SUBTASK 1.22 – MICROBIAL CYCLING OF CH₄, CO₂, AND N₂O IN A WETLANDS ENVIRONMENT

Final Report

(for the period of March 1, 2006, through December 31, 2008)

Prepared for:

AAD Document Control

U.S. Department of Energy
National Energy Technology Laboratory
PO Box 10940, MS 921-107
Pittsburgh, PA 15236-0940

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ACKNOWLEDGMENT

This report was prepared with the support of the U.S. Department of Energy (DOE) National Energy Technology Laboratory Cooperative Agreement No. DE-FC26-98FT40320. However, any opinions, findings, conclusions, or recommendations expressed herein are those of the authors(s) and do not necessarily reflect the views of DOE.

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ACKNOWLEDGMENTS

We thank the U.S. Geological Survey (USGS) Northern Prairie Wildlife Research Center for field gas collection, soil sampling, and column buildup at the DU-12 site in Ipswich, South Dakota. We also thank Ducks Unlimited, Inc., for generously providing the DU-12 site as well as providing the hydraulic hitter for our in situ microcosm study. The soil nitrogen and carbon contents were determined by the U.S. Department of Agriculture Agricultural Research Service North Central Soil Conservation Research Laboratory, and this excellent analytical work is greatly appreciated.

MICROBIAL CYCLING OF CH₄, CO₂, AND N₂O IN A WETLANDS ENVIRONMENT

ABSTRACT

Soil microbial metabolic activities play an important role in determining CO₂, CH₄, and N₂O fluxes from terrestrial ecosystems. To verify and evaluate CO₂ sequestration potential by wetland restoration in the Prairie Pothole Region (PPR), as well as to address concern over restoration effects on CH₄ and N₂O emissions, laboratory and in situ microcosm studies on microbial cycling of CO₂, CH₄, and N₂O were initiated. In addition, to evaluate the feasibility of the use of remote sensing to detect soil gas flux from wetlands, a remote-sensing investigation was also conducted. Results of the laboratory microcosm study unequivocally proved that restoration of PPR wetlands does sequester atmospheric CO₂. Under the experimental conditions, the simulated restored wetlands did not promote neither N₂O nor CH₄ fluxes. Application of ammonia enhanced both N₂O and CH₄ emission, indicating that restoration of PPR wetlands may reduce both N₂O and CH₄ emission by cutting N-fertilizer input. Enhancement of CO₂ emission by the N-fertilizer was observed, and this observation revealed an overlooked fact that application of N-fertilizer may potentially increase CO₂ emission.

In addition, the CO₂ results also demonstrate that wetland restoration sequesters atmospheric carbon not only by turning soil conditions from aerobic to anoxic, but also by cutting N-fertilizer input that may enhance CO₂ flux. The investigation on microbial community structure and population dynamics showed that under the experimental conditions restoration of the PPR wetlands would not dramatically increase population sizes of those microorganisms that produce N₂O and CH₄. Results of the in situ study proved that restoration of the PPR wetland significantly reduced CO₂ flux. Ammonia enhanced the greenhouse gas emission and linearly correlated to the CO₂ flux within the experimental rate range (46–200 kg N ha⁻¹). The results also clarified that the overall reduction in global warming potential (GWP) by the PPR wetland restoration was mainly contributed from reduction in CO₂ flux. These results demonstrate that restoration of currently farmed PPR wetlands will significantly reduce the overall GWP budget. Remote sensing investigations indicate that while the 15-meter resolution of the imagery was sufficient to delineate multiple zones in larger wetlands, it was not sufficient for correlation with the ground-based gas flux measurement data, which were collected primarily for smaller wetland sites (<250 meters) in the areas evaluated by this task. To better evaluate the feasibility of using satellite imagery to quantify wetland gas flux, either higher-resolution satellite imagery or gas flux data from larger wetland sites is needed.

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MICROBIAL CYCLING OF CH₄, CO₂, AND N₂O IN A WETLANDS ENVIRONMENT

EXECUTIVE SUMMARY

Recent studies revealed that restoration of previously farmed wetlands in The Prairie Pothole Region (PPR) may provide a unique and previously overlooked opportunity to sequester atmospheric CO₂. However, land use types also affect CH₄ and N₂O fluxes, and concern was thus raised over CH₄ and N₂O emissions from restored wetlands. Since soil microbial metabolic activities play an important role in determining CO₂, CH₄, and N₂O fluxes from terrestrial ecosystems, to address the concern and to fill the gap, we initiated this project to study microbial CO₂, CH₄, and N₂O cycling in PPR wetland environments. This project consisted of three tasks: the first two tasks were a laboratory and an in situ microcosm study on microbial cycling of CO₂, CH₄, and N₂O in a PPR wetlands environment; and the third task was a feasibility evaluation of the use of remote sensing to detect soil gas flux from wetlands.

The objectives of the laboratory microcosm study were 1) to verify and evaluate the potential of wetland restoration to sequester CO₂; 2) to clarify effects of the restoration on CH₄ and N₂O emission; and 3) to examine the effects of wetland restoration on soil microbial community structure and population dynamics, especially those populations involved in the production and consumption of CH₄ and N₂O. The organic carbon contents results of this task unequivocally proved that restoration of PPR wetlands does sequester atmospheric CO₂. Under the experimental conditions, the simulated restored wetlands did not promote neither N₂O nor CH₄ fluxes. Application of nitrogen fertilizers is a routine agricultural practice in the study region, and the experimental results showed that ammonia enhanced both N₂O and CH₄ emission, indicating that restoration of PPR wetlands may reduce both N₂O and CH₄ emission by cutting N-fertilizer input. Enhancement of CO₂ emission by the N-fertilizer was observed, and this observation revealed an overlooked fact that application of N-fertilizer may potentially significantly increase CO₂ emission. In addition, the CO₂ results also demonstrate that wetland restoration sequesters atmospheric carbon not only by turning soil conditions from aerobic to anoxic, but also by cutting N-fertilizer input that may enhance CO₂ flux. The investigation on microbial community structure and population dynamics showed that under our experimental conditions restoration of the PPR wetlands would not dramatically increase population sizes of those microorganisms that produce N₂O and CH₄.

The in situ microcosm study was an effort to explore a methodology that may provide accurate estimation and prediction on changes in CO₂, N₂O, and CH₄ budget by wetland restoration, as well as to provide a blueprint to foresee what it might occur if the site were restored. This task consisted of two on-site column experiments: one was to quantitate changes in CO₂, N₂O, and CH₄ fluxes from the investigated wetlands “before” and “after” restoration, while the other was to examine the in situ effects of N-fertilizers on CO₂, N₂O, and CH₄ emission from the terrestrial ecosystems. Results of the in situ study proved that restoration of the wetland significantly reduced CO₂ flux. N-fertilizer enhanced greenhouse gas emission and linearly correlated to the CO₂ flux within the experimental rate range (46-200 kg N ha⁻¹). The experimental results also clarified that the overall reduction in global warming potential (GWP) by the wetland restoration was mainly contributed from reduction in CO₂ flux. These results

demonstrate that restoration of currently farmed PPR wetlands will significantly reduce the overall GWP budget.

