

Nitrous Oxide Accumulation in Soils from Riparian Buffers of a Coastal Plain Watershed—Carbon/Nitrogen Ratio Control

P. G. Hunt,* T. A. Matheny, and K. S. Ro USDA-ARS

Riparian buffers are used throughout the world for the protection of water bodies from nonpoint-source nitrogen pollution. Few studies of riparian or treatment wetland denitrification consider the production of nitrous oxide (N_2O). The objectives of this research were to ascertain the level of potential N_2O production in riparian buffers and identify controlling factors for N_2O accumulations within riparian soils of an agricultural watershed in the southeastern Coastal Plain of the USA. Soil samples were obtained from ten sites (site types) with different agronomic management and landscape position. Denitrification enzyme activity (DEA) was measured by the acetylene inhibition method. Nitrous oxide accumulations were measured after incubation with and without acetylene (baseline N_2O production). The mean DEA (with acetylene) was $59 \mu\text{g } N_2O\text{-N kg}^{-1} \text{ soil h}^{-1}$ for all soil samples from the watershed. If no acetylene was added to block conversion of N_2O to N_2 , only $15 \mu\text{g } N_2O\text{-N kg}^{-1} \text{ soil h}^{-1}$ were accumulated. Half of the samples accumulated no N_2O . The highest level of denitrification was found in the soil surface layers and in buffers impacted by either livestock waste or nitrogen from legume production. Nitrous oxide accumulations (with acetylene inhibition) were correlated to soil nitrogen ($r^2 = 0.59$). Without acetylene inhibition, correlations with soil and site characteristics were lower. Nitrous oxide accumulations were found to be essentially zero, if the soil C/N ratios >25 . Soil C/N ratios may be an easily measured and widely applicable parameter for identification of potential hot spots of N_2O productions from riparian buffers.

RIPIARIAN BUFFERS are among the most widely used best management practices for the protection of water bodies from nonpoint-source pollution, particularly nitrogen (Peterjohn and Correll, 1984; Lowrance et al., 1984; Jordan et al., 1993; Stone et al., 1998; Hill et al., 2000; Novak et al., 2002; Hunt et al., 2004). Whereas they are wet by nature, they are often very effective in removing nitrogen via denitrification (Lowrance et al., 1995; Hunt et al., 2004). However, this denitrification in riparian buffers is often spatially uneven because riparian buffers vary considerably in their size and landscape positions as well as their soil, vegetative, and hydrological conditions (Bowden et al., 1992; Hill et al., 2000; Flite et al., 2001). As riparian buffers become more widely used, it is increasingly important to know if their denitrification typically proceeds to completion with the production of dinitrogen gas or if riparian denitrification is incomplete (stopping at nitrous oxide). Incomplete denitrification carries the potential for significant nitrous oxide production and its associated potent greenhouse gas characteristics (Davidson et al., 2000).

Relatively few studies of riparian denitrification consider the production of nitrous oxide. Walker et al. (2002) reported that emissions of nitric oxide, nitrous oxide, and ammonia were lower in an Appalachia riparian zone that was allowed to recover from overgrazing relative to a companion riparian zone that continued to be grazed by cattle (24 vs. $77 \text{ kg ha}^{-1} \text{ yr}^{-1}$ of nitrous oxide). Dhondt et al. (2004) conducted an investigation to determine the extent of potential nitrous oxide production in three different types of riparian zones (mixed vegetation, forest, and grass) of the Molenbeek River of Belgium in an effort to assess the tradeoff of denitrification to improve water quality vs. potential air quality degradation via nitrous oxide emissions. They concluded that observed nitrous oxide emissions in riparian zones were not a significant “pollution-swapping phenomenon.” A similar conclusion was reached for forested riparian buffers in the lower Mississippi Alluvial Valley (Ullah et al., 2005).

Insight into denitrification can also be gained from investigation of nitrous oxide emissions from agricultural and natural ecosystems that are non-riparian in nature. Most of the denitrification proceeds to the production of dinitrogen gas, but some level of

Copyright © 2007 by the American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America. All rights reserved. No part of this periodical may be reproduced or transmitted in any form or by any means, electronic or mechanical, including photocopying, recording, or any information storage and retrieval system, without permission in writing from the publisher.

Published in *J. Environ. Qual.* 36:1368–1376 (2007).
doi:10.2134/jeq2006.0255
Received 30 June 2006.

*Corresponding author (Patrick.Hunt@ars.usda.gov).

© ASA, CSSA, SSSA

677 S. Segoe Rd., Madison, WI 53711 USA

USDA-ARS, Coastal Plains Soil, Water, and Plant Research Center, Florence, SC 29501. Mention of trade name, proprietary product, or vendor is for information only and does not constitute a guarantee or warranty of the product by the USDA and does not imply its approval to the exclusion of other products or vendors that may also be suitable.

Abbreviations: DEA, denitrification enzyme activity.

nitrous oxide production is common for both agricultural and natural ecosystems (Davidson et al., 2000). Several studies have documented higher nitrous oxide production in forested and pasture lands when soils were wet (Erickson et al., 2001; Wick et al., 2005). These two studies also documented the decline in nitrous oxide as leaf litter increased in its C/N ratio. Furthermore, Klemedtsson et al. (2005) found that the C/N ratio of forest soils was a good scaling parameter to predict nitrous oxide emission in forested systems of northern Europe.

We reported high levels of denitrification in a Coastal Plain riparian buffer contiguous to a heavily loaded swine wastewater sprayfield (Hunt et al., 2004). During this investigation we also measured high levels of incomplete denitrification, but we did not report these findings. Hefting et al. (2003) had reported high levels of nitrous oxide emissions in a site that was heavily loaded with nitrate. Hefting et al. (2006) also reported that the nitrous oxide emissions in the riparian buffer were spatially variable. Ullah and Zinati (2006) similarly reported an increase in nitrous oxide production in riparian forested soils when nitrate was added to soils with C/N ratio < 22. The nitrous oxide production was greater for soils that had been exposed to prolonged nitrogen runoff. These investigations provided insight into denitrification in agricultural riparian buffers. However, further investigations are needed to more fully understand the potential for nitrous oxide emissions from riparian buffer soils.

The objectives of this research were to (i) ascertain the level of potential nitrous oxide accumulation in soils of a riparian buffer that was heavily impacted by nitrogen from swine wastewater, (ii) compare this heavily impacted site to other riparian buffer sites within the watershed, and (iii) identify controlling factors for nitrous oxide accumulations in these riparian soils.

