This article was downloaded by: [Louisiana State University]
On: 04 May 2012, At: 06:11
Publisher: Taylor & Francis
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK

Journal of Freshwater Ecology
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/tjfe20

Comparison of $^{15}N_2$ flux and acetylene inhibition denitrification methods in Atchafalaya River basin sediments

Charles W. Lindau [a] , Amy E. Scaroni [b] , Victor H. Rivera-Monroy [a] & John A. Nyman [b]

[a] Department of Oceanography and Coastal Sciences, Louisiana State University, Baton Rouge, LA 70803, USA

[b] School of Renewable Natural Resources, Louisiana State University Agricultural Center, Baton Rouge, LA 70803, USA

Available online: 09 Sep 2011

To cite this article: Charles W. Lindau, Amy E. Scaroni, Victor H. Rivera-Monroy & John A. Nyman (2011): Comparison of $^{15}N_2$ flux and acetylene inhibition denitrification methods in Atchafalaya River basin sediments, Journal of Freshwater Ecology, 26:3, 337-344

To link to this article: http://dx.doi.org/10.1080/02705060.2011.557480

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: http://www.tandfonline.com/page/terms-and-conditions

This article may be used for research, teaching, and private study purposes. Any substantial or systematic reproduction, redistribution, reselling, loan, sub-licensing, systematic supply, or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae, and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand, or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

Journal of Freshwater Ecology
Vol. 26, No. 3, September 2011, 337–344

Taylor & Francis
Taylor & Francis Group

Comparison of $^{15}N_2$ flux and acetylene inhibition denitrification methods in Atchafalaya River basin sediments

Charles W. Lindau[a]*, Amy E. Scaroni[b], Victor H. Rivera-Monroy[a] and John A. Nyman[b]

[a]*Department of Oceanography and Coastal Sciences, Louisiana State University, Baton Rouge, LA 70803, USA;* [b]*School of Renewable Natural Resources, Louisiana State University Agricultural Center, Baton Rouge, LA 70803, USA*

(*Received 23 November 2010; final version received 11 January 2011*)

Flooded swamps in the Atchafalaya River basin, Louisiana, USA, are thought to be a major mechanism for removing excess riverine nitrate that may be causing hypoxia in coastal Louisiana. Two denitrification methods, the $^{15}N_2$ flux and acetylene inhibition technique, were compared and evaluated. Sediment samples were collected from three baldcypress swamp sites located within the Atchafalaya River basin. Highly ^{15}N-labeled (61.7 at.%) and unlabeled nitrate (100 mg NO_3–N L^{-1}) were applied to the floodwater of separate sets of sediment cores. Denitrification potential was measured 2, 6, 10, and 16 days after nitrate addition. Two days after nitrate addition, no significant differences in denitrification rates were observed between methods. On days 6, 10, and 16, denitrification measured by the $^{15}N_2$ method was higher compared to the acetylene inhibition procedure. When significance was observed, $^{15}N_2$ denitrification was approximately 5.3 times greater.

Keywords: denitrification; $^{15}N_2$ flux method; acetylene inhibition technique; Atchafalaya River Basin

Downloaded by [Louisiana State University] at 06:11 04 May 2012

Introduction

The Mississippi–Atchafalaya River basin (MARB) drains approximately 41% of the conterminous United States (all or part of 31 states). Nitrogen fertilizer applied to MARB farmland has increased from less than one million metric tons in 1951 to about seven million metric tons in 1996 (Goolsby et al. 2001).

Approximately 90% of the annual discharge of nitrogen (N) to the Gulf of Mexico (GOM) is transported by the Mississippi River and Atchafalaya River (Dunn 1996). Average annual flux of N (1981–2005) to the GOM was 1,470,000 metric tons of which 61% was inorganic nitrate (Aulenbach et al. 2007). About 90% of the nitrate entering the GOM emanates from nonpoint pollution sources (mainly agriculture), with highest discharges observed in the spring and early summer.