The goal of the third task was to evaluate the feasibility of using satellite-derived imagery to quantify wetland climate-forcing gas fluxes on a regional scale. This was conducted by classifying the wetland morphology in several satellite images to ground-based gas flux measurements collected by the U.S. Geological Survey (USGS) at the corresponding sites. The results indicate that while the 15-meter resolution of the imagery was sufficient to delineate multiple zones in larger wetlands, it was not sufficient for correlation with the ground-based gas flux measurement data, which were collected primarily for smaller wetland sites (<250 meters) in the areas evaluated by this task. To better evaluate the feasibility of using satellite imagery to quantify wetland gas flux, either higher-resolution satellite imagery or gas flux data from larger wetland sites is needed.

MICROBIAL CYCLING OF CH₄, CO₂, AND N₂O IN A WETLANDS ENVIRONMENT

A LABORATORY STUDY ON MICROBIAL CYCLING OF CO₂, CH₄, AND N₂O IN A WETLANDS ENVIRONMENT

Introduction

In terrestrial ecosystems, microbial production and consumption of CO₂, CH₄, and N₂O are the major processes in determining whether an ecosystem is a source or sink of these greenhouse gases. However, these microbial processes are strongly affected by land use types and management practices.

The glaciated North American prairie previously supported the largest wetland complex in North America. However, cultivated agriculture has shifted the function of the wetlands from net sinks to net sources of atmospheric carbon (Euliss et al., 2006). Recently, it has been demonstrated that restoration of previously farmed wetlands in this region resulted in the rapid replenishment of soil organic carbon lost to cultivation (Euliss et al., 2006), revealing that restoration of previously farmed wetlands in the Prairie Pothole Region (PPR) may provide a unique and previously overlooked opportunity to sequester atmospheric CO₂. However, since land use types also affect CH₄ and N₂O fluxes, concern was raised over the CH₄ and N₂O emissions from the restored wetlands. To address the concern and to fill the gap, we initiated this project to study microbial CO₂, CH₄, and N₂O cycling in PPR wetland environments. We hypothesize that restoration of the previously farmed prairie wetlands may not only sequester atmospheric CO₂ but may also mitigate CH₄ and N₂O emissions. This hypothesis is based on the discussion below.

Sequestration of Atmospheric CO₂

In terrestrial ecosystems, soil microorganisms are the major organic carbon decomposers. Under anaerobic conditions, microbial mineralization of organic carbon is much less efficient energetically than under aerobic conditions. Once a soil becomes anoxic, mineralization rates of organic compounds slow down, and organic soils can accumulate and become a sink for atmospheric carbon. Wetland restoration favors the soil conditions becoming more anoxic, hence slowing down organic carbon mineralization, and turns the wetlands from sources to sinks for the atmospheric CO₂.

Mitigation of N₂O and CH₄

Wetland restoration has two major effects on the soils: one is to turn the soils more anoxic and the other is to cut input of nitrogen fertilizers. Even though CH₄ is produced anaerobically, anoxic conditions slow down carbon decomposition and may result in mitigation of CH₄, depending on the environmental conditions (e.g., temperature, carbon availability, etc.). This is mainly because of two reasons: first, methanogens locate on the downstream side of the anaerobic food chain and depend on decomposition of upstream organic compounds for carbon and energy sources. Therefore, a slowdown in decomposition of upstream organic compounds

would result in the production of fewer degradation products that are carbon and energy sources for methanogens. This would consequently mitigate methane production. Second, cutting ammonium N-fertilizer application enhances the methane oxidation (consumption) potential of the farmed soils (Le Mer & Roger, 2001).

Similarly, in spite of the fact that anoxic condition likely increases N_2O production, cutting N-fertilizer input may significantly reduce N_2O production. The European Commission Agricultural Directorate-General concluded that “fertilization of crops significantly contributes to the emission of greenhouse gases, especially the emission of nitrous oxide (N_2O) from soils” (European Commission Agricultural Directorate-General, 2000). Recently, an investigation of wetland restoration in a glaciated region in northeastern Germany similar to the PPR has been reported (Merbach et al., 2002). According to this report, wetland restoration may actually reduce emissions of both CH_4 and N_2O .

Agriculture plays a major role in the global fluxes of the greenhouse gases CO_2 , CH_4 , and N_2O , and maximum mitigation of these gases is provided by removing land from production (Robertson et al., 2000). It appears that restoration of previously farmed prairie wetlands has great potential to sequester atmospheric carbon and to mitigate CH_4 and N_2O emissions.

Goals and Objectives

The goals of this Energy & Environmental Research Center (EERC) investigation are 1) to test the hypothesis that restoration of the previously farmed prairie wetlands may not only sequester atmospheric CO_2 but also mitigate CH_4 and N_2O emissions and 2) to provide experimental data to support the development of action plans and long-term postrestoration management strategies, so that the wetland restoration and management may be economical, sustainable, and environmentally sound.

The objectives of the proposed study are as follows:

- To verify and evaluate the potential of wetland restoration to sequester CO_2 .
- To clarify and to quantitate effects of the restoration on CH_4 and N_2O emission from the wetlands.
- To examine the effects of the wetland restoration on soil microbial community structure and population dynamics, especially those populations involved in the production and consumption of CH_4 , and N_2O .

Experimental

Soil Sampling

The soils were taken from the Nikolaisson area in Cando, North Dakota. The sampling site is located about 3–4 miles north of Cando, and it is a protected site (Figure 1) managed by the Devils Lake Wetlands Office.

A backhoe was used to dig the soil. Soils were sampled from a 0 to 150-cm profile and were taken from six horizon sections of the profile (Figure 2). The top 30 cm of soil was divided into two sections, 15 cm each, while others were divided into four sections, 30 cm each.

Soils from same sections were placed into coolers and drums and were immediately transported to the EERC, and were stored at 4°C in a walk-in cool room. Soil cores from each section were also taken using the standard cylinder method (Figure 3) for determination of the soil bulk densities.

Preparation of the Soil Columns

Eight PVC columns (20 cm × 150 cm or 20 cm × 170 cm) were built up in the lab to reconstruct the 0–150-cm soil section of the site as shown in Figure 4. Four 150-cm-high columns were used to simulate the currently farmed wetlands (F-columns, henceforth), and the four 170-cm-high columns were used to simulate the restored wetlands (R-columns, henceforth). The top 20 cm of the R-columns were the space to hold 20 cm of water on top of the packed soils.

Soil samples from each section were thoroughly mixed to ensure complete homogenization. The soil columns were packed section by section. In order to construct a 0–150-cm soil profile that has the same field bulk density, the amounts of the soils needed to construct the sections were calculated, weighted, and evenly distributed into the columns. After the soils were introduced into the columns, the soil sections were then compressed manually to achieve the field bulk densities.

Figure 1. The sampling site.

Figure 2. The soil was taken from each horizon section of the profile.

EERC DY33704.CDR

Figure 3. Soil cores from each section were also taken using the standard cylinder method for determination of the bulk densities.

EERC DY33705.CDR

(A)

(B)

Figure 4. Soil column design (A) and picture (B).

After the column packing was finished, distilled water was added to the R-columns to fill the 20-cm headspace on top of the packed 150-cm-high soil columns to simulate wetland restoration. All columns were then kept in the lab at room temperature (22°C).

Water was added once a week to replenish the evaporated water and to maintain the water content of the F-column soils at the soil moisture level in the field.

Addition of the Nitrogen Fertilizer

As mentioned above, wetland restoration affects microbial cycling of CO₂, CH₄, and N₂O mainly because of two factors: one is that it turns the soil environment from aerobic to anaerobic and the other is that it cuts nitrogen fertilizer input, which influences microbial production and consumption of N₂O and CH₄. To study the effects of these two factors, the eight columns were first divided into two groups (F- and R-column groups). When the N-fertilizer experiment was started, each group was then further divided into two subgroups: with and without nitrogen fertilizer (N-fertilizer, henceforth). Therefore, there were four experimental groups to simulate farmed and restored wetlands with and without receiving N-fertilizer, and each group had duplicate soil columns.