Materials and Methods

Site Description

The study was conducted within the Herrings Marsh Run Watershed in North Carolina (Stone et al., 1995). The watershed (35°05' N; 77°55' W) had an area of 2360 ha and was located within the Cape Fear River basin. The Herrings Marsh Run watershed was about 43% forested and 57% cropland or pasture. Soil samples were obtained from ten sites with distinctly different combinations of soil, landscape position, and agronomic practices. We called this combination “site type.” (Fig. 1, Table 1).

The site types were: (A) a restored forest riparian buffer downslope of a heavily loaded swine wastewater sprayfield; (B) a forest/shrub riparian buffer across the stream from a heavily loaded swine wastewater sprayfield; (C) a marsh riparian buffer downslope of a cultivated field with row crop production; (D) forested riparian buffers downslope of cultivated fields with row crop production; (E) a forested riparian buffer downslope of a residential area; (F) a forest/shrub riparian buffer downslope of a new swine wastewater sprayfield; (G) grass riparian buffers downslope of cultivated fields with row crop production; (H) in-stream wetlands below a swine wastewater sprayfield; (I) a grass riparian buffer downslope of a livestock feeding pasture; and (J) a forested riparian buffer at the outlet of the watershed.

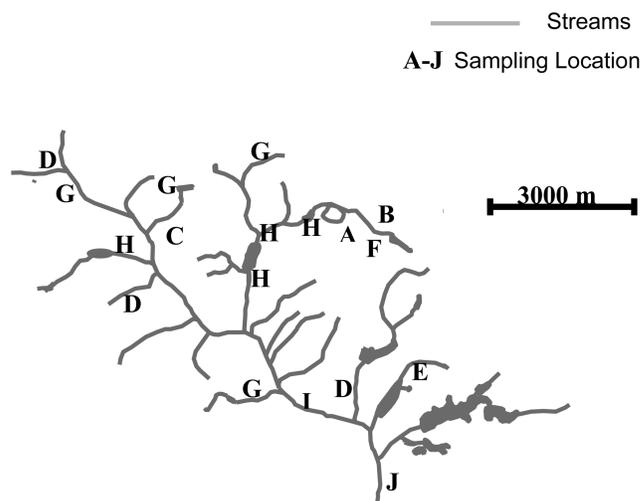


Fig. 1. Site types locations for sampling soil denitrification enzyme activity in riparian buffers of a Coastal Plain watershed.

We had previously conducted specific investigations on water quality and denitrification at site types A and B (Hunt et al. 1999, 2003), and all of the site types were within the watershed studied by Stone et al. (1995, 1998). Site type B (forest/shrub riparian buffer across the stream from the swine wastewater sprayfield) did not receive drainage from the swine wastewater sprayfield. Site A received heavy inputs from a swine wastewater sprayfield. Restoration of site type A (restored riparian buffer) began in April 1993 when the riparian buffer was planted with trees (1 to 1.5 m in height) on 2-m spacing. The trees at the time of this investigation were 5 to 10 m high. Starting at the sprayfield edge and moving toward the stream, species planted were green ash (*Fraxinus pennsylvanica* Marshall), red maple (*Acer rubrum* L.), sycamore (*Platanus occidentalis* L.), water oak (*Quercus nigra* L.), and bald cypress (*Taxodium distichum* L. Rich.). The remaining site types consisted of marsh, grass, and forested riparian buffers. The cultivated fields were planted in either cotton (*Gossypium hirsutum*) and/or soybean [*Glycine max* (L.) Merr.]. The riparian buffers ranged in width from about 30 to 300 m.

The ten site types were selected at locations that were representative of the landscape and agricultural management conditions typical for the Herrings Marsh Run watershed. Site types D, G, and H had three, four, and four different sampling locations, respectively (Fig. 1).

Sample Collection and Analyses

Soil samples were collected in March 2004 and August 2005. Site C was only sampled in 2004, and site I was only sampled in 2005. Samples (5-cm diam. × 15.2-cm length) were collected from three depths at each site: (i) at the upper 15 cm of the soil surface; (ii) midway between the soil surface and the water table; and (iii) 15 cm above the water table. The soil samples were obtained from the same core-hole with a vertical penetration to each respective sample depth. Three cores were taken and composited by depth at each location (1 to 8 locations/site) within a site. The total number of samples taken for analyses was 138 (Table 1). Each composited

Table 1. Physical and chemical characteristics of soils from different riparian buffers in a Coastal Plain watershed.

Site type descriptions	ST†	SYL‡	Taxonomic class	Water table depth	Soil nitrogen	Soil carbon
				cm below surface	mg kg ⁻¹	
Restored riparian buffer downslope of a heavily loaded swine wastewater spray field	A	6	Autryville, loamy, siliceous, subactive, thermic Arenic Paleudults, 0–6% slope	183 to 198	219–804	5133–26 445
		6	Bibb, coarse-loamy, siliceous, active, acid, thermic Typic Fluvaquents, 0–2% slope	46 to 91	622–9190	9620–240 833
Forest/shrub riparian buffer across stream from swine wastewater spray field	B	5	Bibb, coarse-loamy, siliceous, active, Acid, thermic Typic Fluvaquents, 0–2% slope	61 to 76	371–5517	7129–146 300
Marsh riparian buffer downslope of a cultivated field	C	1	Bibb, coarse-loamy, siliceous, active, acid, thermic Typic Fluvaquents, 0–2% slope	46	1883	39 124
Forest riparian buffer downslope of cultivated field	D	1	Norfolk, fine-loamy, kaolinitic, thermic Typic Kandiodults, 0–2% slope	132	357	7567
		1	Bibb, coarse-loamy, siliceous, active, acid, thermic Typic Fluvaquents, 0–2% slope	46	1222	29 220
		3	Gritney-Slagle complex, fine, mixed, semiactive, thermic Aquic Hapludults and fine-loamy, siliceous, subactive, thermic Aquic Hapludults, 0–4% slope	46 to 102	731–1650	20 970–48 000
Forest riparian buffer downslope of residential areas	E	2	Autryville, loamy, siliceous, subactive, thermic Arenic Paleudults, 0–6% slope	61	879–2317	21 653–50 633
Forest/shrub riparian buffer downslope of a new swine wastewater spray field	F	3	Autryville, loamy, siliceous, subactive, thermic Arenic Paleudults, 0–6% slope	168 to 183	311–473	4899–12 366
		1	Bibb, coarse-loamy, siliceous, active, acid, thermic Typic Fluvaquents, 0–2% slope	76	782–4786	19 828–110 957
Grass riparian buffer downslope of cultivated fields	G	2	Autryville, loamy, siliceous, subactive, thermic Arenic Paleudults, 0–6% slope	97	493–905	13 033–31 650
		1	Goldsboro, fine-loamy, siliceous, subactive, thermic Aquic Paleudults, 0–10% slope	87	1222	29 220
In-stream wetlands below swine wastewater spray field	H	3	Autryville, loamy, siliceous, subactive, thermic Arenic Paleudults, 0–6% slope	46 to 137	692–1043	15 012–26 645
		8	Bibb, coarse-loamy, siliceous, active, acid, thermic Typic Fluvaquents, 0–2% slope	46 to 107	160–2843	3939–66 503
Grass riparian buffer downslope of a pasture	I	1	Autryville, loamy, siliceous, subactive, thermic Arenic Paleudults, 0–6% slope	61	1009	21 625
Forest riparian buffer at outlet of watershed	J	2	Bibb, coarse-loamy, siliceous, active, acid, thermic Typic Fluvaquents, 0–2% slope	152	690–856	14 467–19 760