Excess N (mainly nitrate) from the MARB may be one of the major causes of low dissolved oxygen (<2 mg L^{-1}) in a large hypoxic area along the Louisiana–Texas coasts (Rabalais et al. 2002). Hypoxia can cause increased stress or death to coastal

*Corresponding author. Email: clinda1@lsu.edu

ISSN 0270–5060 print/ISSN 2156–6941 online
© 2011 Taylor & Francis
DOI: 10.1080/02705060.2011.557480
http://www.informaworld.com

bottom-dwelling organisms that cannot flee the oxygen depleted zone. The size of the GOM hypoxic zone is highly variable and has increased over the past two decades. The zone averaged approximately 8300 km^2 from 1985 to 1992, but from 1993 to 2001 averaged 16,000 km^2 (Rabalais et al. 2002). The size of the hypoxic zone in 2007 was estimated at 20,500 km^2 and may increase over the next few years due to increased ethanol production from corn in the MARB (USGS 2007).

The Mississippi River/Gulf of Mexico Watershed Nutrient Task Force (2001) developed an action plan to reduce hypoxia and the size of the zone to less than 5000 km^2 by 2015 for the northern GOM. Enhancing and/or restoring natural denitrification has been proposed to reduce nitrogen loads. Remediation actions to increase denitrification include restoring and creating riparian buffers and coastal wetlands and diverting nitrate-elevated river water into Louisiana's coastal ecosystems (USEPA 2001; Mississippi River/Gulf of Mexico Watershed Nutrient Task Force 2008).

Denitrification is the microbial reduction of nitrate (NO_3) and nitrite (NO_2) to gaseous end products nitric oxide (NO), nitrous oxide (N_2O), and dinitrogen (N_2) which represents a major pathway for N removal from aquatic and wetland ecosystems (Mitsch and Gosselink 2000). Denitrification processes are affected by soil moisture and texture, pH, microbial community structure, sediment O_2, NO_3 concentration, organic matter, and temperature (Groffman 1994; Clement et al. 2002; Seitzinger et al. 2006; Hernandez and Mitsch 2007). Denitrification is fueled by NO_3 produced from mineralization of inorganic NH_4^+ and by water column NO_3 diffusion into reduced sediments.

Direct (measurement of N_2O and/or N_2) and indirect procedures have been used to estimate denitrification. Denitrification methods include N_2 quantification, ^{15}N stable isotope tracers, N_2 : Ar ratios, acetylene inhibition, nitrogen mass balance, nitrate disappearance, molecular techniques, and stoichiometric approaches. All denitrification methods have advantages, disadvantages, limitations, and assumptions that must be evaluated prior to method selection (Cornwell et al. 1999; Steingruber et al. 2001; Groffman et al. 2006; Wallenstein et al. 2006).

The objective of this investigation was to compare and evaluate two denitrification methods after NO_3 addition to flooded sediments. Potential denitrification rates were measured using the $^{15}NO_3 \rightarrow {}^{15}N_2$ flux method and the acetylene inhibition technique.

Materials and methods

Baldcypress sediments were collected from three flooded locations within the Atchafalaya River basin. Sites were located along Bayou Cowan (BC), Jones Bayou (JB), and Bee Bayou (BB). Bulk sediment (0–15 cm depth) was manually collected, sealed in polyethylene bottles, and iced. Sediments were transported to the laboratory and stored at 1–2 C. Total sediment organic matter was measured by loss of ignition at 550 C for 2 h and total N was determined with a Leco C and N analyzer (Leco Corp., St. Joseph, Michigan, USA). Clay content was determined by the pipette method. Sediment organic matter, total N, and clay content ranged from 16.8 to 32.3, 0.4 to 0.8, and 53% to 66%, respectively. The lowest concentrations of these components were observed for BB, and highest were for BC. Substrate pH values varied from 4.4 (JB) to 5.5 (BB).

