Denitrification in Alluvial Wetlands in an Urban Landscape

Melanie D. Harrison,* Peter M. Groffman, Paul M. Mayer, Sujay S. Kaushal, and Tamara A. Newcomer

Riparian wetlands have been shown to be effective “sinks” for nitrate N (NO₃⁻), minimizing the downstream export of N to streams and coastal water bodies. However, the vast majority of riparian denitrification research has been in agricultural and forested watersheds, with relatively little work on riparian wetland function in urban watersheds. We investigated the variation and magnitude of denitrification in three constructed and two relict oxbow urban wetlands, and in two forested reference wetlands in the Baltimore metropolitan area. Denitrification rates in wetland sediments were measured with a ¹⁵N-enriched NO₃⁻ “push–pull” groundwater tracer method during the summer and winter of 2008. Mean denitrification rates did not differ among the wetland types and ranged from 147 ± 29 µg N kg soil⁻¹ d⁻¹ in constructed stormwater wetlands to 100 ± 11 µg N kg soil⁻¹ d⁻¹ in relict oxbows to 106 ± 32 µg N kg soil⁻¹ d⁻¹ in relict reference wetlands. High denitrification rates were observed in both summer and winter, suggesting that these wetlands are sinks for NO₃⁻ year round. Comparison of denitrification rates with NO₃⁻ standing stocks in the wetland water column and stream NO₃⁻ loads indicated that mass removal of NO₃⁻ in urban wetland sediments by denitrification could be substantial. Our results suggest that urban wetlands have the potential to reduce NO₃⁻ in urban landscapes and should be considered as a means to manage N in urban watersheds.

Despite long-term efforts to understand and reduce N delivery to coastal waters, there remains great uncertainty about mechanisms of N retention and removal in coastal watersheds (Boesch et al., 2001; Conley et al., 2009). Recently, resource managers have combined watershed restoration strategies with wetland creation as a best management practice (BMP) to reduce the effects of upland nutrient sources and leaky sewer systems in urban landscapes (Verhoeven et al., 2006; CPB 2008; Wenger et al., 2009; Collins et al., 2010). Historically, wetlands have provided important ecosystem services, including flood-storage, nutrient, and sediment attenuation, habitat for flora and fauna, biodiversity, and recreation (Kadlec and Knight, 1996; Mitsch and Gosselink, 2000). Due to their physical location between upland sources and adjacent streams, wetlands have the potential to reduce the magnitude of increased nutrient loading by intercepting inorganic N and transforming it into less bioavailable forms (Valiela et al., 2002).

Permanent removal of N in wetlands occurs primarily through denitrification, an anaerobic microbial process that reduces nitrate N (NO₃⁻) and nitrite (NO₂⁻), to the gases nitric oxide (NO), nitrous oxide (N₂O), and dinitrogen (N₂), resulting in permanent NO₃⁻ loss from the ecosystem (Paul, 2007). There is great interest in the relative production of N₂ and N₂O during this process as N₂O is a greenhouse gas that also contributes to the destruction of stratospheric ozone (Khalil and Rasmussen, 1983).

Generally, wetland soils offer favorable conditions for denitrification due to an abundant organic C supply and saturated conditions that create anaerobic environments (Bowden et al., 1987; Bastviken et al., 2003; Xue et al., 1999; Whitmire and Hamilton, 2005; Burgin and Hamilton, 2007). Denitrification also depends on the presence of NO₃⁻, which may be produced endogenously by nitrifying bacteria or introduced exogenously via surface water runoff and/or groundwater discharge. In the latter case, the physical flux of N into wetland sediments directly stimulates denitrification. Wetlands can have particularly high denitrification potential and thereby function as denitrification hotspots (McClain et al., 2009; Collins et al., 2010).
2003) when aerobic and anaerobic zones coexist in the soil profile, allowing coupled nitrification/denitrification to occur (McClain et al., 2003; Vidon et al., 2010) or when they receive high NO$_3^-$ inputs from agricultural drainage and runoff, or septic system inputs (Groffman et al., 1993; Haycock and Pinay, 1993; McClain et al., 2003; Vidon et al., 2010). Urban riparian wetlands receiving surface runoff or groundwater discharge with high NO$_3^-$ concentrations could thus possibly function as important year-round NO$_3^-$ sinks in urban watersheds (Groffman and Crawford, 2003; Mayer et al., 2007).

Methods used to evaluate or estimate N removal via denitrification in wetlands are problematic and often involve the use of inhibitors, artificially elevated substrate concentrations, and/or physically altered conditions (Groffman et al., 2006). However, relatively new in situ “push–pull” methods allow for measurement of wetland denitrification under more realistic conditions and for evaluation of the ability of different features to affect N flows in the landscape (Istok et al., 1997; Addy et al., 2005; Whitmire and Hamilton, 2005; Kaushal et al., 2008b).

While urban stormwater management is designed to move water away from human settlements and urbanization results in the loss of many natural wetlands, other wetlands are often created coincidentally by the urbanization process. For example, small isolated depressions form as a result of stormwater outfall discharge; floodplain seeps occur as erodible soils and fractures form in degraded stream channels; and, relict stream side channels form as an unintended consequence of geomorphic stream restoration and/or construction of designer ecosystems (Roach et al., 2008). Almost nothing is known about how these coincidental urban wetlands function, but there is great interest in determining if they can function as nutrient sinks and how this function can be enhanced through management and/or restoration activities (Craig et al., 2008).

