due to the strong influence of NO_3^- diffusion and prevented methanogenesis until day 120. The 1-2 and 3-4 cm layers underwent intensified cycling of Fe^{3+} -centered AEAs under NO_3^- diffusion, resulting in substantial reduction of methanogenesis even after these layers had re-developed highly reducing conditions. These intensified intermediate redox processes even effectively lowered the availability of electron donors in the highly reducing 6-7 cm layer, resulting in significant reduction of methanogenesis for at least 13 days.

The effect of cycling of Fe^{3+} -centered AEAs on methanogenesis observed in both control and NO_3^- -diffusing soil columns shows the need of incorporating the role of cycling of Fe^{3+} -centered AEAs in prediction of methanogenesis in wetland soils. The consideration of cycling of Fe^{3+} -centered AEAs is still preliminary in recent model studies on prediction of CH_4 emission in rice paddies (Matthews et al., 2000).

Conclusions

Cycling of Fe^{3+} -centered AEAs in the control flooded soil, primarily regulated by the diffusion of O_2 , was restricted around the aerobic/anaerobic interface but effectively suppressed methanogenesis above the 2-cm depth. Their cycling was vertically expanded and temporally extended under NO_3^- diffusion. Subsequently, primarily through lowering carbon source availability to methanogens, the intensified cycling of Fe^{3+} -centered AEAs resulted in significant suppression of methanogenesis to deeper depths for longer periods. Nitrous oxide production in the control soil was negligible due to NO_3^- depletion. High N_2O production occurred even at Eh around 0 mV under NO_3^- diffusion, partly due to

intensive interaction of Fe^{2+} reoxidation with NO_3^- reduction. The effect of cycling of Fe^{3+} -centered AEAs on the temporal and vertical variations of N_2O and CH_4 production in the flooded soil shows the importance of cycling of Fe^{3+} -centered AEAs in evaluation and prediction of N_2O and CH_4 formation in wetland and aquatic environments.

CHAPTER 4

FATE OF NITRATE IN A FLOODED RICE SOIL AFFECTED BY POOL OF ALTERNATIVE ELECTRON ACCEPTORS

Introduction

Increasing N fertilizer applications to agricultural lands, including rice fields, causes serious NO_3^- pollution in surface and subsurface water bodies (Aulakh and Singh, 1997; Ghosh, 1998). Natural and constructed wetlands are a sink for NO_3^- from waters (Hill, 1996; Reilly et al., 2000; Rutherford and Nguyen, 2004). In wetland environments, the biologically active zone is the functioning zone largely responsible for NO_3^- transformation. Pools of organic carbon sources and oxidative minerals and water movement in this functioning zone are major factors determining the ability of wetlands to remove NO_3^- from waters. The availability of organic carbon sources is important in influencing the rate of NO_3^- transformation (Weisner et al., 1994; Ingersoll and Baker, 1998; Hill and Cardaci, 2004) while water movement controls the contact time for NO_3^- transformation in the functioning zone. Highly reducing conditions may be formed in part of the functioning zone with organic carbon inputs from aquatic plants. A deep highly reducing zone is able to efficiently remove NO_3^- (Kralova et al., 1992) within a range of water residence times.

Nevertheless, the importance of the pool of oxidative minerals in NO_3^- removal by wetlands should also be appreciated, especially when wetlands are seasonally and intermittently flooded or the overlying water is under intensive fluctuation (e.g. riparian

wetlands and rice fields). The capacity of the functioning zone for NO_3^- removal under such conditions may be lowered due to intensive aerobic oxidation, lack of organic carbon inputs and intensive generation and regeneration of Fe^{3+} -centered AEAAs. Simultaneous biological removal of NO_3^- in the process of NO_3^- transport may restrict NO_3^- availability within certain parts of the functioning zone for certain periods while microbial reduction of other AEAAs such as Fe^{3+} and Mn^{4+} may occur in other parts of the functioning zone. Substantial microbial reduction of these AEAAs may lower the availability of carbon sources to heterotrophic denitrification in the functioning zone to promote N_2O formation and NO_3^- transport. As a result, additional N_2O emission from and NO_3^- transport through the functioning zone may occur (Staver and Brinsfield, 1996; Zhu et al., 2000; Dowling et al., 2004).

Oxidized manganese and iron minerals are a major component of the pool of AEAAs in most freshwater wetlands (Yao et al., 1996). While the impact of microbial Fe^{3+} reduction on denitrification, including N_2O production, was observed in the soil slurry experiment (Chapter 2), the significance of this impact under field conditions is yet to be carefully evaluated. As a closer step to more realistic conditions, in this study we used flooded soil columns to study the effects of amendments of oxidative iron and manganese oxides on fate of NO_3^- in a rice soil with and without percolation.

Materials and Methods`

Soil Column Incubation

A Crowley silt loam soil was used in the experiment (Chapter 2). Polyvinyl Chloride (PVC) columns (5.5 x 25 cm), with one end capped, were used for the flooded soil column incubations. One set (n=3) of the PVC columns were used for the non-percolation experiment and another set (n=3), being installed with a 2-cm layer of glass wool and quartz sand at the bottom and an outlet close to the bottom, were used for the percolation experiment. Four hundred g of air-dried soil was mixed with or without MnO₂ and Fe₂O₃ powder (2.7 and 5.0 mg g⁻¹, respectively), and packed into each PVC column to form a 12-cm soil column. The soil columns were flooded with deionized water to form a 4.5-cm layer of overlying water and incubated at 22°C in the dark. Potassium nitrate (0.1 g NO₃⁻-N) was amended to the overlying water (equivalent 70 mM NO₃⁻) of the column 1 day after flooding, and then 10 ml percolate was collected daily by a syringe connected to the bottom of the soil column (equivalent to a moderate percolation rate of 4.2 mm d⁻¹ in irrigated rice fields) and frozen until NO₃⁻ content measurement. Deionized water was added daily after percolate collection to maintain the depth of the overlying water at 4.5 cm for the percolated and unpercolated soil columns. The collection of percolates stopped at week 7 when the NO₃⁻ content in the percolate was found to be below detection at week 6. The incubation of the unpercolated soil columns continued until week 16 for the measurement of NO₃⁻ content remaining in the overlying water.

Redox Profile

The Eh profile in the 0-10 cm layer of the soil column was measured at 0.5-cm (near surface) or 1.0 cm increments at weeks 2, 4, 6 and 8 after NO_3^- addition with a platinum electrode modified from that described by Patrick et al. (1996). Each time before measurement, the platinum electrode was tested using quinhydrone-saturated standard pH solutions (Patrick et al., 1996). A silver chloride reference electrode was used during Eh measurement. The Eh readings were recorded 15 min after the electrode reached a specific depth, and reported after correction against the standard H_2 electrode by adding 200 mV.

Nitrous Oxide Flux

Nitrous oxide fluxes in the soil columns ($n=2$) were measured on days 1, 3, 7, 14, 28, and 42 after NO_3^- addition. After flushing with ambient air, the column was capped with a rubber stopper which was fitted with a septum. Gas samples of 10 ml were taken from the headspace at 0 and 30 min after closing and analyzed with a Tometrics 9001 gas chromatograph using an electron capture detector (ECD) for determining N_2O concentrations.

Nitrate Content in Percolate and in Overlying Water

The frozen percolate samples were thawed at 4°C. The samples collected from the column ($n=2$) in each week were combined as one. Five ml of the combined percolate was filtered and stabilized with 5 ml buffer solution (APHA, 1995) for NO_3^- content determination with an ion meter and a NO_3^- -selective electrode (detection limit 7×10^{-6}

M). Five ml of water was directly sampled from the overlying water at week 6 for the percolated soil columns and at the end of the incubation (week 16) for the unpercolated soil columns and stabilized with 5 ml buffer solution for NO_3^- content determination.

Data Analysis

Statistical analysis was conducted with SAS (SAS Institute Inc., 1999-2001).

Variations in N_2O emission and NO_3^- percolation between treatments were analyzed with one-way analysis of variance. The statistical level of significance was chosen at $\alpha = 0.05$ for all statistical analysis.

Results

Redox Profile

Different redox profiles were observed in the control and amended soil columns (Figure 4.1). The control soil developed intermediate reducing conditions at the 1-7 cm depths at week 2 and weakly reducing conditions from week 4 to 8. The amended treatment maintained weakly to intermediately reducing conditions at the 1-7 cm depths from week 2 to 8. The redox discontinuity (a rapid change in Eh slope before and after a depth) in the control soil, occurring around the 7-cm depth at week 8, was sharper than in the amended soil. Percolation greatly flattened the redox profiles in both treatments in the first 6 weeks. At week 8 the redox discontinuity zone was moved to the 5-cm depth in the control treatment and the 4-cm depth in the amended treatment.

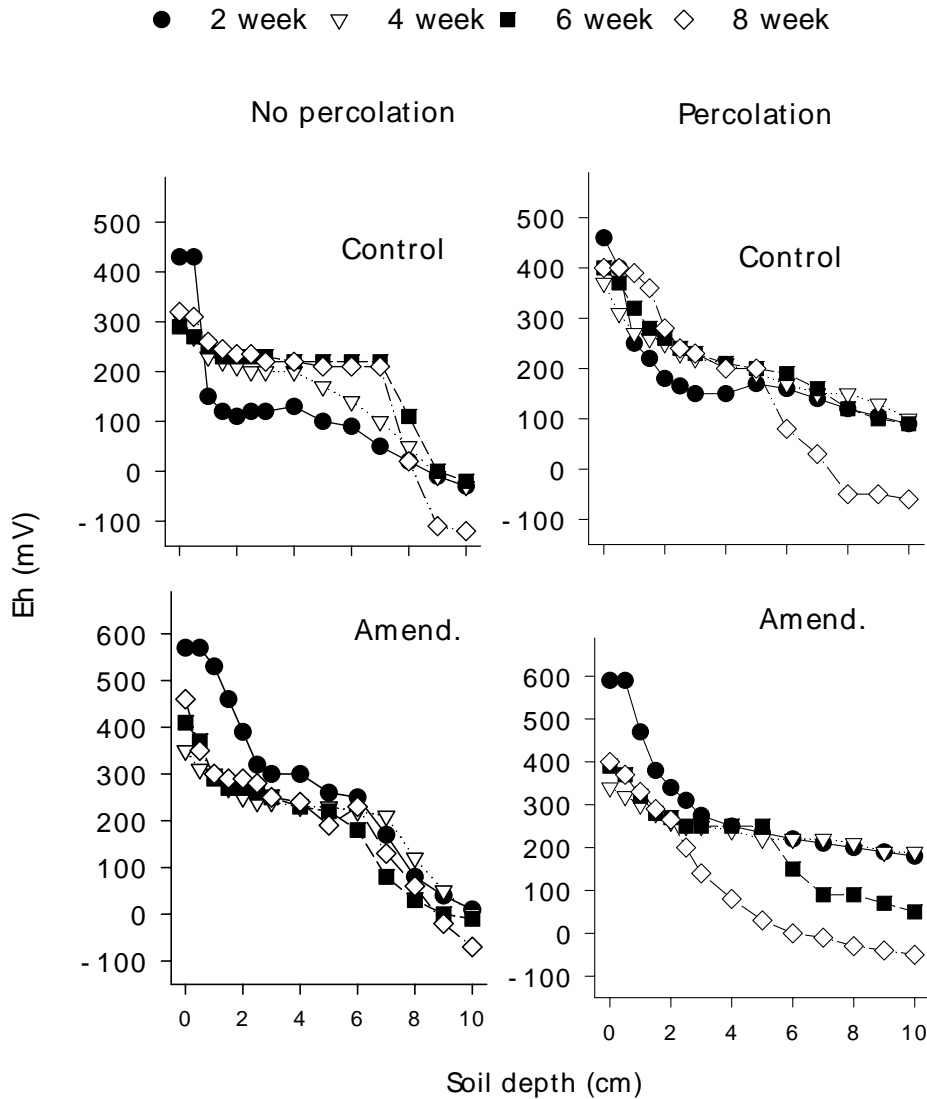


Fig. 4.1 Redox profiles in the flooded soil columns affected by amendments of Fe^{3+} and Mn^{4+} and percolation. The soil columns were amended with potassium nitrate ($0.1 \text{ g NO}_3^- \text{-N}$) to the 4.5-cm overlying water (equivalent to 70 mM NO_3^-) 1 day after flooding, with or without amendments of MnO_2 and Fe_2O_3 powder before flooding (2.7 and 5.0 mg g^{-1} , respectively), and percolated at 0 or 4.2 mm d^{-1} .

Nitrous Oxide Emission

Without percolation, N_2O flux was higher in the amended treatment than in the control treatment on each date until day 42 when N_2O emission measurement stopped (Fig. 4.2). Percolation significantly reduced N_2O emissions in both treatments. The N_2O

emission in the 42-day measurement was significantly increased by amendments of Mn^{4+} and Fe^{3+} in both unpercolated and percolated soil columns ($p < 0.05$) (Table 4.1).