Downloaded by [Louisiana State University] at 06:11 04 May 2012

For each sediment type (site), six glass incubation jars (9 cm diameter by 16 cm height) were filled with sediment to a height of 6 cm, and the sediment was then packed to field bulk density. A 4-cm layer of nitrate-free distilled water was added, and cores were allowed to incubate (22 C) for 1 week to re–establish the thin surface oxidized layer (observed in the field) overlaying the reduced zone. Then, 180 mg of ^{15}N labeled (61.7 at.%) KNO_3 (dissolved) was added to the floodwater of two sediment cores, and 180 mg of dissolved KNO_3 (unlabeled) was added to the floodwater of the remaining four cores. A high concentration of NO_3–N was required to assure sediment cores were not nitrate-limited and to detect $^{15}N_2$ in the headspace volume (containing 78% N_2) at the end of the 6-hour incubation. Labeled nitrate was only added to two cores due to cost. Gas samples were collected at 2, 6, 10, and 16 days after nitrate addition.

Downloaded by [Louisiana State University] at 06:11 04 May 2012

Acetylene inhibition method

The acetylene (C_2H_2) inhibition method was used to estimate potential denitrification (Groffman 1994). After unlabeled nitrate (dissolved) was added to the floodwater, the incubation jars were sealed (screw caps fitted with rubber septa), and purified C_2H_2 was then added to the headspace volume (10% v/v) and floodwater. Headspace gas samples were collected 2 and 6 h after C_2H_2 addition with a 2-mL gas-tight syringe to estimate the increase of N_2O. Two hours were allowed for C_2H_2 diffusion through the floodwater into the sediment before gas collection. After 6 h, screw caps were removed and cores were exposed to the atmosphere. Floodwater/sediment cores were only exposed to C_2H_2 for a short time to minimize effects on microorganisms (Watts and Seitzinger 2000). Nitrous oxide fluxes were calculated using the closed chamber equation of Rolston (1986): $F = (V/A)(273/T)(\Delta C/\Delta T)$, where V is core headspace volume, A the core sediment area, T the absolute temperature of headspace gases, and $\Delta C/\Delta T$ the change in N_2O concentration per unit of time. The Bunsen absorption coefficient was used to estimate N_2O dissolved in the floodwater. Nitrous oxide flux is reported as mg N evolved $m^{-2} d^{-1}$ which represents total denitrification ($N_2O + N_2$).

Nitrous oxide concentrations were determined with a Shimadzu GC-14A gas chromatograph (Shimadzu Scientific Instruments, Inc., Columbia, Maryland, USA) fitted with a 1-mL sample loop, Poropak Q 1.8 m ss column, and electron capture detector (ECD) and calibrated with certified N_2O reference standards (Scott Specialty Gases, Inc., Plumsteadville, Pennsylvania, USA). Nitrogen was the carrier gas and the instrument operated at 40, 100, and 290 C for the oven, injector, and ECD, respectively (Lindau et al. 1998).

$^{15}N_2$ method

Highly ^{15}N-labeled nitrate was added to the floodwater of two sediment cores collected from each of the three sites. Incubation jars were sealed and headspace gas samples were collected at 0 (background) and 6 h with a gas-tight syringe. Collected gas samples were immediately transferred into evacuated glass Vacutainers (100 mm length by 16 mm i.d.). Prior to gas collection, Vacutainers (Becton Dickinson, Franklin Lakes, New Jersey, USA) were placed on a high-vacuum system to remove residual gases. A slight over-pressure of headspace gas was injected into each

Vacutainer to prevent atmospheric gas contamination. Screw caps were removed at the end of each 6 h incubation.

Stable $^{15}N_2$ isotopic distribution (28, 29, and 30 masses) was determined with a gas chromatograph coupled to a triple collector 20/20 isotope ratio mass spectrometer (GC–IRMS, Robo Prep-G Plus, Europa Scientific, SerCon Ltd., UK). Fluxes of $^{15}N_2$ from the floodwater/sediment cores were calculated using the equations and $^{15}X_N$ and d values outlined by Hauck and Bouldin (1961) and Mulvaney and Boast (1986). Emission of $^{15}N_2$ is reported as mg N evolved $m^{-2} d^{-1}$.

