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Influence of natural and novel organic carbon sources on denitrification in forest, degraded urban, and restored streams

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Abstract. Organic carbon is important in regulating ecosystem function, and its source and abundance may be altered by urbanization. We investigated shifts in organic carbon quantity and quality associated with urbanization and ecosystem restoration, and its potential effects on denitrification at the riparian–stream interface. Field measurements of streamwater chemistry, organic carbon characterization, and laboratory-based denitrification experiments were completed at two forested, two restored, and two unrestored urban streams at the Baltimore Long-Term Ecological Research site, Maryland, USA. Dissolved organic carbon (DOC) and nitrate loads increased with runoff according to a power-law function that varied across sites. Stable isotopes and molar C:N ratios suggested that stream particulate organic matter (POM) was a mixture of periphyton, leaves, and grass that varied across site types. Stable-isotope signatures and lipid biomarker analyses of sediments showed that terrestrial organic carbon sources in streams varied as a result of riparian vegetation. Laboratory experiments indicated that organic carbon amendments significantly increased rates of denitrification (35.1 ± 9.4 ng N·[g dry sediment]$^{-1}$·h^{-1}; mean $\pm$ SE) more than nitrate amendments (10.4 ± 4.0 ng N·[g dry sediment]$^{-1}$·h^{-1}) across streamflow conditions and sites. Denitrification experiments with naturally occurring carbon sources showed that denitrification was significantly higher with grass clippings from home lawns (1244 ± 331 ng N·g dry sediment^{-1}·h^{-1}), and overall unrestored urban sites showed significantly higher denitrification rates than restored and forest sites. We found that urbanization influences organic carbon sources and quality in streams, which can have substantial downstream impacts on ecosystem services such as denitrification.

Key words: Baltimore County, Maryland, USA; C:N ratio; denitrification; dissolved organic carbon; grass clippings; lipid biomarkers; nitrogen; organic carbon; stable isotopes; stream restoration; urbanization; urban stream.

Introduction

Organic carbon plays a key role in regulating ecosystem functions (Fisher and Likens 1973, Vannote et al. 1980). In streams and rivers, dissolved organic carbon (DOC) serves as an energy source for microorganisms and influences nutrient cycling (Edwards and Meyer 1987, McDowell and Likens 1988, Bernhardt et al. 2002), forms complexes with metals (Perdue et al. 1976), absorbs ultraviolet light (Frost et al. 2005), and can stimulate production of disinfection by-products in drinking water during chlorination (Krasner et al. 1989,

Kraus et al. 2008). Availability of dissolved and particulate organic carbon can limit denitrification, a microbial process critical to maintaining water quality (Sobczak et al. 2003, Mayer et al. 2010).

Many streams and rivers in the United States have elevated concentrations of nitrogen (Carpenter et al. 1998, Howarth et al. 2006). The Chesapeake Bay watershed has elevated NO_3^- concentrations in many streams and rivers because of agricultural and urban land use and fossil fuel combustion (Boesch et al. 2001, Kemp et al. 2005, Kaushal et al. 2008a). We need to enhance N management within watersheds to reduce downstream delivery to sensitive coastal waters (Boesch et al. 2001, Kemp et al. 2005, Kaushal et al. 2008b). A key ecosystem service that naturally removes reactive N is denitrification. Denitrification is an anaerobic micro-

bial process that typically requires organic carbon as an electron donor (Davidson and Schimel 1995, Groffman et al. 2005, Boyer et al. 2006). The riparian–stream interface, an area where streamwater and groundwater mix, is a "hot spot" for denitrification because it has low levels of dissolved oxygen and high levels of dissolved organic carbon (DOC; e.g., Hedin et al. 1998, Kaushal et al. 2008b, Mayer et al. 2010).

Dissolved organic carbon in streams is a mixture of both recalcitrant and labile fractions, with the labile fraction being important to biogeochemical processes (Findlay and Sinsabaugh 1999, Kaushal and Lewis 2003). Therefore, it is critical to understand which watershed organic carbon sources enter streams and the role that various terrestrial vs. aquatic sources play in influencing denitrification, metabolism, and organic carbon export. In forest ecosystems, riparian vegetation surrounding streams can influence DOC and comprise a substantial proportion of stream organic carbon budgets (Fisher and Likens 1973, McDowell and Likens 1988). The effects of organic carbon sources on ecosystem functions have been quantified for forested streams (McDowell and Likens 1988, McCutchan and Lewis 2002) and agricultural streams (Schaller et al. 2004, Royer and David 2005, Griffiths et al. 2009, Warrner et al. 2009). However, there has been little assessment of the relative importance of sources of natural and anthropogenic organic carbon sources on denitrification in urban streams (Paul and Meyer 2001, Ulseth and Hershey 2005, Paul et al. 2006, Petrone et al. 2011).

Variations in organic carbon from autochthonous (in-stream) and allochthonous (watershed) sources can be pronounced in urban streams due to flashy hydrology, wastewater inputs, and anthropogenically enhanced sources (Hook and Yeakley 2005, Kaushal et al. 2010, Petrone 2010). Urban watersheds and riparian zones may also have extensively modified vegetation such as home lawns, and this vegetation can have a strong effect on the supply of organic carbon to streams (Ryder and Miller 2005, Pouyat et al. 2009, Kaushal and Belt 2012). Therefore, we need to elucidate how denitrification in sediments may vary in response to stormflow vs. baseflow conditions and in response to changes in the relative importance of terrestrial vs. aquatic sources in urban watersheds.

We investigated the relative importance of different organic carbon sources to denitrification at the riparian–stream interface of forested, restored, and unrestored urban streams. During urbanization, large areas that were once forest or agricultural fields are often converted to lawns. In addition, urban stream restoration can influence carbon sources through tree removal and riparian reforestation. Our overall objective was to investigate how urbanization and restoration affect C and N delivery to and cycling within streams. Our secondary objective was to explore how uniquely urban organic matter sources such as lawn clippings vs. more natural organic matter sources affect denitrification. Our

study objectives were to: (1) determine the influence of land use and restoration status on amounts and sources of organic carbon reaching streams, (2) measure denitrification potential rates associated with baseflow and stormflow conditions, (3) evaluate whether nitrate or carbon amendments stimulated larger denitrification potential rates, and (4) characterize the relative importance of naturally occurring organic carbon sources (leaves, grass, and periphyton) for fostering denitrification across land use and restoration status.

METHODS

Our project design included six Baltimore County, Maryland, USA, streams (two forested, two urban restored, and two unrestored urban). At each site, we monitored discharge and concentrations of nitrate and dissolved organic carbon for two years. We conducted a field survey on how particulate organic matter (POM) and organic carbon sources varied across land use and restoration status by analyzing stable-isotope ratios and molar C:N ratios. We also used laboratory experiments to measure microbial responses to three organic carbon sources typical of the study systems: grass clippings from home lawns, decomposed leaves taken from debris dams, and periphyton (which was a mixture of filamentous algae and terrestrial detritus). Our laboratory experiments examined changes in denitrification potential rates in sediments from the riparian–stream interface with water taken at baseflow vs. stormflow conditions and in response to different organic carbon sources.

Site description: forest, unrestored urban, and restored streams

Study sites included six low-order streams (two forested, two restored, and two unrestored urban) in the Baltimore metropolitan area, which is situated in the Piedmont region of Maryland, in the Chesapeake Bay Watershed (Figs. 1 and 2; Appendix B). These sites have been studied as part of the Baltimore Ecosystem Study (BES), one of two urban study sites in the U.S. National Science Foundation Long-Term Ecological Research network (Pickett et al. 2011).