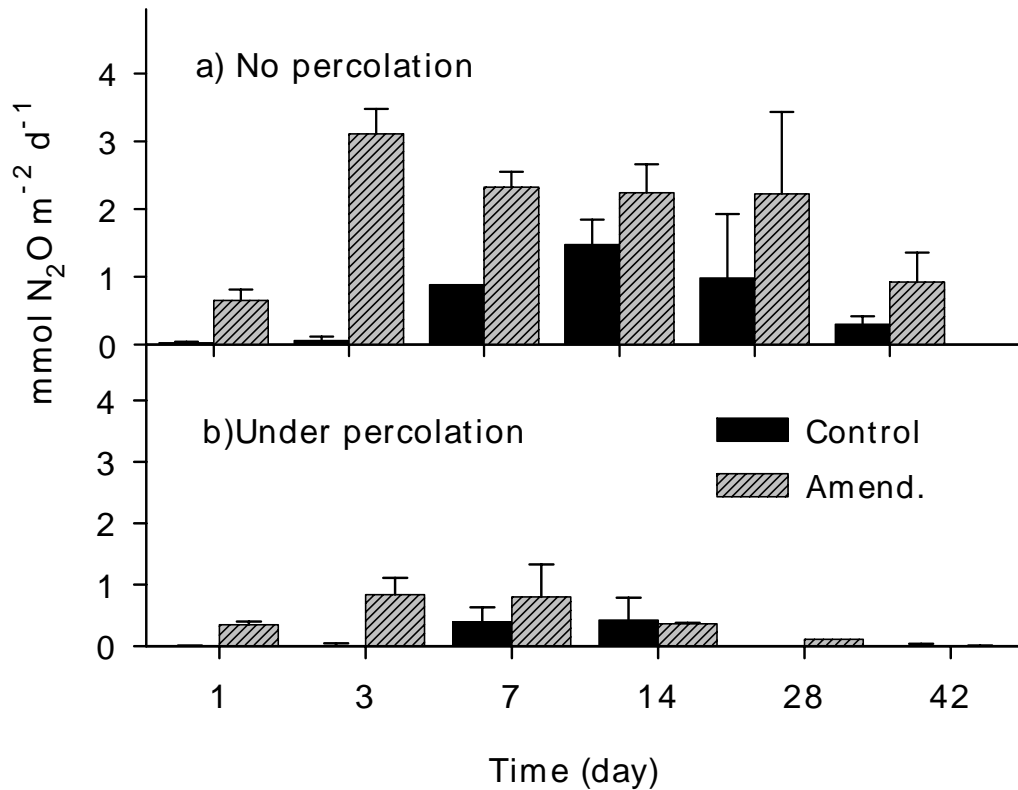


Fig. 4.2 Fluxes of N_2O in the flooded soil columns affected by amendments of Mn^{4+} and Fe^{3+} and percolation (mean \pm SE, $n=2$). For explanation, see Fig. 4.1.

Nitrate Transformation and Transport

Under no percolation, the levels of NO_3^- remaining in the overlying water at week 16 were 12.0 mM in the control treatment and 22.8 mM in the amended treatment, accounting for 17.2 and 32.5% of NO_3^- applied, respectively. Under percolation, the levels of NO_3^- remaining in the overlying water at week 6 were 0.7 and 0.9 mM in the control and amended treatments, respectively. Nitrate percolation in the control treatment

compared to the amended treatment was significantly lower ($p < 0.01$) and started 1 week later (Fig. 4.3). Nitrate removal by percolation mainly occurred between weeks 3 to 5 in both treatments. The 6-week cumulative amounts of NO_3^- percolated were 10.9 and 24.3% of NO_3^- applied in the control and amended treatments, respectively. Without considering the amounts of NO_3^- remaining within the flooded soils, the averaged rates of NO_3^- transformation were higher in both treatments under percolation (Table 4.1).

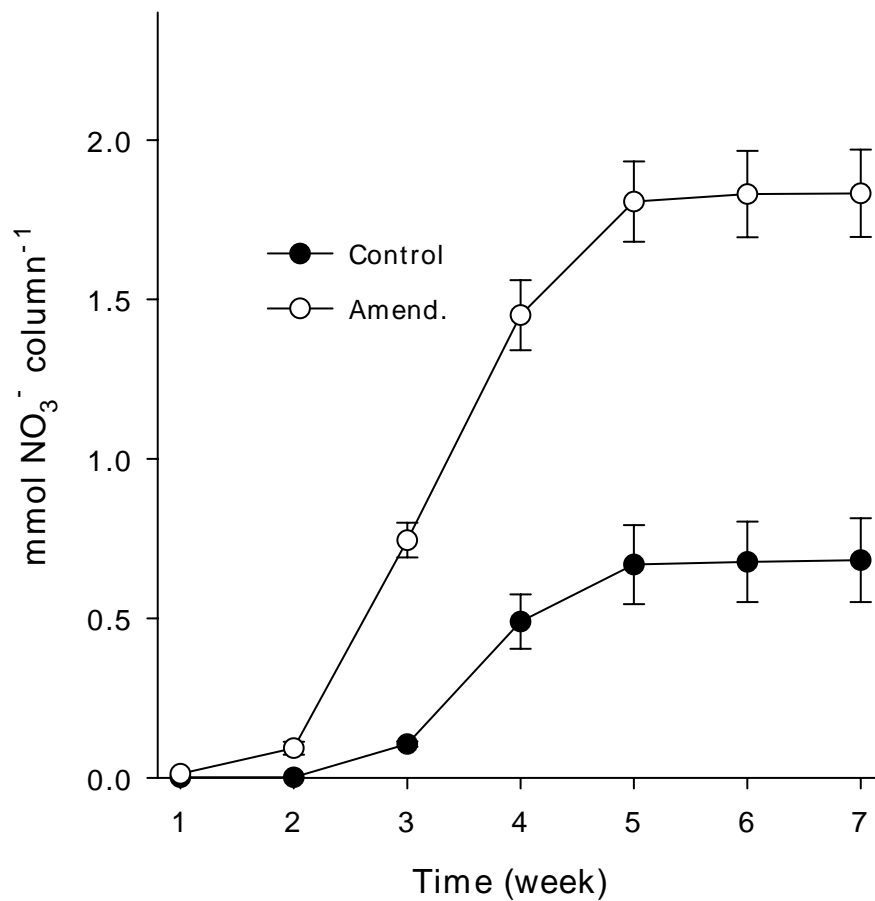


Fig. 4.3 Cumulative NO_3^- percolation from the flooded soil column affected by amendments of Mn^{4+} and Fe^{3+} . For explanation, see Fig. 4.1.

Table 4.1 Fate of NO_3^- in the flooded soil as affected by amendments of Mn^{4+} and Fe^{3+} . The soil columns were amended with potassium nitrate ($0.1 \text{ g NO}_3^- \text{-N}$) to the 4.5-cm overlying water (equivalent to 70 mM NO_3^-) 1 day after flooding, with or without amendments of MnO_2 and Fe_2O_3 powder before flooding (2.7 and 5.0 mg g^{-1} , respectively), and percolated at 0 or 4.2 mm d^{-1} .

Percolation (mm d^{-1})	Treatment	Duration (d)	NO_3^- removal ($\text{mmol m}^{-2} \text{ d}^{-1}$)	NO_3^- percolated ($\text{mmol m}^{-2} \text{ d}^{-1}$)	N_2O emission ($\text{mmol m}^{-2} \text{ d}^{-1}$)
0	Control	112	23.3	-	0.87
0	Amend.	112	19.0	-	2.01
4.2	Control	42	66.1	8.1	0.16
4.2	Amend.	42	55.8	18.2	0.30

Discussion

Oxygen in the soil columns was depleted soon after flooding. Oxygen diffusion might only have a direct effect on microbial activity in the surface layer of the soil columns even under percolation according to the redox profile. The size and composition of the pool of AEAs became important in determination of the succession of anaerobic redox processes in the flooded soil columns. The amendments of Fe^{3+} and Mn^{4+} increased the pool of AEAs and consequently changed redox conditions and NO_3^- fate in the soil columns.

Redox Profile

The development of redox conditions at a specific depth in the soil columns was microbially mediated and controlled by the availability of AEAs relative to electron donors. The NO_3^- diffusion plume stimulated heterotrophic denitrification increasing carbon source limitation (see Chapter 4). The downward diffusion of NO_3^- also promoted

the regeneration of AEAs, increasing Eh from week 2 to 4 at the 2-7 cm depths in the control soil column. The amendments of iron and manganese oxides, increasing the pool of AEAs, further increased carbon limitation for denitrification and greatly delayed Eh changes at the 1-8 cm depths from week 2 to 8. Under percolation, NO_3^- was transported much deeper, resulting in greatly flattened redox profiles in both treatments in the first 6 weeks.

Fate of Nitrate

Soil physical properties such as texture are important in controlling NO_3^- transport in flooded soils. Nevertheless, in the same soil and under a specific percolation rate, the availability of electron donors and Fe^{3+} -centered intermediate AEAs largely controlled the ability of the soil to transform (mainly denitrify) NO_3^- and NO_3^- transport by diffusion and percolation. Dissimilatory reduction of NO_3^- to ammonium, occurring when electron donor availability is high (King and Nedwell, 1985), was not expected to be an important pathway for NO_3^- transformation in this experiment.

Indigenous soil NO_3^- was depleted soon after flooding in these incubations. Nitrate transport from the overlying water to the soil should not affect the reduction of Mn^{4+} , Fe^{3+} , SO_4^{2-} , and/or CH_4 formation in the deep soil layer immediately. So, before NO_3^- reached a deeper depth, some readily available carbon sources had been consumed by Fe^{3+} reducers, etc. The amount of available carbon at a specific soil depth consumed by anaerobes other than heterotrophic denitrifiers was dependent on the time needed for NO_3^- to reach that depth and the availability of other AEAs such as Mn^{4+} and Fe^{3+} at that

depth. With greater time and more AEAs, more available carbon would be consumed to limit heterotrophic denitrification and promote N_2O emission and NO_3^- transport.

Under no percolation, NO_3^- still remained in the overlying water at week 16. Passive NO_3^- diffusion appeared to be slow and could not reach below the 8-cm depth according to the redox profile while the upper soil layer (e.g. 0-2 cm depths) underwent continuous NO_3^- transformation with N_2O being emitted as the end product at high rates under increasing carbon limitation (Benckiser et al., 1996). The amendments of Mn^{4+} and Fe^{3+} , increasing the pool of AEAs, further increased the limitation of carbon source to heterotrophic denitrifiers. While the amendments of Mn^{4+} and Fe^{3+} were expected to intensify redox cycling of Mn^{4+} and Fe^{3+} to promote chemical and/or chemo-autotrophic NO_3^- reduction, this effect appeared to be less efficient than heterotrophic denitrification, decreasing NO_3^- transformation overall to promote more N_2O emission.

The effect of amendments of Fe^{3+} and Mn^{4+} on NO_3^- transformation was also evidenced in the percolated soil column. Although the redox profiles were quite similar in the first 6 weeks and microbial reduction of Fe^{3+} -centered AEAs would be suppressed in the whole soil columns of both treatments from week 3 to 5 when NO_3^- percolation mainly occurred, the amendments of iron and manganese oxides were still effective in lowering NO_3^- transformation while increasing NO_3^- percolation. The significant reductions in N_2O emission under percolation in both treatments were ascribed to high solubility of N_2O in water (Handbook of Chemistry and Physics, 1991).

It should be noted that while percolation increased the risk of NO_3^- transport to deeper groundwater, it also promoted NO_3^- removal in both control and amended soil columns through promoting deeper transport of NO_3^- which increased the effectiveness of the flooded soil column for NO_3^- removal. While it is possible that strong percolation decreases NO_3^- removal through dramatically shortening the residence time, it is more likely that percolation at slow to moderate rates increases the efficiency of the functioning zone to remove NO_3^- under field conditions. Besides the pool of organic carbon sources (Weisner et al., 1994; Ingersoll and Baker, 1998; Hill and Cardaci, 2004), the availability of AEA's other than NO_3^- and water movement need to be considered with the processes of groundwater discharge and recharge for NO_3^- removal by wetlands, specifically under seasonal and intermittent flooding conditions. Such considerations have been limited in evaluating NO_3^- removal by treatment wetlands and NO_3^- leaching/percolation in rice paddies (Zhu et al., 2000). More studies are needed on the interrelations between pools of carbon sources and AEA's and water movement in NO_3^- removal and N_2O emission in treatment wetlands.