More recently, there has been interest in the deliberate creation of urban wetlands to address N problems in coastal cities (Mitsch et al., 2005; Mitsch and Day, 2006; Craig et al., 2008). The evaluation of wetland function for N removal is particularly important in the Chesapeake Bay Watershed, where rapid expansion of urban, suburban, and exurban land (Jantz et al., 2005) has coincided with increases in eutrophication, hypoxia, and harmful algal blooms in coastal waters (Howarth et al., 2002; Kemp et al., 2005; Kaushal et al., 2008a). Resource managers in this region are interested in the effectiveness of wetlands in urban landscapes to function as N sinks, particularly as pressure to reduce N delivery to the Chesapeake Bay increases with the implementation of new total maximum daily load (TMDL) and stormwater regulations (Boesch et al., 2001; Roberts et al., 2009).

The objective of this study was to evaluate sediment denitrification rates in five urban and two seminatural (hereafter referred to as forested) wetland sediments in the Baltimore Metropolitan region. We quantified in situ rates of N$_2$O and N$_2$ production by denitrification using a $^{15}$N-enriched NO$_3^-$-based push–pull method in both winter and summer. We also characterized physicochemical variables known to influence rates of denitrification (NO$_3^-$ concentrations, dissolved oxygen [DO], dissolved organic C [DOC] levels, and temperature). For two sites, we calculated the amount of NO$_3^-$ removed on an areal basis to evaluate the importance of denitrification relative to the standing stock of NO$_3^-$ in the water column of the wetlands and the load of NO$_3^-$ in the adjacent stream. We hypothesized that urban wetlands have the potential to promote substantial denitrification year round and that the highest rates of denitrification would be observed in wetlands with the highest NO$_3^-$-N concentrations.

**Materials and Methods**

**Site Descriptions**

We evaluated in situ denitrification rates in three types of floodplain wetlands located in the Maryland Piedmont Region (Fig. 1a). We studied two relict oxbow wetlands adjacent to Minebank Run, an urban restored stream that drains an 8.46 km$^2$ subwatershed of the Gunpowder Watershed located in Baltimore County, MD (39°49’29” N, 76°49’58” W) (Fig. 1c). Suburban development in the watershed led to high flows that created substantial erosive forces in the stream channel leading to severe degradation of the stream banks, exposure of sewer lines, and fractures within the riparian zone (Doheny et al., 2006). The study reach was restored in 2005 to improve geomorphic stability and reduce stream channel incision. Restoration techniques included reshaping the slope to reconnect the channel to the floodplain, lowering of the banks to promote overbank flooding, installing engineered pool-riffle sequences in channel, armor ing of the banks with boulders to increase the stability of the stream channel, and revegetation of the riparian zone.

During the reconstruction phase, stone boulders were placed in channel to create a berm to stop erosion of the degraded stream bank and stabilize the stream channel. As a result, two barriers were created between existing meander bends and the adjacent stream channel forming the relict oxbow floodplain wetlands. Thus, the oxbow wetlands occur in the riparian floodplain in association with the stream channel. The oxbows are hydraulically connected to the adjacent stream channel via overbank flow from the channel or subsurface flow. Additional water sources to the oxbows include interflow and return flow from adjacent uplands, occasional overland flow, as well as direct inputs from precipitation.

The downstream oxbow (OX1) is a 412 m$^2$ wetland that receives overbank flow when the water level in the stream channel rises above the 1.5 m berm (1.5 m is the threshold elevation to initiate the exchange of water between the oxbow and stream) (Fig. 2a). A water-level elevation of 1.5 m is equivalent to a peak discharge of 2.8 m$^3$·s$^{-1}$ (based on a stage-discharge relationship developed from a rating curve for water year 2008 by USGS for stream gauge 0158397967, Minebank Run near Glen Arms, MD). A storm of this magnitude occurs twice in any given year (Table 1). There is also continuous seepage into the wetland through subsurface flow from the adjacent stream channel as the water table slopes from the stream to the oxbow over a distance of ~1.5 m (Fig. 2a).

The upstream oxbow (OX2) is a 296 m$^2$ wetland that receives input from surface water flow more frequently than OX1, due to a low-gradient open connection with the adjacent stream channel (Fig. 2b). Water flows passively from the stream channel to the inlet of the oxbow when the water level in the stream is >0.67 m. Peak discharge required to initiate overbank flooding ranges from 0.1 to 0.14 m$^3$·s$^{-1}$. Oxbow 2 may also occasionally receive inflow from a nearby (<10 m) upstream tributary.
Continuous water-level measurements taken from April through July 2008 indicate that while both sites were continuously inundated, OX1 received overbank flooding just once, whereas OX2 received floodwaters ~12 different times. Discharge during this time period ranged from 0.4 to 3.8 m$^3$ s$^{-1}$. Therefore, compared with OX1, OX2 has a greater extent of connectivity with the adjacent stream reach over a larger range of moderate to high flow conditions (Table 1), thus capturing a greater percentage of the total annual NO$_3^-$ flux transported downstream during storm events.

The oxbows contain a mix of fine sediments, silt, and sand, and support emergent vegetation, mainly cattails (Typha L.), sawgrasses (Cladium P. Br.), common reed (Phragmites australis), and skunk cabbage (Symplocarpus foetidus L.). Common duckweed (Lemma minor L.) and filamentous algae (cyanobacteria) cover most of the wetlands during the summer months. Algae were abundant and dense in OX1 but sparse in OX2. The wetlands also collected leaves and therefore received organic C inputs from riparian trees.