† Site types (ST); see Fig. 1 for the locations within the watershed; site types C and I were only sampled in 2004 and 2005, respectively.

‡ Site-year-location (SYL); samples were taken from each of three soil depths at each of the 46 SYL, for a total of 138 samples.

soil sample was manually homogenized, placed in plastic bags, stored on ice, transported to the laboratory, and stored at 4°C.

Denitrification Enzyme Activity

Denitrification enzyme activity (DEA) was measured by the acetylene inhibition method (Tiedje, 1994). Field moist soil (10–15 g) from each sampling location was placed in 60-mL serum bottles (four bottles per sample per replication). All analyses were performed in triplicate. The treatments were: (i) 5 mL of chloramphenicol (1 g L⁻¹) to inhibit protein synthesis and to measure incomplete denitrification; (ii) 5 mL of chloramphenicol (1 g L⁻¹) and 15 × 10⁻³ L of acetylene (produced from calcium carbide) to block denitrification at the nitrous oxide phase for measuring total denitrification; (iii) 5 mL of chloramphenicol (1 g L⁻¹) and 5 mL of amendment (200 mg L⁻¹ NO₃-N and 600 mg L⁻¹ glucose-C) to measure potential, incomplete denitrification; and (iv) 5 mL of chloramphenicol (1 g L⁻¹), 15 × 10⁻³ L of acetylene, and 5 mL of

amendment (200 mg L⁻¹ NO₃-N and 600 mg L⁻¹ glucose-C) to measure potential denitrification. By injecting acetylene to half of the treatments, we were able to determine complete and incomplete denitrification. With the addition of acetylene, the denitrification process was stopped (chemical inhibition of the enzyme) at the N₂O step. This provided a measure of the total denitrification. Without the addition of acetylene, the denitrification process could proceed to completion with the production of N₂. Thus, with this treatment, any N₂O production was the result of natural incomplete denitrification. By comparing N₂O produced with and without acetylene, we were able to determine the quantity of N₂O produced by natural incomplete denitrification. The treatments without glucose or nitrate (i and ii) provided measurements for actual denitrification (complete or incomplete). The treatments with addition of glucose and nitrate N (iii and iv) provided sufficient glucose for energy and nitrate for electron acceptors to measurements of the potential denitrification (complete or incomplete).

Thus, the treatment with the addition of glucose and nitrate without acetylene provided an estimate of what we refer to as potential, incomplete denitrification. However, it must be noted that DEA provides a measure of the nitrous oxide accumulated in the soil under the anaerobic incubation conditions. As such, it does not provide a measure of the aerobic denitrification or the actual emission of nitrous oxide from the soil surface.

The serum bottles were capped with rubber septa, evacuated, and purged with purified nitrogen gas three times. The serum bottles were incubated on a horizontal shaker at 1.5 cycles s^{-1} and 24°C. After 1, 5, and 24 h of incubation, 5 × 10⁻³ L of the headspace gases were removed from the serum bottles with a syringe (Plastipak, Franklin Lakes, NJ) and injected into vials (borosilicate glass, crimp top with butyl septum). For our analyses, we used the incubation period (1, 5, or 24 h) with the highest N₂O concentration per unit of time.

The N₂O-N in the headspace gas was measured with a Model 3600 CX gas chromatograph (Varian, Palo Alto, CA) equipped with a 15-m Ci⁶³ Ni electron capture detector operating at 350°C. Chromatographic separation of the headspace gases was obtained by use of a 1.8-m by 2-mm i.d. stainless steel column packed with Poropak Q (80–100 mesh; Alltech Associates, Deerfield, IL); the column and injector temperatures were 70°C; and the carrier gas was purified nitrogen. Samples were injected into the column by a Model 8200 auto sampler (Varian). Rates of N₂O-N accumulation were expressed on a dry soil weight basis.

Field moist soil samples were dried at 100°C for 72 h and weighed to determine moisture content. Total soil nitrogen and carbon were determined on a Model CN2000 carbon/nitrogen analyzer (LECO Corporation, St. Joseph, MI).

Data Analyses

Data were analyzed using the general linear model (GLM) for analysis of variance (ANOVA). For the analysis of variance, we pooled sampling locations for a site type into a single mean for each site type. We used a split plot in time analysis with years as replication to evaluate treatments and interactions, including site type, soil layers, and amendments. The main plot treatment was site type (A–J), the subplot treatment was soil layers, and the sub-subplot treatment was DEA amendments. Analysis of variance indicated that there were no significant interaction effects with soil layers or amendment treatments ($P \leq 0.05$). Furthermore, the results of the ANOVA were not significantly altered by log, square root, or Box-Cox transformations of the data. The site type and treatment differences were central to our interest in nitrous oxide accumulation in the soils of the watershed. Therefore, soil layers were pooled for an ANOVA of the site types for each treatment so that the site types within a treatment could be compared by the least significant difference (LSD). Similarly, soil layers were pooled for an ANOVA of the treatments within each site type so that they could be compared by LSD. For insight into the impact of soil characteristic on denitrification, we used stepwise regression. The data were analyzed for each of the amendment treatments at all sampling locations and depth (138 total samples; Table 1). The stepwise regression

Table 2. Mean and median denitrification enzyme activity of soil in a Coastal Plain watershed.