Conclusions

The flooded soil column was a strong sink for NO_3^- but also a strong source of N_2O under simulated conditions of elevated nitrate in the overlying water. Simultaneous biological NO_3^- removal during the slow process of NO_3^- passive diffusion restricted NO_3^- within the upper part of the soil column. Percolation at a moderate rate resulted in the occurrence of NO_3^- percolation but also promoted NO_3^- removal and lessened N_2O

emission, through promoting deep transport of NO_3^- to increase the effectiveness of the soil column for NO_3^- removal.

An increase in pool of AEAs through amendments of iron and manganese oxides increased the limitation of carbon sources, especially in the deep layer, to heterotrophic denitrification and hence lowered NO_3^- transformation, but increasing AEAs promoted N_2O formation and NO_3^- transport. Besides the availability of organic carbon sources, the pool of AEAs and percolation (more generally water movement) need to be considered to predict NO_3^- removal and N_2O emission in wetlands, especially under seasonal and intermittent flooding conditions.

CHAPTER 5

MICROBIAL ACTIVITY IN DIFFERENT REDOX ZONES OF A FLOODED SOIL

Introduction

Most redox processes in anaerobic environments are microbially mediated.

Assessment of microbial activity under different redox conditions is important in understanding of biogeochemical cycling of natural and pollutant substances in anaerobic environments. Redox gradients formed in anaerobic environments are normally too steep, from aerobic to weakly reducing through intermediately reducing to highly reducing conditions within a distance from a few millimeters to a few centimeters, to carry out in situ measurements of microbial activity relating to specific redox processes under specific redox conditions. It is even more difficult to estimate microbial activity in iron cycling-centered intermediately reducing zones due to their normally transitional occurrence in many anaerobic environments. In a few environments, e.g., subsurface soils, intermediately reducing zones are found as long flat redox profiles, due to an excess of iron-centered intermediate AEAs relative to electron donors (Ludvigsen et al., 1998). Flat redox profiles may be also formed in surface soils under certain conditions. Low levels of soil organic carbon, abundant presence of one or more types of alternative electron acceptors (e.g. NO_3^- and Fe^{3+}) and low temperatures may all create flat redox profiles in wetland and aquatic environments.

The redox potential-controlled soil slurry system (Patrick et al., 1973) has been

intensively used to study redox processes under different redox conditions, including the intermediately reducing condition. The redox potential is controlled by adjustment of the availability of electron donors (e.g. glucose) and electron acceptors, normally O₂, which is toxic to anaerobes, especially obligate anaerobes (Roy et al., 1997). Redox processes have also been studied along redox gradients in anoxic soils (Ludvigsen et al., 1998), although redox conditions are rarely controlled. It is possible to study microbial activity in different redox zones of a flooded soil after the redox profile is flattened using some approach, e.g., NO₃⁻ diffusion (Chapter 3).

Multiple functional groups of microorganisms, e.g., denitrifiers, Fe³⁺ reducers and methanogens are involved in microbial activity in anaerobic soils. The relative importance of these functional groups varies temporally and spatially under different redox conditions. For example, denitrification activity may dominate in a weakly reducing condition (Dassonville et al., 2004) while fermenting bacteria and methanogens are important in the methanogenic condition (Conrad, 1999). This difference needs to be considered when a specific method for microbial activity estimation is adopted.

Compared to measurements of ATP, microbial biomass, etc (Inubushi et al., 1991; Karl, 1993), measurement of glucose- or acetate-induced respiration is more widely used for microbial activity estimation in anaerobic soils. Nevertheless, their applications in different redox zones dominated by specific redox processes are rarely compared. In this study, respiration induced by acetate and glucose was comparatively evaluated for microbial activity estimation in different redox zones of a rice soil formed with and

without NO_3^- diffusion. Oxygen-induced respiration was also measured to reflect the availability of carbon sources for microbial activity in the different redox zones.

Materials and Methods

Soil Column Incubation

A silt loam soil was used in the experiment (see Chapter 2). PVC columns (5.5 x 25 cm), with one end capped, were used for the flooded soil column incubations. Four hundred g of dry soil (<1mm) was packed into each PVC column to form a 12-cm soil column. Deionized water was added to the soil columns to achieve saturation and maintain a 4.5-cm layer of overlying water. The flooded soil columns were incubated at 22°C in the dark. Potassium nitrate was amended at 0 or 70 mM (n=2) into the overlying water of the column 1 day after flooding

Redox Profile

The Eh profile in the 0-10 cm layer was measured at a step of 0.5 cm (or 1.0 cm at greater depths) at week 16 for the control soil column and at weeks 8, 12, and 16 for the amended soil column with a platinum electrode as described in Chapter 4.

Microbial Activity in Different Redox Zones

When a stable flat redox profile was formed in the NO_3^- -diffusing zone at week 16, soil layers of 0-1, 5-6 and 9-10 cm from the control and NO_3^- -diffusing soil columns were sectioned in a glove bag under a N_2 atmosphere and transferred into 125 ml flasks containing 80 ml deionized water where a soil slurry was maintained with a magnetic stirrer under a N_2 atmosphere. Four ml of the soil slurry was transferred to a test tube (16

mm x 125 mm) to which had been added 1 ml solution containing: 1) no substrate, 2) 10 mM acetate, or 3) 25 mM glucose. The tube was capped under a N₂ atmosphere and incubated at 22°C with shaking (120 oscillations per min). The incubation time was 4-5 h to also consider the response of fermenting bacteria in the different redox zones. Another set of the test tubes with no substrate addition was aerobically incubated under the above condition. Carbon dioxide and CH₄ concentrations in the headspace of the tube were analyzed (See Chapter 2) for determination of CO₂ and CH₄ production rates expressed as nmol g⁻¹ h⁻¹. The CO₂ production rate was calculated with consideration of CO₂ dissolution in the soil slurry. In addition, pH and NO₃⁻ content in the soil slurry prepared from each layer were also measured. Five ml of filtered water (< 0.4 μm) from the soil slurry was analyzed for NO₃⁻ content with a NO₃⁻-selective electrode (detection limit 7 x 10⁻⁶ M) (APHA, 1995).

Data Analysis

The student t test was used for comparing production of CH₄ and CO₂ as affected by redox zones and substrate addition. Bivariate correlation was performed for establishing the relationship among substrate-induced CO₂ and CH₄ production in all redox zones.

Results

Redox Profile

The Eh profiles were stable in the control and NO₃⁻-diffusing soil columns at week 16 (Fig. 5.1). In the control soil, the redoxcline (redox discontinuity zone from oxic to anoxic) was observed within the 0-2 cm layer and the deeper soil was under highly

reducing conditions. The NO_3^- diffusion plume flattened the Eh profile in the 0-7 cm layer with the Eh slope being $< 50 \text{ mV cm}^{-1}$ and a redox discontinuity zone was formed at the 7- to 8-cm depth. Based on the Eh profile and with consideration of pH and NO_3^- availability in the sectioned layers (Table 5.1), the soil layers sectioned from the control and NO_3^- -diffusing soil columns were categorized into four redox zones: the 0-1 cm layer of the control soil was the whole-redox-range zone; the 0-1 cm layer of the NO_3^- -amended soil was the weakly reducing zone; the 5-6 cm layer of the NO_3^- -diffusing soil was the intermediately reducing zone; the 9-10 cm layer of the NO_3^- -diffusing soil and the 5-6 and 9-10 cm layers of the control soil were the highly reducing zones.

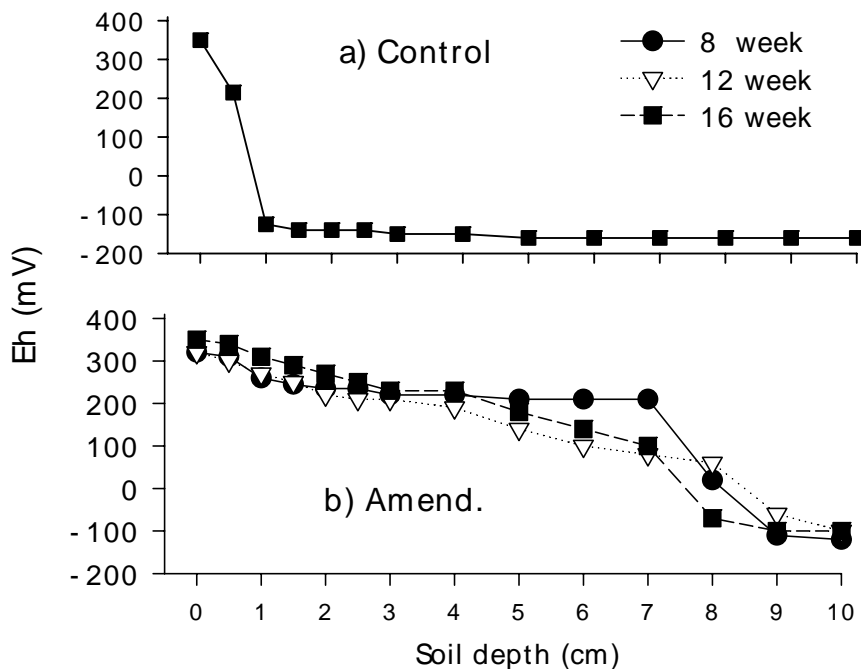


Fig. 5.1 Redox profiles of the control and NO_3^- -diffusing soil columns

Table 5.1 Values of pH and NO₃⁻ contents in the sectioned soil layers at week 16.

Soil layer (cm)	pH		NO ₃ ⁻ (μmol g ⁻¹)	
	Control	Amend.	Control	Amend.
0-1	8.0	8.1	ND†	0.34 ± 0.3
5-6	7.7	7.8	-	ND
9-10	7.7	7.7	-	ND

†: Not detectable.

Carbon Dioxide and Methane Production

After the long-term incubation and without external amendment of carbon sources, CO₂ production in the whole-redox-range zone was slightly lower than in the weakly reducing zone but much lower than in the other redox zones (Fig. 5.2). Methane production was negligible in the weakly and intermediately reducing zones, and relatively small in the whole-redox-range zone, but high in all highly reducing zones (Fig. 5.3). Differences in CH₄ production among the highly reducing zones were also observed, with the rates from the control soil being even higher than that from the NO₃⁻-diffusing soil.

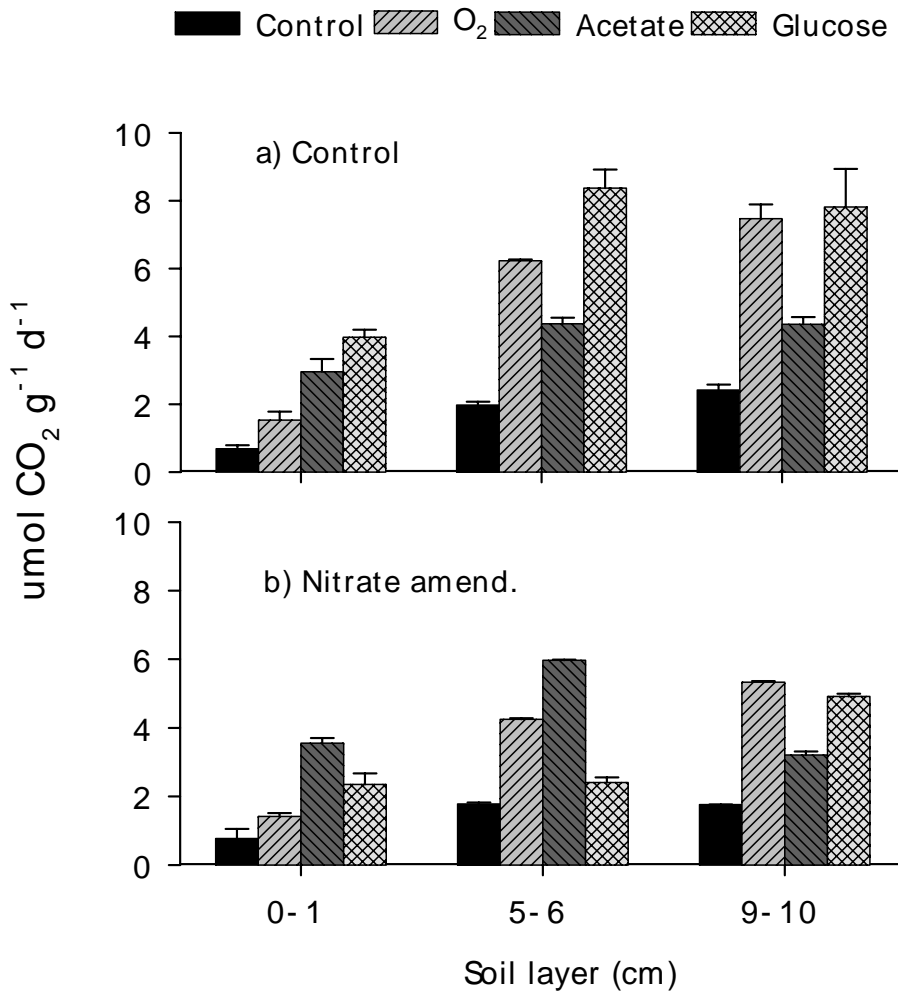


Fig. 5.2 CO₂ production in different redox zones of the flooded soil. The control, acetate and glucose treatments were anaerobic incubations, while O₂ indicates an aerobic incubation with no carbon substrate addition.