Three additional urban wetland sites were located adjacent to Stony Run, an urban restored (2006) stream located in north central Baltimore, MD (39°12′44″ N, 76°22′29″ W), which is a tributary to the Jones Falls Watershed (Fig. 1d). Land use in the 9.06 km$^2$ watershed was 85% commercial/residential, with the remaining 15% classified as open space or forest. Before restoration, high storm flows in the watershed created lateral and vertical instability of the stream channel causing severe bank erosion and channel incision. We evaluated three-constructed stormwater wetlands (CW) located in the Upper Stony Run subregion that were created to intercept stormwater runoff conveyed directly from discharge outfall pipes. Occasionally, the wetlands received inputs of overbank flow during storm events. Organic C inputs were primarily from cattails, sawgrasses, and common reed, and some mixed riparian vegetation planted during the restoration phase. These wetlands contain a mixture of fine sediments and silt, as well as unconsolidated layered gray clay material. There were minimal C inputs from leaf litter due to tree removal during the early stages of restoration. The wetlands were designed to receive stormwater runoff, which can contain a variety of C sources, including leaf litter from urban trees and sewage from leaking pipes.

Two forested reference wetland sites were located adjacent to Baisman Run, a second order stream draining a 3.80 km$^2$ watershed located in Baltimore County, MD (39°17′3″ N, 76°24′19″ W).
W) (Fig. 1b). The headwaters of Baisman Run are 80% forested and include some residential land use with septic sanitary infrastructure (no sewers). We studied two forested floodplain wetlands (sloughs) at this site that were created when in-stream flows cut across or behind point bars in the stream channel, or when obstructions in the stream channel (possibly large woody debris) caused flows to be rerouted onto the adjacent floodplain. These are ephemeral features (i.e., there is little to no standing water in the wetlands during the summer months) that received inputs of water primarily during snowmelt periods and storm events. These systems were occasionally flooded but otherwise dry for varying portions of the growing season. Carbon inputs were from a dense riparian canopy and the dominant emergent vegetation was skunk cabbage.

Analytical Methods

Monthly groundwater, surface water, and stream water samples were collected during base flow at each study site from January 2008 to June 2009. Groundwater samples were collected from mini piezometers installed to 0.5 m depth below the surface of the sediment using a Master flex L/S portable peristaltic pump (Cole Parmer, Vernon Hills, IL). Samples were collected in 125-ml high-density polyethylene bottles (Fisher Scientific, Pittsburgh, PA) and were analyzed for ammonium (NH$_4^+$), NO$_3^–$-total N, total P, DO, DOC, temperature, and conductivity (Table 2). All samples were placed on ice and transported to the laboratory and filtered using Whatman 45-μm glass fiber filters GF/F (0.7 nominal pore size) (Fisher Scientific). Surface and groundwater temperature, and DO were measured using a YSI Model 55 DO/temperature meter (YSI, Yellow Springs, OH). Concentrations of NO$_3^–$ were analyzed on a Dionex LC20 series ion chromatograph (Dionex, Sunnyvale, CA). Total N and P were analyzed by persulfate digestion followed by analysis of NO$_3^–$ and phosphate on a Lachat Quikchem 8100 flow injection analyzer (Lachat Instruments, Loveland, CO). Dissolved organic C (mg L$^{-1}$) was measured by infrared analysis using an O.I. Corporation Model 1010 carbon analyzer (College Station, TX).

In situ Denitrification

Twenty-two minipiezometers (AMS, 300 series, American Falls, ID), consisting of small steel-probe well points (slot zone ~0.64 cm, slot hole 0.05 cm for groundwater entry) attached to gas permeable Teflon tubing and fitted with a fluoropolymer umbrella and #50 mesh screen (equivalent to 297 μm, 1.27 cm diam., depth 2.2 cm) to keep out fine-grain sediment during groundwater sampling, were installed with a retractable groundwater sampler to a depth of 0.5 m below the wetland sediment surface in late fall 2007, early spring 2008, and early summer 2008. Piezometers were sealed at the surface with plastic caps to prevent surface water intrusion and contamination. Minipiezometers were positioned approximately 6 m apart along observed surface water flow paths at each wetland site. Two minipiezometers were installed at the inflow and outflow of the

Table 1. Hydraulic characteristics of each oxbow wetland relative to the Minebank Run stream reach from April through July 2008, indicating the extent of connectivity with the adjacent stream reach.

<table>
<thead>
<tr>
<th></th>
<th>Oxbow 1</th>
<th>Oxbow 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Threshold elevation (TF)</td>
<td>1.55 m</td>
<td>0.67 m</td>
</tr>
<tr>
<td>Distance from gauge</td>
<td>91 m (downstream)</td>
<td>30 m (upstream)</td>
</tr>
<tr>
<td>Discharge required for exchange†</td>
<td>~2.8 m$^3$ s$^{-1}$</td>
<td>~0.12 m$^3$ s$^{-1}$</td>
</tr>
<tr>
<td>Recurrence interval for exchange‡</td>
<td>twice a year</td>
<td>&gt;8 times a year</td>
</tr>
<tr>
<td>Exceedance probability for exchange§</td>
<td>~0.26 (%)</td>
<td>~9 (%)</td>
</tr>
</tbody>
</table>

† Rating curve to determine stage–discharge relationship taken from USGS stream gauge # 0158397967, Minebank Run near Glen Arms, MD, water year (WY) 2008.
‡ Estimated recurrence interval (yr) calculated from available peak discharge values for WY 2002–2008 (n = 8).
§ Estimated exceedance probability calculated from 5-min instantaneous discharge values for WY 2008.
Baisman Run and Stony Run study wetlands. There were seven minipiezometers in OX2 and five at OX1 at Minebank Run.

In situ rates of denitrification were measured in summer (June through August) and winter (late November through December) 2008 using a push–pull method, where a single minipiezometer was used for both dosing and sampling (Istok et al., 1997; Addy et al., 2002). We conducted, on average, two push–pull experiments per day, i.e., one individual denitrification rate per piezometer on a single date during summer and winter (44 push–pull experiments total). Before each push–pull on the day of the experiment, the dosing solution was prepared (20 atom percent $^{15}$N-enriched KNO$_3$, 32 mg N L$^{-1}$) in the laboratory and 10 L of ambient groundwater pulled from each minipiezometer and collected in 15 L carboys. When pretested piezometer groundwater extraction rates were slow, i.e., <1 L h$^{-1}$, the ambient groundwater was collected in 15 L carboys. When pretested piezometer groundwater was collected ~24 to 48 h before the experiment and stored at 4°C until the push–pull experiment. Each carboy was sealed tightly during storage to minimize changes in ambient groundwater concentrations of chloride and bromide are observed, as in the plant uptake, and is recommended in areas where high ambient concentrations of chloride and bromide are observed, as in urban wetlands in this study.