Treatment	C ₂ H ₂	Mean	Median	Std. dev.
		μg N kg ⁻¹ soil h ⁻¹		
Control	no	15	0	54
Control	yes	59	23	120
N+C†	no	66	1	261
N+C	yes	146	51	332

† Addition of nitrate and glucose.

components were soil nitrogen, carbon, C/N ratio, and depth along with depth to water table. Data were also evaluated based on ranges of soil C/N ratios in increments of 5 from 15 to 50 (i.e., 15–20 ... 45–50). All data analyses were conducted with Version 6.12 of Statistical Analysis System (SAS Institute, 1999).

Results and Discussion

Nitrous Oxide Accumulation in Soils within the Entire Watershed

The mean DEA for all soil samples from the entire watershed for the control treatment (no carbon or nitrogen amendments) was 59 μg N₂O-N kg⁻¹ soil h⁻¹ (Table 2). The high standard deviation (120 μg N₂O-N kg⁻¹ soil h⁻¹) associated with this mean is consistent with reports of the highly spatially variable nature of DEA (Bowden et al., 1992; Hill et al., 2000; Flite et al., 2001). If no acetylene was added to block conversion of nitrous oxide to dinitrogen gas, 15 μg N₂O-N kg⁻¹ soil h⁻¹ was accumulated, which constituted 25% of the total denitrification. This percentage of incomplete denitrification (stopping at nitrous oxide rather than being fully converted to dinitrogen gas) was considerably higher than the <5% commonly found in agricultural fields and forested lands (Davidson et al., 2000). The nature and distribution of this incomplete denitrification were more apparent when the median values were examined. The median value of DEA for the control treatment was 23 μg N₂O-N kg⁻¹ soil h⁻¹, which was about 40% of the mean value. However, the median value of the control treatment for the nitrous oxide accumulated without addition of acetylene was 0 μg N₂O-N kg⁻¹ soil h⁻¹. If the soils were amended with nitrate and glucose, the mean DEA was increased to 146 μg N₂O-N kg⁻¹ soil h⁻¹. If no acetylene was added the increase was 66 μg N₂O-N kg⁻¹ soil h⁻¹. This represented slightly more incomplete denitrification, 44%. The median value for DEA of the soils when nitrate and glucose were added was 51 μg N₂O-N kg⁻¹ soil h⁻¹. The median value for the soils when nitrate and glucose were added in the absence of acetylene was 1 μg N₂O-N kg⁻¹ soil h⁻¹. These low medians of zero and one indicated that the incomplete denitrification was very unevenly distributed in the watershed. Half of the samples were producing essentially no nitrous oxide. In contrast to the zero nitrous oxide, some sites were producing substantial amounts of nitrous oxide. One of the potential causes of this variation in incomplete denitrification was the variation among site types.

Table 3. Denitrification enzyme activity of soils from different riparian buffers in a Coastal Plain watershed.

Site types descriptions	ST†	Control		NO ₃ + C		LSD _{0.10}	LSD _{0.05}
		No C ₂ H ₂	C ₂ H ₂	No C ₂ H ₂	C ₂ H ₂		
		—µg N ₂ O-N kg ⁻¹ soil h ⁻¹ —					
Restored riparian buffer downslope of a heavily loaded swine wastewater spray field	A	23	95	175	217	82	98
Forest/shrub riparian buffer across stream from a heavily loaded swine wastewater spray field	B	33	105	68	172	55	66
Marsh riparian buffer downslope of a cultivated field	C	24	170	172	742	103	124
Forest riparian buffer downslope of cultivate field	D	37	44	76	209	85	101
Forest riparian buffer downslope of residential area	E	11	58	30	213	66	79
Forest/shrub riparian buffer downslope of a new swine wastewater spray field	F	2	10	2	31	8	10
Grass riparian buffer downslope of cultivated fields	G	1	46	18	65	11	14
In-stream wetlands below swine wastewater spray field	H	1	25	6	56	12	15
Grass riparian buffer downslope of a pasture	I	1	2	2	5	3	4
Forest riparian buffer at outlet of watershed	J	1	21	4	49	11	14
LSD _{0.10} ‡		19	43	67	84		
LSD _{0.05}		23	51	80	100		

† Site types (ST).

‡ LSD, least significant difference.

Nitrous Oxide Accumulation in Soils from Different Site Types

There was an increase in potential DEA for the soils of nearly all of the site types on the addition of glucose and nitrate (Table 3). This indicated that most of these riparian buffer soils had significant denitrification potential, but they lacked sufficient carbon or nitrate to express this potential at the time of sampling.

Three site types (A, B, and C) had soil with both high DEA (95 to 170 µg N₂O-N kg⁻¹ soil h⁻¹) and high nitrous oxide accumulation (23 to 33 µg N₂O-N kg⁻¹ soil h⁻¹) (Table 3). A fourth site type (D) had high nitrous oxide accumulation (37 µg N₂O-N kg⁻¹ soil h⁻¹), but lower DEA (44 µg N₂O-N kg⁻¹ soil h⁻¹).

The riparian buffer contiguous to a heavily loaded swine wastewater sprayfield (site type A) had a mean soil DEA of 95 µg N₂O-N kg⁻¹ soil h⁻¹ (Table 3). These values, which were a mean of all three soil layers, were in line with those reported in an earlier investigation of this site (Hunt et al., 2004). The accumulation of nitrous oxide in the absence of acetylene for soils of site type A was 23 µg N₂O-N kg⁻¹ soil h⁻¹. These nitrous oxide accumulation and DEA values placed the sprayfield riparian buffer among the highest for both DEA and nitrous oxide accumulation. Furthermore, the values are consistent with the high levels of carbon and nitrogen as well as the high water table throughout much of this location. The accumulated 23 µg N₂O-N kg⁻¹ soil h⁻¹ represented about 24% of the site's total DEA. This percentage of denitrification going to nitrous oxide was almost identical to the mean percentage for the watershed. Thus, despite the fact that this riparian buffer was high in nitrous oxide accumulation, it was not atypical for the watershed.

