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Article

Determination of Initial Denitrification in Intact Cores under Various Freshwater Wetland Types

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Denitrification rate was determined for various freshwater wetland types in the Mississippi River Coastal delta plain. Site 1 and 4 were collected from forested-tupelo dominated wetland, and site 2 and 3 were from floating emergent marsh. The maximum N_2O emission was 7.47 mg N m^{-2} for site 1 at day 6 after the addition of nitrate, 6.96 mg N m^{-2} for site 2 at day 4, 6.63 mg N m^{-2} for site 3 at day 3, and 9.64 mg N m^{-2} for site 4 at day 4. The denitrification rate was determined using the acetylene inhibition method 1.24 mg N m^{-2} d^{-1} for site 1, 1.93 mg N m^{-2} d^{-1} for site 2, 2.24 mg N m^{-2} d^{-1} for site 3, and 2.78 mg N m^{-2} d^{-1} for site 4. The maximum denitrification rate was in the order of site 4 > site 3 > site 2 > site 1.

Key words: Denitrification, Nitrous oxide emission, Intact core, Acetylene inhibition

Introduction

Denitrification, which removes nitrogen in such systems, is important because it represents a direct loss to the atmosphere (Knowles, 1982). Nitrogen gas is the major product of denitrification, with N_2O as an intermediate product some of which is released to the atmosphere (Firestone et al., 1980). Denitrification occurs mainly at the sediment-water interface. Nitrate in river water entering Louisiana Wetlands, when in contact with anaerobic soil or sediment surface, can be biologically reduced to gaseous nitrogen (Gale et al., 1993). However, the sediment may also serve as a source of nitrate to the water column. Mineralization of organic-N to ammonium-N and the subsequent nitrification in the surface oxidized layer can also be a source of nitrate to the water column (Miao et al., 2006).

Previous studies have used the acetylene inhibition method or stable isotopic techniques to quantify nitrate removal and denitrification in wetland sediment (Herrman and White, 2008; Miao et al., 2006; Seo and DeLaune, 2010). None of these studies considered the influence of wetland type and removal velocity in intact core on denitrification and nitrate removal. Especially, the removal velocity of nitrate is an important parameter for estimating the amount of nitrate removal with considering the influence of various wetland types in Louisiana marsh.

The objective of this study was to evaluate the denitrification rate under various wetland types in intact core from Louisiana Marsh. The specific objectives were to determine the N_2O emission and denitrification rate in intact cores under different wetland types using acetylene inhibition method.

Materials and Methods

Study site The Bayou Boeuf Basin is located in southern Louisiana in the Barataria Basin approximately 50 km southwest of New Orleans (Fig. 1). The Boeuf Basin includes a network of bayous and streams with a combined area of approximately 311 km^2. The overall drainage pattern is towards Lac des Allemands, with inflow from outside the basin occurring at the western end of Grand Bayou (a distributary of Bayou Citamon), and to some extent through Bayou Boeuf via Lac des