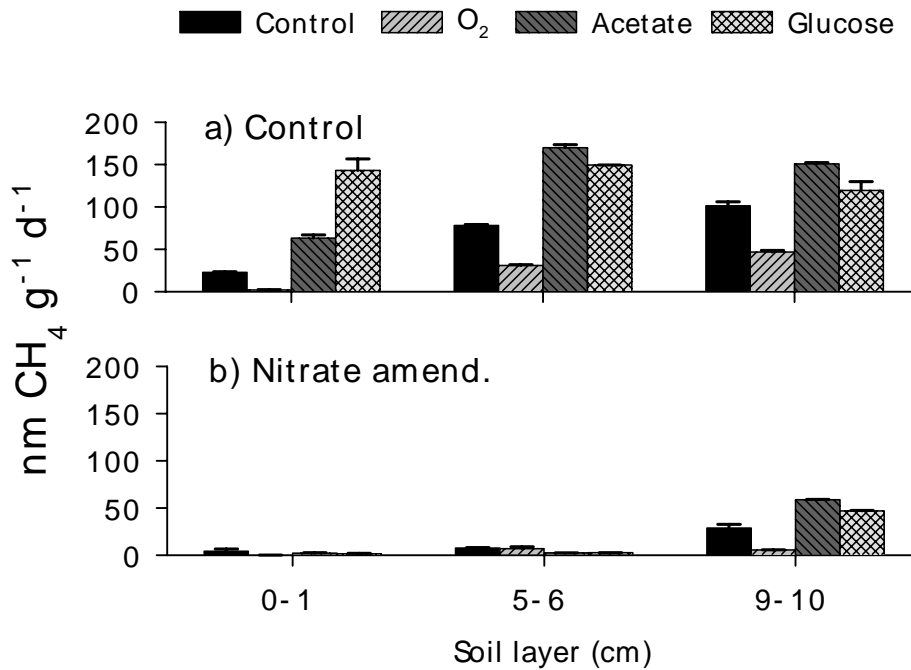


Fig. 5.3 CH₄ production in different redox zones of the flooded soil (same explanation as for Fig. 5.2)

Substrate-Induced Carbon Dioxide and Methane Production

Glucose compared to acetate induced more CO₂ production in the whole-redox-range and highly reducing zones, and vice versa in intermediately reducing zones ($p < 0.01$) (Fig. 5.2). The amounts of CO₂ induced by glucose and acetate in the weakly reducing zone were not significantly different. Oxygen induced more CO₂ production in the intermediate and highly reducing zones than in the weakly reducing and whole-redox-range zones ($p < 0.05$).

Glucose and acetate both significantly stimulated CH₄ production in the whole-redox-range and highly reducing zones. Acetate compared to glucose induced even more CH₄ production in the highly reducing zones and the opposite was observed in the

whole-redox-range zone. Methane production was not stimulated by glucose or acetate addition in the weakly and intermediately reducing zones. Oxygen addition significantly suppressed CH₄ production in the highly reducing zones.

Correlation among Substrate Induced Carbon Dioxide and Methane Production

Correlation analysis showed that glucose- or acetate- induced CO₂ production had a poor correlation while O₂-induced CO₂ production had a significant correlation with the control CO₂ production (Table 5.2). The correlation was low between acetate- and glucose- induced CO₂ production. Glucose-induced CO₂ production compared to acetate-induced respiration had a better correlation to O₂- induced respiration.

Table 5.2 Correlation (R², p) among substrate-induced CO₂ and CH₄ production in all redox zones.

	Control	O ₂	Acetate
CO ₂			
O ₂	0.97 (0.001)		
Acetate	0.53 (0.28)	0.37 (0.47)	
Glucose	0.65 (0.16)	0.78 (0.07)	-0.07 (0.89)
CH ₄			
O ₂	0.97 (0.001)		
Acetate	0.95 (0.003)	0.87 (0.03)	
Glucose	0.63 (0.18)	0.48 (0.34)	0.77 (0.07)

Acetate-induced CH₄ production was significantly correlated to the background CH₄ production. The correlation of glucose-induced CH₄ with the background CH₄ production was relatively low. The inhibition effect of O₂ on CH₄ production was highly correlated to the background CH₄ production.

Discussion

Glucose- or acetate-induced CO₂ and CH₄ production is widely used to estimate microbial activity in anaerobic soils. The applicability of these techniques needs to be evaluated in different redox zones when or where specific anaerobic redox processes dominate. Facultative anaerobes are mainly responsible for denitrification activity and obligate anaerobes are mainly involved in other anaerobic redox processes. Facultative anaerobes use a wide range of substrates, including acetate and glucose, while obligate anaerobes, including methanogens, SO₄²⁻ reducers and Fe³⁺ reducers, mainly live on acetate-based simple organic carbon and H₂ derived from fermentation processes as electron donors but cannot directly use or can only use glucose at low efficiencies. Estimates of respiration limited to only glucose- or acetate-induced respiration may not be sufficient to reflect microbial activity in different redox zones as evidenced by this study.

In the intermediate reducing zone dominated by iron-centered redox processes, acetate induced much more CO₂ production than glucose. This indicated that Fe³⁺ reducers could not efficiently use glucose as an electron donor. Also, the relatively small increase of CO₂ induced by glucose indicated that fermenting activity was weak in this zone, likely due to carbon limitation, and could not rapidly initiate glucose fermentation providing electron donors for Fe³⁺ reduction. Such delay in the initiation of fermenting activity was also observed after the depletion of NO₃⁻ in the slurry experiment (Chapter 2). In contrast, in the highly reducing zones, glucose induced much more CO₂ production

and similar CH₄ production compared to acetate, indicating glucose fermentation was rapidly initiated and provided sufficient substrates for methanogens. In the weakly reducing and whole-redox range zones, the responses of anaerobes to acetate and glucose were not significantly different, due to the important role of facultative anaerobes in the two zones. While microbial activity in the two zones was low due to carbon limitation under O₂ diffusion, the relative increases in CO₂ production by acetate and glucose additions were comparable to or even higher than those in the other redox zones.

Thus, both glucose and acetate had advantages and disadvantages for estimation of microbial activity in the different redox zones. Acetate-induced respiration did not reflect fermenting activity, but was better in estimating microbial activity in the intermediately reducing zone. Overall, glucose-induced respiration was better in the other redox zones, as supported by its better correlation to O₂-induced respiration, which indicated the availability of carbon sources and significantly correlated to the background anaerobic respiration. Acetate- and glucose- induced respiration may be both measured to better account for the effect of redox conditions, or more specifically, the relative importance of specific redox processes, on microbial activity in anaerobic soils.

Conclusions

Both glucose- and acetate-induced CO₂ and CH₄ production had disadvantages and advantages for microbial activity estimation in different redox zones when specific anaerobic redox processes dominated. Glucose induced respiration was as effective as acetate to reflect the activity of obligate anaerobes in the highly reducing zone, but was

not in the intermediately reducing zone where fermenting activity could not be quickly initiated after glucose addition. While acetate-induced respiration did not reflect fermenting activity in the highly reducing zone, it did not significantly differ from glucose-induced respiration in the weakly reducing and whole-redox-range zones where facultative anaerobes dominated.

Acetate appeared to better reflect microbial activity in the intermediately reducing zone where iron-centered intermediate redox processes dominated while glucose was better overall in the other redox zones. To better account for the effect of redox condition on microbial activity, respiration induced by acetate and glucose may be both used for microbial activity assessment in anaerobic soils under different redox conditions.

CHAPTER 6

BIOELECTROCHEMICAL NITRATE REMOVAL FROM WATER USING FLOODED SOIL AS A REDUCTANT SOURCE

Introduction

Huge amounts of energy sources other than fossil fuels exist at low densities in anaerobic environments but difficult to exploit (Reimers et al., 2001; Bond et al., 2002). Redox gradients are established in anaerobic environments where a difference in redox potential typically ranging around 0.8 V can be found within a distance as short as a few centimeters centering at oxic and anaerobic interfaces. This is sufficient to support electricity generation through a fuel cell process (Reimers et al., 2001; Bond et al., 2002). Many groups of microorganisms and electron shuttling substances are involved in the process of bioelectricity generation (Reimers et al., 2001; Bond et al., 2002; Rabaey et al., 2003; Chaudhuri and Lovley, 2003; Gregory et al., 2004; Liu et al., 2004). The microbially harvested power normally ranges from 10 to 100 mW m⁻² (Liu et al., 2004) and is able to drive low-power instruments in marine environments (Reimers et al., 2001; Bond et al., 2002). Difficulties exist in methods to collect, concentrate and store this bioelectricity for practical applications.

Nevertheless, since the internal resistance of a biobattery is relatively high, such relatively low bioelectricity may not be harvested through an external load but generated directly for bioelectrochemical pollutant removal. During bioelectricity generation from wastewater, organic matter is bioelectrochemically oxidized by O₂ (Liu et al., 2004).

Oxidized pollutants such as NO_3^- can be bioelectrochemically reduced when O_2 is not available (Gregory et al., 2004). Nitrate as a major pollutant in drinking water can be removed with physico/chemical and biological processes (Kapoor and Viraraghavan, 1997). While a biological process is cost-effective compared to expensive physico/chemical processes such as reverse osmosis and ion-exchange, a readily reducible carbon substrate such as ethanol normally needs to be added to the water being treated (Fuchs et al., 1997). With the biobattery approach, a wide range of organic carbon sources (e.g. agricultural biomass) may be applicable for NO_3^- removal without direct contact with the water. The use of organic matter as an energy source for microbes with the biobattery approach also makes it different from electrochemical/bioelectrochemical NO_3^- removal by an electric current field generated by typical electricity generating sources (Feleke and Sakakibara, 2001; Zaveri and Flora, 2002; Beschkov et al., 2004).

Relatively little research has been done on the use of microbially mediated reductants for bioelectrochemical NO_3^- removal. A recent report shows that an electrode poised at -300 mV (against the standard H_2 electrode) with a potentiostat as the source of electron donors reduces NO_3^- with nitrite being the dominant end product (Gregory et al., 2004). While wastewaters and soils/sediments may develop low redox potentials close to -300 mV, bioelectrochemical reduction of NO_3^- by microbially mediated reductants from these environments may differ from that by a poised electrode.

In this study, the ability of a flooded soil as a source of reductants to bioelectrochemically reduce NO_3^- in carbon-limiting water was evaluated. The role of

N₂O as an intermediate product of NO₃⁻ reduction in bioelectricity generation was also studied.

Materials and Methods

Microbial Fuel Cell Set Up

The microbial fuel cell using a flooded soil as a reductant source is shown in Fig. 6.1.

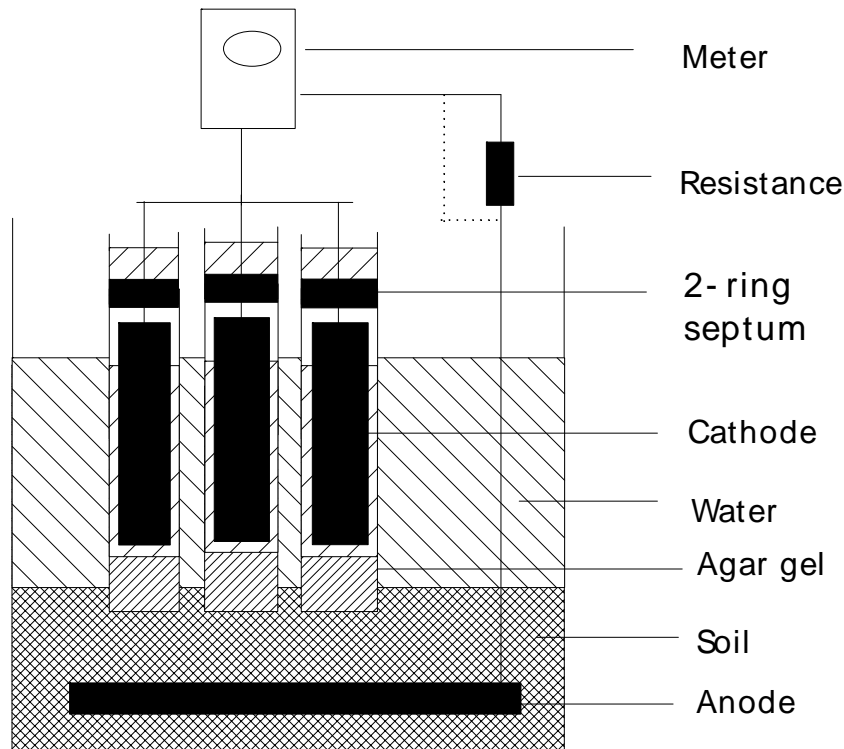


Fig. 6.1 A microbial fuel cell using a flooded soil as a reductant source

The electrode placed in the reducing soil layer is the anode and the cathode is placed in the overlying water. The flooded soil, already under a 2-month flooding preincubation, was mixed with 0.05% glucose and put into a plastic vessel (25 cm x 30 x 18 cm) to form a 6-cm soil layer. For properties of the soil, see the Louisiana soil from Table 2.1 in Chapter 2. A graphite plate (15 mm x 100 mm x 150 mm), coarsened with sand paper, electrically connected to a copper wire with silver conductive epoxy covered with

nonconductive epoxy (Reimers et al., 2001), was placed at the 4-cm depth of the flooded soil to serve as the anode.

