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

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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 (N₂O). The objectives of this research were to ascertain the level of potential N₂O production in riparian buffers and identify controlling factors for N₂O 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 N₂O production). The mean DEA (with acetylene) was 59 μg N₂O-N kg⁻¹ soil h⁻¹ for all soil samples from the watershed. If no acetylene was added to block conversion of N₂O to N₂, only 15 μg N₂O-N kg⁻¹ soil h⁻¹ were accumulated. Half of the samples accumulated no N₂O. 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 (r² = 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 N₂O productions from riparian buffers.

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Abbreviations: DEA, denitrification enzyme activity.

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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, Klemetsson 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.

**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.

<table>
<thead>
<tr>
<th>Site type descriptions</th>
<th>ST†</th>
<th>SYL‡</th>
<th>Taxonomic class</th>
<th>Water table depth</th>
<th>Soil nitrogen</th>
<th>Soil carbon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Restored riparian buffer downslope of a heavily loaded swine wastewater spray field</td>
<td>A 6</td>
<td></td>
<td>Autryville, loamy, siliceous, subactive, thermic Arenic Paleudults, 0–6% slope</td>
<td>132</td>
<td>357</td>
<td>7567</td>
</tr>
<tr>
<td>Forest/shrub riparian buffer across stream from swine wastewater spray field</td>
<td>B 5</td>
<td></td>
<td>Bibb, coarse-loamy, siliceous, active, acid, thermic Typic Fluvaquents, 0–2% slope</td>
<td>61 to 76</td>
<td>371–5517</td>
<td>7129–146300</td>
</tr>
<tr>
<td>Marsh riparian buffer downslope of a cultivated field</td>
<td>C 1</td>
<td></td>
<td>Bibb, coarse-loamy, siliceous, active, acid, thermic Typic Fluvaquents, 0–2% slope</td>
<td>46</td>
<td>1883</td>
<td>39124</td>
</tr>
<tr>
<td>Forest riparian buffer downslope of cultivated field</td>
<td>D 1</td>
<td></td>
<td>Bibb, coarse-loamy, siliceous, active, acid, thermic Typic Fluvaquents, 0–2% slope</td>
<td>46 to 102</td>
<td>731–1650</td>
<td>20970–48000</td>
</tr>
<tr>
<td>Forest riparian buffer downslope of residential areas</td>
<td>E 2</td>
<td></td>
<td>Autryville, loamy, siliceous, subactive, thermic Arenic Paleudults, 0–6% slope</td>
<td>61</td>
<td>879–2317</td>
<td>21653–50633</td>
</tr>
<tr>
<td>Forest/shrub riparian buffer downslope of a new swine wastewater spray field</td>
<td>F 3</td>
<td></td>
<td>Autryville, loamy, siliceous, subactive, thermic Arenic Paleudults, 0–6% slope</td>
<td>168 to 183</td>
<td>311–473</td>
<td>4899–12366</td>
</tr>
<tr>
<td>Grass riparian buffer downslope of cultivated fields</td>
<td>G 2</td>
<td></td>
<td>Autryville, loamy, siliceous, subactive, thermic Arenic Paleudults, 0–6% slope</td>
<td>97</td>
<td>493–905</td>
<td>13033–31650</td>
</tr>
<tr>
<td>In-stream wetlands below swine wastewater spray field</td>
<td>H 3</td>
<td></td>
<td>Bibb, coarse-loamy, siliceous, active, acid, thermic Typic Fluvaquents, 0–2% slope</td>
<td>46 to 137</td>
<td>692–1043</td>
<td>15012–26645</td>
</tr>
<tr>
<td>Grass riparian buffer downslope of a pasture</td>
<td>I 1</td>
<td></td>
<td>Autryville, loamy, siliceous, subactive, thermic Arenic Paleudults, 0–6% slope</td>
<td>61</td>
<td>1009</td>
<td>21625</td>
</tr>
<tr>
<td>Forest riparian buffer at outlet of watershed</td>
<td>J 2</td>
<td></td>
<td>Bibb, coarse-loamy, siliceous, active, acid, thermic Typic Fluvaquents, 0–2% slope</td>
<td>152</td>
<td>690–856</td>
<td>14467–19760</td>
</tr>
</tbody>
</table>

† 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.

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, we were able to determine the quantity of N₂O produced by 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 (iii and iv) provided sufficient glucose for energy and nitrate for electron acceptors to measure the potential denitrification (complete or incomplete).

soil sample was manually homogenized, placed in plastic bags, stored on ice, transported to the laboratory, and stored at 4°C.
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 N$_2$O concentration per unit of time.

The N$_2$O-N in the headspace gas was measured with a Model 3600 CX gas chromatograph (Varian, Palo Alto, CA) equipped with a 15-m C$^{60}$ 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 N$_2$O-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 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 N$_2$O-N kg$^{-1}$ soil h$^{-1}$ (Table 2). The high standard deviation (120 μg N$_2$O-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 N$_2$O-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 N$_2$O-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 N$_2$O-N kg$^{-1}$ soil h$^{-1}$. If the soils were amended with nitrate and glucose, the mean DEA was increased to 146 μg N$_2$O-N kg$^{-1}$ soil h$^{-1}$. If no acetylene was added the increase was 66 μg N$_2$O-N kg$^{-1}$ soil h$^{-1}$. This represented slightly more complete denitrification, 44%. The median value for DEA of the soils when nitrate and glucose were added was 51 μg N$_2$O-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 N$_2$O-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>
<thead>
<tr>
<th>Treatment</th>
<th>C$_2$H$_2$</th>
<th>Mean</th>
<th>Median</th>
<th>Std. dev.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control no</td>
<td>15</td>
<td>0</td>
<td>54</td>
<td></td>
</tr>
<tr>
<td>Control yes</td>
<td>59</td>
<td>23</td>
<td>120</td>
<td></td>
</tr>
<tr>
<td>N+C† no</td>
<td>66</td>
<td>1</td>
<td>261</td>
<td></td>
</tr>
<tr>
<td>N+C† yes</td>
<td>146</td>
<td>51</td>
<td>332</td>
<td></td>
</tr>
</tbody>
</table>

† Addition of nitrate and glucose.