Statistical methods

All statistical analyses were conducted using SAS 9.1.3 (SAS Institute Inc., Cary, North Carolina). Denitrification data was evaluated by the analysis of variance (ANOVA) test procedure and significant differences between treatment means were calculated by Duncan's multiple range test. An alpha level of 0.05 was used for all statistical analyses.

Downloaded by [Louisiana State University] at 06:11 04 May 2012

Results

Denitrification rates measured by the $^{15}N_2$ method generally increased (after NO_3 addition) from day 2 to day 10 and then decreased on day 16. Denitrification potential measured by the C_2H_2 technique steadily decreased over the 16 day incubation. On days 6, 10, and 16, $^{15}N_2$ denitrification fluxes were higher compared to C_2H_2 treatment means (Figure 1).

BC $^{15}N_2$ denitrification ranged from 17.1 to 26.7 mg N evolved $m^{-2} d^{-1}$ and averaged 21.3 mg N $m^{-2} d^{-1}$ over the 16-day incubation. With the C_2H_2 procedure, BC denitrification ranged from 0.8 to 35.7 mg N $m^{-2} d^{-1}$ with an average flux of 12.6 mg N $m^{-2} d^{-1}$. Over the entire incubation, $^{15}N_2$ denitrification was approximately 1.7 times greater. On day 2, denitrification potential $^{15}N_2$ and C_2H_2 flux means were not significantly different. On days 6, 10, and 16, denitrification measured using the $^{15}N_2$ method was significantly higher (Figure 1).

Denitrification measured on BB sediment varied from 15.4 to 39.1 mg N $m^{-2} d^{-1}$ and averaged 28.0 mg N $m^{-2} d^{-1}$ ($^{15}N_2$ method). Potential denitrification ranged from 4.4 to 14.4 mg N $m^{-2} d^{-1}$ with an average of 9.3 mg N evolved $m^{-2} d^{-1}$ using the C_2H_2 method (Figure 1). Dinitrogen fluxes from ^{15}N cores were about 3.0 times higher compared to C_2H_2 method denitrification means. On days 6, 10, and 16, $^{15}N_2$ denitrification means were significantly higher, but on day 2, no significant differences were observed between the methods.

Using the $^{15}N_2$ method, JB denitrification rates varied from a low of 16.5 to a maximum of 46.7 mg N $m^{-2} d^{-1}$ and averaged 29.9 mg N $m^{-2} d^{-1}$ over the sampling period. Acetylene inhibition denitrification means were lower and ranged from 3.3 to 20.3 mg N $m^{-2} d^{-1}$ (Figure 1) and averaged 11.2 mg N $m^{-2} d^{-1}$ over the 16 days. ^{15}N-labeled denitrification values were approximately 2.7 times greater compared to the C_2H_2 procedure. JB denitrification fluxes measured by the $^{15}N_2$ method were significantly higher on days 10 and 16.