However, if glucose and nitrate were added to the soils of site type A, the data were radically different. The potential DEA was 217 µg N₂O-N kg⁻¹ soil h⁻¹. This level of potential DEA was the second highest in the watershed. The large potential DEA of the sprayfield soils was matched by a large accumulation of nitrous oxide in the absence of acetylene

(175 µg N₂O-N kg⁻¹ soil h⁻¹). All other site types, except C, were statistically lower in nitrous oxide accumulation for the glucose- and nitrate-amended soils when acetylene was absent (LSD_{0.05}). This nitrous oxide (potential, incomplete denitrification) represented 81% of the potential DEA of site type A. No other site had such a high percentage of the potential DEA as incomplete denitrification. These values indicate that there was not only a very high potential for denitrification, but there was a high potential for incomplete denitrification. Moreover, this very high percentage of potential incomplete denitrification was consistent with unpublished data from an earlier investigation of DEA at this site (Hunt et al., unpublished data, 2004). The data indicate that site type A may differ from the other site types in microbial populations and gene activation conditions (Baumann et al., 1996).

Without nitrate and glucose amendments, somewhat similar values were found for both nitrous oxide accumulations and DEA in site type B, a forest/shrub riparian buffer across the stream from the heavily loaded swine wastewater sprayfield. In the absence of acetylene, the control treatment had 33 µg N₂O-N kg⁻¹ soil h⁻¹. This represented 31% of its DEA (105 µg N₂O-N kg⁻¹ soil h⁻¹) as incomplete denitrification; a percentage slightly higher than the riparian buffer nearer the sprayfield. When nitrate and glucose were added, site type B had a potential DEA of 172 µg N₂O-N kg⁻¹ soil h⁻¹ and a potential, incomplete denitrification of 68 µg N₂O-N kg⁻¹ soil h⁻¹. While this was a very high amount of potential, incomplete denitrification (40%), it was not nearly as high as the 81% measured in the riparian buffer near the sprayfield.

Site type C (marsh riparian buffer downslope of a cultivated field) had the highest DEA, 170 µg N₂O-N kg⁻¹ soil h⁻¹. Most of this denitrification was complete. The nitrous oxide accumulation in the absence of acetylene was 24 µg N₂O-N kg⁻¹ soil h⁻¹, 14% of the total denitrification. Site type C also had by far the highest potential DEA, 742 µg N₂O-N kg⁻¹ soil h⁻¹. The nitrous oxide accumulation in the absence of acetylene was also higher when

nitrate and glucose were added, $172 \mu\text{g N}_2\text{O-N kg}^{-1} \text{ soil h}^{-1}$. This represented 23% of the total denitrification, which was similar to the mean of the watershed. The cause of this very high potential DEA is likely related to the marsh environment. The soil nitrogen was adequate to support denitrification, and it could have been that most of the nitrogen was in a form that could be readily denitrified. It could also have been that the site was often exposed to flooding and drying conditions, which could have enhanced the DEA potential. This possibility is supported by the fact that we were unable to sample the site after 2004 because it was underwater from a beaver dam.

Site type D was downslope of cultivated fields similar to the site type C, but it joined forested riparian buffers rather than a riparian marsh. It had a distinctly lower DEA than site type C, $44 \mu\text{g N}_2\text{O-N kg}^{-1} \text{ soil h}^{-1}$. Furthermore, a large portion of this DEA was incomplete denitrification. In the absence of acetylene in the control treatment, the nitrous oxide accumulation was $37 \mu\text{g N}_2\text{O-N kg}^{-1} \text{ soil h}^{-1}$ —84% incomplete denitrification. The soils of this site type had potential, incomplete denitrification and potential DEA values of 76 and $209 \mu\text{g N}_2\text{O-N kg}^{-1} \text{ soil h}^{-1}$, respectively—36% incomplete denitrification. While the specific causes of this incomplete denitrification are not clear, they may be, as site A, related to the microbial population or gene activation conditions of the site type (Baumann et al., 1996).

In variance to the agriculturally impacted site types A through D, site type E was a forested riparian buffer downslope of a residential area. It had a DEA of $58 \mu\text{g N}_2\text{O-N kg}^{-1} \text{ soil h}^{-1}$. The nitrous oxide accumulated in the absence of acetylene was $11 \mu\text{g N}_2\text{O-N kg}^{-1} \text{ soil h}^{-1}$, 19% of the total denitrification. When nitrate and glucose were added, the percentage of potential, incomplete denitrification was slightly lower, 14%. The potential DEA was $213 \mu\text{g N}_2\text{O-N kg}^{-1} \text{ soil h}^{-1}$, and the accumulation of nitrous oxide in the absence of acetylene was $30 \mu\text{g N}_2\text{O-N kg}^{-1} \text{ soil h}^{-1}$.

The remaining site types F through J were much lower in nitrous oxide accumulations in the absence of acetylene ($\leq 2 \mu\text{g N}_2\text{O-N kg}^{-1} \text{ soil h}^{-1}$). The DEA values were also generally lower (2 to $46 \mu\text{g N}_2\text{O-N kg}^{-1} \text{ soil h}^{-1}$). The potential DEA and potential, incomplete denitrification values were also lower. When nitrate and glucose were added, the accumulation of nitrous oxide in the absence of acetylene for these site types was $\leq 18 \mu\text{g N}_2\text{O-N kg}^{-1} \text{ soil h}^{-1}$. The potential DEA was $\leq 65 \mu\text{g N}_2\text{O-N kg}^{-1} \text{ soil h}^{-1}$. The management and landscape features of these site types were not radically different from the site types with higher levels of incomplete denitrification. Thus, it was important to consider if soil characteristics could better explain the variation in both DEA and incomplete denitrification.

Soil Depth Impact on Nitrous Oxide Accumulation

The surface layer of the soil was highest in DEA in nearly all of the sites throughout the watershed (Fig. 2). This is in agreement with other investigations (Ambus and Lowrance, 1991; Dhondt et al., 2004) as well as the results of the earlier investigation of the riparian buffer near the sprayfield (Hunt et al., 2004). In the study by Hunt et al. (2004), they report-

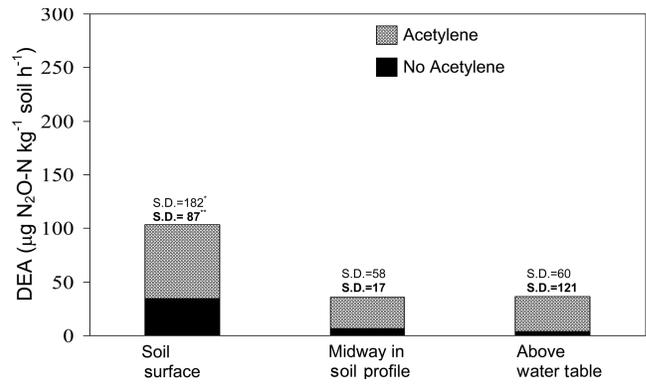


Fig. 2. Denitrification enzyme activity (DEA) in riparian buffers of a Coastal Plain watershed by soil profile depth. *, Standard deviation for DEA with acetylene; **, standard deviation for DEA without acetylene.