Pond Branch (39°28′49″ N, 76°41′16″ W; 32.3 ha) is a forested, first-order stream with no impervious surfaces in its watershed. Pond Branch is a tributary of Baisman Run (39°28′45″ N, 76°40′42″ W; 381 ha), which is a third-order stream within a watershed that was 66% forested, 1% agriculture, 34% residential with septic systems, and 1% total impervious surface coverage (Groffman et al. 2004). Discharge in both streams was monitored continuously by the U.S. Geological Survey (USGS) gaging stations.

The two restored streams, Spring Branch (39°26′43.9″ N, 76°37′12.9″ W) and Minebank Run (39°24′36″ N, 76°33′23″ W), are low-order streams in close proximity to the Loch Raven drinking-water reservoir. Both restorations incorporated a combination of standard
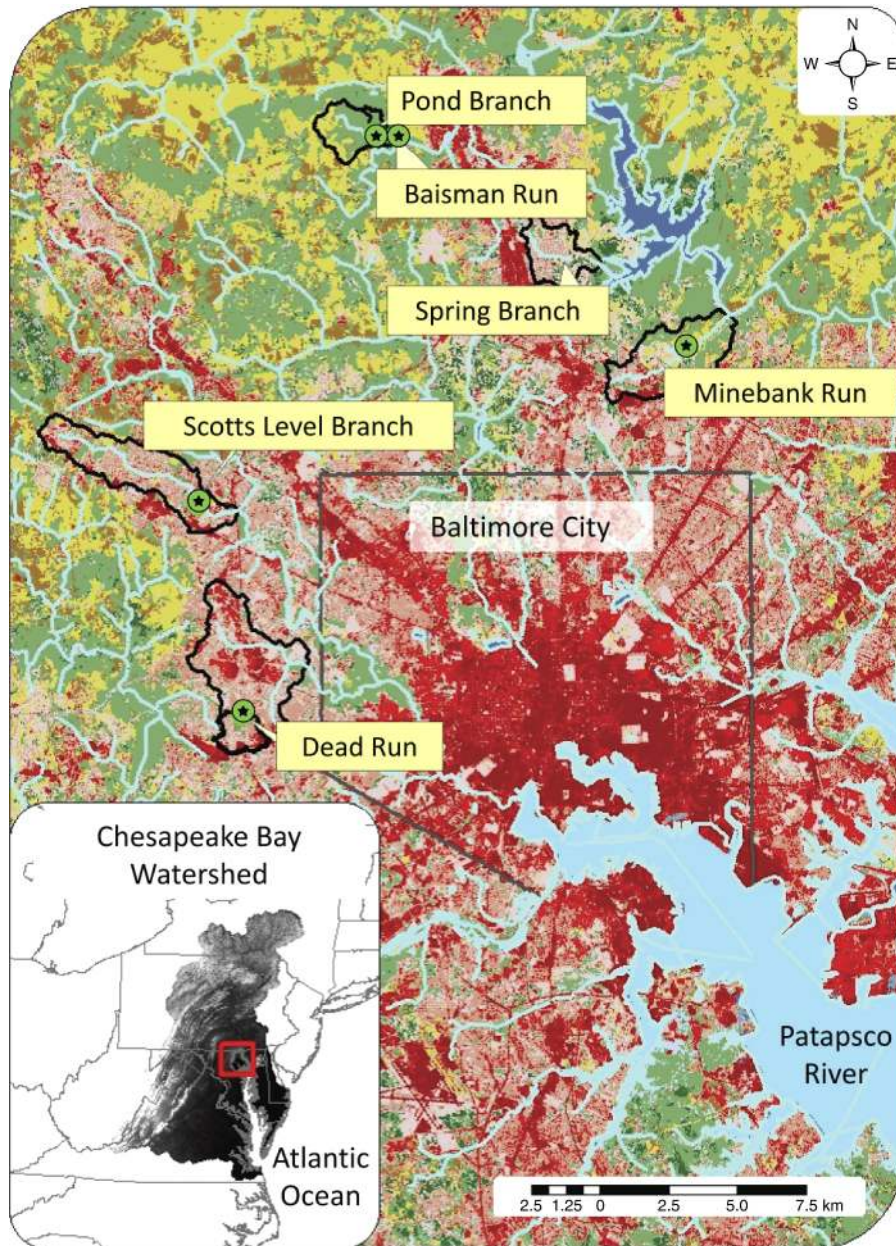
Fig. 1. Land cover map of study sites (outlined with black lines) at the Baltimore Ecosystem Study Long-Term Ecological Research Site, Maryland, USA. Coloration is from the 2001 National Land Cover Database (red indicates urban areas, and green indicates forested areas). Green circles with stars indicate USGS gage locations.

natural channel design techniques (Rosgen 1994) and integrated stormwater management such as hydrologically connected floodplains (Minebank Run; Kaushal et al. 2008b, Klocker et al. 2009) or stormwater management areas including wetlands and ponds below a storm drain outfall (Spring Branch; DEPRM 2008a, b, U.S. EPA 2011). Spring Branch (407 ha) was the first restoration site in Baltimore County, and 3.2 km of stream length were restored during 1994–1997. Spring Branch was restored by removal of concrete channels, creation of a series of step-pools, tree and shrub

plantings for bank stabilization, and creation of multiple-cell stormwater management areas in the headwaters (DEPRM 2008a, b, U.S. EPA 2011). The Spring Branch watershed has a total impervious surface coverage of 18.6%, and land use composition is 91.5% residential with varying degrees of density (33% low, 54.8% medium, and 3.7% high), 1.7% institutional (a school), and 6.7% forest (DEPRM 2008a, b). At Minebank Run (207 ha), 2.4 km of stream length were restored during 1998–1999 and 2.9 km were restored from 2004 to 2005 (U.S. EPA 2006). Land use in

FIG. 2. Photographs showing the (A) forested Pond Branch, (B) forested Baisman Run, (C) restored Minebrook Run, (D) restored Spring Branch, (E) unrestored urban Dead Run, and (F) Scotts Level Branch streams.

Minebank Run is 17% forest, 2% agriculture, and 81% urban/suburban, including 30–35% total impervious surface coverage (Doheny et al. 2006, 2012). Discharge is continuously monitored at Minebank Run by the USGS.

The two unrestored urban streams are Scotts Levels Branch (39°21′41.8″ N, 76°45′42.3″ W) and Dead Run (39°17′45.2″ N, 76°44′38.7″ W) on the boundary of urban Baltimore City and suburban Baltimore County. In contrast to the forested streams, Scotts Level Branch

(836.5 ha) and Dead Run (204.6 ha) have sections where the riparian zone is forested and reaches where lawns are managed to the edge of the stream. Total impervious coverage is 29% at Scotts Level Branch and 40% at Dead Run (Ryan et al. 2010). Dead Run is part of the lower Gywnns Falls watershed, which has a land use composition of 2% agriculture, 14% forested, 75% urban, 8% suburban, and 1% other (Klocker et al. 2009). Discharge at both streams is monitored continuously by USGS gaging stations.