The amended solution was adjusted to ambient groundwater DO concentrations by bubbling sulfur hexafluoride (SF$_6$, 100 μg L$^{-1}$, balance in He) (Matheson Trigas, Gloucester, MA) through the solution (20–25 min). SF$_6$ also served as a conservative tracer to account for dilution, dispersion, and degassing, and to provide information about groundwater residence time in each well. Ambient DO and temperature were measured in groundwater before SF6 bubbling and during bubbling by placing a DO probe and sparge stone into the carboy with the dosing solution and sealing the carboy air tight. After bubbling, the 10-L groundwater-amended solution was “pushed” (i.e., injected) into the same minipiezometer from which it was originally taken and then “pulled” (i.e., extracted) again after incubation for 4 to 5 h. On average, the amended solution was pushed at a rate of 16 L h$^{-1}$ and pulled at a rate of 6 L h$^{-1}$. Several site factors, including hydraulic conductivity (K) and hydraulic gradient, can affect push–pull rates (Addy et al., 2002). In our study sites, soil type varied from a mix of very fine sediment, sand, and silt in the forest and oxbow wetlands, to a mix of fine sediment and silt, as well as unconsolidated gray clay material in the CWs. Although we did not measure the hydraulic conductivity (K) of soils in our study sites, K values mostly likely ranged from 10$^{-3}$ to 10$^{-6}$ cm s$^{-1}$ in forest and oxbow wetland sediments, and from 10$^{-4}$ to 10$^{-10}$ cm s$^{-1}$ in the CW sediments (Bear, 1972).

To minimize the effects of dilution and dispersion when estimating denitrification, rates were estimated from only the “core” of the plume (i.e., the first 3 L pulled from the minipiezometer after the incubation period). This portion of the plume consistently exhibits the highest conservative tracer recovery rate (Addy et al., 2002).

Many studies have used bromide rather than SF$_6$ as the conservative tracer in NO$_3^-$ enrichment studies (Nelson et al., 1995; Starr and Gillham, 1993). However, bromide is subject to uptake by plants, a biological process that may affect concentration values. SF$_6$ is a gaseous tracer that has been shown to behave conservatively in coastal sediments (Wilson and Mackay, 1996) and riparian aquifers (Addy et al., 2005), is not subject to plant uptake, and is recommended in areas where high ambient concentrations of chloride and bromide are observed, as in urban wetlands in this study.

**Gas Extraction from Groundwater and Conservative Tracer Recovery Estimates**

The N$_2$, N$_2$O, and SF$_6$ gases were extracted from ambient, pushed, and pulled samples, using a phase equilibrium headspace extraction technique (Lemon and Lemon, 1981; Davidson and Firestone, 1988). Groundwater samples were collected for N$_2$, N$_2$O, $^{15}$N$_2$, $^{15}$N$_2$O, and SF6 analysis with an air-tight sampling apparatus made of IV tubing connected to a peristaltic pump. Next, samples were injected by syringe into an evacuated serum bottle and the headspace was filled with high-purity He gas. After incubating overnight at 4°C and shaking, the bottle

<table>
<thead>
<tr>
<th>Parameter†</th>
<th>Minebank Run</th>
<th>Stony Run</th>
<th>Baisman Run</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Oxbow 1</td>
<td>Oxbow 2</td>
<td>CW1</td>
</tr>
<tr>
<td></td>
<td>GW</td>
<td>SW</td>
<td>GW</td>
</tr>
<tr>
<td>NH$_4^+$ (mg N L$^{-1}$)</td>
<td>0.06</td>
<td>0.12</td>
<td>0.39</td>
</tr>
<tr>
<td>NO$_3^-$ (mg N L$^{-1}$)</td>
<td>1.3</td>
<td>0.4</td>
<td>1.0</td>
</tr>
<tr>
<td>TN (mg N L$^{-1}$)</td>
<td>1.4</td>
<td>0.5</td>
<td>1.3</td>
</tr>
<tr>
<td>TP (μg P L$^{-1}$)</td>
<td>13.0</td>
<td>4.0</td>
<td>10.0</td>
</tr>
<tr>
<td>DOC (mg L$^{-1}$)</td>
<td>0.62</td>
<td>0.75</td>
<td>1.2</td>
</tr>
<tr>
<td>DO (mg L$^{-1}$)</td>
<td>0.92</td>
<td>8.0</td>
<td>1.8</td>
</tr>
<tr>
<td>Temp. (°C)</td>
<td>18.6</td>
<td>19.6</td>
<td>20.83</td>
</tr>
<tr>
<td>Cond. (μs cm$^{-1}$)</td>
<td>980</td>
<td>960</td>
<td>1100</td>
</tr>
<tr>
<td>Depth (cm)</td>
<td>≈</td>
<td>16</td>
<td>≈</td>
</tr>
</tbody>
</table>

† NH$_4^+$, ammonium; NO$_3^-$, nitrate; TN, total nitrogen; TP, total phosphorus; DOC, dissolved organic carbon; DO, dissolved oxygen; Temp, temperature; Cond., conductivity; Depth, surface water level of the overlying water column in the wetlands the month of September 2008.

‡ Zero indicates no measurement of GW depth.