ed DEA means for the surface, middle, and water table layers of 147, 83, and $67 \mu\text{g N}_2\text{O-N kg}^{-1} \text{ soil h}^{-1}$, respectively. The surface was also the highest layer for the accumulation of nitrous oxide (Fig. 2). The percentages of incomplete denitrification for the surface, middle, and water table layers were 34, 17, and 11%, respectively. This higher percentage of apparent incomplete denitrification is likely related to the lower C/N ratio in the surface layer. The C/N ratios were 21, 27, and 31, respectively, for the surface, middle, and water table layers. These ratios may have resulted from high nitrogen content of leaf and other plant litter falling on the surface of the soil as plants senesce (Hunt et al., 2004; Ambus and Lowrance, 1991); this would produce a lower C/N ratio and higher nitrous oxide accumulations (Erickson et al., 2001).

When glucose and nitrate were added (Fig. 3), there was an even higher percentage of potential, incomplete denitrification: 58, 35, and 24%, respectively, for the surface, middle, and water table layers. This is likely related to the exacerbation of the low soil C/N ratios by the low C/N ratio (3:1) of the glucose/nitrate amendment.

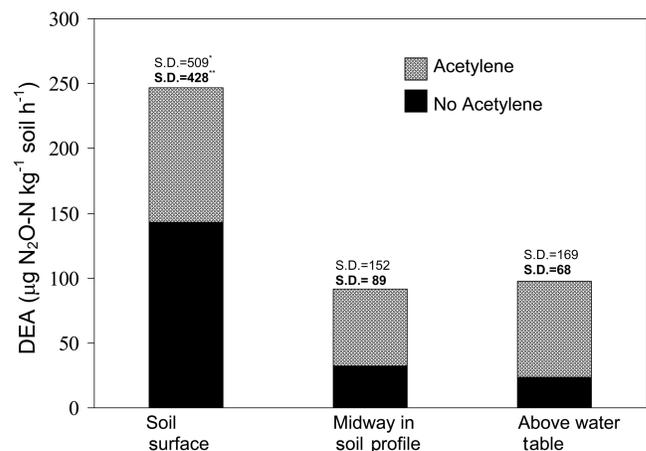


Fig. 3. Denitrification enzyme activity (DEA) in riparian buffers of a Coastal Plain watershed by soil profile depth for glucose- and nitrate-amended soil. *, Standard deviation for DEA with acetylene; **, standard deviation for DEA without acetylene.

Table 4. Stepwise regression analysis for denitrification enzyme activity of soil in a Coastal Plain watershed.

Treatment/regression variable	Partial r^2	Model r^2	C(p)	Pr > F
Control				
Soil nitrogen	0.15	0.15	41	<0.0001
Soil carbon	0.08	0.23	2	<0.0001
Control, C ₂ H ₂				
Soil nitrogen	0.59	0.59	20	<0.0001
Soil carbon	0.03	0.62	4	<0.0001
NO ₃ + glucose				
Soil nitrogen	0.40	0.40	30	<0.0001
Soil carbon	0.04	0.44	2	<0.0001
NO ₃ + glucose, C ₂ H ₂				
Soil nitrogen	0.43	0.43	19	<0.0001
Soil carbon	0.03	0.46	3	<0.0001

Relationships between Nitrous Oxide Accumulation and Soil Characteristics

The previous analyses provided some good insight into total denitrification. However, they were not as enlightening in the explanation of the large number of sites which had no nitrous oxide accumulations in the absence of acetylene—incomplete denitrification. To gain some further insights, we used soil nitrogen, carbon, C/N ratio, and depth along with depth to water table as the parameters in stepwise regressions.

In the control treatment with C₂H₂, DEA values were moderately well correlated to the soil nitrogen (Table 4). The r^2 was 0.59 with a $P < 0.01$, but the C(p) value of 20 was high. Including soil carbon in the analyses improved the r^2 to 0.62 as well as lowered the C(p) value to 4. No other parameters were significant at the 0.05 level. These results are generally similar to the results reported for the sprayfield riparian buffer (Hunt et al., 2004). The soil parameters were only somewhat correlated to nitrous oxide accumulation of the control treatment in the absence of acetylene (Table 4). Stepwise regression with soil nitrogen plus carbon resulted in an r^2 of 0.23, a C(p) value of 2, and a P value of < 0.01 .

When data from treatment with glucose, nitrate, and acetylene were analyzed by stepwise regression, the potential DEA

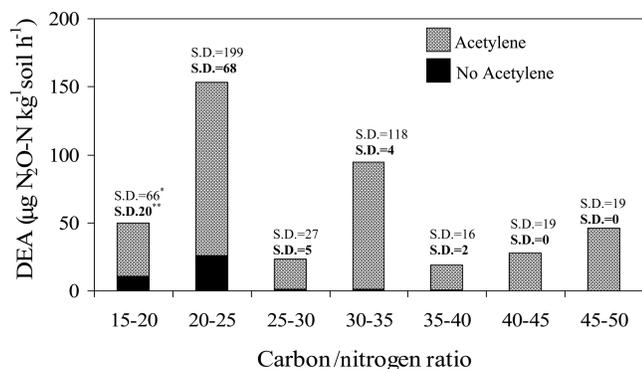


Fig. 4. Denitrification enzyme activity (DEA) versus C/N ratio for riparian buffer soils in a Coastal Plain watershed. *, Standard deviation for DEA with acetylene; **, standard deviation for DEA without acetylene.

was also most related to soil nitrogen. The r^2 was 0.43 with a $P < 0.01$, but the C(p) value of 19 was high. Including soil carbon in the analyses improved the r^2 to 0.46 and lowered the C(p) value to 3. When nitrate and glucose were added to the soils in the absence of acetylene, there was a better correlation of accumulated nitrous oxide to soil nitrogen than in the control treatment. The r^2 was 0.40 with a C(p) value of 30. With the addition of carbon to the stepwise analysis, the r^2 was 0.44 with a C(p) value of 2 and a P of < 0.01 .