Temporal changes in NO_3^- and DOC concentrations and daily fluxes

From April 2008 to April 2010, we collected monthly surface water samples to characterize temporal changes in nitrate (NO_3^-) and DOC concentrations at all six streams over a range of hydrologic conditions. Surface water samples were collected in high-density polyethylene (HDPE) Nalgene bottles that were rinsed five times with sample water prior to sample collection. Samples were filtered within 24 h using pre-combusted Whatman 0.45-μm glass fiber filters (GF/F) and kept frozen until analysis at the University of Maryland Center for Environmental Science, Chesapeake Biological Laboratory, Solomons, Maryland, USA. Analysis of NO_3^- was performed with a Dionex ion chromatography system (ICS-1500; Dionex, Sunnyvale, California, USA), and analysis of DOC was performed with a Shimadzu total organic carbon analyzer (TOC-272 V CPH/CPN; Shimadzu, Columbia, Maryland, USA; Kaushal and Lewis 2003, 2005). Daily fluxes were calculated by multiplying concentration (mg/L) by stream flow (L/d) to get mass transport per day. We used mean daily stream flow recorded at five sites with USGS gages (forested Pond Branch is gage 01583570, forested Baisman Run is 01583580, unrestored urban Scotts Level Branch is 01589290, unrestored urban Dead Run is 01589312, and restored Minebank Run is 0158397967) and measured instantaneous stream flow at the restored Spring Branch site with a Marsh McBirney 2000 (Hach Company, Loveland, Colorado, USA) velocity meter.

Organic matter sources: C:N ratios, $\delta^{15}N$ and $\delta^{13}C$ isotopic analysis, lipid biomarkers

We analyzed $\delta^{15}N$ and $\delta^{13}C$ isotopic ratios and molar C:N ratios on triplicate samples of sediment, grass, periphyton, leaves, and POM that we collected at two locations at five streams: forested Pond Branch and Baisman Run, restored Spring Branch and Minebank Run, and unrestored urban Dead Run (unrestored urban Scotts Level Branch was omitted because of time and cost constraints). Particulate organic carbon (POC) and particulate nitrogen (PN) samples were collected by filtering 750–1000 mL of streamwater (collected at the surface using 2-L amber HDPE bottles) through a 125-μm sieve followed by filtering onto a pre-combusted Whatman GF/F filter (0.8 μm; 25 mm diameter; 500°C for 2 h). The POM filters were rinsed with 10% HCl to

remove carbonates, and then rinsed with deionized water. The filters were placed in combusted foil and frozen at −80°C until subsequent analysis. Sediments were collected at the riparian–stream interface from a depth of ~0.5 m below the baseflow water surface elevation using a soil auger at each stream. Samples were placed in washed, combusted (450°C, 4.5 h) 0.12-L (4-oz) Qorpak jars and put on dry ice in the field until returned to the laboratory and stored at −80°C. Organic carbon sources (grass, leaves, and periphyton) were collected at each site and were placed into the Alconox-washed, combusted (450°C, 4.5 hours) 4-oz Qorpak jars and put on dry ice in the field until returned to the laboratory and stored at −80°C. Grass samples were typically cut from as near the stream as possible, leaves were collected from debris dams within the stream channel, and periphyton samples were collected from within the stream. Before final analysis, filters, sediment, and vegetation samples were rinsed, dried, milled, and acidified according to protocols of the Stable Isotope Facility, University of California, Davis, California, USA (UC Davis; details *available online*).[8] Samples were shipped to UC Davis for analysis on a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon, Cheshire, UK). The stable-isotope ratios ^{13}C:^{12}C and ^{15}N:^{14}N are reported in delta (δ) units as per-mil difference between the ratio of the sample to the standard (Pee Dee belemnite [PDB] and air, respectively).

Lipid biomarker analyses were conducted to further investigate the sources of organic carbon in stream sediments. We used lipid biomarkers to examine contributions from terrestrial (percentage of long-chain fatty acids, percentage of long-chain alcohols, and percentage of plant sterols) and aquatic-source indicators (percentage of short-chain alcohols and percentage of diatom sterols). Two sediment samples were collected from each of three sites: forested Pond Branch, restored Spring Branch, and restored Minebank Run during May and June 2008. We were unable to conduct lipid biomarker analyses at all sites due to cost restraints. Sediment samples were extracted in a 2:1 solution of CH_2Cl_2:CH_3OH (DCM:MeOH) using an accelerated solvent extraction-200 (ASE; Dionex) at 80°C and 12.4 MPa (1800 psi; 2 × 10 min cycles) following a modification of the Bligh and Dyer (1959) method (Waterson and Canuel 2008). Frozen sediments were thawed, homogenized, and dried with hydromatrix prior to extraction. Surrogate standards including a fatty acid methyl ester (FAME), methyl nonadecanoate (C_{19} FAME), nonadecanol, a wax ester (myristyl arachidate) that yielded a C_{14} alcohol and a C_{20} FAME following saponification, and androstanol were added to each sample prior to extraction. Extracts were partitioned into two phases, and the lower organic phase collected. The aqueous phase was back-extracted into hexane and

the combined organic phases placed over anhydrous Na_2SO_4 overnight to reduce traces of H_2O. The samples were concentrated to 1 mL using turbo-evaporation (Zymark Turbo Vap 500; Caliper Technologies, Hopkinton, Maryland, USA). The mass of each total lipid extract (TLE) was determined gravimetrically using aliquots representing ~10% of the TLE. A portion of the extract was saponified using 1 mol/L KOH in aqueous methanol (110°C for 2 h). Neutral and acidic lipids were extracted into hexane from the saponified sample following Canuel and Martens (1993). Fatty acids were converted to methyl esters using BF_3-MeOH. Both fatty acids (as methyl esters) and neutral lipids were separated from other lipid classes by silica gel chromatography following Canuel and Martens (1993). Sterols were derivatized to trimethylsilyl (TMS) ethers using BSTFA and acetonitrile and heating at 70°C for 30 min. Fatty acids (as methyl esters) and alcohols/sterols (as TMS ethers) were analyzed using gas chromatography (GC; Hewlett Packard 5890 Series II Plus, Palo Alto, California, USA) with flame ionization detection using a 40 m × 0.18 mm DB5 column (J&W Scientific; Agilent Technologies, Santa Clara, California, USA). Peak areas were quantified relative to internal standards: C_{21} FAME was used for fatty acids and 5(α)-H-cholestane for alcohols/sterols. A GC interfaced to a mass selective detector (Hewlett Packard 6890 GC-MSD) operated in electron impact mode was used to verify the identification of individual compounds using similar conditions as for GC analysis.

Experimental design for denitrification experiments

Denitrification experiments were related to three of our four overall study objectives: (1) evaluate whether nitrate or carbon availability produced a greater denitrification potential response, (2) measure the denitrification potential rates associated with baseflow and stormflow, and (3) characterize the relative importance of naturally occurring organic carbon sources to denitrification across land use and restoration status.

We conducted two types of denitrification experiments. The first involved amending sediment and streamwater with glucose or nitrate to determine how denitrification potential rates were affected by N and C availability, hydrologic conditions (stormflow vs. baseflow), and restoration status. The second experiment involved incubating sediment and streamwater with naturally occurring organic carbon sources (grass, periphyton, or leaves) to assess their differential impacts on denitrification. The first experiment had a factorial design with three factors: (1) site type (forested, restored, and unrestored urban), (2) streamwater type (collected during baseflow and stormflow conditions), and (3) amendment type (glucose and nitrate). The second experiment had a factorial design with two factors: (1) site type (forested, restored, and unrestored urban) and (2) naturally occurring organic carbon source type (control, periphyton, grass, and leaves).