§ — indicates feature was dry during the sampling period.
headspace was sampled to extract SF6 and gas (N2 and N2O) produced by denitrifying microbes. Samples were sent to the stable isotope laboratory at the University of California at Davis for N2 and 15N:14N ratio analysis by mass spectrometry using a Europa Integra continuous flow isotope ratio mass spectrometer (Europa Scientific, Seron, Cheshire, UK) coupled to an in-line elemental analyzer. Concentrations of N2O and SF6 were analyzed by electron-capture gas chromatography on a Shimadzu GC 14 gas analyzer. Concentrations of N2O and SF6 were analyzed by the mean in situ denitrification rate (pooled across seasons and sites) and the average NO3− concentration of the overlying water column in OX1 and OX2, during the time of the study, ~0.2 mg N L−1 and 0.3 mg N L−1, respectively (n = 12). Daily average removal estimates were also compared with estimates of NO3− export/load in the Minebank Run stream reach adjacent to the wetlands, which was calculated by multiplying the average discharge (129,000 L d−1) for water year 2008 (n = 11) by the average NO3− stream concentration (0.93 mg N L−1) sampled during April, July, and October 2008 (n = 18).

Statistical Analysis

We evaluated differences in NO3− concentrations and denitrification rates across wetland type (OX, CW, and forested [FW]), site (total of seven wetlands), and season (summer and winter), and their interactions using general linear models for unbalanced analysis of variance (ANOVA) followed by the Tukey-Kramer post hoc analysis to examine differences among site least squared means (n = 37). Separate one-way ANOVAs were run to compare the variation in denitrification and NO3− concentrations among sites (total of seven sites). Regression analyses were used to examine relationships between denitrification, NO3−, temperature, and DO on pooled data across all sites. To examine the spatial variation in denitrification rates, the effect of distance (minipiezometer location) from the inlet was compared among the Minebank Run oxbow wetlands. Differences were accepted as significant at the α = 0.05 level. All statistical analyses were performed in SAS (SAS Institute, 2004).

Results

Average groundwater NO3− concentrations in the wetlands ranged from undetectable to 11 mg L−1 (Fig. 3). The highest average groundwater NO3− concentrations were observed in the Stony Run CWs (3.1 ± 5.9 mg L−1), followed by the Minebank Run oxbows (1.6 ± 1.3 mg L−1) and Baisman Run FWs (1.3 ± 1.8 mg L−1). However, these differences were not significant (F = 1.2, P = 0.31) (mean ± SD). The main effect of site was significant in the model (F = 8.0, P = 0.002) and comparisons of means by site revealed that the second CW at Stony Run (CW2) had significantly higher groundwater NO3− concentrations than all other wetlands (F = 7.0, P < 0.0001) (Fig. 3). Groundwater NO3− concentrations were generally higher in the winter compared with summer (2.4 ± 4.4 and 1.4 ± 2.5 mg L−1, respectively), but the differences were not statistically significant (F = 0.62, P = 0.43). Regression analysis showed no significant relationship between groundwater NO3− in the wetland and distance from the wetland inlet (P = 0.2), and there was no significant relationship between NO3− concentrations and denitrification (F = 2.55, P = 0.11) when data were pooled across all study sites (Fig. 4).

Ambient groundwater DO and temperature ranged from 0.2 to 4.7 mg L−1 and 6.1 to 27.5°C, respectively, and showed no significant relationship with denitrification rates (Fig. 4). We observed minimal changes in ambient groundwater DO and temperature during in situ push–pull experiments (data...
not shown). For instance, during winter push–pull experiments the difference between mean ambient groundwater DO before the injection and DO concentrations after bubbling in OX1 and OX2 were 0.36 ± 0.28 and 0.10 ± 0.04 (mean difference ± SD), respectively.

Recovery of Conservative Tracer SF6
Tracer recovery in the plume of all the minipiezometers for all sites exceeded 70%, with the exception of OX2 during the winter, which had a tracer recovery of 60% (Fig. 5), indicating minimal loss due to physical processes. The SF$_6$ concentrations dropped steadily after the first 1.5 L extracted. The high recovery rates for each sampling period at all sites demonstrate that the portion of the plume analyzed was not significantly altered by physical processes (Addy et al., 2005). The high plume recovery suggests that the push–pull method should be effective in determining denitrification rates across all sites.

Denitrification Rates
Denitrification rates ranged from <0.1 to 193 μg N kg soil$^{-1}$ d$^{-1}$. The highest denitrification rates were observed in the CWs (147 ± 29 μg N kg soil$^{-1}$ d$^{-1}$) compared with the urban oxbow wetlands (100 ± 11 μg N kg soil$^{-1}$ d$^{-1}$) and the forested wetlands (106 ± 32 μg N kg soil$^{-1}$ d$^{-1}$) (mean ± SE). However, these differences were not significant by type ($F = 0.25, P = 0.77$) (Fig. 6) or season ($F = 0.35, P = 0.55$). Comparison of means by site showed that the OX2 wetland had significantly ($P < 0.05$) higher denitrification rates in winter than in summer, whereas the CW2 had higher ($P < 0.05$) denitrification rates in summer than winter (Fig. 6). Across all wetland sites, the CW2 wetland had highest ($P < 0.05$) denitrification rates.

In situ denitrification rates varied spatially throughout the oxbow wetlands located at Minebank Run from <0.1 to 100.9 μg N kg soil$^{-1}$ d$^{-1}$ (Fig. 7). There was no significant pattern in denitrification rates along surface water flow paths ($F = 4.33, P = 0.092$).