Ammonia was chosen as a representative N-fertilizer in this experiment since it is the most commonly used N-fertilizer in this region. Ammonium hydroxide (50%, v/v) aqueous solution (Ricca Chemical Co., Arlington, Texas) was used as the N-fertilizer, and the application rate of the N-fertilizer was 200 kg N/ha. To simulate agricultural injection, a screwdriver was used to puncture holes in the soil to a depth of 4.5 inches, and the liquid ammonia was then added using a 9-inch glass Pasteur pipette. The tip of the pipette reached the bottom of the holes. As soon as the fertilizer was added, the holes were immediately refilled with soil.

Gas Sampling and Analysis

The gas emitted from the soil columns were periodically sampled with static gas chambers (Livingston & Hutchinson, 1995). The gas chambers are made of 20-cm × 10-cm PVC columns, and each chamber has a pressure vent and a rubber stopper sampling port (Figure 5). To collect the gas samples, the top of the columns were sealed with the chambers for 1 hour. Then 40 mL of gas was taken from each chamber using a syringe, and the gas sample was then immediately injected into a 10-mL anaerobic vial sealed with a butyl stopper and aluminum crimper. Prior to being used, the vials were placed in a vacuum chamber of an anaerobic glove box and experienced three vacuum–air cycles to ensure the same gas contents in all vials. Before collection of the gas samples, environmental gas was injected into the vials, 40 mL for each vial, to quantitate the background CO₂, CH₄, and N₂O concentrations.

Gas samples were analyzed by a gas chromatography (Agilent GC 6890) with a Haysep DB 100/120 column (30' × 1/8" × 0.85" SS, Altech, Deerfield, Illinois). Three detectors were used to analyze the gas samples: a thermal conductivity detector (TCD), an electron capture detector (ECD), and a flame ionization detector (FID) for analysis of CO₂, N₂O, and CH₄, respectively. The carrier gas for CO₂ and CH₄ analysis was helium, while the carrier gas for N₂O analysis was argon blended with 5% methane.

Figure 5. Gas-sampling chamber.

Analysis of Soil Carbon Contents

After the soil columns were packed and maintained in the lab for 2 years, soil samples were taken from the top 0–15-cm section of each column, and the samples were analyzed for soil carbon contents.

The soil samples were air-dried, grounded, and the carbon contents were determined by the dry combustion method (Nelson & Sommers, 1996).

The Glassware Experiment

To further examine the effects of the N-fertilizer on methane oxidation in the soil, a glassware experiment was conducted. The soil used in the glassware experiment was the same soil (from the 0–15-cm section) used for the column experiment, which was preserved at 4°C in a cool room. Prior to setting up this experiment, the soil had been incubated at 25°C for 3 weeks.

Serum bottles (125 mL) were used as the experimental vessels. Four experimental groups received different concentrations of the N-fertilizer 0 (the control group), 200, 400, and 800 kg N ha⁻¹. Each group consisted of three bottles (three replicates), and each bottle contained 10 g (dry weight) of soil. Similar to the column experiment, ammonia hydroxide was used as the N-fertilizer. After the fertilizer was introduced, the water content of the soil in each bottle was 23.8%. The bottles were then sealed with butyl stoppers and aluminum crimpers. After the bottles were sealed, 50 mL of the headspace gas was taken from each bottle using a 60-mL syringe, and then 10 mL of O₂ (99.994%) and 40 mL of 5% CH₄ in argon were injected into the bottles. The bottles were then incubated at 25°C in the dark.

After 7 days of incubation, 1 mL of headspace gas was taken from each bottle to analyze for CH₄ on gas chromatograph (GC)/FID, then another 20 mL of O₂ was injected, and the bottles were continuously incubated.

Changes in Soil Microbial Community Structure

Changes in microbial community structure in the soil columns were studied. Since in terrestrial environments most microorganisms are in the topsoil, the samples were taken from the top 0 to 15 cm soils. This activity mainly focused on the microorganisms that are directly involved in production and consumption of CO₂, CH₄, and N₂O. The investigated microbial physiological groups were heterotrophic aerobic bacteria (HAB), heterotrophic anaerobic plus facultative anaerobic bacteria (HANfB), nitrifying bacteria (autotrophic ammonium- and nitrite-oxidizing bacteria), denitrifying bacteria, and methanogenic bacteria.

HAB counts were obtained by plate count method (Page et al., 1982) using plate count agar (PCA; Difco, Detroit Michigan). HANfB population size was determined with a five-tube most probable number (MPN) method (Page et al., 1982) with thioglycollate medium (Difco, Detroit Michigan). The tubes were sealed, and the tubes with evident growth in the lower portion were counted as positive tubes. Nitrifying bacteria, autotrophic ammonia- and nitrite-oxidizing bacteria, denitrifying bacteria, and methanogenic bacteria were all enumerated by MPN method. NH₄⁺ oxidizer and NO₂⁻ oxidizer media were used for ammonium- and nitrite-oxidizing bacteria, respectively (Schmidt & Belser, 1994). Nutrient broth (Difco, Detroit, Michigan) was used as the enumeration medium for denitrifying bacteria, and the presence of denitrifiers was confirmed by bubble formation. When nutrient broth is used as the growth medium, the bubbles produced are virtually always N₂ (Tiedje, 1994). Methanogenic bacteria were enumerated using anaerobic culture tubes. Barker's medium (Man & Smith, 1981.) was chosen as the basal medium with the addition of methanol and acetate; the headspace gas was H₂/CO₂. Positive tubes were those confirmed by the production of CH₄ (detected by GC/FID).

Results and Discussion

The Laboratory Microcosm Experiments

Establishment of the Simulated Farmed and Restored Wetlands Microcosms

The bulk density of the on-site soil is listed in Table 1. When the soil columns were packed to the top two sections (0–15 cm and 15–30 cm), it was difficult to further compress the soil; therefore, each column was only loaded with 75% of the calculated amount of the top 30 cm of soils.

After the column packing was finished, the R-columns were filled with water to the top, and then all columns were kept in the lab (22°C). The nitrogen fertilizer was added 3 months after the columns were packed. The 3 months were a time period to allow the columns to be “conditioned” to approach the field conditions of the “farmed” and “restored” wetlands. During the 3 months, gas samples from the columns were taken biweekly, and the samples were analyzed for CO₂, N₂O, and CH₄. Since no columns received N-fertilizer in these 3 months,

Table 1. Bulk Density of the On-Site Soil

Depth, cm	Bulk Density, g cm ⁻³
0–15	1.55
15–30	1.38
30–60	1.75
60–90	1.72
90–120	1.65
120–150	1.66

there were only two experimental groups during this time period: the F-column group and the R-column group. When the N-fertilizer experiment started, each group was then subdivided into another two groups: with (the FN and RN groups) and without (the F and R groups) N-fertilizer application groups.

CO₂ Flux

As shown in Figure 6a, CO₂ emission from the F-columns was more than that from the R-columns; however, the amount of CO₂ evolved from the F-columns decreased with time. Since during the experiment no nutrients were added, it is likely that as some nutrient(s) were consumed and became a limiting factor, the biodegradation would consequently be slowed down.