Sample collection for denitrification experiments

Sediments were collected from each stream at the riparian–stream interface using a gas-powered auger. Sediment samples were taken at two locations at each stream at a distance ~1 m from the wetted channel perimeter and at a depth of ~0.5 m below the baseflow water surface elevation. All samples were refrigerated <2 weeks before analysis. Organic carbon sources (grass, periphyton, and leaves) were collected from the riparian zone at each site and refrigerated in re-sealable plastic bags for <2 weeks before the experiments. Grass samples were typically cut from as near the stream as possible, leaves were collected from debris dams within the stream channel, and periphyton samples were collected from within the stream. Leaves, periphyton, and grass clippings were rinsed in the laboratory with deionized water to remove possible silt or debris. In a few cases, periphyton or grass were not available from a particular study site, and were used from a nearby site or location. Samples were collected during June 2006. We collected stormflow streamwater during a storm on 25 June 2006 and baseflow streamwater four days later. Organic carbon sources change over time, so these experiments should be considered a snapshot of field conditions at the time of collection.

Denitrification potential rate methodology

Denitrification enzyme activity assays are widely used to compare sites and treatments (Smith and Tiedje 1979, Groffman et al. 1999, 2005, 2006, Roach and Grimm 2011). Briefly, we amended 5.0 g of sediment and 10 mL of streamwater with a media made of organic carbon (glucose), nitrate (KNO_3^-), and chloramphenicol (Groffman et al. 1999). We added enough organic carbon and nitrate to ensure denitrification was not limited and added chloramphenicol to block the production of new enzymes during incubation. This mixture was sealed in 125-mL Erlenmeyer flasks using rubber stoppers, and the headspace was evacuated and replaced with N_2 gas. Acetylene was added to each flask to block the final step of denitrification, the transformation of N_2O to N_2. Gas samples were taken at 30 min and 90 min. Samples were stored in evacuated glass vials, and N_2O concentrations were analyzed by gas chromatography using a Shimadzu GC 14 gas chromatograph outfitted with an electron capture detector at the Cary Institute for Ecosystem Studies, Millbrook, New York, USA.

Denitrification experiment 1: glucose vs. nitrate amendment

In this first experiment, we conducted denitrification enzyme assays where we amended sediments with either glucose or nitrate. For glucose amendments, glucose concentrations were increased by 500 mg/L so that we could measure denitrification potential rates associated with ambient nitrate in streamwater samples collected under different hydrologic conditions (baseflow or
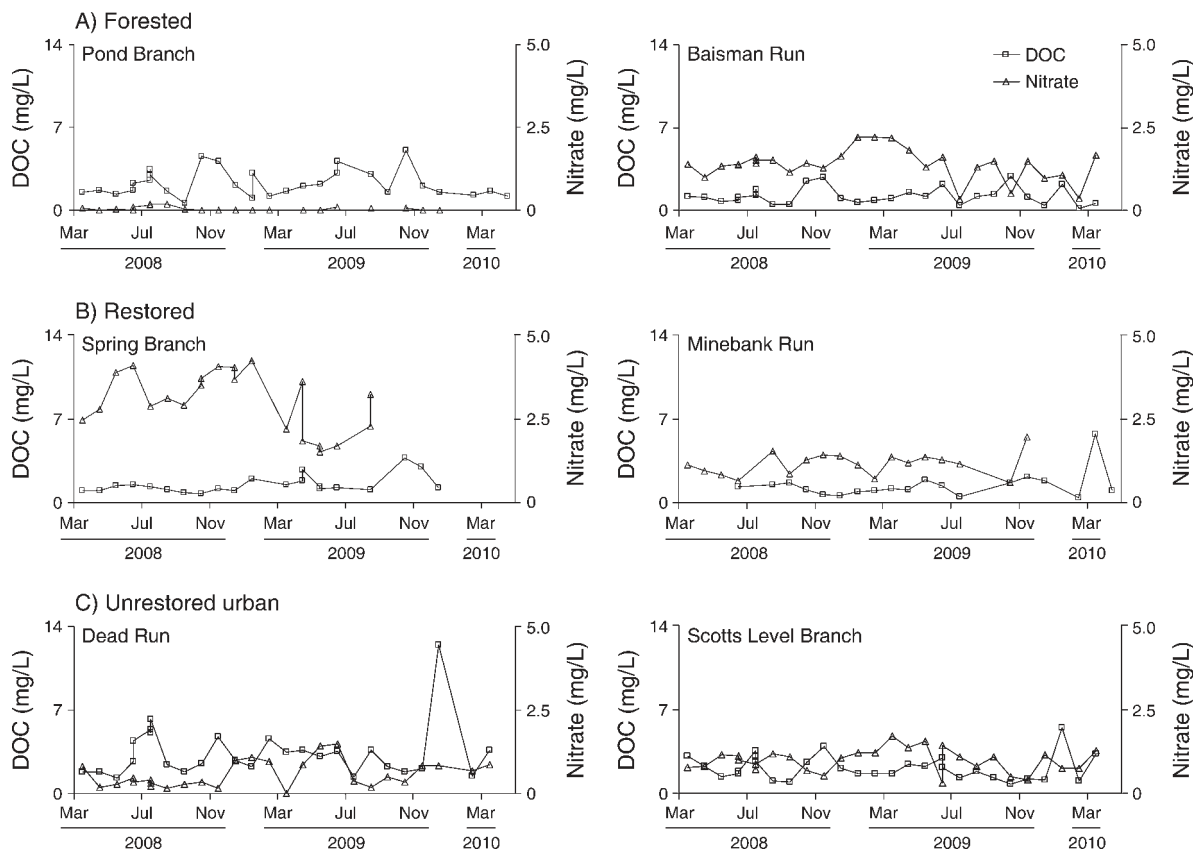
FIG. 3. Monthly dissolved organic carbon (DOC) and nitrate concentrations from (A) forested, (B) restored, and (C) unrestored urban watersheds.

stormflow) across study sites. For the nitrate amendment experiment, we increased the KNO_3 concentration by 720 mg/L so that we could measure denitrification potential rates associated with ambient organic carbon. Our experimental design for comparing glucose, nitrate, and stormwater included 96 samples (6 stream sites × 2 locations per stream × 2 amendment types [organic carbon and nitrate] × 2 hydrologic conditions [baseflow and stormflow] × 2 duplicates).

Denitrification experiment 2: effects of naturally occurring carbon sources on denitrification

A second denitrification enzyme activity experiment was conducted to investigate how different naturally occurring organic carbon sources affected denitrification potential rates. This experiment used media that included nitrate but omitted glucose to induce carbon limitation. The dry mass equivalent of 0.2 g of local organic carbon (grass, periphyton, or leaves) was made into a slurry in a blender and added to the incubations in place of glucose as an organic carbon source. Slurries were incubated with sediment, streamwater, and media in ~200-mL (half-pint) Mason jars. Controls contained only sediment, streamwater, and media. Our experimental design for comparing the effects of naturally

occurring organic carbon sources on denitrification rates across streams included 96 samples (6 streams × 2 locations per stream × 4 organic carbon sources [control, grass, periphyton, or leaves] × 2 duplicates).

Statistical analysis

Statistical analyses were performed using SAS Analyst (SAS Institute 2003). Differences in streamwater chemistry, denitrification potential rates, and C:N ratios, and lipid biomarkers were evaluated using an analysis of variance (ANOVA) followed by Tukey's test with a significance level (α) of 0.05. We evaluated differences in denitrification potential rates across site type (forest, restored, and unrestored urban), organic carbon source (periphyton, leaves, and grass), and flow conditions (baseflow streamwater and stormflow streamwater).