Dinitrogen production was the dominant denitrification end product at all sites (Fig. 8a), ranging from 0.27 to 1.17 μg. The highest individual mass of N$_2$O was observed in the winter at BR–FW1 and OX2 (0.1 and 0.2 μg, respectively). When ratios were averaged across all wetland types, the forested reference wetlands had the highest N$_2$O:N$_2$ ratio (0.17 ± 0.04) compared with the relict oxbow (0.02 ± 0.002) and constructed wetlands (0.11 ± 0.05) (mean ± SD) (Fig. 8b). The N$_2$O yields (N$_2$O/N$_2$O+N$_2$) ranged from 0.020 to 0.205 and averaged 0.102.

Scaling Up: Mass NO$_3^-$ Removal
The mass of N removed by sediment denitrification ranged from to 24 to 43 mg N m$^{-2}$ d$^{-1}$ in OX1 and OX2, respectively (Table 3). This flux was equal to 23% of the standing stock of NO$_3^-$ in the water column of OX1 and 28% of the stock in OX2. The OX1 was denitrifying an amount of N equivalent to 8.3% of the average daily load of NO$_3^-$ transported by Minebank Run and OX2 was denitrifying an equivalent of 11% of the daily load.

Discussion
In situ Denitrification Rates among Wetlands
Our results clearly show that urban wetlands have the potential to function as denitrification sinks for NO$_3^-$ . Denitrification rates (<0.1 to 193.2 μg N kg$^{-1}$ d$^{-1}$) at our sites are similar to those reported for both urban and nonurban sites (Addy et al., 2002, 2005; Kellogg et al., 2005; Whitmire and Hamilton, 2005; Watson et al., 2010; Kaushal et al., 2008b). The rates here were similar to previous denitrification measurements in restored and unrestored stream banks at Minebank Run (<1 to 262 μg N kg$^{-1}$ d$^{-1}$) (Kaushal et al., 2008b) that were determined to be significant relative to stream water NO$_3^-$ loads. In situ stream $^{15}$N studies have also suggested that denitrification is a significant sink for N in Minebank Run (Klocke et al., 2009).

The lack of difference between summer and winter denitrification rates suggests that these urban and forested wetlands are sinks for NO$_3^-$ year round. Created oxbow wetlands have been shown to be net sinks for N and P on an annual basis (Fink and Mitsch, 2007), and riparian wetlands have demonstrated year-round NO$_3^-$ removal, even in cold climates (Phipps and Crompton, 1994; Hosomi et al., 1994). Our results were unexpected because denitrification is strongly influenced by temperature in wetland sediments (Kadlec and Reddy, 2001). However, winter temperatures in Baltimore are high enough (Brazel et al., 2000) to allow biogeochemical processes such as denitrification to occur (Kaushal et al., 2010). Moreover, the factors that regulate denitrification, such as the availability of NO$_3^-$ and C, are often higher during the nongrowing season due to inputs from leaf litter during autumn and a lack of plant assimilation of NO$_3^-$ during the dormant season. There is great interest in winter denitrification, because in urban (and many
other) watersheds, the majority of total N and NO$_3^-$ export occurs during the dormant season (Shields et al., 2008).

Because the addition of NO$_3^-$ may stimulate denitrification in low NO$_3^-$ systems and denitrification rates in this study were quantified under high NO$_3^-$ enrichment (32 mg N L$^{-1}$), they may be considered to represent potential, rather than in situ, rates. However, all of our study sites are exposed to relatively high NO$_3^-$ concentrations (>1 mg N L$^{-1}$) such that denitrification is likely zero order with respect to NO$_3^-$ in many cases (Myrold and Tiedje 1985). Thus, our reported rates are likely more similar to in situ than potential rates. And more fundamentally, the fact that the sediments denitrified at high NO$_3^-$ concentrations supports our conclusion that these urban wetlands have the potential to function as significant denitrification sinks for anthropogenic NO$_3^-$.

We expected low in situ denitrification rates in the forested reference wetlands compared with urban wetlands, as we expected the reference sites to have low NO$_3^-$ concentrations. However, these sites had similar NO$_3^-$ concentrations and denitrification rates as the urban sites—likely due to high NO$_3^-$ inputs from septic systems in the headwaters of the Baisman Run Watershed (Groffman et al., 2004; Kaushal et al., 2008b; Shields et al., 2008). Because our forest sites received anthropogenic inputs of N, our comparison of wetland types may be more accurately described as urban versus seminatural wetlands in terms of N inputs. Still, these sites were not physically disturbed and were dominated by intact, mature forest, whereas the urban sites had highly altered physical, chemical, and biological conditions. The forested wetlands are thus optimal environments for denitrification as high inputs of leaf litter provide a rich organic C substrate that drives denitrification of the N supplied by the stream/septic systems. Hydrologic connectivity between these wetlands and the stream could be important to reducing NO$_3^-$ outputs from this watershed as in-stream retention of N has been found to be minimal (Claessens et al., 2009), whereas previous work has suggested that in situ denitrification rates may be relatively high in the Minebank Run stream during baseflow conditions (Klocker et al., 2009).

The CW2 located at Stony Run had the highest NO$_3^-$ concentrations and highest denitrification rates. Our results are somewhat puzzling in that if NO$_3^-$ was a strong driver of denitrification in our systems, we might expect to see a strong positive correlation between denitrification rates and NO$_3^-$ concentrations. One explanation for the lack of correlation may be that we are comparing ambient groundwater NO$_3^-$ concentrations with denitrification rates measured under high NO$_3^-$ enrichment concentrations (32 mg N L$^{-1}$), which may obscure ambient relationships. Another explanation may be that denitrification is controlled more strongly by NO$_3^-$ supply from the overlying water than by the small and transient sediment NO$_3^-$ pool.