It was unexpected that, under our experimental conditions, the N-fertilizer promoted CO₂ flux from the soil columns (Figure 6b). As illustrated in Figure 6b, 10 days after receiving the N-fertilizer, the CO₂ flux from both FN- and RN-columns significantly increased; both were tripled compared to their control groups without the addition of the N-fertilizer (the F- or R-columns). It is possible that activities of the heterogeneous microorganisms might be limited by the available ammonium or nitrogen source. Since deficiency of nitrogen is quite common in agricultural soils and the application of N-fertilizer is a routine agricultural practice, this result revealed an overlooked fact that the application of N-fertilizer to nitrogen-deficient soils may potentially significantly increase CO₂ emission. Since wetland restoration converts fertilizer-demanding agricultural lands to non-fertilizer-demanding wetlands, our results showed that wetland restoration sequesters atmospheric carbon not only by turning soil conditions from aerobic to anoxic but also by cutting N-fertilizer input, which may significantly enhance CO₂ flux from nitrogen-deficient soils.

Soil Organic Carbon Contents

After the soil columns were packed and maintained in the lab for 2 years, soil samples were taken from the top 0 to 15-cm section of each column, and the samples were analyzed for carbon content.

Significant differences in soil carbon content between the simulated “farmed wetland” (the F- and FN-columns) and the “restored wetland” (the R- and RN-columns) were observed. Despite the fact that the soil used to pack the columns was thoroughly homogenized and the

Figure 6. CO₂ flux from the soil columns: (A) before addition of the nitrogen fertilizer and (B) after addition of the N-fertilizer. Error bars represent Standard Error (SE).

columns were randomly assigned to simulate “farmed” or “restored” wetlands, after 2 years sitting in the lab, the plant debris (mainly grass stems and root hairs) partially persisted in the soils of the “restored-wetland” columns, while completely disappeared from the “farmed-wetland” columns (Figure 7, Table 2).

Results of soil organic carbon contents are listed in Table 3. As shown in Table 3, soils in R- and RN-columns contained 38.1% and 36.1% more organic carbon, respectively, than their corresponding control columns (F- and FN-columns). If 1) carbon of the plant debris is included as “organic carbon in the soils” and 2) the FN-columns represent the “Farmed” wetlands while the R-columns represent the “Restored” wetlands, then the “Restored” wetlands contained 42.1% more organic carbon (calculation not shown). Since the more organic carbon accumulated the more atmospheric carbon sequestered, the results of the soil organic carbon analysis unequivocally proved that wetland restoration in this region does sequester atmospheric CO₂.

N₂O Flux

Before the addition of the N-fertilizer, during the first 3-month period, no significant difference in the N₂O flux between the F- and R-columns was detected (Figure 8a). In soil environments, both microbial nitrification and denitrification processes produce N₂O as an intermediate metabolic product (Groffman, 1991). Despite the fact that nitrification mainly occurs under aerobic conditions while denitrification is generally associated with anaerobic conditions, anoxic nitrification has been reported (Robertson & Kuenen, 1991). Similarly, denitrification has also been demonstrated to occur in the presence of O₂ (Groffman, 1991). Under our experimental conditions, the simulated restored wetlands (the R-columns) did not produce more N₂O than the simulated currently farmed wetlands (the F-columns).

Figure 7. Photograph of the air-dried soil samples from the different columns. Plant debris (mainly grass stems and root hairs) partially persisted in the simulated “restored-wetland” columns but completely disappeared from the “farmed-wetland” columns.

Table 2. Weight Percentage of Plant Debris in the Soil Samples

Columns	F	FN	R	RN
Weight, ^a %	0	0	0.24	0.13
SE ^b	0	0	0.05	0.01

^a Dry weight; average of two samples from replicate columns.

^b Standard Error (SE).

Table 3. Soil Organic Carbon of the Soil Samples

Columns	F	FN	R	RN
% Organic Carbon	2.85	2.78	3.88	3.84
SE	0.03	0.00	0.05	0.10

^a Weight percentage; average of two samples from replicate columns.

Addition of N-fertilizer resulted in a significant increase in the N_2O in both F- and R-columns (Figure 8b). Enhanced N_2O flux from the N-fertilizer-amended columns (FN- and RN-columns) was observed 7 days after application of the N-fertilizer. At Day 10, the N_2O flux from the FN- and RN-columns was 8.6 and 13.6 times of that produced from the F- and R-columns, respectively.

Figure 8. N_2O flux from the soil columns: (A) before addition of the nitrogen fertilizer and (B) after addition of the N-fertilizer. Error bars represent Standard Error (SE).

Application of nitrogen fertilizers, especially ammonia, is a routine agricultural practice in this region. Since wetland restoration cuts N-fertilizer input, our experimental results demonstrate that wetland restoration in this region may reduce N_2O emission.

CH₄ Flux

During the first 3 months before the N-fertilizer was introduced, little difference in CH_4 flux between the F- and R-column groups was observed (Figure 9a), suggesting that under our experimental conditions, the simulated restored wetlands did not produce more CH_4 .

Wetland restoration makes the soils more anoxic, which may have two contradictory effects on methane formation. Methanogenic bacteria are strictly anaerobes; therefore, anoxic conditions promote methanogens. On the other hand, however, methanogens locate on the downstream side of the anaerobic food chain and depend on decomposition of upstream organic compounds for carbon and energy sources. Anoxic conditions slow down carbon decomposition, resulting in limitation of available food for methanogens and, thereby, may consequently mitigate CH_4 formation, whether the net effect is to increase or decrease CH_4 production, depending on other environmental conditions, such as temperature, nitrogen source, etc. (Grill et al., 1991). As shown in Figure 7 and Table 2, under our experimental conditions (22°C, no organic carbon added), significant decrease in organic decomposition rate was evidenced, indicating potential limitation on CH_4 formation.

In soil environments, once CH_4 is produced, it will migrate upward. It has been well documented that during the migration process, 60% to more than 90% of CH_4 is reoxidized by methylotrophic microorganisms before it emits to the atmosphere (Le Mer & Roger, 2001). The fact that there was no significant difference in CH_4 flux between the F- and R-columns may result from either one or both of the following two possible reasons: 1) the CH_4 oxidation activity in the F-columns was high and 2) the methane formation rate in the R-columns was limited by the slow decomposition of organic carbon.

A significant increase in CH₄ flux was observed after addition of the N-fertilizer (Figure 9b). It has been generally accepted that the consumption of methane in upland as well as lowland systems is inhibited by nitrogenous fertilizer additions (Bodelier & Laanbroek, 2004). Despite the fact that the soil in the R-columns was under anoxic conditions, the soil–water interface as well as some micro aerobic niche may support CH₄ oxidizing microorganisms (methanotrophs). In fact, methanotrophs are quite common in both freshwater and deep-sea sediments; in addition, anaerobic methane oxidation plays an important role in marine sediments (Kotelnikova, 2002). Liebner and Wagner reported that addition of ammonium to the water layer on top of the soil core reversibly increased the aerobic CH₄ flux because of inhibition of CH₄ oxidation in the soil surface layer (Liebner & Wagner, 2007).

The Glassware Experiment

A glassware experiment was conducted to further examine the effects of nitrogen fertilizer on methane consumption. The experiment was conducted with 125-mL serum bottles, and methane was injected into the bottles. Aerobic conditioning in this close system was achieved by injecting oxygen into the bottles.

No methane consumption was detected at Day 7. However, after 21 days of incubation, the GC results showed that addition of the N-fertilizer at all experimental concentrations completely inhibited CH₄ consumption (Table 4).