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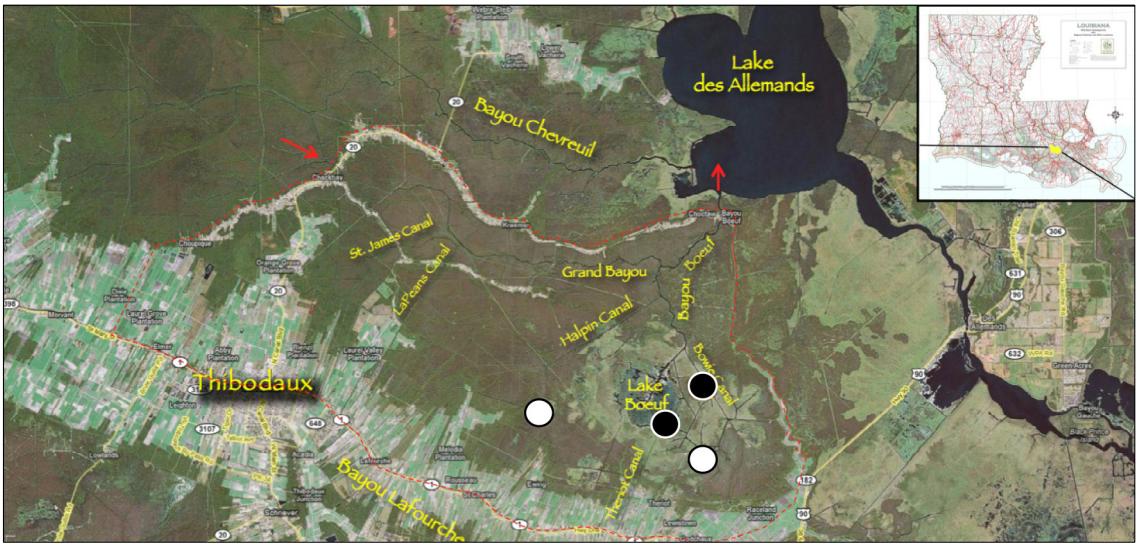

Fig. 1. Major waterways in the Boeuf Basin. The red dashed line indicates watershed boundary, and red arrows indicate breaches in the boundary where water exchange occurs with the greater Barataria Basin (direction of arrows indicates net flow). White circles indicate the location of core sampling sites (1 and 4) in forested wetlands, and Black circles indicate the location of core sampling sites (2 and 3) in floating emergent wetlands.

Allemands during storms or wind induced high water in the greater Barataria Basin. The riot Canal connects to Bayou Lafourche, but hydrological exchange is limited by a gated structure that is normally closed during low flow periods except to allow boats to pass through its opening, which is approximately 8 ft wide. The basin is predominantly forested wetland, with emergent floating marsh at the interior surrounding Lake Boeuf, and agriculture dominating the surrounding uplands. *Panicum hemitomon* and *Sagittaria lancifolia*-dominated floating marshes occupy regions in the upper Barataria Basin near Lake Boeuf and Lake Salvador (Swarzenski et al., 1991). Since much of Lake Boeuf is covered by relatively thick densities of floating and/or rooted vegetation, a large portion of Lake boeuf (over 67%) was classified as marsh rather than water (LDEQ, 2004).

The soil cores collected for this investigation were from four sites (Site 1: N29°47′6.09″, W90°41′1.44″; Site 2: N29°46′51.02″, W90°37′27.08″; Site 3: N29°47′49.63″, W90°36′11.86″; Site 4: N29°46′9.20″, W90°36′14.75″; Fig. 1). Sites 1 and 4 were forested-tupelo dominated wetlands, and sites 2 and 3 were from floating emergent marshes.

Preliminary preparations Intact soil cores for nitrous oxide emission and denitrification experiments were taken from the four study sites on May, 2009. PVC columns (40 cm in height × 15 cm inside diameter) with sharpened ends were inserted approximately 20 cm into the sediment or plant root complex with emphasis on minimizing compaction. Four cores were taken, stored on ice, and immediately transported back to the laboratory for incubation. The heights of the sediment/root in all cores were adjusted to the same height (17 cm) in the laboratory. The sediment/root-water columns were preincubated at 23℃ for one week in order to allow for equilibration and establishment of a thin surface-oxidized layer at the sediment/root-water interface. After the sediment/root-water columns had equilibrated, overlying water was removed.