When the soil had developed highly reducing conditions (e.g. redox potentials below the 1-cm depth < -200 mV) after 1 more week of incubation at 25°C, a 140-ml plastic syringe (inner diameter 3.7 cm) was placed at the 1-cm depth of the flooded soil to serve as the cathode chamber after modification. The tip near the body of the syringe was cut to get a 2-cm diameter hole. A rubber stopper temporarily sealed the hole while hot agar gel (4%) was poured into the syringe to form a 3-cm gel layer upon cooling, and then the stopper was removed. The gel layer was used as the separator between the cathode chamber and the flooded soil. Fifty ml of water was poured on top of the agar gel in the syringe. One piece of carbon paper (170 μm x 3.5 cm x 6 cm, AvCarb-p50, Ballard Power System) as a cathode was put into the syringe and was partly submerged (3.5 cm x 4 cm). The depth of overlying water in the vessel was adjusted to be same as that in the syringe during electricity generation as described below.

Electricity Generation Coupled to Oxygen Reduction

The cathode chamber was filled with 50 ml of tap water and kept aerobic. Three cathodes were connected in parallel and then were connected to the anode with the option of going through a 465-ohm external resistor or bypassing this resistor during electricity generation. The ratio of the anode area relative to the total effective cathode area was 3.6 to facilitate electron transfer by the anode from the static flooded soil. The electric current was measured once per minute for 1 day using a digital multimeter (RadioShack

22-812) serially connected between the anode and cathodes and was recorded by a computer using Meter View 1.0 through a RS232 port.

Electricity Generation Coupled to Nitrate Reduction

The cathode chamber was filled with 50 ml solution containing 0.8% sodium chloride and 5 mM potassium nitrate and inoculated with soil at 0.2 or 1.0% (from the top aerobic layer of the flooded soil without glucose addition) under a N₂ atmosphere and capped with a two-ring septum leaving a headspace of 47 ml. The septum was covered with a 1-cm layer of overlying water to ensure anaerobic conditions in the cathode chamber. Then the cathode chamber was anaerobically incubated for 3 d with or without electricity generation. No external resistance was used during electricity generation. A control with no soil inoculation and no current generation was included to account for NO₃⁻ change due to diffusion into the gel layer.

Based on the current variation, gas samples (2 ml) were taken from the headspace of the cathode chamber at hours 20 and 60 at the low inoculation rate and at hours 20 and 40 at the high inoculation rate and analyzed with a Tremetrics 9001 gas chromatograph using an electron capture detector (ECD) for determining N₂O concentrations. At the end of the experiment, water samples from the cathode chambers were taken, filtered (<0.4 μm), and analyzed for NO₃⁻ content by a NO₃⁻ selective ion electrode (APHA, 1995). The electrons transferred into the cathode chamber through current flow were calculated according to 1 coulomb = 1 ampere x 1 second and 1 mole = 96,485 coulombs.

Electricity Generation Coupled to Nitrous Oxide Reduction

The procedure was the same as for “electricity generation coupled to NO_3^- reduction” except that the cathode chamber was filled with 50 ml of solution containing 20 mM potassium nitrate and 5 mM glucose and inoculated with 4.0% soil and that the headspace of the cathode chamber was amended with acetylene at 20% for inhibition of enzymatic N_2O reduction to N_2 (Tiedje, 1982). Half an hour after the addition of acetylene, the current was measured for 1 d with and without a 465-ohm external resistor in the circuit.

Results

Electricity Generation

Electricity was consistently generated when the cathode chamber was kept aerobic (Fig. 6.2a). The current was 32.9 mA m^{-2} with no external resistance and 21.4 mA m^{-2} with a 465-ohm external resistance based on the anode area. The power harvested through the external resistance was 3.2 mW m^{-2} based on the anode area, but 11.4 mW m^{-2} based on the cathode area.

The current coupling to NO_3^- reduction was variable with different patterns at the two inoculation rates and averaged $\sim 70.0\%$ lower than when coupled to O_2 reduction (Fig. 6.2b). The current at the high inoculation rate was high with repeated peaks in the first 40 hours and became small thereafter. The current at the low inoculation rate decreased rapidly soon after the start of electricity generation, gradually increased to $\sim 0.16 \text{ mA}$ at hour 10, and then was stable around this level until hour 35 and became variable with two peaks thereafter.

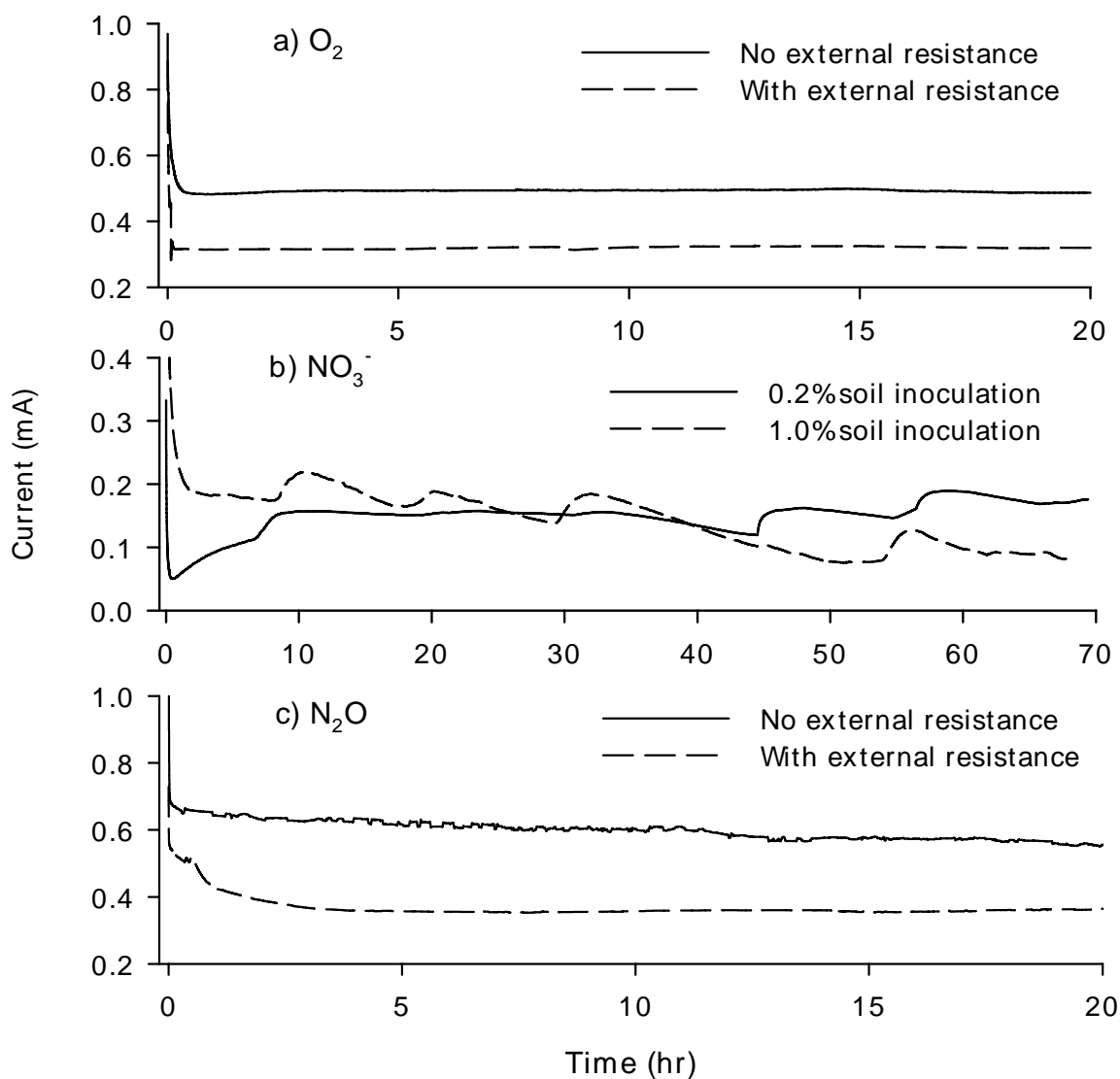


Fig. 6.2 Current generation coupled to O_2 reduction (a), NO_3^- reduction (b), and N_2O reduction (c) with or without a 465-ohm external resistance. No external resistance was used when nitrate was the oxidant.

Nitrous oxide was detected ($> 2.5 \mu L L^{-1}$) in the headspace of the cathode chambers with the higher concentration of N_2O corresponding to the higher current at each inoculation rate (Table 6.1). When N_2O accumulated under the inhibition by acetylene of

the enzymatic reduction of N₂O to N₂, the current generated was stable and was 21% higher than that coupled to O₂ reduction with no external resistance and 15% higher with a 465-ohm external resistance (Fig. 6.2c).

Table 6.1 Nitrous oxide concentrations (mean \pm SE, n=3) in the headspace of the cathode chambers under current generation.

Inoculation	Hour	N ₂ O (μ L L ⁻¹)	Current (mA)
0.2%	20	2.68 \pm 0.87	0.15
	60	3.92 \pm 0.68	0.19
1.0%	20	3.44 \pm 0.73	0.18
	40	2.98 \pm 0.54	0.12

Nitrate Removal under Electricity Generation

Using the decrease in NO₃⁻ content in the non-inoculation treatment to account for the effect of NO₃⁻ diffusion in the inoculation treatments, the NO₃⁻ removal under electricity generation was faster than under no electricity generation at each inoculation rate (Table 6.2). With or without electricity generation, the NO₃⁻ removal at the high inoculation rate was >3 times faster than that at the low inoculation rate. The ratios of electrons transferred through current flow to the amount of current flow-induced NO₃⁻ removal were 2.2 at the high inoculation rate and 6.1 at the low inoculation rate.

Table 6.2 Nitrate contents (mean \pm SE, n=3) in the cathode chambers. The chambers were amended with 5 mM potassium nitrate and anaerobically incubated for 3 days with or without current generation.

Inoculation	Treatment	Decrease in NO ₃ ⁻ content (mM)
-	Control	1.1 \pm 0.1
0.2%	No current	1.3 \pm 0.0
	With current	1.7 \pm 0.1
1.0%	No current	2.1 \pm 0.2
	With current	3.3 \pm 0.1

Discussion

Electricity Generation

The ability of the static flooded soil as a source of reductants to support bioelectricity generation coupled to O₂ reduction was low based on the anode area, but was within the normal reported range of 10-100 mW m⁻² based on the cathode chamber (Liu et al., 2004). The use of a 3-cm layer of agar gel instead of a proton exchange membrane (e.g., Nafion) as the separator for the cathode chamber and the reducing soil increased the internal resistance between them. A Nafion membrane would not function well under direct contact with bulk soil. The static states of the reducing soil and the cathode chamber may also lower the ability of bioelectrochemically active anaerobes to transfer electrons on the anode and cathode surfaces (Bond et al., 2002; Gregory et al., 2004).

The lower currents coupled to NO₃⁻ reduction compared to that coupled to O₂

reduction showed that NO_3^- reduction was not as efficient as O_2 reduction in accepting electrons from current flow. Further, the current coupled to NO_3^- reduction was variable. The anode was not expected to be a factor for the current variations since it constantly transferred electrons when the cathode chamber was aerobic. Such current variations would be largely attributed to the variations in NO_3^- reduction and N_2O accumulation in the cathode chambers affected by the inoculation rate. The electrons transferred through current flow directly stimulated NO_3^- reduction to nitrite (Gregory et al., 2004), but more likely promoted the reduction of N_2O and NO due to their higher redox potentials (Handbook of Chemistry and Physics, 1991) when they were accumulated in the cathode chambers under carbon limitation (Benckiser et al., 1996). According to the concentrations of N_2O in the headspace, N_2O dissolved in the solution was even sufficient as the oxidant to accept the electrons transferred through current flow. Nevertheless, the slower reduction of NO_3^- at the low inoculation rate due to a greater extent of carbon limitation and a lower density of microorganisms would delay the accumulation of N_2O and NO resulting in the lower currents in the first 10 hours and vice versa at the high inoculation rate, and, the faster removal of NO_3^- at the high inoculation rate would lower the accumulation of N_2O and NO later resulting in the lower currents in the late hours.