These results confirmed that ammonia inhibits CH₄ consumption in the soils in this region; in other words, application of ammonia fertilizer in this region increases CH₄ emission.

Figure 9. CH₄ flux from the soil columns: (A) before addition of the nitrogen fertilizer and (B) after addition of the nitrogen fertilizer. Error bars represent Standard Error (SE).

Table 4. Effects of the N-Fertilizer on Methane Consumption

Application Rate of Ammonium (kg N ha ⁻¹)	0	200	400	800
% CH ₄ Remaining	0.018	100.1	100.1	96.2
SE	0.00	2.9	1.4	3.0

Changes in Soil Microbial Community Structure

After 3 months of sitting in the lab (22°C), compared to the F columns, the R-columns had more heterotrophic (obligate plus facultative) anaerobic microorganisms and less aerobic heterotrophic microorganisms, and this trend remained for the 18 months to completion of the microbiology work (Figure 10). The N-fertilizer did not change the total counts of either anaerobic or aerobic heterotrophic bacteria (data not shown).

Both denitrifying bacteria (microorganisms that are able to convert nitrate to N₂) and nitrifying bacteria (microorganisms that are able to convert ammonia to nitrite and nitrate) are involved in soil N₂O production processes. It appeared that R-columns had less nitrifying bacteria (Figure 11). However, during the 18 months of incubation at room temperature (22°C), the population sizes of denitrifying bacteria (these bacteria are facultative anaerobic) in both R- and F-columns were comparable (Figure 11).

Figure 10. Population dynamics of heterotrophic bacteria in the soils: (A) CFU (colony-forming unit) per gram of dry soil of the heterotrophic aerobic bacteria and (B) MPN per gram of dry soil of the heterotrophic obligate plus facultative anaerobic bacteria.

Figure 11. Population dynamics of nitrifying and denitrifying bacteria in the soils.

The MPN results on enumeration of methanogenic bacteria (Figure 12) showed that under our experimental conditions (22°C, without addition of organic carbon), after 18 months of incubation, the population levels of methanogenic bacteria in both columns simulated to wetlands before and after restoration were roughly comparable. Generally speaking, upland soils emit less CH₄ than water-logged soils, however, the difference is mainly due to their CH₄ oxidation ability instead of CH₄ production capability. An upland soil may produce much more CH₄ than many water-logged soils and yet still emit less CH₄ or even function as a CH₄ sink if it has high CH₄ oxidation activity.

Figure 12. Population dynamics of methanogenic bacteria in the soils.

MPN method is valid to quantitate methanotrophs in aerated soils. However, it has been proposed that this method might not be suitable to quantitate methanotrophs in anaerobic environments because microorganisms or consortia responsible for anaerobic methane oxidation have not yet been cultured, although diverse aerobic methanotrophs have been isolated from a variety of underground niches (Kotelnikova, 2002). Since methanotrophs play an equally important role as methanogens in determining CH₄ flux from terrestrial environments, to avoid any possible misinterpretation of the experimental results, we did not conduct MPN enumeration on methanotrophs. In fact, the best way to monitor the population dynamics of methanotrophs is to use gene probe and DND hybridization methods. However, because of budget limitations, we were unable to conduct this investigation, so the effects of wetland restoration on methanotrophs remain to be clarified for our future investigation. Nevertheless, except for the methanotrophs, results of this microbiology study showed that under our experimental conditions, restoration of the wetlands in this region would not dramatically increase population sizes of those microorganisms that produce N₂O and CH₄.

Conclusions

Results of the analysis on organic carbon contents unequivocally proved that wetland restoration in this region does sequester atmospheric CO₂. Under our experimental conditions, the simulated restored wetlands did not promote neither N₂O nor CH₄ fluxes from the soils.

The fact that the simulated wetland did not promote CH₄ emission, coupled with the fact that mineralization of organic carbon was significantly slowed down in the simulated wetland soils, suggests that CH₄ production was probably limited by the decreased rate in degradation of organic carbon.

Application of nitrogen fertilizers, especially ammonia, is a routine agricultural practice in this region. Our experimental results confirmed that ammonia enhances both N₂O and CH₄ emission, indicating that wetland restoration in this region may reduce both N₂O and CH₄ emission by cutting N-fertilizer input.

Enhancement of CO₂ emission by the N-fertilizer was observed, and we postulate that the soil might be ammonium- or nitrogen-limited. Deficiency of nitrogen is quite common in agriculture soils, and application of N-fertilizer is a routine agricultural practice. This observation revealed an overlooked fact that application of N-fertilizer to nitrogen-deficient soils may potentially significantly increase CO₂ emission. Since wetland restoration converts fertilizer-demanding agricultural lands to non-fertilizer-demanding wetlands, our results showed that wetland restoration sequesters atmospheric carbon not only by turning soil conditions from aerobic to anoxic, but also by cutting N-fertilizer input which may significantly enhance CO₂ flux from nitrogen-deficient soils.

Except for population dynamics of methanotrophs that remain for future investigation, results of the microbial community study showed that under our experimental conditions, restoration of the wetlands in this region would not dramatically increase population sizes of the microorganisms that produce N₂O and CH₄.

AN IN SITU MICROCOSM STUDY ON MICROBIAL CYCLING OF CO₂, CH₄, AND N₂O IN A WETLANDS ENVIRONMENT

Introduction

In terrestrial ecosystems, fluxes of CO₂, CH₄, and N₂O are mainly determined by soil microbial metabolic activities, which are, in turn, strongly affected by environmental conditions such as soil types, land use type, temperature, water contents, and plant covers, etc. In Part I of this report, a laboratory microcosm study was reported to investigate the effects of wetland restoration on microbial cycling of CO₂, CH₄, and N₂O in the glaciated North American prairie ecosystem. The laboratory experiments are the Phase I study of Project 1.22. The laboratory experiments eliminated impacts of some environmental variables (e.g., temperature, water content, plant cover, etc.) on microbial production and consumption of CO₂, CH₄, and N₂O, and the results may provide some information that might give an insight into the mechanisms regarding the effects of wetland restoration on microbial CO₂, CH₄, and N₂O cycling. However, laboratory conditions are different from in situ conditions, and results of the laboratory study cannot be directly extrapolated to field evaluation and prediction. In order to accurately quantitate the real effects of wetland restoration on field microbial CO₂, CH₄, and N₂O cycling, it is necessary to initiate an in situ microcosm study (the Phase II study) so that a high level of accuracy in evaluating and predicting effects of wetland restoration on carbon sequestration and greenhouse gas mitigation can be obtained. The essential difference between Phase I and Phase II studies is that Phase I mainly focused on verification of the effects and elucidation of the possible mechanisms, while Phase II mainly focused on evaluation of the real effects in situ.

Currently, study on wetland restoration mainly uses a survey and field gas monitoring method to provide quantitative information for evaluation and prediction of potential changes in greenhouse gas fluxes. This widely used method is very useful; however, it is limited by the fact that, in natural environments, the field conditions are different from site to site and the dynamic nature plus interactions among the on-site environmental parameters make predictions even more complicated. An on-site microcosm study, however, provides an alternative protocol that may overcome the drawbacks of the current methods.

Soil columns built on-site can be viewed as a miniature terrestrial ecosystem of the test site. If a soil column is built on the test site by pushing an empty column into the soil to isolate the enclosed soil from its surroundings, then the soil inside the column will experience the same environmental conditions as the site soil. Under this condition, the CO₂, CH₄, and N₂O fluxes from the columns should be very close to those from the site. Therefore, if the columns are built on a currently farmed wetland to mimic “wetlands,” and the top of the columns are then filled with water, the columns thus become simulated restored wetlands and may provide information about what may happen if this site is restored.