Table 2. Mean and median denitrification enzyme activity of soil in a Coastal Plain watershed.
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 (LSD0.05). 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. This DEA was nearly 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.

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

<table>
<thead>
<tr>
<th>Site types descriptions</th>
<th>Control</th>
<th>NO₂ + C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ST‡</td>
<td>No C₂H₂</td>
</tr>
<tr>
<td></td>
<td>μg N₂O-N kg⁻¹ soil h⁻¹</td>
<td>μg N₂O-N kg⁻¹ soil h⁻¹</td>
</tr>
<tr>
<td>Restored riparian buffer downslope of a heavily loaded swine wastewater sprayfield</td>
<td>A</td>
<td>23</td>
</tr>
<tr>
<td>Forest/shrub riparian buffer across stream from a heavily loaded swine wastewater sprayfield</td>
<td>B</td>
<td>33</td>
</tr>
<tr>
<td>Marsh riparian buffer downslope of a cultivated field</td>
<td>C</td>
<td>24</td>
</tr>
<tr>
<td>Forest riparian buffer downslope of cultivate field</td>
<td>D</td>
<td>37</td>
</tr>
<tr>
<td>Forest riparian buffer downslope of residential area</td>
<td>E</td>
<td>11</td>
</tr>
<tr>
<td>Forest/shrub riparian buffer downslope of a new swine wastewater spray field</td>
<td>F</td>
<td>2</td>
</tr>
<tr>
<td>Grass riparian buffer downslope of cultivated fields</td>
<td>G</td>
<td>1</td>
</tr>
<tr>
<td>In-stream wetlands below swine wastewater spray field</td>
<td>H</td>
<td>1</td>
</tr>
<tr>
<td>Grass riparian buffer downslope of a pasture</td>
<td>I</td>
<td>1</td>
</tr>
<tr>
<td>Forest riparian buffer at outlet of watershed</td>
<td>J</td>
<td>1</td>
</tr>
<tr>
<td>LSD0.10‡</td>
<td>19</td>
<td>43</td>
</tr>
<tr>
<td>LSD0.05</td>
<td>23</td>
<td>51</td>
</tr>
</tbody>
</table>

† Site types (ST).
‡ LSD, least significant difference.
nitrates and glucose were added, 172 μg N₂O-N kg⁻¹ 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 μg N₂O-N kg⁻¹ 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 μg N₂O-N kg⁻¹ soil h⁻¹—84% incomplete denitrification. The soils of this site type had potential, incomplete denitrification and potential DEA values of 76 and 209 μg N₂O-N kg⁻¹ 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 μg N₂O-N kg⁻¹ soil h⁻¹. The nitrous oxide accumulated in the absence of acetylene was 11 μg N₂O-N kg⁻¹ 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 μg N₂O-N kg⁻¹ soil h⁻¹, and the accumulation of nitrous oxide in the absence of acetylene was 30 μg N₂O-N kg⁻¹ soil h⁻¹.

The remaining site types F through J were much lower in nitrous oxide accumulations in the absence of acetylene (≤2 μg N₂O-N kg⁻¹ soil h⁻¹). The DEA values were also generally lower (2 to 46 μg N₂O-N kg⁻¹ 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 ≤18 μg N₂O-N kg⁻¹ soil h⁻¹. The potential DEA was ≤65 μg N₂O-N kg⁻¹ 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.](image)

...ed DEA means for the surface, middle, and water table layers of 147, 83, and 67 μg N₂O-N kg⁻¹ 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 linked 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.](image)
Stepwise regression analysis for denitrification enzyme activity of soil in a Coastal Plain watershed.

<table>
<thead>
<tr>
<th>Treatment/regression variable</th>
<th>Partial $r^2$</th>
<th>Model $r^2$</th>
<th>C(p)</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.15</td>
<td>0.15</td>
<td>41</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Soil nitrogen</td>
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<td>0.23</td>
<td>2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Control, $\text{C}_2\text{H}_2$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soil nitrogen</td>
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<td>0.59</td>
<td>20</td>
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</tr>
<tr>
<td>Soil carbon</td>
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<td>0.62</td>
<td>4</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>$\text{NO}_3 + \text{glucose}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soil nitrogen</td>
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<td>0.40</td>
<td>30</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Soil carbon</td>
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<td>0.44</td>
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<td>&lt;0.0001</td>
</tr>
<tr>
<td>$\text{NO}_3 + \text{glucose}, \text{C}_2\text{H}_2$</td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>Soil nitrogen</td>
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<td>0.43</td>
<td>19</td>
<td>&lt;0.0001</td>
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<tr>
<td>Soil carbon</td>
<td>0.03</td>
<td>0.46</td>
<td>3</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

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 $\text{C}_2\text{H}_2$, 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 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 Klemedtsson 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 DEAs 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 μg N\(_2\)O-N kg\(^{-1}\) soil h\(^{-1}\). If no acetylene was added to block conversion of nitrous oxide to dinitrogen gas, only 15 μg N\(_2\)O-N kg\(^{-1}\) 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 μg N\(_2\)O-N kg\(^{-1}\) soil h\(^{-1}\)) and nitrous oxide accumulation in the absence of acetylene (23 μg N\(_2\)O-N kg\(^{-1}\) 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.

References