RESULTS

Concentrations and fluxes of NO_3^- and DOC

Pond Branch (forested) had significantly lower and Spring Branch (restored) had significantly higher mean nitrate-N concentrations than other sites ($F_{5, 150} = 94.33$, $N = 159$, $P < 0.01$; Fig. 3, Table 1). The low-density residential forested site, Baisman Run, had significantly lower and Dead Run (unrestored urban) had signifi-

TABLE 1.   Dissolved organic carbon (DOC) and nitrate concentration (mg/L) in forested, restored, and unrestored urban Baltimore, Maryland, USA, streams from April 2008 to April 2010.

Restoration status and stream	Analysis	Tukey comparison	Mean ± SE	Range	Sample size (N)
Forested					
Pond Branch	DOC	a	2.13 ± 0.14	0.62–5.07	47
	NO_3^-	A	0.045 ± 0.010	0.001–0.188	31
Baisman Run	DOC	b	1.23 ± 0.13	0.20–2.87	28
	NO_3^-	B	1.374 ± 0.089	0.323–2.215	28
Restored					
Spring Branch	DOC	a, b, c	1.50 ± 0.16	0.71–3.74	25
	NO_3^-	C	2.898 ± 0.222	0.704–4.218	22
Minebank Run	DOC	b, c	1.38 ± 0.12	0.40–5.72	48
	NO_3^-	B, D	1.083 ± 0.060	0.470–1.532	22
Unrestored urban					
Dead Run	DOC	d	3.42 ± 0.43	1.33–12.42	27
	NO_3^-	E	0.568 ± 0.076	0.007–1.469	27
Scotts Level Branch	DOC	a, c	2.14 ± 0.20	0.80–5.50	29
	NO_3^-	D	0.982 ± 0.062	0.316–1.717	29

Notes: Values are means ± SE. Letters represent comparisons that are significant ($\alpha = 0.05$) according to Tukey's studentized range (HSD) test; lowercase letters correspond to DOC concentration, and uppercase letters correspond to nitrate concentration.

cantly higher mean DOC concentrations than other sites ($F_{5, 198} = 14.14$, $N = 204$, $P < 0.01$; Fig. 3, Table 1).

At all six streams, the daily loads of DOC and nitrate (mg/d) increased according to a power function with runoff (Fig. 4). There were substantial differences in mean daily runoff normalized by watershed area between the different stream types during the 25 June 2006 storm when denitrification was measured (Fig. 5). The forested sites, Pond Branch and Baisman Run, displayed peak flows that were an order of magnitude lower than the flashy peak flows at the unrestored urban sites, Dead Run and Scotts Level Branch. Minebank Run (restored) had a flashy peak (230 L·sec^{-1}·km^{-2}) that was intermediate between forested and unrestored urban streams.

Organic carbon source characterization

Overall, there was a significant difference in molar C:N ratios among site types ($F_{2, 240} = 23.14$, $N = 245$, $P < 0.01$) and organic carbon sources ($F_{4, 240} = 21.89$, $P < 0.01$), and there was a significant interaction between site type and organic carbon source ($F_{8, 240} = 3.25$, $P < 0.01$; Fig. 6). The forested sites had a significantly higher mean C:N ratio, 22.9 ± 1.1, than the restored sites, 16.4 ± 0.8 ($t = 5.99$, $P < 0.01$), and the unrestored urban sites, 16.6 ± 0.9 ($t = 5.44$, $P < 0.01$). Mean molar C:N ratios associated with leaves, 26.5 ± 1.7, were significantly higher than for periphyton, 19.3 ± 1.5 ($t = 4.67$, $P < 0.01$), or grass, 18.3 ± 0.8 ($t = 4.90$, $P < 0.01$), and stream POM, 11.9 ± 0.4 ($t = 9.32$, $P < 0.01$). Mean molar C:N ratios associated with stream POM were significantly lower than for grass ($t = 4.41$, $P < 0.01$), periphyton ($t = 4.64$, $P < 0.01$), and sediment ($t = 5.26$, $P < 0.01$).

Isotopic C and N signatures of grass clippings, leaf litter, and periphyton typically showed distinct separation with no overlap among sources in all five streams; But POM and sediment overlapped at Baisman Run (forested) and POM and leaves overlapped at Minebank Run (restored; Fig. 7). Across sites, mean $\delta^{13}C$ signatures for grass clippings ranged from −32.01‰ at Baisman Run (forested) to −28.76‰ at Dead Run (unrestored urban); mean $\delta^{15}N$ for grass clippings ranged from −1.69‰ at Pond Branch (forested) to 4.96‰ at Minebank Run (restored). Mean $\delta^{13}C$ of leaf litter ranged from −29.47‰ at Pond Branch (forested) to −25.10‰ at Spring Branch (restored); mean $\delta^{15}N$ of leaf litter ranged from −0.86‰ at Pond Branch (forested) to 2.69‰ at Spring Branch (restored). Mean $\delta^{13}C$ of periphyton ranged from −29.75‰ at Pond Branch (forested) to −23.68‰ at Minebank Run (restored); mean $\delta^{15}N$ of periphyton ranged from −2.63‰ at Pond Branch (forested) to 7.87‰ at Dead Run (unrestored urban). The isotopic signatures for POM and sediment were intermediate between the grass, leaf, and periphyton sources indicating a mixture of sources. Isotope biplots showed that at the forested sites, Pond Branch and Baisman Run, the POM and sediment were closest to the $\delta^{15}N$ and $\delta^{13}C$ values of the leaf litter. This suggested that decayed leaves were an important source for POM and sediment among all site types, but especially at the forested sites (Fig. 7). At Minebank Run (restored), the isotopic signature for POM was similar to the leaf source. Isotopic signatures at Spring Branch (restored) and Dead Run (unrestored urban) also suggested that POM appeared to be a mixture of grass, periphyton, and decayed-leaf isotope signatures. At most sites, the POM signature was similar to the sediment signature except at Minebank Run (restored).
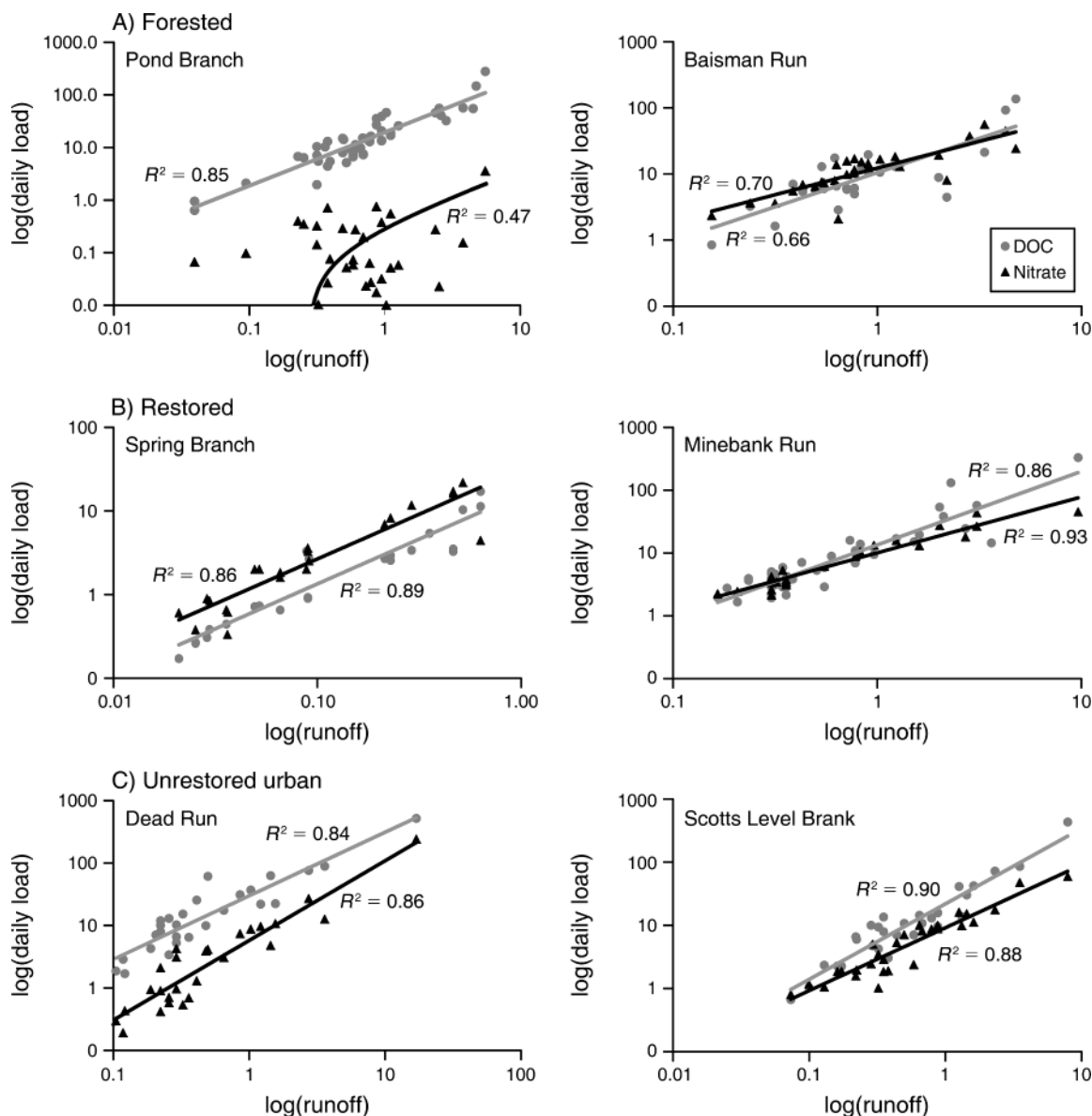
FIG. 4.   Daily loads of DOC and nitrate (log-transformed; originally measured in g·ha^{-1}·d^{-1}) vs. daily runoff (log-transformed; originally measured in mm/d) from (A) forested, (B) restored, and (C) unrestored urban watersheds. Daily loads of DOC and nitrate increased with daily runoff according to a power-law function at all sites, except for nitrate at the forested Pond Branch, which increased linearly.