Similarly, if a series of soil columns are built on a test site and the columns receive different concentrations of a nitrogen fertilizer (N-fertilizer, henceforth), correlation of the measured CO₂, N₂O, and CH₄ fluxes with the fertilizer concentrations will give a regression equation, which can be used to predict the effects of different amounts of the fertilizer on the greenhouse gas emission from the site. The quantitative greenhouse gas effects of the fertilizer,

plus the energy consumed in production and application of the fertilizer, can be converted to CO₂-equivalent global warming potential (GWP, henceforth). In this way, a clear picture of GWP effects from the fertilizer is obtained. Reduction in GWP from cutting input of nitrogen fertilizer is one of the components that contribute to overall GWP budget reduction from wetland restoration.

It is expected that the in situ microcosm study may provide experimental data for an accurate estimation and prediction of the carbon sequestration and greenhouse gas mitigation by wetland restoration in the PPR. In addition, this study will also provide a blueprint to foresee the future performance of the restored wetlands. Information obtained from this study is, therefore, expected to be important to decision-making and strategy development.

Goals and Objectives

The goal of this project is to explore a methodology that may provide accurate estimation and prediction of changes in the CO₂, N₂O, and CH₄ budget by wetland restoration in the PPR.

This project consists of two on-site column experiments as follows:

1. To quantitate changes in CO₂, N₂O, and CH₄ fluxes from the investigated wetlands in the PPR “before” and “after” wetland restoration.
2. To examine effects of N-fertilizers on CO₂, N₂O, and CH₄ emission from terrestrial ecosystems.

Experimental

Site Description

The experimental site is in Ipswich, South Dakota. This site (DU-12) was a farmed land that is currently grassland (Figure 13) managed by Ducks Unlimited (Memphis, Tennessee), a habitat conservation organization. A midslope field of the DU-12 was chosen for the on-site column experiments.

Column Installation

PVC columns (8", Schedule 40) were used for this study. The columns were cut into 63-cm-high pieces. One end of the columns was sharpened with sand to facilitate pushing the columns into the ground. The columns were thoroughly washed to remove residuals from the manufacturing process as well as any other residues that might have accumulated on them. The columns were installed using a hydraulic heavy hitter (Figure 14) and were pushed into the ground to a depth of ~33 cm. This is the maximum depth the columns could go into the ground without causing compression of the soil inside the columns. This depth should satisfy the experimental needs since production and consumption of CO₂, CH₄, and N₂O in soil are mainly determined by soil microbial activities, and this depth covers >95% of the soil's microbial activities.

Figure 13. The experimental site in Ipswich, South Dakota.

Eighteen columns were installed. Three columns (three replicates) have portions of 73 cm above the ground and were filled with water to simulate the restored wetlands (the “after” restoration group, or R-group, henceforth), while the other 15 columns have portions of 30 cm above the ground and were used to simulate the currently farmed wetlands (Figure 15). The 15 columns were randomized and separated into five treatments: one group did not receive any treatment and served as a control (the F-group, henceforth), while the other four groups received different application rates of N-fertilizer (the FN-groups, henceforth). The four application rates chosen in this study were 46, 100, 160, and 200 kg N ha⁻¹, respectively. To further facilitate the description, based on the N-fertilizer rates, we designated the four groups as FN-46, FN-100, FN-160, and FN-200, respectively.

N-Fertilizer Treatment

Nitrogen fertilizers are typically applied in two seasons: fall and spring. In this region, fall application is normally conducted no earlier than the end of September when the soil temperature drops to below 50°F to avoid loss of the fertilizer from application processes as well as from microbial nitrification and denitrification processes. In this study, the N-fertilizer was added in late October 2007 and late April 2008 (Figure 16), respectively.

Similar to the laboratory column experiment described in Part I of this report, ammonia was chosen as a representative N-fertilizer since it is the most commonly used N-fertilizer in this

EERC DY33715.CDR

Figure 14. A hydraulic hitter was used to push the columns into the ground.

Figure 15. An on-site F-column.

EERC DY33716.CDR

Figure 16. Application of the N-fertilizer in late April 2008.

region. The ammonium used in October 2007 was prepared from hydroxide aqueous solution (50%, v/v), and ammonium used in April 2008 was prepared from a 16.7% (v/v) aqueous solution; both were purchased from Ricca Chemical Company, Arlington, Texas.

To simulate agricultural injection, a screwdriver was used to puncture holes in the soil to a depth of 4.5 inches, and the liquid ammonia was then added using a 9-inch glass Pasteur pipette. The tip of the pipette reached the bottom of the holes. As soon as the fertilizer was added, the holes were immediately refilled with soil.

Gas Sampling and Sample Analysis

During each sample period, airtight extensions (20 cm × 20 cm) with a septum port were sealed to the chamber base to trap gases in the chamber headspace (Figure 17). Gases accumulated in the headspace for 30 minutes were sampled using a syringe inserted through the septum port. At the start and end of each sampling period, atmospheric gas samples were obtained as the background gas concentration. All gas samples were transferred and stored in overpressurized 10-mL preevacuated (<10 torr) serum bottles fitted with butyl stoppers and aluminum crimpers. The samples were then shipped to an EERC lab and analyzed by gas chromatography (Agilent GC 6890) with a Haysep DB 100/120 column (30' × 1/8" × 0.85" SS, Altech, Deerfield, Illinois). Three detectors were used to analyze the gas samples: a TCD, an ECD, and an FID for analysis of CO₂, N₂O, and CH₄, respectively. The carrier gas for CO₂ and

Figure 17. An F-column was capped with a static gas chamber for gas collection.

CH₄ analysis was helium, while the carrier gas for N₂O analysis was argon blended with 5% methane.

Soil temperature and moisture within and outside of the columns were recorded by lysimeters, and the soil bulk density was also analyzed. In late October 2007 after the fertilizer was added, gas samples were collected from the columns at Day 1, 2, 3, 5, and 7. The samples were shipped to an EERC lab and analyzed for CO₂, CH₄, and N₂O. As temperature dropped and winter approached, no significant microbial activity was expected, and the sampling activity was stopped. The gas sampling was resumed in May 2008, 2 weeks after the spring application of the N-fertilizer (late April 2008), and continued biweekly to the end of August. The gas sampling was then conducted once more in September 2008.

Results and Discussion

During the experimental period, we did not simulate any tilling activity; therefore, results reported here represent simulation of wetland restoration from “no-till” farmed wetlands.

Three R-group columns were built. However, one column failed to hold water and had to be discarded. Therefore, there were only duplicate columns in the R-group.

Analysis on the samples collected in October 2007 showed that 7 days after addition of the N-fertilizer, a slight increase in CH₄ emission from the FN-200 columns was detected, whereas little discernible differences in CO₂ and N₂O flux among different groups was observed (data not shown). This was probably because the temperature was low and the soil microbial activities slowed down. In fact, this is one of the major reasons that the N-fertilizer application is instructed be conducted when the soil temperature drops below 50°F. The reason is that low temperature not only minimizes evaporation of the fertilizer (i.e., ammonia) but also prevents significant loss of the N-fertilizer from soil microbial nitrification and denitrification processes.