Downloaded by [Louisiana State University] at 06:11 04 May 2012

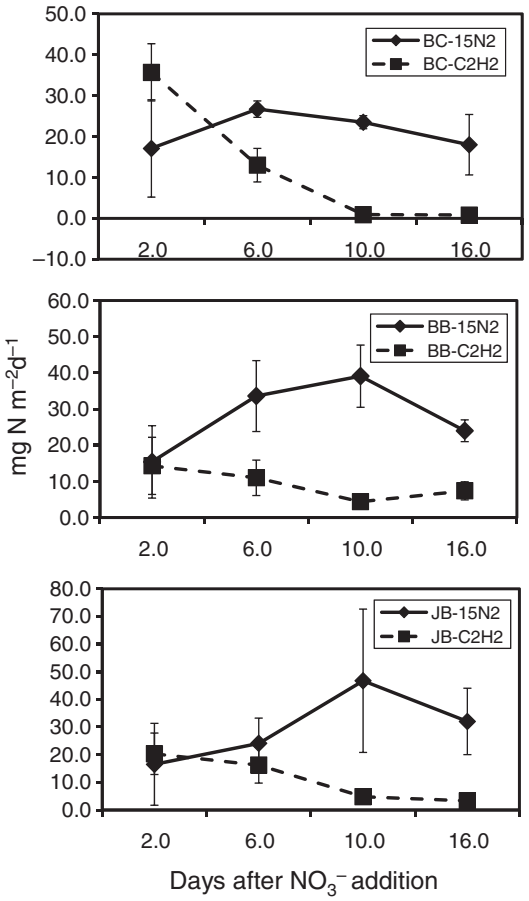

Figure 1. $^{15}N_2$ and C_2H_2 methods potential denitrification means (mg N m ^{2}d ^{1}) for Bayou Cowan (BC), Bee Bayou (BB), and Jones Bayou (JB) days after NO_3–N addition to sediment cores. Error bars are one standard deviation.

Discussion

Across locations, denitrification rates measured by the $^{15}N_2$ flux and C_2H_2 methods were not significantly different 2 days after nitrate addition. For the remaining days (6, 10, and 16), $^{15}N_2$ denitrification was significantly higher eight out of nine sampling times and was approximately 5.3 times greater than C_2H_2 method denitrification. Denitrification $^{15}N_2$ fluxes evolved into the floodwater/sediment core headspace may significantly underestimate sediment $^{15}N_2$ production. In a 1988 study, Lindau et al. (1988) observed approximately 34% of the applied ^{15}N was entrapped as $^{15}N_2$ in flooded rice soil 33 days after ^{15}N addition. The $^{15}N_2$ flux rates are a function of production rate, solubility, mass flow, and diffusion (Well and Myrold 1999).

The C_2H_2 inhibition and $^{15}N_2$ flux methods have limitations and assumptions that need to be considered for flooded sediments. Limitations of the C_2H_2 technique include: inhibition of nitrification by C_2H_2, which can lead to underestimation of

total denitrification; incomplete blockage of N_2O to N_2 by C_2H_2; presence of hydrogen sulfide; slow diffusion of C_2H_2 through the floodwater and sediment; sediment respiration enhancement; metabolism and/or decomposition of C_2H_2; and contaminated C_2H_2 (Seitzinger et al. 1993; Cornwell et al. 1999; Groffman et al. 2006). Assumptions for the $^{15}N_2$ method include: ^{14}N and ^{15}N atoms are randomly distributed between N_2 molecules produced during the denitrification process; total amount of $^{28}N_2$ in core headspace does not change over the incubation period; and the ^{15}N label of the added nitrate in the sediment is uniform (Siegel et al. 1982; Mulvaney and Boast 1986).

Numerous sediment denitrification methods have been compared, including: ^{15}N isotope pairing and C_2H_2 inhibition (Lohse et al. 1996); N_2 flux and C_2H_2 blockage (Watts and Seitzinger 2000); N_2 flux, ^{15}N isotope pairing and mass balance techniques (van Luijn et al. 1996); membrane inlet mass spectrometry and isotope pairing mass spectrometry (Smith et al. 2006); and isotope pairing and N_2:Ar ratios (Ferguson and Eyre 2007). Comparison of our denitrification methods ($^{15}N_2$ flux and C_2H_2 inhibition) to published research is difficult due to a wide variety of laboratory procedures, sediment properties, applied nitrate and ammonium concentrations, denitrification calculations, incubation methods and times, and gas collection techniques used. In a 1993 experiment, Seitzinger et al. compared three denitrification methods (N_2 flux, ^{15}N tracer, and C_2H_2 inhibition) using aquatic sediments. Denitrification rates due to addition of nitrate to the sediment core floodwater using the ^{15}N tracer method were about 35% of those measured by the C_2H_2 inhibition method.