Thus, regression analyses of the DEA were reasonably related to soil nitrogen and carbon as previously reported (Hunt et al., 2004). Regression analyses provided less insight into the large variation in nitrous oxide accumulation in the absence of acetylene. Soil depths and C/N ratios provided no significant improvements in the regression models. This is likely related to the fact that many factors can influence the production of nitrous oxide at low C/N ratios even in relatively homogenous systems such as wastewater treatment systems (Hwang et al., 2006).

Carbon/Nitrogen Ratio as a Threshold Controlling Factor

Without acetylene, nitrous oxide accumulation was found to be essentially zero (Fig. 4) in the control treatment when the soil C/N ratio exceeded 25. This ratio of 25 was a threshold, and significant nitrous oxide accumulation occurred only in soils with lower C/N ratios. Similar suppression of nitrous oxide emissions from soils has also been recently reported by Klemetsson et al. (2005) when the soil C/N ratios were >25 . They found that soil C/N ratio could be used as a scalar parameter to predict nitrous oxide emissions in forested histosols of northern Europe. They used a different technique (chamber emission) in a different ecosystem, yet they found very similar results. When the C/N ratio was above 25, nitrous oxide emissions were essentially zero. This similarity between the results of their chamber emission investigation and this soil DEA investigation indicates that soil C/N ratio may be a robust threshold controller of nitrous oxide production.

Nitrous oxide production in wastewater treatment has also been found to be controlled by the C/N ratio. Although the type and availability of carbon and nitrogen varies between wastewater and soil, parallel insights can be gained from the C/N ratio findings. For instance, Hwang et al. (2006) showed the clear sensitivity of denitrification in anaerobic wastewater treatment reactors to the C/N ratio of the wastewater. When the C/N ratio was 3, the system produced very little nitrous oxide over a wide range of ammonia and hydraulic loading conditions. However, when the C/N condition ratio was 1, denitrification produced large portions of nitrous oxide.

It is also possible that the higher amounts of carbon were affecting the portions of aerobic and anaerobic microsites. Baumann et al. (1996) found that transitional aerobic and anaerobic conditions produced all denitrification intermediates including nitrous oxide. However, under steady anaerobic conditions, the predominant product was dinitrogen. This finding relates to the reduction in nitrous oxide with increased soil pore water content reported by Ullah et al. (2005). It might be that the higher C/N ratio conditions were more generally related to anaerobic soils.

The precise cause of this threshold C/N ratio control of nitrous oxide accumulation is not known, but it may likely be related to the bio-energetic preference for the lower amount of energy required for partial denitrification. Thus, when sufficient carbon is available, there would be sufficient energy to push the reaction to completion. This explanation is supported by the fact that addition of the glucose to nitrate at a C/N ratio of 3 caused nitrous oxide to be accumulated in soils with higher C/N ratios (Fig. 5). Addition of the glucose and nitrate according to the method caused the threshold soil C/N ratio to move to 35. This phenomenon may partially explain the seemingly high values of predicted potential nitrous oxide production vs. the actual measured values in wastewater treatment wetlands (Hunt et al., 2003; Teiter and Mander, 2005).

Conclusions

1. The mean DEA for the watershed was $59 \mu\text{g N}_2\text{O-N kg}^{-1} \text{ soil h}^{-1}$. If no acetylene was added to block conversion of nitrous oxide to dinitrogen gas, only $15 \mu\text{g N}_2\text{O-N kg}^{-1} \text{ soil h}^{-1}$ was accumulated. The surface layer had the highest DEA and nitrous oxide accumulation. However, DEA and nitrous oxide accumulations were highly variable; the median value for nitrous oxide accumulation was zero. Thus, half of the soils accumulated no nitrous oxide while others produced high levels.
2. The riparian buffer that was heavily impacted by nitrogen from swine wastewater (site type A) was among the three site types with both high DEA ($95 \mu\text{g N}_2\text{O-N kg}^{-1} \text{ soil h}^{-1}$) and nitrous oxide accumulation in the absence of acetylene ($23 \mu\text{g N}_2\text{O-N kg}^{-1} \text{ soil h}^{-1}$), incomplete denitrification.
3. Site type A was singularly high in the percentage of potential, incomplete denitrification at 81%. These results suggest that this site may have both different microbial populations and gene activation conditions.
4. When analyzed via stepwise regression, among soil nitrogen, carbon, C/N ratio, and depth as well as water table depth; the best predictor for DEA levels was soil nitrogen.
5. Nitrous oxide accumulations in the absence of acetylene were not well predicted by soil or landscape characteristics. However, they seemed to be controlled by a threshold level of soil C/N ratio >25 . Thus, the soil C/N ratio may be an easily measured and widely applicable parameter for identification of potential hot spots of nitrous oxide production in riparian buffers.
6. Whereas the threshold C/N ratio value of 25 has been found in both the USA and Europe in very different ecosystems, there is a need to better understand and use the soil C/N ratios as a controlling factor for nitrous oxide production in riparian buffers across a range of watersheds and ecosystems.
7. Research is needed for both the soil microbial production and gas emission aspects.

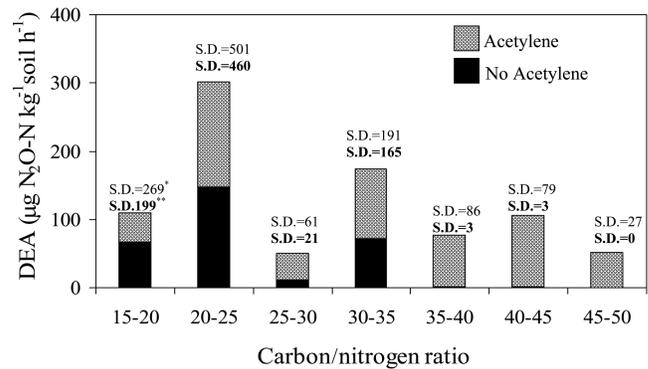


Fig. 5. Denitrification enzyme activity (DEA) versus C/N ratio for riparian buffer soils in a Coastal Plain watershed for glucose- and nitrate-amended soil. *, Standard deviation for DEA with acetylene; **, standard deviation for DEA without acetylene.