The gas sampling was resumed May 2008, 2 weeks after the spring application of the N-fertilizer, and continued till late September. During this experimental period, significant differences in CO₂, CH₄, and N₂O emission from columns of different experimental groups were observed.

CO₂ Flux

Before June 17, no statistical difference in CO₂ flux among the experimental groups was detected. Starting from June 17, discernible differences in CO₂ flux between the F- and R-groups were observed (Figure 18); at this time, the CO₂ flux from the F-group was 44.7 ± 3.4 kg C ha⁻¹ day⁻¹ while the flux from the R-group was 32.6 ± 4.6 kg C ha⁻¹ day⁻¹. The difference between the F- and R-group increased with time and reached a peak on July 29, and the CO₂ flux from the F-group was about 10 times that of the R-group (161.2 ± 11.3 vs. 15.4 ± 0.5 kg C ha⁻¹ day⁻¹). The soil temperature and moisture profile curves (the lower panel in Figure 18) suggest that temperature played an important role in CO₂ production; in contrast, it seems that soil moisture

Figure 18. CO₂ flux from the on-site soil columns of different experimental groups. Error bars represent Standard Error (SE). Data for soil temperature and moisture are the average of 18 samples. SE of the soil temperature and moisture range from 0.2% to 1.3% and 1.9% to 7.2%, respectively, depending on the sampling date.

had no appreciable effect on CO₂ flux within the experimental range (7.9%–34.4%). These results confirmed that wetland restoration turns soil conditions from aerobic to anaerobic, resulting in a slowing down of decomposition of organic carbon, hence a sequestering of CO₂.

Application of ammonia significantly enhanced CO₂ production. GC analysis on July 15 samples showed that CO₂ produced in FN-46, FN-100, FN-160, and FN-200 groups was 2.8, 5.7, 5.2, and 6.4 times that produced in the F-group. Since application of N-fertilizer is a routine agricultural activity, these results suggest that addition of N-fertilizer may enhance CO₂ emission.

Positive correlation between the CO₂ fluxes and ammonia application rates was observed. Figure 19 is the regression plot, and the data are the CO₂ flux results of the samples collected on August 10, 2008. Results of the CO₂ flux from the two R-group columns are also shown in Figure 19; however, they are not included in the regression. The CO₂ flux data in Figure 20 are the average CO₂ flux of each group over the entire sampling period (from May 5 to September 22, 2008). The regression results demonstrate that within the ammonium rate range (46–200 kg N ha⁻¹), CO₂ flux was linearly correlated to the application rate.

N₂O Flux

Application of the N-fertilizer strongly affected the N₂O fluxes (Figure 21). N₂O emission from the FN-200 group reached a peak on July 15 and then subsequently declined. In the September 22 samples, no statistical difference in N₂O fluxes was found. In the July 15 samples, N₂O flux from the FN-200 group was 20.8 g N ha⁻¹ day⁻¹ more than that from the F-group.

Figure 19. Regression of effects of N-fertilizer on CO₂ flux. These are the results of the samples collected on August 10, 2008. The regression does not include the data on two R-columns.

Figure 20. Regression of average effects of N-fertilizer on CO₂ flux. Each Y value is the average of all sampling dates for that group. Error bars represent Standard Error (SE) of the samples from triplicate columns.

Since after restoration the farmed wetlands will be removed from agriculture and will no longer need N-fertilizer, restoration of currently farmed wetlands will reduce the portion of N₂O flux contributed by N-fertilizer application.

CH₄ Flux

As shown in Figure 22, before June 17 the CH₄ fluxes from different experimental groups were either negative (consuming CH₄ in the air) or fairly low. Starting from June 17, a steady increase in CH₄ emission from all FN-groups was detected. Compared to the flux from the R-group, the CH₄ emission from FN-groups was one order of magnitude higher. For example, the CH₄ flux from the July 15 R-group was 58 kg C ha⁻¹ day⁻¹; in contrast, the flux from the RN-160 was 814 kg C ha⁻¹ day⁻¹ (Figure 22).

It has generally been accepted that the consumption of methane in upland as well as lowland systems is inhibited by nitrogenous fertilizer additions. Hence, mineral nitrogen has conceptually been treated as a component with the potential to enhance emission of methane from soils and sediments to the atmosphere, and results from numerous studies have been interpreted as such. Recently, a few reports reported reduction in CH₄ emission by N-fertilizer under certain circumstances. Survey of available literature showed that despite some exceptions, enhancement of CH₄ emission by N-fertilizer is a dominant, most commonly observed fact. Stimulation of CH₄ emission by ammonia was observed in our laboratory microcosm study and

Figure 21. N₂O flux from the on-site soil columns of different experimental groups. Error bars represent Standard Error (SE). Data of soil temperature and moisture are the average of 18 samples. SE of the soil temperature and moisture range from 0.2% to 1.3% and 1.9% to 7.2%, respectively, depending on sampling date.

Figure 22. CH₄ flux from the on-site soil columns of different experimental groups. Error bars represent Standard Error (SE). Data of soil temperature and moisture are the average of 18 samples. SE of the soil temperature and moisture range from 0.2% to 1.3% and 1.9% to 7.2%, respectively, depending on sampling date.

was also confirmed by our glassware experiment (refer to Part I of this report). In the laboratory experiments soils sampled from Cando, North Dakota, were used, while in this field study, the on-site Ipswich, South Dakota, soil was used. Despite the fact that two different regional soils were used, the experimental results were consistent and are also in agreement with popular observations. These results showed that wetland restoration in this region may reduce CH₄ emission by cutting N-fertilizer input.

GWP

Differences in average fluxes of CO₂, N₂O, and CH₄ between the R- and other experimental groups are listed in Table 5. As listed in Table 5, restoring wetland at the experimental site does sequester CO₂. The restoration will also mitigate N₂O and CH₄ emission if the land is currently in agricultural use and with N-fertilizer application. Even if no N-fertilizer were used, a potential increase in CH₄-contributed GWP is actually negligible compared to the GWP reduced by CO₂ sequestration.

The flux values in Table 5 were converted to CO₂-equivalent GWP, using Intergovernmental Panel on Climate Change (IPCC) factors of 1 for CO₂, 275 for N₂O, and 62 for CH₄ (20-years GWP; Houghton et al. 2001). The conversion results are listed in Table 6. As shown in Table 6, it turned out that neither the values of N₂O nor CH₄ were at the same level of magnitude as those of CO₂, indicating that the overall reduction in GWP by restoring the PPR wetland was mainly contributed from CO₂ sequestration.

Effects of wetland restoration vary from region to region depending on the regional climate, soil types, current land use, etc. In some areas, wetland restoration may not reduce overall GWP; however, results of this in situ microcosm study demonstrate that restoration of currently farmed wetlands in the PPR will significantly reduce the total GWP budget.

Conclusions

Results of this in situ microcosm study showed that restoration of the wetland site would significantly reduce CO₂ flux. N-fertilizer enhanced the CO₂, N₂O, and CH₄ emissions and linearly correlated to the CO₂ flux within the experimental rate range (46–200 kg N ha⁻¹). The

Table 5. Differences in Average Fluxes of CO₂, N₂O, and CH₄ Between the R- and Other Experimental Groups^{1,2} (Negative values indicate reduction in the fluxes.)