In highly urban settings, such as Stony Run, the sources of N delivery to wetlands vary greatly and often depend on the wetlands' location in the landscape, i.e., close proximity to adjacent leaking sewer pipes and/or direct connection to discharge outfall pipes conveying stormwater runoff. Although high denitrification rates suggest that the CWs at Stony Run have the potential to be significant sinks for N in the urban environment, a more detailed characterization of the hydrology of these systems is necessary to determine if these sites remove significant amounts of N relative to stormwater inputs and/or stream N loads.

Variability in C inputs may be driving at least some of the variability in denitrification in the Stony Run wetlands. Allochthonous inputs from leaf litter along the stream that reach adjacent to these wetlands may be reduced due to tree removal during the restoration phase. However, even though these wetlands are <5 yr old, riparian and emergent vegetation is well established and provides adequate C substrate for
denitrifying microbes (Gift et al., 2010). In urban landscapes, other upstream sources of C and N to CW may be important. Even though many urban streams are buried or channelized (Elmore and Kaushal, 2008), they transport leaf litter from trees and sewage from leaking pipes throughout the watershed to riparian wetlands. The substrate used to create the wetlands may also have contained some organic C. Still, these results

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**Fig. 5.** Example relative concentration profiles of the conservative tracer SF$_6$ from (a) oxbow 1 and (b) oxbow 2 during summer and winter 4-h push–pull incubations at the Minebank Run study site. Rectangle = the core of the introduced plume (e.g., the first two pulled samples collected) after the 4-hr incubation period was complete.

**Fig. 6.** Summer and winter in situ denitrification rates for each study wetland site: MBR = Minebank Run, SR = Stony Run, and BR = Baisman Run wetlands. Values are mean ± standard error of minipiezometers sampled in each site between June 2008 and December 2008. Comparison of means by site showed that the oxbow 2 wetland had significantly ($P < 0.05$) higher denitrification rates in winter than in summer, whereas the constructed stormwater wetlands (CW) 2 had higher ($P < 0.05$) denitrification rates in summer than winter (Fig. 8). Across all wetland sites, the CW2 wetland had highest ($P < 0.05$) denitrification rates.
are somewhat surprising (i.e., the high denitrification rates) because denitrification in restored/created wetlands is often limited by organic C (Duncan and Groffman, 1994; Hunter and Faulkner, 2001; Ullah and Faulkner, 2006), particularly in the early phases of restoration. Moreover, previous wetland restoration studies have found that the establishment of organic matter levels similar to natural wetlands in these systems can take several decades (Craft et al., 2003; Kasahara and Hill, 2007). Although we could not determine whether or not C was a factor limiting denitrification rates, the results presented here reveal that there is enough available organic matter present in these systems to sustain denitrification year round.

We observed high spatial variability in denitrification rates in the Minebank Run oxbows and no trend in denitrification rates with distance from inlet. Denitrification rates can decrease along flowpaths as NO₃⁻ and C that enter at the wetland inlet can be consumed, leading to lower rates. Residence time is thus a key controller of wetland denitrification efficiency (Tomaszek et al., 1997; Poe et al., 2003). In our sites, spatial variability in denitrification rates is likely a product of variation in conditions in the immediate vicinity of our sampling points (Kjellin et al., 2007; Kaushal et al., 2008b). Key factors that could have varied include the patchiness of particulate organic material in the sediments (Parkin, 1987), vegetation density (Bachand and Horne, 2000), and the nature and extent of the aerobic/anaerobic interface in the sediments (Reddy and Patrick, 1984).

**Mass Removal of Nitrate in Oxbow ‘Relict’ Wetlands**

Comparison of measured denitrification rates with the standing stock of NO₃⁻ suggests that mass removal of NO₃⁻ in urban wetland sediments could be substantial and that denitrification underlies the NO₃⁻ sink function of these wetlands (Table 3). The sediment denitrification rates that we measured could remove between 23 and 28% of the NO₃⁻ standing stock in the overlying water column of the Minebank Run oxbow wetlands (Table 3). Thus, a residence time of ~4 to 3.5 d would result in complete removal of any NO₃⁻ that enters these wetlands.

Daily sediment denitrification in the oxbow wetlands was also significant relative to the load of N being transported by Minebank Run, i.e., an amount of N equal to 8 to 11% of the daily stream load was denitrified in each of the oxbow wetlands. The idea that oxbow denitrification is consuming significant amounts of streamwater N is also supported by the fact that NO₃⁻ concentrations in surface water in the oxbows are much lower than concentrations in the stream.

Given that we do not have detailed data on the nature and extent of hydraulic connectivity between the wetlands and the adjacent stream channel (discussed below), our calculations may overstate the importance of denitrification as we compared our areal denitrification mass removal with the average annual daily stream N export, while the majority of export occurs during storm events (Shields et al., 2008). Furthermore, we may have somewhat overestimated denitrification rates by using high NO₃⁻ concentrations during our push–pull incubations (discussed above).

A wetlands’ denitrifying efficiency depends both on its denitrification capacity and water residence time, i.e., long residence time fosters greater interaction between NO₃⁻-rich water and denitrifying sediments (Xue et al., 1999; Spieles and Mitsch, 2000). Our results support the idea that urban wetlands that are hydrologically connected to the adjacent stream, whether through groundwater or surface water flows, have the potential to remove a substantial amount of N. This potential will only be realized, however, if residence time is long enough to allow denitrification to occur. Successful (in terms of the retention of nutrients, such as N and P) constructed wetlands have often featured an oxbow design that increases water residence time in the wetland (Fink and Mitsch, 2007). Earlier studies at Minebank Run (Kaushal et al., 2008b) showed that hydrologic connectivity and residence time influenced denitrification and mass removal of N and that groundwater–surface water hydrology controlled nutrient patterns, including C and N, in this stream (Mayer et al., 2010). Residence times were highly variable, seasonally controlled, and influenced by drought (Striz and Mayer, 2008).