Lipid biomarker results showed that the source and quality of organic matter varied across sites (Table 2). Long-chain alcohols and plant sterols serve as proxies for vascular plant (terrigenous) sources (Canuel and Martens 1993, Waterson and Canuel 2008). The percentages of long-chain alcohols were higher at Pond Branch (forested) than at Minebank Run (restored; $t =$ 2.23, $P = 0.03$), while the percentage of plant sterols was higher at Spring Branch (restored) than at Pond Branch (forested; $t = 2.68$, $P > 0.01$) and Minebank Run (restored; $t = 3.89$, $P = 0.02$). The percentages of diatom

sterols were lower at Pond Branch (forested) than at Minebank Run (restored; $t = 2.09$, $P = 0.04$).

Denitrification experiment 1: glucose vs. nitrate amendment

Denitrification potential was significantly higher when sediments were amended with glucose (35.1 $\pm$ 9.4 ng N·[g dry sediment]$^{-1}$·h^{-1}) than when amended with nitrate (10.4 $\pm$ 4.0 ng N·[g dry sediment]$^{-1}$·h^{-1}; $F_{1,46} =$ 94.33, $N = 48$, $P = 0.02$; Fig. 8). Denitrification potential was too low to detect in the nitrate amendment
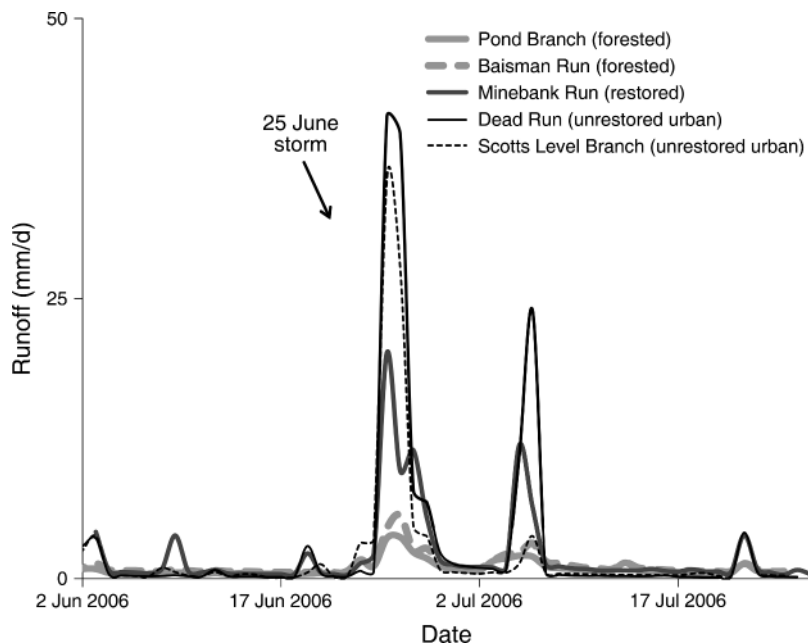
Fig. 5. Hydrograph of mean daily runoff of the storm that was sampled as part of the denitrification experiments (mm/d). Unrestored urban and restored sites have higher peak flows than forested sites. Data are lacking for Spring Branch because it does not have a real-time USGS flow monitoring station.

experiment at the forested site. Denitrification potential rates at forested streams (2.2 ± 1.0 ng N·[g dry sediment]$^{-1}$·h^{-1}) were significantly lower than at restored streams (36.0 ± 12.3 ng N·[g dry sediment]$^{-1}$·h^{-1}) and urban streams (30.1 ± 8.8 ng N·[g dry sediment]$^{-1}$·h^{-1}), but the rates measured at the restored and the unrestored urban sites were not significantly different ($F_{2,45} = 4.29$, $N = 48$, $P = 0.02$; Fig. 8). Denitrification potential rates associated with incubating sediments with stormflow streamwater (29.6 ± 9.4 ng N·[g dry sediment]$^{-1}$·h^{-1}) were consistently higher than with baseflow streamwater (15.9 ± 5.1 ng N·[g dry sediment]$^{-1}$·h^{-1}), but the difference was not statistically significant ($F_{2,45} = 1.67$, $N = 48$, $P = 0.20$; Fig. 8).

Denitrification experiment 2: effects of naturally occurring carbon sources on denitrification

Denitrification potential rates from the experiment comparing the effects of naturally occurring organic carbon sources (control, periphyton, leaves, and grass), differed across site type ($F_{2,81} = 3.79$, $N = 86$, $P = 0.01$; Fig. 9B) and organic carbon source ($F_{3,60} = 8.78$, $N = 86$, $P < 0.01$; Fig. 9A). There was also a significant interaction between site type and organic carbon source ($F_{6,70} = 2.33$, $N = 86$, $P = 0.02$; Fig. 9).