Denitrification experiments The denitrification experiment consisted of four cores are shown in Fig. 2. To provide a nitrate source for measuring nitrous oxide emission and denitrification in cores, 2 mg NO_3-N L^{-1} was added to the all cores. In all cores, initial NO_3-N + NH_4-N concentrations in the water were mostly below the detection limit (0.01 mg L^{-1}). Denitrification potential of sediment was measured using the acetylene (C_2H_2) inhibition technique, which inhibits reduction of N_2O to N_2 (Tiedje, 1982; Yoshinari et al., 1977). The cores were sealed with a lid containing a septum for sampling and pure C_2H_2 was injected replacing 250 mL (10% C_2H_2 based on the volume of headspace) of the headspace volume of each core. The

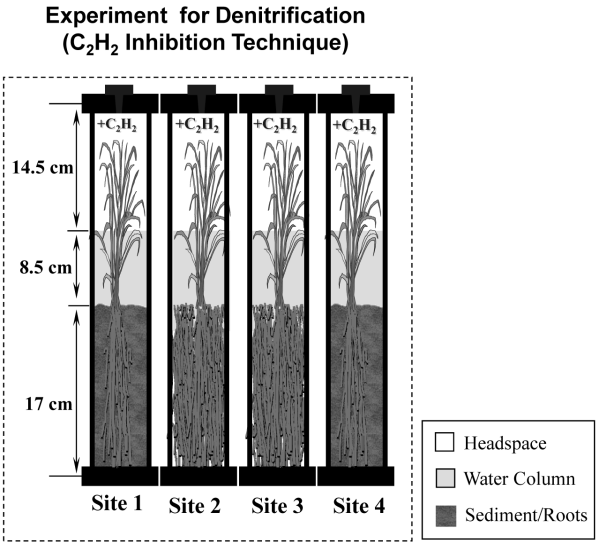

Fig. 2. Experimental design used in this study. Each site in experiments consisted of three replicate cores (n=3). Nitrous oxide emission and denitrification rates were estimated in the water column over the incubation period using acetylene inhibition technique.

cores were incubated at room temperature (23℃) and gas samples (2 mL) were collected from headspace of the cores at day 0, 1, 3, 4, 5, 6, 8, 10, 14, 21, and 31 after the addition of acetylene for N_2O analysis. Denitrification rates were calculated using N_2O concentration by the acetylene (C_2H_2) inhibition technique.

Sample analysis Nitrous oxide concentrations in gas samples were analyzed using a Tremetrics 9001 gas chromatograph (GC) with an electron capture detector (ECD). Gas samples were calibrated using certified N_2O standard gas mixture (Scott Specialty Gases, Inc. Plumsteadville, PA). The GC was equipped with stainless steel columns packed with Porapak-Q. The system had a back-flush mechanism operated by 10-port Valco valves to prevent moisture in the samples from entering the detectors. Operating temperature was 50℃ for the oven and 310℃ for the ECD. Gas samples were injected through a 2.0 mL and a 0.5 mL sample loop connected to the Valco valves for ECD detections. N_2O gas analysis was subject to conventional quality control with a standard spike in every third sample with a standard curve $R^2 > 0.999$. All data were presented as dry weight of sediment.

Statistical analysis Statistical analysis of the data was conducted using SAS software (version 9.1, 2008, SAS Institute, Cary, NC). Simple linear regression using PROC REG (SAS 9.1, SAS Institute Inc. Cary, NC, USA) was conducted to determine if the slope of a regression was significantly different from a theoretical model ($p < 0.05$).

Results and Discussion

Change of N_2O emission in intact cores for determining denitrification rate For determining initial denitrification rate, nitrous oxide (N_2O) emissions from intact cores using acetylene inhibition technique are shown in Fig. 3. In site 1, nitrous oxide emission showed a linear increase with time during the 6-day incubation. After that, N_2O emission slightly decreased at incubation time between day 6 and day 10 in site 1. Nitrous oxide emission in site 1 rapidly decreased after day 10. In site 1, the maximum N_2O emission was 7.47 mg N m^{-2} at day 6 after incubation.

Nitrous oxide emission in site 2 showed a linear increase as the incubation time increased during the 4-day incubation. From day 4 to day 8 after incubation, N_2O emission rapidly decreased with incubation time in site 2. Nitrous oxide emission reached 0 mg L^{-1} on the 15 days. The maximum N_2O emission was 6.96 mg N m^{-2} in site 2 at day 4 after the addition of nitrate.