The importance of N_2O in electricity generation was directly reflected by the electricity generation coupled to N_2O reduction (Fig. 2c). Sufficient glucose was added into the cathode chamber to stimulate NO_3^- reduction but N_2O accumulated due to the

inhibition of acetylene on the enzymatic reduction of N_2O to N_2 (Tedje, 1982). However, N_2O is a strong oxidant comparable to O_2 but is much more soluble (Handbook of Chemistry and Physics, 1991). Nitrous oxide reduction, compared to O_2 reduction, resulted in generation of the even higher currents.

Bioelectrochemical Nitrate Removal

An imbalance in electron transfer between current generation and NO_3^- removal was observed. The ratio of the amount of electrons transferred through current flow to that of NO_3^- removed due to current generation was 6.1 at the low inoculation rate, close to the ratio 5 for reduction of NO_3^- to N_2 and this ratio became 2.2 at the high inoculation, close to the ratio 2 for reduction of NO_3^- to NO_2^- or NO to N_2 . In other words, NO_3^- removal under current generation was more efficient at the high inoculation rate. Such inconsistencies have been reported elsewhere (Gregory et al., 2004). Due to a greater extent of carbon limitation and a lower density of microorganisms, NO_3^- removal under current generation at the low inoculation rate was largely associated with current flow (Gregory et al., 2004). In contrast, NO_3^- removal at the high inoculation rate under no current generation was even higher than that at the low inoculation rate under current generation. The bioelectrochemical reduction of NO_3^- and N_2O at the high inoculation rate likely lowered the extent of carbon limitation in the biological NO_3^- reduction, e.g. the step from N_2O to N_2 , to stimulate biological reduction overall. The high inoculation rate also favored the cloning of bioelectrochemically active microorganisms on the cathode (Gregory et al., 2004) contributing to the more efficient NO_3^- removal.

The function of NO_3^- as an oxidant in electricity generation provides the promise of bioelectrochemical NO_3^- removal from carbon-limiting environments such as pretreated drinking waters. The rates of bioelectrochemical NO_3^- removal in this study are within the low part of the range of denitrification in natural environments (Davidsson and Stahl, 2000; Hunter and Faulkner, 2001) but are considerable for NO_3^- removal from drinking water. The efficiency of NO_3^- removal by the biobattery approach may be further increased when new proton exchange media functioning well in contact with bulk soils/sediments become available to increase bioelectricity generation. The biobattery approach may be combined with the biological approach to lower the requirement of readily reducible substrates for NO_3^- removal from drinking water.

Conclusions

Through increasing the anode area relative to the cathode area, microbially mediated reductants from the static flooded soil were able to support current generation coupled to the reduction of O_2 dissolved in the overlying water. The current was lowered when NO_3^- was substituted for O_2 , but NO_3^- removal was significantly stimulated compared to that under no current generation suggesting the applicability of a wide range of organic carbon sources for bioelectrochemical NO_3^- removal from drinking water. The current generation with NO_3^- as the oxidant not only coupled the reduction of NO_3^- to nitrite but also the reduction of N_2O to N_2 . The current varied with microbial NO_3^- reduction and N_2O accumulation affected by the inoculation rate. When N_2O accumulation was high

under the inhibition of acetylene on the enzymatic reduction of N_2O to N_2 , the current generated was even higher than that coupled to O_2 reduction.

CHAPTER 7

EFFECTS OF FERRIC IRON AMENDMENT ON NITRATE PERCOLATION AND EMISSIONS OF NITROUS OXIDE AND METHANE IN A RICE SOIL

Introduction

Iron redox cycling plays a key role in organic carbon decomposition, metal mobilization and immobilization, nutrient release, and biogeochemistry of NO_3^- and CH_4 in non-sulfidogenic anaerobic environments (Lovley, 1991; Lovley et al., 1994; Roden and Wetzel, 1996; Yao et al., 1999; Senn et al., 2002).

There is increasing interest in the role of iron redox cycling in CH_4 biogeochemistry in rice fields (Roden and Wetzel, 1996; Frenzel et al., 1999; Matthews et al., 2000). Rice fields are a major biogenic source of CH_4 , an atmospheric trace gas second only to CO_2 in its contribution to global warming (IPCC, 2001). Ferric iron generally constitutes the major part of the alternative electron acceptor (AEA) pool in rice soils (Yao et al., 1999). Microbial Fe^{3+} reduction suppresses CH_4 production in flooded soils, especially under substrate-limiting conditions (Achthich et al., 1995). Therefore, increasing Fe^{3+} reduction intensity, e.g. through Fe^{3+} amendment, reduces CH_4 emission from rice fields (Watanabe and Kimura, 1999; Jäckel and Schnell, 2000; Furukawa and Inubushi, 2002).

More importantly, iron redox cycling affects CH_4 dynamics in rice fields through Fe^{3+} regeneration. Ferric iron is the dominant AEA capable of regeneration in rice soils. Ferric iron regeneration at the oxygenated soil-water interface and in the oxidized rhizosphere is important in controlling root exudation and CH_4 dynamics (Frenzel et al.,

1999; King and Garey, 1999; Ratering and Schnell, 2000). Ferric iron regeneration stimulated by drainage has been suggested to be important in drainage-induced CH₄ mitigation (Sigren et al., 1997; Ratering and Conrad, 1998). Nevertheless, while drainage is most effective and promising in reducing CH₄ emission (Yagi et al., 1997), few experimental evaluations are available on the role of Fe³⁺ regeneration in drainage-induced CH₄ mitigation. Such evaluations are especially needed before adoption of a short-term drainage-based recommendation for CH₄ mitigation while lowering the risks of reducing rice yield and increasing the emission of another very important greenhouse gas, N₂O (Yagi et al., 1997; Henckel and Conrad, 1998; Cai et al., 1999).

Iron redox cycling also affects NO₃⁻ biogeochemistry in anaerobic environments. Anaerobic oxidation of Fe²⁺ may be coupled to NO₃⁻ reduction (Benz et al., 1998; Nielsen et al., 1998; Senn et al., 2002) and promote N₂O formation (Brons et al., 1991; van Cleemput et al., 1996). Aerobic Fe²⁺ reoxidation at the aerobic/anaerobic interfaces of wetland soils may regulate O₂ availability (van Bodegom et al., 2001; Neubauer et al., 2002) and thereby affect NO₃⁻ reaction dynamics and N₂O formation. Fate of NO₃⁻ in wetland soils may also be altered by intensified reduction of Fe³⁺-centered AEAs in the deep soil layer (Chapter 4).

Here we present a study on the effect of intensity of Fe³⁺ reduction on NO₃⁻ percolation, N₂O and CH₄ fluxes in a potted rice soil. The effect of Fe³⁺ reduction and regeneration intensity on N₂O and CH₄ fluxes was evaluated in control and ferrihydrite-amended rice pots before and after short-term drainage.

Materials and Methods

Experimental Setup

A Louisiana rice soil was used in the experiment. The soil sample was collected from the field top layer (0-20 cm), air-dried, stored at room temperature (20 °C), and sieved through 4-mm mesh. See Table 2.1 in Chapter 2 for the soil properties.

The pot experiment was carried out outside in early June, 2003. The pot was placed with or without a 3-cm layer of quartz sand and glass wool at the bottom. Three kilograms of soil (dw) was mixed with 1000 ml fertilizer solution in a bucket, transferred into the pot, and stabilized under flooded conditions for 2 days. The fertilizer solution contained 0.98 g KH_2PO_4 and 0.48 g urea with or without amendment of 10.50 g ferrihydrite-Fe. The ferrihydrite-containing fertilizer solution was prepared by mixing ferrihydrite suspension with the fertilizers just before mixing with the soil. The addition rate of Fe was equivalent to $63 \mu\text{mol g}^{-1}$, a rate comparable to indigenous reducible iron content in the soil. Two days after flooding, each pot was planted with three 21-day-old rice seedlings (*Oryza sativa* L.). The pots were watered if needed to maintain a 2-4 cm layer of overlying water in the first month after flooding, and 5-7 cm afterwards. Weeds in the pots were manually removed weekly. Urea (0.32 g) was applied twice as top dressing on days 37 and 67.

The pots with quartz sand and glass wool placed at the bottom were used for the experiment on NO_3^- percolation (see below). The other pots were used for the experiments on N_2O and CH_4 emission. These pots were drained by removal of overlying water either once (on Day 55 after flooding for 2 days) or twice (on days 55 and 85, both for 2 days).

The selections of drainage dates were based on the observance of large increases of CH₄ emissions from the pots with consideration of rice growth stage. All the pots were drained on day 114 for harvest on day 127. Thus, there were 4 combinations of ferrihydrite amendment and drainage treatments to study emissions of CH₄ and N₂O, and each combination was done in triplicate.

Nitrate Percolation

From day 3 to 22 after flooding, 110 ml water was collected each day from the bottom of the pot (n=3) with a 140-ml syringe, equivalent to a percolation rate of 4.0 mm d⁻¹. Percolates taken on days 3, 6, 16 and 22 were filtered (<0.4 μm) for NO₃⁻ content determination by a NO₃⁻ selective electrode (APHA, 1995).

Nitrous Oxide and Methane Emissions

The closed chamber method was used for measurement of N₂O and CH₄ emissions from the pots. Transparent cylindrical plastic chambers (inner diameter 12.5 cm) were used during different rice-growing periods. Each chamber was installed with a small battery-operated fan for gas mixing and a rubber septum for gas sampling. An equilateral triangle-shape wood frame was permanently fixed onto the inner sidewall of the pot just above the soil-water interface to be used as the base to hold the chamber during gas sampling. The air in the chamber was mixed by the fan and gas samples were taken by a 10-ml air-tight syringe at 0, 10, 20 and 30 min after positioning the chamber onto the base. Gas sampling was carried out between 9:30 and 11:00 in the morning on days 4, 6 and 10 after flooding, and weekly or biweekly afterwards. Methane emissions from the pots were

measured until day 113. Nitrous oxide emissions were measured during the first 22 days, and 1 day after the reflooding of the drained soil in the mid- and late-seasons. Within one day after sampling, gas samples were analyzed with a Tometrics 9001 gas chromatograph using an electron capture detector (ECD) and a flame ionization detector (FID) for determination of N_2O and CH_4 concentrations, respectively. The N_2O and CH_4 fluxes were determined as the amounts of gas accumulated versus time over a unit of surface area. The cumulative CH_4 emission from the pot was computed as the sum of the production rate on each sampling date multiplied by the time interval which was calculated from the midpoints of neighboring sampling dates.

Relationship between Ferric Iron Regeneration and Methane Emission

Reliable estimates of Fe^{3+} reduction or regeneration in the potted soil before and after drainage was not possible due to soil heterogeneity (Ratering and Schnell, 2000). The effect of ferrihydrite amendment on reducing CH_4 emission before the midseason drainage was not considered since CH_4 emission from each treatment in this period was similarly small and accounted for <10% of the cumulative emission during the 113-day measurement. Ferric iron regeneration during flooded periods was assumed to be largely controlled by O_2 diffusion but not by the availability of reoxidizable Fe^{2+} and hence not by Fe^{3+} amendment. Then, regression analysis was conducted between potential drainage-induced Fe^{3+} regeneration and cumulative CH_4 emission during the whole measurement period. The potential Fe^{3+} regeneration during drainage was calculated as 100% regeneration of reducible iron in the soil. The amount of the reducible iron was

estimated according to the reducibility of indigenous soil iron and/or ferrihydrite from the slurry experiment (see Table 2.2 in Chapter 2).

Rice Yield and Aboveground Biomass

After harvest, rice yield per pot was determined by weighing. Aboveground biomass (excluding rice seeds) per pot was determined by weighing after drying at 105 °C for 24 h.

Statistical Analysis

Statistical analysis was conducted with SAS (SAS Institute Inc., 1999-2001). Differences in NO_3^- percolation, aboveground biomass and rice yield between the treatments were evaluated with Student t test, and seasonal variation of CH_4 emission between the treatments was evaluated with one-way analysis of variance (ANOVA). A linear regression was conducted between potential of drainage-induced Fe^{3+} regeneration and cumulative CH_4 emission in the potted soil. The statistical level of significance was chosen at $\alpha = 0.05$ for all statistical analysis.

Results

Nitrate Percolation

Nitrate percolation was detected at $>1.0 \text{ mM NO}_3^-$ in both treatments on day 1 after percolation (Fig. 7.1). Nitrate percolation was negligible in the control soil by day 4 but continued until between day 14 and 20 in the amended soil.