Over a short incubation time (2 days), our denitrification results measured with the C_2H_2 method were comparable to the $^{15}N_2$ flux method; however, over extended incubation periods (6, 10, and 16 days), the C_2H_2 inhibition method significantly underestimated potential denitrification. Coupled nitrification–denitrification would not be captured due to C_2H_2 inhibition of nitrification in sediments. In addition, slow diffusion of C_2H_2 through the floodwater, incomplete blockage of N_2O by C_2H_2, and C_2H_2 metabolism by microorganisms may also be responsible for underestimating denitrification. For our study, denitrification due to coupling was thought to be a minor component compared to the denitrification contribution due to the high level of NO_3–N added (100 mg N L^{-1}).

Further studies are needed to compare denitrification methods at Mississippi River water *in situ* NO_3–N concentrations (1–3 mg N L^{-1}) entering the Atchafalaya River basin and across all basin habitats. In addition, denitrification methods need to be evaluated for incubation periods less than 2 days. If the C_2H_2 inhibition method can be calibrated against the $^{15}N_2$ flux procedure, considerable expense and time can be saved on denitrification analyses. Quantification of denitrification is needed to evaluate the contribution of denitrification to nitrogen removal within the Atchafalaya River basin.

Acknowledgements

This project was funded by the Louisiana Governor's Office of Coastal Activities and the US Army Corps of Engineers.

Downloaded by [Louisiana State University] at 06:11 04 May 2012

Downloaded by [Louisiana State University] at 06:11 04 May 2012

References

Aulenbach BT, Buxton HT, Battaglin WA, Coupe RH. 2007. Streamflow and nutrient fluxes of the Mississippi–Atchafalaya River basin and subbasins for the period of record through 2005. United States Geological Survey. Open File Report 2007-1080.

Clement JC, Pinay G, Marmonier P. 2002. Seasonal dynamics of denitrification along topohydrosequences in three different riparian wetlands. Journal of Environmental Quality. 31(3):1025–1037.

Cornwell JC, Kemp WM, Kana TM. 1999. Denitrification in coastal ecosystems: methods, environmental controls, and ecosystem level controls, a review. Aquatic Ecology. 33(1):41–54.

Dunn DD. 1996. Trends in nutrient inflows to the Gulf of Mexico from streams draining the conterminous United States 1972–93. Austin, Texas: USGS Report 96-4113.

Ferguson AJP, Eyre BD. 2007. Seasonal discrepancies in denitrification measured by isotope pairing and N_2:Ar techniques. Marine Ecology Progress Series. 350(3707):19–27.

Goolsby DA, Battaglin WA, Aulenbach BT, Hooper RP. 2001. Nitrogen input to the Gulf of Mexico. Journal of Environmental Quality. 30(2):329–336.

Groffman PM. 1994. Denitrification in freshwater wetlands. Current Topics in Wetland Biogeochemistry. 1:15–36.

Groffman PM, Altabet MA, Bohlke JK, Butterbach-Bahl K, David MB, Firestone MK, Giblin AE, Kana TM, Nielsen LP, Voytek MA. 2006. Methods for measuring denitrification: diverse approaches to a difficult problem. Ecological Applications. 16(6):2091–2122.

Hauck RD, Bouldin DR. 1961. Distribution of isotopic nitrogen in nitrogen gas during denitrification. Nature. 191:871–872.

Hernandez ME, Mitsch WJ. 2007. Denitrification potential and organic matter as affected by vegetation community, wetland age, and plant introduction in created wetlands. Journal of Environmental Quality. 36(1):333–342.

Lindau CW, Delaune RD, Collins JW, Bollich PK, Scott LM, Lambremont EN. 1998. Methane and nitrous oxide evolution and N-15 and Ra-226 uptake as affected by application of gypsum and phosphogypsum to Louisiana rice. Agriculture, Ecosystems and Environment. 68:165–173.