References

- Ambus, P., and R.R. Lowrance. 1991. Comparison of denitrification in two riparian soils. *Soil Sci. Soc. Am. J.* 55:994–997.
- Baumann, B., M. Snozzi, A.J.B. Zehnder, and J.R.D. Van Meer. 1996. Dynamics of denitrification activity of *Paracoccus denitrificans* in continuous culture during aerobic-anaerobic changes. *J. Bacteriol.* 178(15):4367–4374.
- Bowden, W.B., W.H. McDowell, C.E. Ashbury, and A.M. Finley. 1992. Riparian nitrogen dynamics in two geomorphologically distinct tropical forest watersheds: Nitrogen oxide fluxes. *Biogeochemistry* 18:77–99.
- Davidson, E.A., M. Keller, H.E. Erickson, L.V. Verchot, and E. Veldkamp. 2000. Testing a conceptual model of soil emissions of nitrous and nitric oxides. *Bioscience* 50(8):667–680.
- Dhondt, K., P. Boechx, G. Hofman, and O. Van Cleemput. 2004. Temporal and spatial patterns of denitrification enzyme activity and nitrous oxide fluxes in three adjacent vegetated riparian buffer zones. *Biol. Fertil. Soils* 40:243–251.
- Erickson, H., M. Keller, and E.A. Davidson. 2001. Nitrogen oxide fluxes and nitrogen cycling during postagricultural succession and forest fertilization in the humid tropics. *Ecosystems* 4(1):67–84.
- Flite, O.P., III, R.D. Shannon, R.R. Schnabel, and R.R. Parizek. 2001. Nitrate removal in a riparian wetland of the Appalachian Valley and ridge physiographic province. *J. Environ. Qual.* 30:254–261.
- Hefting, M.M., R. Bobbink, and H. De Caluwe. 2003. Nitrous oxide emission and denitrification in chronically nitrate-loaded riparian buffer zones. *J. Environ. Qual.* 32(4):1194–1203.
- Hefting, M.M., R. Bobbink, and M.P. Janssens. 2006. Spatial variation in denitrification and N₂O emission in relation to nitrate removal efficiency in a N-stressed riparian buffer zone. *Ecosystems* 9(4):550–563.
- Hill, A.R., K.J. Devito, S. Campagnolo, and K. Sanmugas. 2000. Subsurface denitrification in a forest riparian zone: Interactions between hydrology and supplies of nitrate and organic carbon. *Biogeochemistry* 51:193–223.
- Hunt, P.G., T.A. Matheny, and K.C. Stone. 2004. Denitrification in a Coastal Plain riparian zone contiguous to a heavily loaded swine wastewater sprayfield. *J. Environ. Qual.* 33:2367–2374.
- Hunt, P.G., T.A. Matheny, and A.A. Szogi. 2003. Denitrification in constructed wetlands used for treatment of swine wastewater. *J. Environ. Qual.* 32:727–735.
- Hunt, P.G., K.C. Stone, F.J. Humenik, T.A. Matheny, and M.H. Johnson. 1999. In-stream wetland mitigation of nitrogen contamination in a USA Coastal Plain stream. *J. Environ. Qual.* 28:249–256.
- Hwang, S., K. Jang, H. Jang, J. Song, and W. Bae. 2006. Factors affecting nitrous oxide production: A comparison of biological nitrogen removal processes with partial and complete nitrification. *Biodegradation* 17:19–29.
- Jordan, T.E., D.L. Correll, and D.E. Weller. 1993. Nutrient interception by a riparian forest receiving inputs from adjacent cropland. *J. Environ. Qual.* 22:467–473.
- Klemmedtsson, L., K. Von Arnold, P. Weslien, and P. Gundersen. 2005. Soil C/N ratio as a scalar parameter to predict nitrous oxide emissions. *Glob. Change Biol.* 11:1142–1147.
- Lowrance, R.R., R.L. Todd, and L.E. Asmussen. 1984. Nutrient cycling in an agricultural watershed: II. Stream flow and artificial drainage. *J.*

- Environ. Qual. 13:27–32.
- Lowrance, R.R., G.R. Vellidis, and R.K. Hubbard. 1995. Denitrification in a restored riparian forested wetland. *J. Environ. Qual.* 24:808–815.
- Novak, J.M., P.G. Hunt, K.C. Stone, D.W. Watts, and M.H. Johnson. 2002. Riparian zone impact on phosphorus movement to a Coastal Plain black water stream. *J. Soil Water Conserv.* 57:127–133.
- Peterjohn, W.T., and D.L. Correll. 1984. Nutrient dynamics in an agricultural watershed: Observations on the role of a riparian forest. *Ecology* 65:1466–1475.
- SAS Institute. 1999. The SAS system for Windows. Release 8.02. SAS Inst., Cary, NC.
- Stone, K.C., P.G. Hunt, S.W. Coffey, and T.A. Matheny. 1995. Water quality status of a USDA water quality demonstration project in the eastern Coastal Plain. *J. Soil Water Conserv.* 59:567–571.
- Stone, K.C., P.G. Hunt, F.J. Humenik, and M.H. Johnson. 1998. Impact of swine waste application on ground and stream water quality in an eastern Coastal Plain watershed. *Trans. ASAE* 41(6):1665–1670.
- Teiter, S., and Ü. Mander. 2005. Emission of N_2O , N_2 , CH_4 , and CO_2 from constructed wetlands for wastewater treatment and from riparian buffer zones. *Ecol. Eng.* 25:528–541.
- Tiedje, J.M. 1994. Denitrifier enzyme activity (DEA). p. 256–257. *In* R.W. Weaver et al. (ed.) *Methods of soil analysis*. Part 2. 2nd ed. SSSA Book Ser. 5. SSSA, Madison, WI.
- Ullah, S., G.A. Breitenbeck, and S.P. Faulkner. 2005. Denitrification and N_2O emission from forested and cultivated alluvial clay soil. *Biogeochemistry* 73:499–513.
- Ullah, S., and G.M. Zinati. 2006. Denitrification and nitrous oxide emissions from riparian forests soils exposed to prolonged nitrogen runoff. *Biogeochemistry* 81:253–267.
- Walker, J.T., C.D. Geron, J.M. Vose, and W.T. Swank. 2002. Nitrogen trace gas emissions from a riparian ecosystem in southern Appalachia. *Chemosphere* 49:1389–1398.
- Wick, B., E. Veldkamp, W.Z. de Mello, M. Keller, and P. Crill. 2005. Nitrous oxide fluxes and nitrogen cycling along a pasture chronosequence in Central Amazonia, Brazil. *Biogeosciences* 2:175–187.