Experimental Group	CO ₂ (g C ha ⁻¹ day ⁻¹)	N ₂ O (g N ha ⁻¹ day ⁻¹)	CH ₄ (g C ha ⁻¹ day ⁻¹)
F	-44,065	-0.4	53
F-46	-105,584	-4.8	-58
FN-100	-146,354	-6.1	-97
FN-160	-150,373	-6.6	-173
FN-200	-182,370	-8.5	-183

¹ Fluxes from the experimental group are defined as 0 g C/N ha⁻¹ day⁻¹.

² The data are averages of the fluxes over the entire sampling period (from May 5 to September 22, 2008).

Table 6. Relative GWPs for the R-group When Compared to Other Experimental Groups.^{1,2,3} Units Are CO₂-Equivalents (g m⁻² year⁻¹, 20 years GWP; negative values indicate a global warming mitigation potential)

Experimental Group	CO ₂	N ₂ O	CH ₄
F	-5,897	-6	160
F-46	-14,131	-76	-175
FN-100	-19,587	-96	-293
FN-160	-20,125	-104	-522
FN-200	-24,407	-134	-552

¹ Based on IPCC conversion factors (Houghton et al, 2001).

² GWPs for the experimental groups are defined as 0 g m⁻² year⁻¹.

³ The data are converted from averages of each flux over the entire sampling period (from May 5 to Sept 22, 2008).

experimental results also clarified that the overall reduction in GWP by wetland restoration was mainly contributed from CO₂ sequestration. These results demonstrate that restoration of currently farmed wetlands in the PPR will significantly reduce the GWP budget.

FEASIBILITY EVALUATION OF THE USE OF REMOTE SENSING TO DETECT SOIL GAS FLUX FROM WETLANDS

Introduction and Background

Several parameters may affect the production of CH₄ and N₂O from wetlands. Soil moisture (degree of saturation) is a key factor that determines whether gas flux is dominated by CH₄ or N₂O.¹ Other studies have shown that gas flux type and rate are affected by vegetation type and extent (Sahagian & Melack, 1996). Quantifying soil moisture conditions surrounding a wetland and determining dominant vegetation type and extent typically requires time-consuming, ground-based surveying; however, through the use of remote sensing, these physical parameters can be determined quite easily. For example, synthetic aperture radar (SAR) is quite sensitive to the dielectric properties of different media, rendering it a highly effective tool for delineating wetlands and for quantifying soil moisture (Kasischke & Bourgeau-Chavez, 1997). Visible and near-infrared sensors, such as those onboard the Landsat, ASTER, and SPOT satellites, have been used extensively to delineate and classify vegetation. Currently, the coupling of ground-based measurements of wetland trace gases with satellite image classifications of land use and soil moisture has not been investigated for the Northern Plains region of the United States. If a positive correlation were found between these techniques, it would allow for a regional estimation of wetland gas flux based on remote sensing.

The goal of this task was to evaluate the feasibility of using satellite-derived imagery to quantify wetland climate-forcing gas fluxes on a regional scale. This was conducted by classifying the wetland morphology in several satellite images to ground-based gas flux

¹ Robert Gleason, personal communication, 12/13/2005.

measurements collected by the U.S. Geological Survey (USGS) at the corresponding sites. The results indicate that while the 15-meter resolution of the imagery was sufficient to delineate multiple zones in larger wetlands, it was not sufficient for correlation with the ground-based gas flux measurement data, which were collected primarily for smaller wetland sites (<250 meters) in the areas evaluated by this task. To better evaluate the feasibility of using satellite imagery to quantify wetland gas flux, either higher-resolution satellite imagery or gas flux data from larger wetland sites is needed.

Methodology

Site Selection

Study sites were selected based on the availability of wetland gas flux data previously collected by the USGS in 2004 and/or 2005. Preference was given to areas with two or more gas measurement sites in close proximity so that as many areas as possible were encompassed by a single satellite scene.

Initially, efforts focused on acquiring imagery for several USGS field sites located in north-central North Dakota, near the town of Cando. The satellites targeted for this inventory included Landsat, SPOT, ASTER, and RADARSAT. Unfortunately, no cloud-free Landsat-ETM, SPOT, or ASTER scenes were available for the 2004 and 2005 summer seasons for the North Dakota study area, and no RADARSAT data were available.

The lack of available satellite imagery for North Dakota resulted in the selection of an alternate study area to correspond with USGS wetland sampling sites near Fergus Falls, Minnesota. Only one year of field data, collected during 2005, was available for these sites. An inventory of satellite imagery for these sites revealed that while no SPOT or RADARSAT data were available, there were Landsat and ASTER scenes available for the summer of 2005. Since the ASTER imagery has a higher resolution than the Landsat imagery, only ASTER imagery was purchased.

Data Acquisition and Processing

ASTER images of the Minnesota field sites were purchased for May and August of 2005 from the Earth Resources Observation and Science (EROS) laboratory. ASTER imagery has three bands that cover the visible and near infrared portions of the spectrum (from 0.52 to 0.86 μm) with a horizontal resolution of 15 meters. It has five bands in the short-wave portion of the electromagnetic spectrum (1.600–2.430 μm); however, the resolution of these bands is only 30 meters. ASTER also has five thermal bands (8.125–11.65 μm) with a resolution of 90 meters.

Using the ERDAS software package, the satellite images were processed to account for variations in radiance due to sun angle. The images were then spectrally enhanced to better distinguish between different wetland zones, such as wet areas and the vegetative fringe. A supervised classification of the images was conducted, with a focus on delineating pixels associated with the wetlands in the imagery.

Results

The results indicate that the 15-meter resolution of the ASTER imagery was sufficient to distinguish and classify different vegetation types and/or the upland versus open water zones in wetlands larger than about 250 meters. However, the 15-meter resolution was insufficient for classification of wetlands smaller than 250 meters. In some instances, the different wetland zones and vegetation types in smaller wetlands were distinguishable in the satellite images, especially those with open water; however, these zones were rarely differentiated in the classified image.

For example, a comparison of an aerial photograph (Figure 23) and an ASTER scene (Figure 24) of four of the USGS wetland sites demonstrates how well the wetlands can be distinguished in the satellite imagery, despite the fact that the aerial photograph's resolution is five times better than the ASTER image. The wetland sites shown in Figure 24 display different-colored pixels, indicating differences in vegetation and/or soil moisture. However, after the satellite image was classified (Figure 25), many of the features are no longer distinguishable. For example, the two smaller wetland sites on the left side of the image are not delineated within the classified image.

Because most of the USGS wetland field sites were smaller than 250 meters and are not always defined within the classified image, a comparison between the gas flux data and the classified wetlands was not conducted. To better evaluate the potential coupling of satellite imagery with ground-based gas flux measurements, higher-resolution satellite imagery or gas flux data from larger wetland field sites is needed. In addition, advanced image classification software, such as eCognition, could be used to obtain a more accurate delineation of wetland zones and vegetation.

Figure 23. A 2004 aerial photograph of four of the wetland field sites (circled in red) located near Fergus Falls, Minnesota (photo from the Farm Service Agency). The red dots indicate the edge of the wetland sites as defined by USGS.

Figure 24. A 2005 ASTER image of the wetland sites (circled in red) depicted in Figure 23. Image is displayed in false color using ASTER bands 7, 4, and 1. The red dots indicate the edge of the wetland sites as defined by the USGS.

Figure 25. The results of the classification performed on the ASTER image shown in Figure 24. Blue represents water, while pink and purple represent different types of wetland vegetation. The remaining colors represent woodland vegetation, grassland, crops, roads, and/or bare soil. The red dots correspond to the wetland sites depicted in Figures 23 and 24. The black and green colors on the right side of the image mark the edge of the satellite scene.

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