Denitrification potential rates (ng N·[g dry sediment]$^{-1}$·h^{-1}) were greatest when grass clippings were added as the naturally occurring organic carbon source (1200 ± 300) compared to periphyton (410 ± 110; $t = 2.9$, $P < 0.01$), leaves (170 ± 30; $t = 4.21$, $P < 0.01$), and control treatments (3.1 ± 1.7; $t = 4.63$, $P < 0.01$; Fig. 9A). The highest denitrification potential rates were

observed when sediments from Scotts Level Branch (unrestored urban) were incubated with the grass extract (7200 ng N·[g dry sediment]$^{-1}$·h^{-1}). In addition, mean denitrification potential rates at the urban sites (1000 ± 470 ng N·[g dry sediment]$^{-1}$·h^{-1}) were significantly higher than denitrification potential rates from the forested sites (92 ± 36 ng N·[g dry sediment]$^{-1}$·h^{-1}; $t = 3.20$, $P < 0.01$) and the restored sites (290 ± 90 ng N·[g dry sediment]$^{-1}$·h^{-1}; $t = 2.51$, $P = 0.01$; Fig. 9B). Mean denitrification potential rates across naturally occurring organic carbon sources were not significantly different between the forested and restored sites.

DISCUSSION

Variations in organic carbon amounts and sources across land use

Our results demonstrate that urbanization causes shifts in organic carbon quantity, sources, and quality. We observed higher organic carbon concentrations and daily fluxes in urbanized streams than in forest streams. Urban streams can receive inputs from natural and/or anthropogenically enhanced organic carbon sources including leaf litter, autochthonous production, materials deposited on impervious surfaces, human and animal waste, and grass clippings from home lawns (Lofton et al. 2007, Sickman et al. 2007, Kaushal et al. 2011). Furthermore, urbanization can decrease canopy cover (Paul and Meyer 2001), and canopy coverage of riparian flow paths can influence the quantity and character of DOC delivered to streams and alter DOC bioavailability (Findlay et al. 2001, Pernet-Coudrier et al. 2010).

Molar C:N ratios served as a second organic carbon source tracking method; higher values indicate potential terrestrial sources (Kaushal and Binford 1999). Both stable-isotope signatures and molar C:N ratios suggested that POC sources and quality varied with watershed land use. Mean molar C:N ratios of organic carbon samples at the forested site were significantly higher than at the restored and the unrestored urban sites, indicating that organic matter at the forested sites may be lower in quality and more recalcitrant. The molar C:N ratios suggest that the forested site receives terrestrial organic matter such as leaves, while the restored and the unrestored urban sites receive a mixture of higher quality organic matter sources such as grass clippings, periphyton, and wastewater. Ratios of C:N in streams influence N cycling (Dodds et al. 2004), and increasing the quantity of available DOC can enhance whole-stream N uptake (Johnson et al. 2009) when N is not limiting heterotrophic production (e.g., Bernhardt and Likens 2002). Furthermore, these differential C:N ratios may affect denitrification and respiration rates in streams. Isotopic signatures suggested that terrestrial leaf sources contributed to POM in forested streams. In contrast, the POM in restored and unrestored urban streams was a mixture of periphyton, leaves, and grass. The $\delta^{15}N$ of the sediment in Minebank Run was considerably higher than other sites, and these high values may indicate denitrification or contamination from previous $\delta^{15}N$ tracer studies (Kaushal et al. 2008b). We used stable-isotope signatures of POM and sediment to indicate organic matter sources.

Lipid biomarker data provided another line of evidence that the source and amount of organic matter varied across land use and restoration status. Forested Pond Branch and Spring Branch (the older restoration) showed higher contributions from terrestrial sources than Minebank Run (the newer restoration). We speculate that Minebank Run showed lower contributions from terrestrial sources because trees planted in the restored riparian zone have not yet matured to full canopy coverage. The Minebank Run study reach that was restored between 2004 and 2005 had a significantly higher relative abundance of diatom carbon, indicating higher aquatic source contributions. Though lipid biomarker data were only available for a limited number of sites, these data provide further evidence that urbanization influences the source and quality of organic carbon in streams and the proportion of terrestrial vs. aquatic contributions.

Residential landscaping decisions like replacing forested areas with mowed lawns can also lead to considerable variability in riparian vegetation (Larson et al. 2009). For example, some riparian zones at our study sites consisted of managed lawns near streams with little or no tree canopy. Previous work analyzing stable isotopes from streams draining non-forested sites at the BES LTER site suggested an organic carbon contribution from lawns (Kaushal et al. 2011). There is
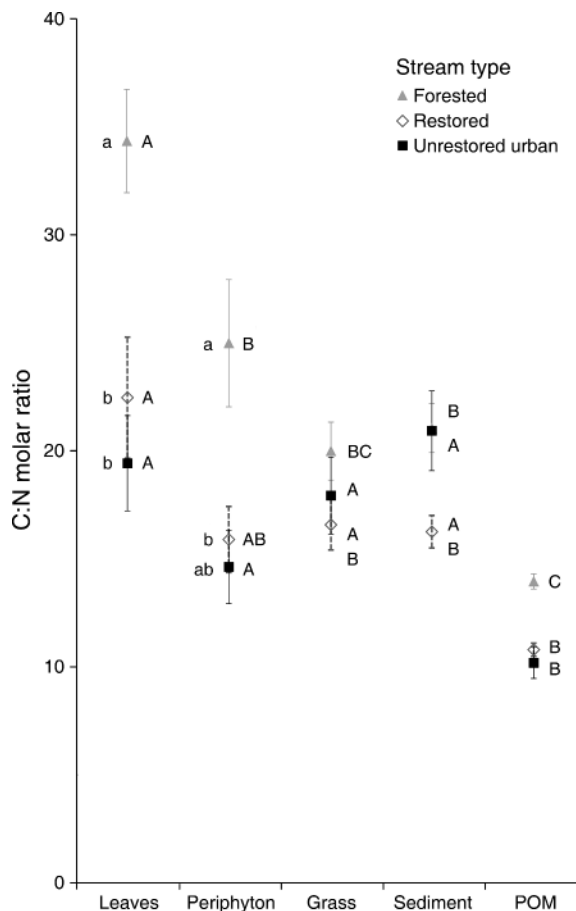

FIG. 6. Comparison of C:N molar ratios for leaves, periphyton, grass, sediment, and particulate organic matter (POM) across forested ($N = 20$), restored ($N = 14$), and unrestored urban sites ($N = 13$). Values are means ± SE. Letters represent comparisons (lowercase is inter-site type, and uppercase is intra-site type) that are significant ($\alpha = 0.05$) according to Tukey's studentized range (HSD) test.