Lindau CW, Patrick WH, Delaune RD, Reddy KR, Bollich PK. 1988. Entrapment of nitrogen-15 dinitrogen during soil denitrification. Soil Science Society of America Journal. 52:538–540.

Lohse L, Kloosterhuis HT, van Raaphorst W, Helder W. 1996. Denitrification rates as measured by the isotope pairing method and by the acetylene inhibition technique in continental shelf sediments of the North Sea. Marine Ecology Progress Series. 132:169–179.

Mississippi River/Gulf of Mexico Watershed Nutrient Task Force. 2001. Action plan for reducing, mitigating, and controlling hypoxia in the northern Gulf of Mexico. Washington, DC.

Mississippi River/Gulf of Mexico Watershed Nutrient Task Force. 2008. Gulf hypoxia action plan 2008 for reducing, mitigating, and controlling hypoxia in the northern Gulf of Mexico and improving water quality in the Mississippi River basin. Washington, DC.

Mitsch WJ, Gosselink JG. 2000. Wetlands. New York: Van Nostrand Reinhold.

Mulvaney RL, Boast CW. 1986. Equations for determination of nitrogen-15 labeled dinitrogen and nitrous oxide by mass spectrometry. Soil Science Society of America Journal. 50:360–363.

Rabalais NN, Turner RE, Wiseman WJ. 2002. Gulf of Mexico, aka ''the dead zone''. Annual Review of Ecology and Systematics. 33:235–263.

Rolston DE. 1986. Gas flux In: Methods of soil analysis, Part 1. Klute A, editor, Physical and mineralogical methods. Madison, Wisconsin: Soil Science Society of America. p. 1103–1119.

Seitzinger SP, Harrison JA, Bohlke JK, Bouwman AF, Lawrance R, Peterson B, Tobias C, van Drecht G. 2006. Denitrification across landscapes and waterscapes: a synthesis. Ecological Applications. 16:2064–2090.

Seitzinger SP, Nielsen LP, Caffrey J, Christensen PB. 1993. Denitrification measurements in aquatic sediments: a comparison of three methods. Biogeochemistry. 23:147–167.

Siegel RS, Hauck RD, Kurtz LT. 1982. Determination of $^{30}N_2$ and application to measurement of N_2 evolution during denitrification. Soil Science Society of America Journal. 46:68–74.

Smith LK, Voyek MA, Bohlke JK, Harvey JW. 2006. Denitrification in nitrate-rich streams: application of N_2:Ar and ^{15}N-tracer methods in intact cores. Ecological Applications. 16:2191–2207.

Steingruber SM, Friedrich J, Gachter R, Wehrli B. 2001. Measurement of denitrification in sediments with the ^{15}N isotope pairing technique. Applied and Environmental Microbiology. 67:3771–3778.

USEPA 2001. Action plan to reduce the size of the "dead zone" in the Gulf of Mexico. Washington, DC. Office of Water (4503F), EPA 841-F-01-001.

USGS 2007. The Gulf of Mexico hypoxic zone [Internet]. [Accessed 2010 October 7]. Available from: http://toxics.usgs.gov./hypoxia/hypoxic_zone.html

van Luijn F, Boers PCM, Lijklema L. 1996. Comparison of denitrification rates in lake sediments obtained by the N_2 flux method, the ^{15}N isotope pairing technique and the mass balance approach. Water Resources. 30:893–900.

Wallenstein MD, Myrold DD, Firestone M, Voytek M. 2006. Environmental controls on denitrifying communities and denitrification rates: insights from molecular methods. Ecological Application. 16:2143–2152.

Watts SH, Seitzinger SP. 2000. Denitrification rates in organic and mineral soils from riparian sites: a comparison of N_2 flux and acetylene inhibition methods. Soil Biology and Biochemistry. 32:1383–1392.

Well R, Myrold DD. 1999. Laboratory evaluation of a new method for in situ measurement of denitrification in water-saturated soils. Soil Biology and Biochemistry. 31:1109–1119.

Downloaded by [Louisiana State University] at 06:11 04 May 2012