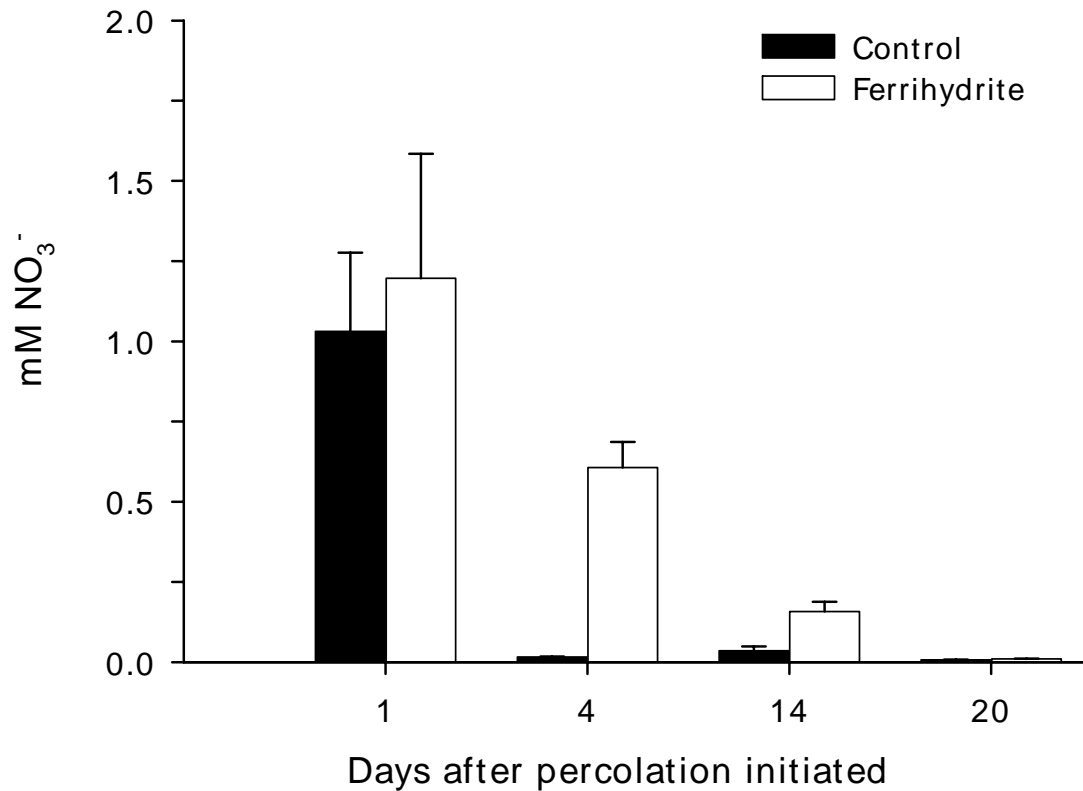


Fig. 7.1 Nitrate percolation (mean \pm SE, n=3) from the potted soil affected by ferrihydrite amendment. Percolation started at 4.0 mm d⁻¹ 2 days after flooding.

Nitrous Oxide and Methane Emissions

Significant N₂O fluxes were observed, which were increased by ferrihydrite amendment at early phase of flooding (Fig. 7.2). Nitrous oxide fluxes were small in both treatments after flooding for 10 days. No significant N₂O fluxes were observed 1 day after reflooding of the drained pots in the mid- and late-seasons.

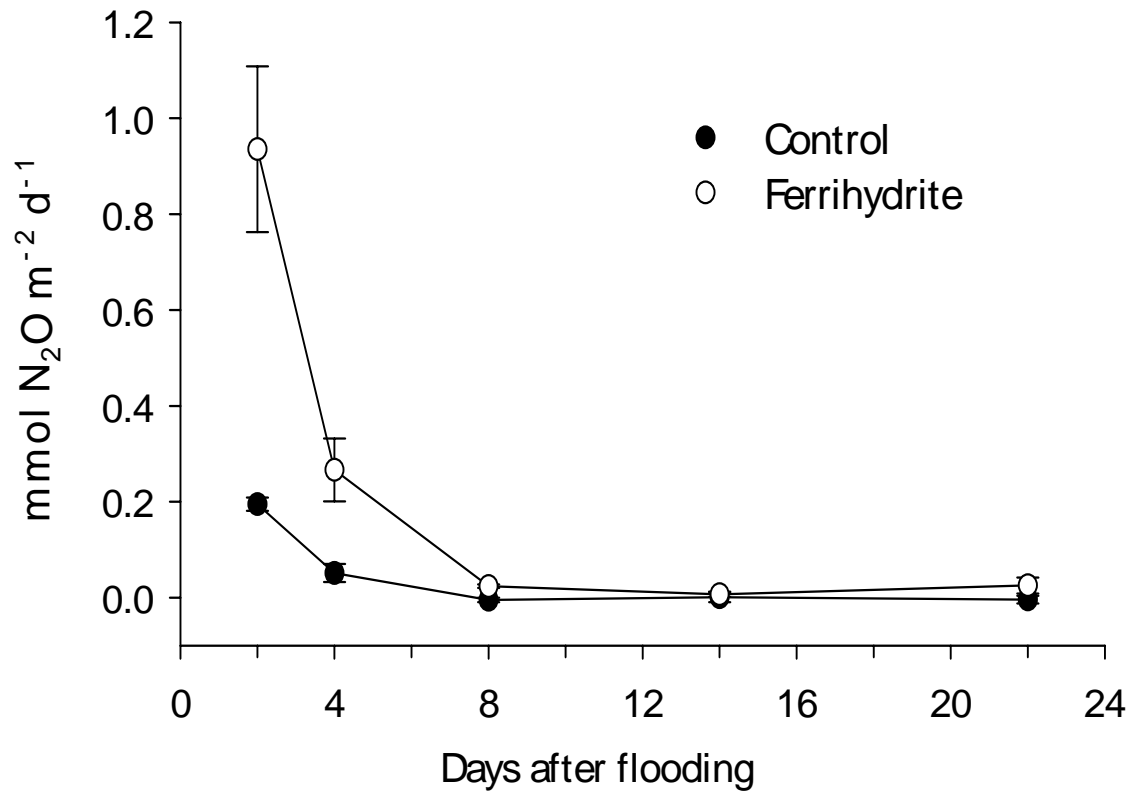


Fig. 7.2 Fluxes of N₂O in the potted soil.

The initiation of CH₄ emissions from the pots was relatively slow. Methane was emitted at low rates of less than 1.5 mmol CH₄ m⁻² d⁻¹ in all the pots until day 40 after flooding (Fig. 7.3). Iron amendment had little effect on delaying and reducing CH₄ emission before the midseason drainage on day 55.

Methane emission from the amended pots was significantly lowered by the single midseason drainage compared to the control pots through day 77 ($p < 0.05$). Thereafter, similar high fluxes of more than 40 mmol CH₄ m⁻² d⁻¹ were observed in both treatments on most dates. Ferrihydrite amendment generally lowered CH₄ emission when the pot was drained twice until the end of measurements (Fig. 7.3). Seasonal CH₄ emission was

reduced by 26% from 2.94 to 2.19 mol m⁻² by the single drainage (p=0.47) with ferrihydrite amendment and 69% from 1.81 to 0.56 mol m⁻² with the double drainage treatment (p < 0.01). A linear equation $y = -0.014 x + 3.665$ ($R^2 = 0.82$; p<0.01) was obtained (Fig. 7.4) between the potential drainage-induced Fe³⁺ regeneration and the cumulative CH₄ emission during the 113-day measurement.

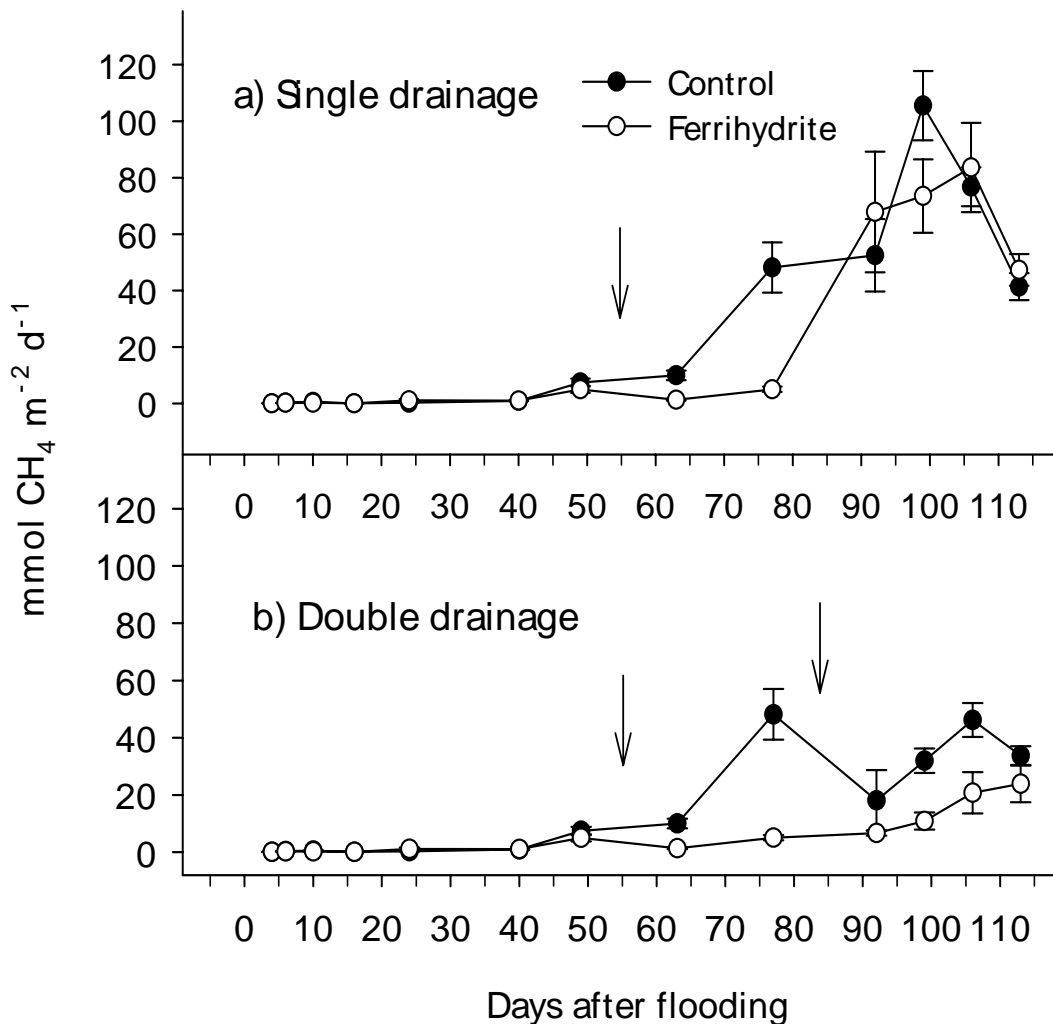


Fig. 7.3 Methane emission from the potted soil affected by ferrihydrite amendment before and after drainage. The arrows show the dates when drainage was carried out.

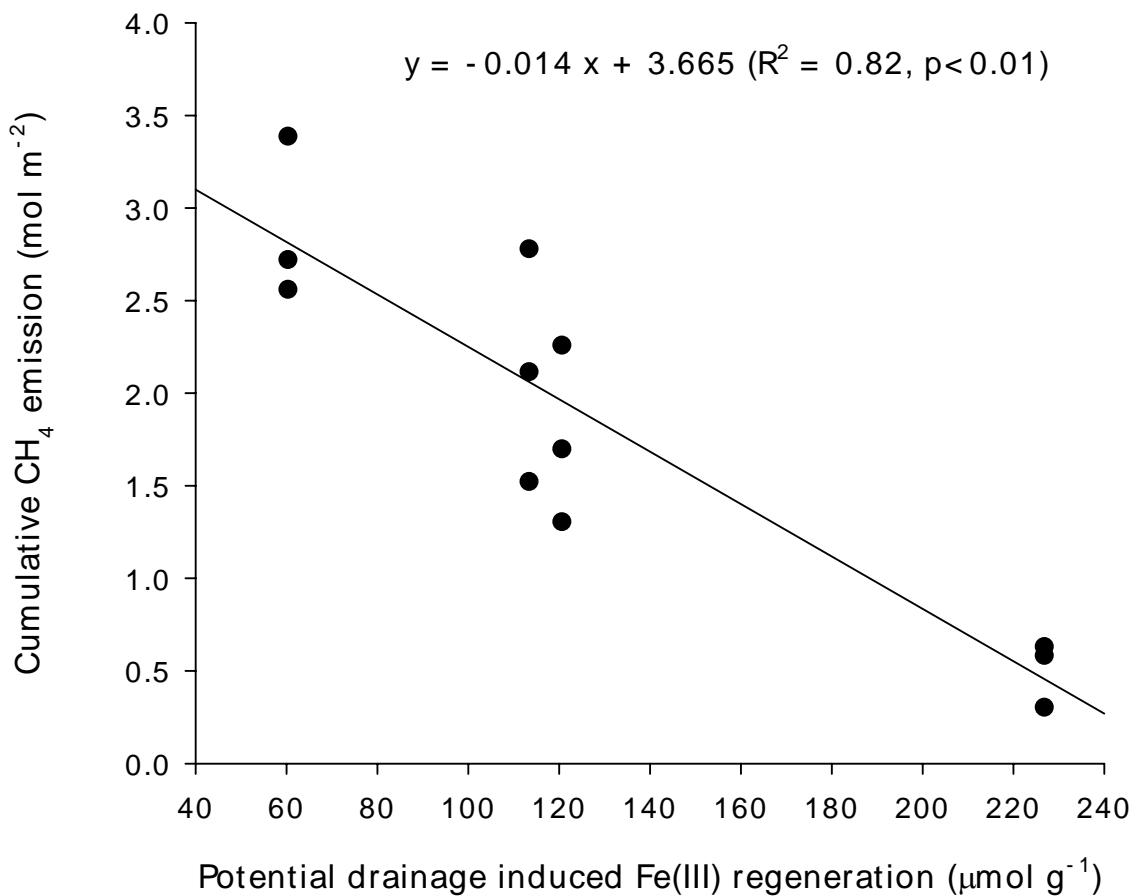


Fig. 7.4 Relationship between potential of drainage induced Fe³⁺ regeneration and cumulative CH₄ emission (113 days) in the rice pots. The potential Fe³⁺ regeneration during each 2-day drainage was calculated as 100% regeneration of reducible iron in the soil, which was calculated according to the reducibility of indigenous soil iron and/or ferrihydrite from the slurry experiment (see Table 2.2 in Chapter 2).