In site 3, nitrous oxide emission showed a linear increase as the incubation time increased during the 3-day incubation after the addition of nitrate. Nitrous oxide emission rapidly decreased with incubation time (from day 3 to day 6) in site 3. At day 3 after the addition of nitrate, the maximum N_2O emission was 6.63 mg N m^{-2} in site 3.

In site 4, N_2O emission showed a linear increase with incubation time during the 4-day incubation. From day 4 to day 8 after incubation, N_2O emission in site 4 rapidly decreased as the incubation time increased. In site 4, the maximum N_2O emission was 9.64 mg N m^{-2} at day 4 after the addition of nitrate.

After maximum N_2O emission in all sites, N_2O emission rapidly decreased as the incubation time increased because most NO_3-N for denitrification were consumed by microorganisms and water plants in all tested cores. If nitrous oxide emission will be observed continuously in all cores, there may be a need for additional nutrient (such as NO_3-N, organic matter, etc.) for denitrification.

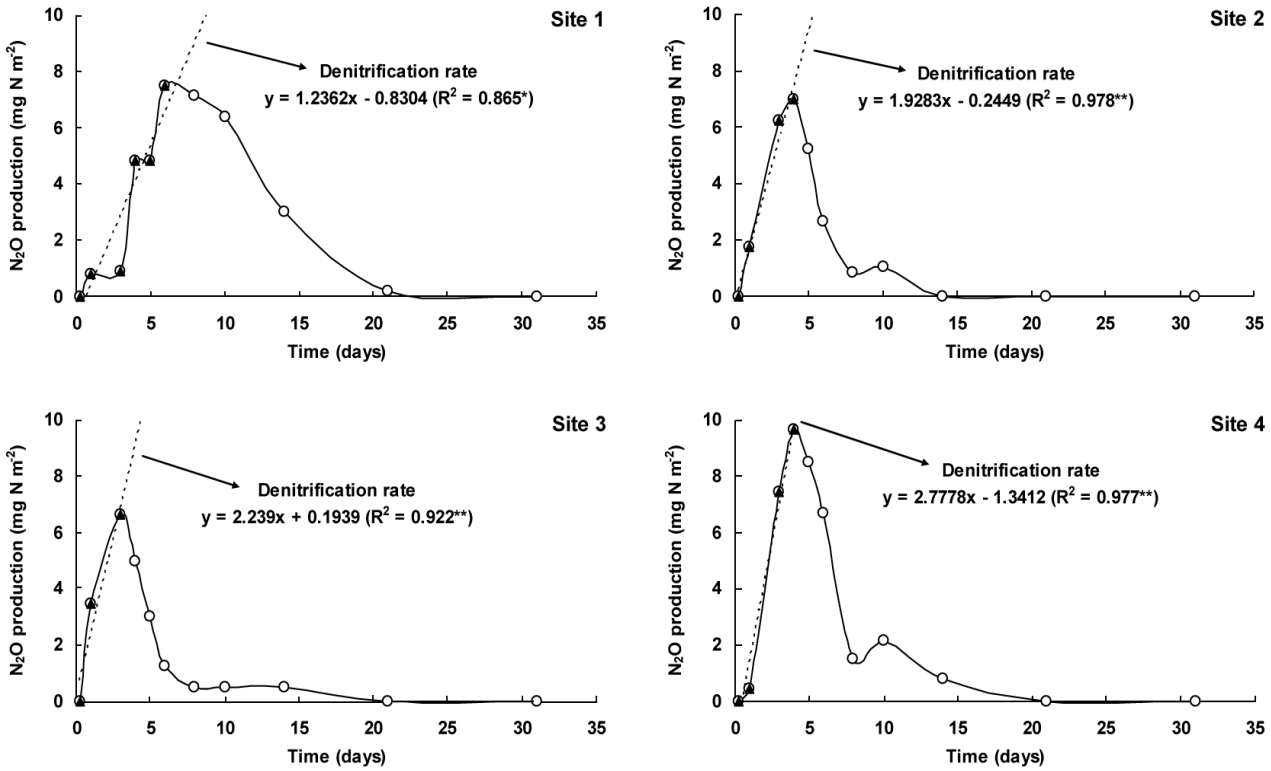

Fig. 3. N_2O production using acetylene inhibition technique and denitrification rate in intact cores.