There was considerable variability in hydrologic connectivity between the two oxbow wetlands in this study. In OX1, there was continual subsurface flow from the stream channel, whereas inputs from overbank flow and precipitation played a less important role. The OX2 was more directly connected to the stream and therefore more influenced by precipitation and overbank flooding.
events than OX1. Detailed hydrologic modeling of these sites is ongoing and will help to more accurately quantify the ability of these sites to remove a substantial amount of the NO$_3^-$ transported in urban watersheds.

The Importance of Nitrous Oxide

There is great interest in the N$_2$O:N$_2$ ratio during denitrification in wetlands, especially those relied on for water quality improvement due to the need to determine if these improvements come at the expense of air quality or increased greenhouse gas emissions (Ullah et al., 2005). In our sites, N$_2$O:N$_2$ ratios were low and were higher in forested wetlands than in urban oxbow and constructed wetlands with similar rates of denitrification. The N$_2$O yields averaged 0.102, which is similar to the global synthesis value (0.082 ± 0.024) for “freshwater wetlands and flooded soils” reported by Schlesinger (2009), and is much lower than the global synthesis values for “agricultural soils” (0.375 ± 0.035) or “soils under natural or recovering vegetation” (0.492 ± 0.066). Gale et al. (1993) also found that the ratio of N$_2$O:N$_2$ evolved during denitrification in constructed wetlands was low (0.13–0.18) relative to natural wetlands (0.86). Hernandez and Mitsch (2007) observed N$_2$O:N$_2$ in created wetlands ranging from 0.02 to 0.30. Our results suggest that N$_2$O emissions were a small percentage of the total denitrification from the wetlands in our study sites and that these wetlands could not be considered to be a significant source of N$_2$O in regional greenhouse gas inventories for the city or county of Baltimore, or the state of Maryland.

Conclusions

Our results suggest that stream restoration that includes the creation of wetlands, either by design for stormwater management or by coincidence, can be important for increasing denitrification in urban landscapes year round. The urban wetlands in this study had rates of denitrification that could remove amounts of N that are significant relative to N loads carried by urban streams. These results are particularly relevant in the Chesapeake Bay Watershed, where there is great interest in the development of BMPs in urban areas that have the potential for nutrient removal to meet new TMDL water quality standards (USEPA, 2009, 2010).

While the wetlands in this study had significant capacity for denitrification, there is a clear need for evaluation of the

<table>
<thead>
<tr>
<th>Site</th>
<th>Wetland area</th>
<th>Denitrification rate†</th>
<th>Soil NO$_3^-$ denitrified in the wetland</th>
<th>NO$_3^-$ standing stock OWC‡</th>
<th>NO$_3^-$ removal</th>
<th>% removal</th>
<th>Minebank Run Stream</th>
</tr>
</thead>
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<tr>
<td>Oxbow 1</td>
<td>412</td>
<td>31.4</td>
<td>24.3</td>
<td>42,840</td>
<td>4.3</td>
<td>23.4</td>
<td>119,970</td>
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<tr>
<td>Oxbow 2</td>
<td>296</td>
<td>56.2</td>
<td>43.4</td>
<td>44,400</td>
<td>3.5</td>
<td>28.3</td>
<td>119,970</td>
</tr>
</tbody>
</table>

† Average denitrification rates pooled for summer and winter (n = 10).
‡ Nitrate-standing stock obtained from NO$_3^-$ concentrations in oxbow 1 and oxbow 2 OWC during time of the experiment, -0.2 mg L$^{-1}$ and 0.3 mg L$^{-1}$, respectively.
§ Stream NO$_3^-$ load calculated by multiplying the average annual discharge (129,000 L d$^{-1}$) for water year 2008 from the USGS Minebank Run stream gauge 015839795 by the average stream NO$_3^-$ concentration (0.93 mg L$^{-1}$); data from Paul Mayer USEPA collected April, July, and October 2008.

Fig. 8. The masses of N$_2$O-N and N$_2$ ($\mu$g) produced by each wetland pooled across seasons (a) and the mean ratio of N$_2$O:N$_2$ evolved from the oxbow, constructed, and forested wetland types (b). Denitrification rates in this study reflect the sum production of N$_2$O + N$_2$ via denitrification expressed as a mass in units ($\mu$g).
ability of these and other urban structural BMPs to function as “hotspots” for nutrient removal at the reach and ultimately the watershed scale. Demonstrated effectiveness at scales that influence receiving water quality and/or that are subject to regulation is necessary to convince managers and decision makers that these practices are useful aids in achieving water quality standards at the watershed scale (Palmer and Bernhardt, 2006; Craig et al., 2008; Palmer, 2008; Beechie et al., 2010).

The ultimate mechanisms controlling NO$_3$ removal in urban wetland ecosystems remain uncertain because of the spatial and temporal variability of physical and biogeochemical processes that affect N transformations. Nutrient removal in wetlands can vary considerably by growing season and during similar hydrologic conditions (Rucker and Schraufnagel, 2010). For example, spatial and seasonal variation in NO$_3$ loading may affect the capability of urban wetlands to remove N. This variation may also influence the N$_2$O yield and air quality implications of urban wetland construction and restoration.

One of the key mechanistic uncertainties in urban wetlands is the nature and extent of C supply to fuel denitrification (Groffman et al., 2005; Gift et al., 2010; Mayer et al., 2010; Sivirichi et al., 2011). The sources of C driving denitrification in urban wetlands are variable and incompletely understood, and include leaf litter, sediment, and soil substrates used during construction, sewage, and algal production. Noncarbon energy sources capable of supporting denitrification, e.g., sulfur (Burgin and Hamilton, 2007), can also be important. Further research to understand these mechanisms and controls is critical for designing stream and watershed restoration projects to optimize denitrification in urban landscapes.

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References