Rice Growth

The growth of rice seedlings was delayed by ferrihydrite amendment in the first four weeks after transplanting. No observable differences were found in rice growth between the control and amended pots in other periods. Rice yield and aboveground biomass (including rice seeds) in the amended pots were $9.3 \text{ g} \pm 0.8$ and $35.5 \text{ g} \pm 1.5$ per pot, not

significantly different from $9.4 \text{ g} \pm 0.7$ ($p = 0.92$) and $34.6 \text{ g} \pm 0.3$ per pot ($p = 0.52$) in the control pots, respectively.

Discussion

In the potted soil, NO_3^- that accumulated under dry conditions contributed to NO_3^- percolation, but denitrification and N_2O emission were promoted in the early days after flooding. The effects of ferrihydrite amendment on increasing NO_3^- percolation and N_2O emission were consistent with those found in soil column and slurry experiments (Chapters 4 and 2). Nitrous oxide emission may be increased during short-term drainage (Ratering and Conrad, 1998). However, NO_3^- generated during the short-term drainage could only be transitionally accumulated due to rapid denitrification/utilization and hence, no high N_2O emission occurred soon upon reflooding.

While ferrihydrite amendment considerably reduced CH_4 production in the same soil for 23 days under an anoxic slurry incubation (Chapter 2), it had little effect on CH_4 emission from pot experiments for 40 days after flooding. Methane emission from the soil-rice system is the net result of CH_4 production and oxidation, greatly mediated by the rice plant. Rice influence on production and transport of CH_4 in the pots in the early period was expected to be weak as found by Aulakh et al (2000, 2001). The soil, without organic amendment, may not provide sufficient energy sources to support high CH_4 production, but CH_4 oxidation was expected to be active in this period (Krüger et al., 2002). A large part of CH_4 produced in this period would be oxidized before it diffused or was transported into the atmosphere, leading to the low emission rates in all pots.

Thus, ferrihydrite amendment may reduce CH₄ production as found under anoxic incubation, but had only a small effect on reducing CH₄ emission from the pots in the early season.

However, ferrihydrite amendment significantly improved short-term drainage-induced CH₄ mitigation in the mid- and late-seasons when high CH₄ fluxes occurred. Drainage-induced CH₄ mitigation was mainly due to the toxic/inhibitory effects on methanogens of drainage-induced deeper O₂ diffusion, aerobic carbon oxidation and, generation and regeneration of AEAs (Sigren et al., 1997; Ratering and Conrad, 1998). Ferric iron is the dominant AEA capable of regeneration. The generation and regeneration of other AEAs such as NO₃⁻ and SO₄²⁻ in the soil is likely to be less important. The onset of redox conditions to support methanogenesis upon reflooding was largely controlled by the amount of Fe³⁺ generated during drainage. Ferrihydrite amendment at a relatively low rate of 3.5 g Fe per kg soil, compared to the rate of 10 or 20 g Fe per kg soil (15 or 30 g ferrihydrite per kg soil) applied by Jäckel and Schnell (2000), did not affect rice growth after the early weeks, but almost doubled the soil reducible iron content. This presumably increased Fe³⁺ regeneration during drainage, resulting in more effective delay and reduction of CH₄ emission upon reflooding. This was also supported by the negative relation between the potential of drainage-induced Fe³⁺ regeneration and the cumulative CH₄ mitigation.

When short-drainage is effective in CH₄ mitigation (Yagi et al., 1996; Ratering and Conrad, 1998), it is more desirable than long-term drainage because it also lowers the risk

of reducing rice yield and increasing N₂O emission (Yagi et al., 1997; Henckel and Conrad, 1998; Cai et al., 1999). Nevertheless, factors affecting the mitigation extent need to be more carefully evaluated to ensure effective CH₄ mitigation by short-term drainage. In general, iron redox cycling in rice paddies is poorly quantified. More studies are needed for better evaluation of the role of Fe³⁺ reduction and regeneration in CH₄ biogeochemistry and short-term drainage-based CH₄ mitigation in rice paddies.

Conclusions

Microbial Fe³⁺ reduction as a major alternative electron accepting process affected NO₃⁻ dynamics and methanogenesis in the potted soil. An increase in reducible Fe³⁺ content by ferrihydrite amendment temporally but significantly increased NO₃⁻ percolation and N₂O flux in the early days after flooding. Ferrihydrite amendment had little effect on CH₄ emission in the early growing season when CH₄ flux is low. Instead, ferrihydrite amendment effectively improved short-term drainage-induced CH₄ mitigation through increasing Fe³⁺ regeneration in the mid- and late-seasons. Hence, the intensity of Fe³⁺-regeneration should be considered in short-term drainage-based options for effective CH₄ mitigation in rice paddies.

CHAPTER 8 SUMMARY AND FUTURE RESEARCH RECOMMENDED

Summary

Dissimilatory microbial Fe^{3+} reduction was a major intermediate alternative electron accepting process in anaerobic carbon decomposition. Controls on NO_3^- reduction and CH_4 formation by iron-centered intermediate redox processes occurred to various extents in the soil slurry, soil column and rice pot experiments.

The roles of microbial Fe^{3+} reduction in denitrification and methanogenesis were first investigated in the homogenized anoxic soil slurries (Chapter 2). Ferric iron reduction was a major redox process in anaerobic carbon decomposition. More reducible iron and less favorable pH caused slower redox potential decrease, slower denitrification with more N_2O production, and later methanogenesis in the Texas soil compared to the Louisiana soil. An increase in Fe^{3+} reduction through Fe^{3+} amendment temporally stimulated N_2O production and considerably suppressed CH_4 production in both soils, but had a small effect on the redox range within which both N_2O and CH_4 are produced at low rates.

The roles of cycling of Fe^{3+} -centered AEAs in N_2O and CH_4 production were also investigated in flooded soil columns (Chapter 3). Under the influence of O_2 diffusion, iron-centered intermediate redox processes occurred around the aerobic/anaerobic interface and subsequently lowered methanogenic activity at upper depths. Nitrous oxide production was generally low in the soil column due to NO_3^- depletion. While under the

additional influence of downward diffusion of NO_3^- , iron-centered intermediate redox processes were vertically expanded and temporally extended, and caused relatively high N_2O production even at Eh levels around 0 mV partly due to intensive interactions of Fe^{2+} reoxidation with NO_3^- reduction, and, significantly suppressed methanogenesis to deeper depths for prolonged periods largely due to lowered substrate availability to methanogens.

Another flooded soil column experiment was carried out to investigate whether the competition for carbon sources of iron-centered intermediate redox processes in the flooded soil column would affect the fate of NO_3^- in the overlying water with and without percolation (Chapter 4). Moderate percolation caused the occurrence of NO_3^- percolation but promoted NO_3^- removal and lowered N_2O emission. Amendments of iron and manganese oxides decreased NO_3^- removal but promoted N_2O emission and NO_3^- percolation, primarily through intensifying iron-centered intermediate redox processes to lower carbon source availability, especially at the deeper depths, to heterotrophic denitrification. The pool of Fe^{3+} -centered AEAs needs to be considered in evaluation of NO_3^- removal and N_2O emission in seasonally and intermittently flooded wetlands.

Microbial activity involved in reduction of Fe^{3+} -centered intermediate AEAs was estimated using substrate induced respiration from a Fe^{3+} -reduction dominated intermediately reducing zone with comparison to other redox zones (Chapter 5). Acetate compared to glucose induced more CO_2 production in the intermediate reducing zone, due to the dominance of obligate anaerobes and the weak role of fermenting activity,

which was active and responsible for comparable stimulation of CH₄ production in highly reducing zones by acetate and glucose additions. Measuring both acetate and glucose induced respiration may be useful to account for the effect of redox conditions on microbial activity.

Ferric iron reducers are bioelectrochemically active microorganisms capable of using electrodes as electron donors and acceptors. Nitrate is a strong oxidant.

Bioelectrochemical reduction of NO₃⁻ in the overlying water was also investigated through a fuel cell process without direct contact with the flooded soil, the source of reductants (Chapter 6). Nitrate removal was faster under bioelectricity generation than under no electricity generation suggesting the applicability of a wide range of organic carbon sources for bioelectrochemical NO₃⁻ removal from drinking water. The current varied with microbial NO₃⁻ reduction and N₂O accumulation in the cathode chamber affected by the inoculation rate. When the enzymatic reduction of N₂O to N₂ was inhibited by acetylene, the current generated was higher than that coupled to O₂ reduction.

The effect of Fe³⁺ reduction and regeneration in NO₃⁻ percolation and fluxes of N₂O and CH₄ in a soil-rice system was investigated in the pot experiment (Chapter 7). Nitrate percolation and N₂O emission were stimulated by Fe³⁺ amendment in the early days after flooding. The effect of Fe³⁺ amendment on reducing CH₄ emission was small in the early season when CH₄ production was low, but was significant after 2-day drainage intervals in the mid- and late-seasons. The improved CH₄ mitigation was mainly attributed to more

Fe³⁺ regeneration during drainage in amended pots, delaying the onset of favorable soil conditions to support CH₄ production upon reflooding. No side effect of Fe³⁺ amendment on rice yield was observed. This experiment shows that Fe³⁺ regeneration plays an important role in short-term drainage-based CH₄ mitigation in rice soils.

Conclusions

Reduction of Fe³⁺-centered intermediate AEAs was mainly mediated by obligate anaerobes relying on fermentation products.

Ferric iron reducers are important as bioelectrochemically active microorganisms, supporting bioelectricity generation from the flooded soil coupled to the reduction of oxidants O₂ and NO₃⁻ in the overlying water.

Microbial Fe³⁺ reduction was a major electron accepting process in flooded rice soils.

Ferric iron reduction temporally affected N₂O production but its interaction with NO₃⁻ reduction was obscured in homogenized soil slurries. In heterogeneous soil columns and rice pots, intensification of iron-centered intermediate redox processes changed the fate of NO₃⁻ in the overlying water, decreasing heterotrophic denitrification and increasing NO₃⁻ percolation and N₂O emission.

Ferric iron reduction competitively suppressed methanogenic activity in homogenized soil slurries. Temporal and vertical variations of iron-centered intermediate redox processes subsequently controlled temporal and vertical variations of methanogenic activity in flooded soil columns. In a soil-rice system, Fe³⁺ regeneration effectively reduced CH₄ emission after midseason drainage intervals.

Future Research Recommended

The importance of cycling of Fe^{3+} in anaerobic carbon decomposition and CH_4 emission in wetland environments, especially rice fields, has been acknowledged in recent studies. Nevertheless, reliable quantitative evaluation and predication of cycling of Fe^{3+} in wetland environments is yet to be established. Studies on cycling of Fe^{3+} at the soil-water interface and the rhizosphere in field conditions are needed to account for the role of cycling Fe^{3+} in anaerobic carbon mineralization and CH_4 emission in rice fields.

The role of non-nitrate AEAs is poorly understood in denitrification in wetland environments. However, due to soil heterogeneity, AEAs other than NO_3^- do affect fate of NO_3^- in the flooded soil, especially under carbon limitation. Studies under more realistic conditions are needed to evaluate these effects on the fate of NO_3^- in seasonally and intermittently flooded wetlands, typically rice fields.

The possibility of using a wide range of organic carbon sources for bioelectrochemical removal of oxidized pollutants, including NO_3^- from drinking water, also deserves further investigation.

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