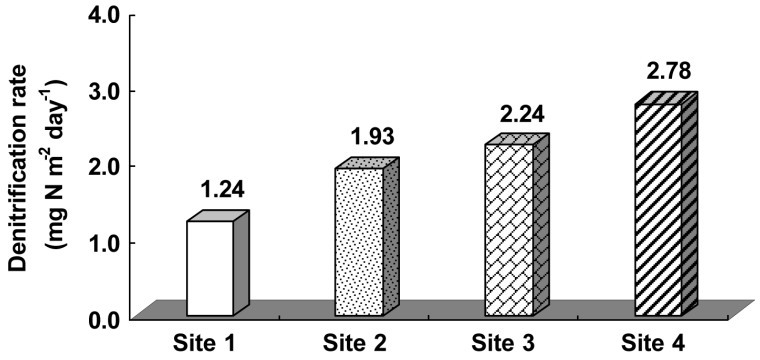

Fig. 4. Denitrification rate using acetylene inhibition technique in intact cores (Site 1, and 4: forested-tupelo dominated wetland; Site 2, and 3: floating emergent marsh).

Denitrification rate in intact cores Based on the above results until incubation time for maximum N_2O emission after the addition of nitrate, denitrification rates in all tested cores are shown in Fig. 3 and 4. Linear regression for denitrification rates were N_2O emission (mg N m^{-2}) = 1.2362 × Time (day) − 0.8304 (R^2=0.865*) for site 1, N_2O emission (mg N m^{-2}) = 1.9283 × Time (day) − 0.2449 (R^2=0.978**) for site 2, N_2O emission (mg N m^{-2}) = 2.239 × Time (day) + 0.1939 (R^2=0.922**) for site 3, and N_2O emission (mg N m^{-2}) = 2.7778 × Time (day) − 1.3412 (R^2 = 0.977**) for site 4. The maximum denitrification rate was 1.24 mg N m^{-2} d^{-1} for site 1, 1.93 mg N m^{-2} d^{-1} for site 2, 2.24 mg N m^{-2} d^{-1} for site 3, and 2.78 mg N m^{-2} d^{-1} for site 4. The maximum denitrification rate was in the order of site 4 > site 3 > site 2 > site 1.

According to Maio et al. (2006), the denitrification rate was 45.53 mg N m^{-3} d^{-1} in the sediment-water column. Herrman and White (2008) report that denitrification rates from sediment cores were 0.21-0.92 mg N m^{-2} h^{-1}. Yu and Patrick (2003, 2004) found a similar result for denitrification rate.

Conclusions

In order to evaluate and estimate the amount of nitrate removal with considering the influence of various wetlands types in Louisiana marsh, the nitrous oxide emission and denitrification rate in intact core from Louisiana marsh were determined under various wetland types. Site 1 and 4 were from forested-tupelo dominated wetland, and site 2 and 3 were from floating emergent marsh. The maximum N_2O emission in site 1, 2, 3 and 4 were 7.47 (at day 6 after the addition of nitrate), 6.96 (at day 4), 6.63 (at day 3), and 9.64 mg N m^{-2} (at day 4), respectively. The maximum denitrification rate was 1.24 mg N m^{-2} d^{-1} for site 1, 1.93 mg N m^{-2} d^{-1} for site 2, 2.24 mg N m^{-2} d^{-1} for site 3, and 2.78 mg N m^{-2} d^{-1} for site 4. The maximum denitrification rate was in the order of site 4 > site 3 > site 2 > site 1.

Acknowledgments

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