evidence of organic carbon inputs to streams shifting from C_3 plants (trees) to C_4 plants (grasses) and changes in carbon quality with increasing urbanization (Kaushal et al. 2011). Our results from stable isotopes and C:N ratios are consistent with grass clippings contributing as a carbon source in urban streams. The total estimated area of urban lawns for the contiguous United States is $163\,800 \pm 35\,850$ km^2, which is three times greater than the area covered by irrigated corn (Milesi et al. 2005). Home lawns may have unique organic carbon dynamics when compared to native ecosystems (Kaye et al. 2005, Golubiewski 2006, Yesilonis et al. 2008, Groffman and Pouyat 2009). For example, lawn clippings are more labile than leaves from trees. Organic carbon from lawns may quickly turnover and enter food webs or may be rapidly decomposed by microbes and bypass higher trophic levels in food webs completely. Grassy riparian areas may alter biogeochemical cycling, food webs, and ecosystem functions like decomposition in ways that
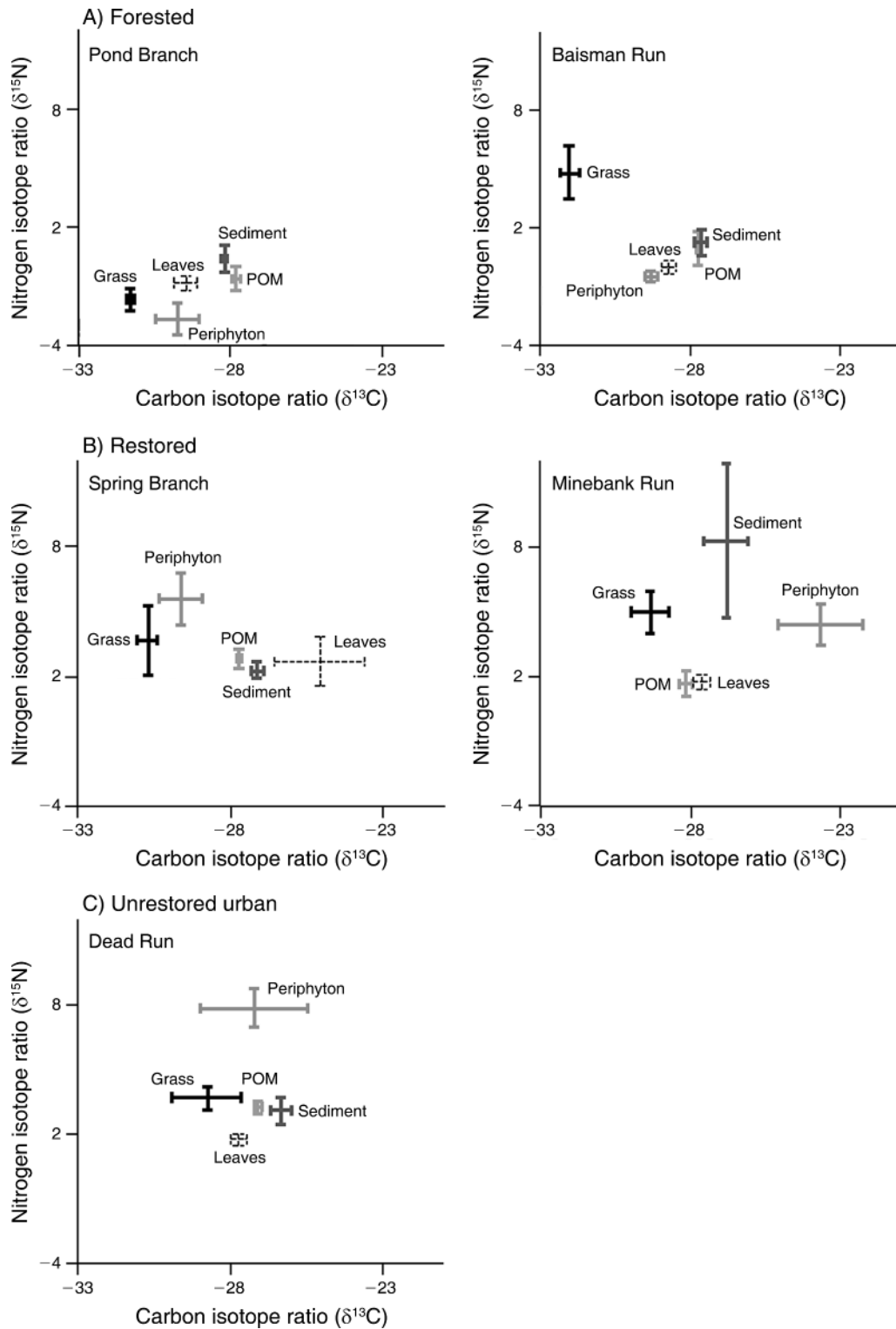
FIG. 7. Comparison of $\delta^{13}C$ and $\delta^{15}N$ isotopic ratios in periphyton, leaves, grass, and particulate organic matter (POM) from (A) forested ($N = 20$), (B) restored ($N = 14$), and (C) unrestored urban sites ($N = 13$). Values are means ± SE. Scotts Level Branch was omitted because of cost and time constraints.
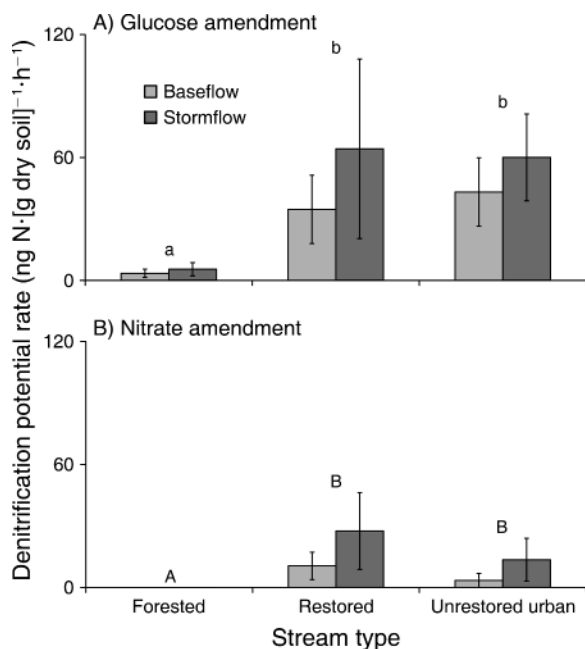
FIG. 8. Carbon amendments produced higher denitrification potential rates than nitrogen amendments to sediments incubated with streamwater collected during baseflow and stormflow conditions. Comparison of denitrification potential rates associated with sediments amended with (A) glucose and (B) nitrate. Values are means ± SE; $N = 4$ denitrification rate measurements for each bar. Letters above the bars represent comparisons between site types that are significant ($\alpha = 0.05$) according to Tukey's studentized range (HSD) test.

diverge from the river continuum model, which highlights the importance of leaf litter from trees (Vannote et al. 1980, Wiley et al. 1990, Kaushal and Belt 2012).

Relative importance of organic carbon sources to denitrification in urban streams

Urbanized streams are more likely to have flashy hydrology (Striz and Mayer 2008), and storms that carry nutrient- and DOC-rich water to the stream (Paul and Meyer 2001, Allan 2004). We did not observe a significant difference in denitrification rates between stormflow vs. baseflow. Our laboratory experiments showed that labile organic carbon availability can be more important in limiting denitrification than nitrate availability in our urban streams during baseflow and stormflow conditions. Previous studies suggest that nitrate concentrations are an important regulator of differences in denitrification potential; however, once levels of nitrate are sufficient (which occurs at fairly low suburban densities) organic carbon availability becomes important (Arango et al. 2007, Inwood et al. 2007).

Our results also suggest that shifts in natural organic matter may influence denitrification in urbanized and restored streams (Dosskey et al. 2010). The question of how different organic carbon sources can differentially influence denitrification rates extends broadly to fields as

varied as prairie ecosystems, agriculture, and wastewater treatment (see Appendix A). We found that grass clippings from urban areas stimulated the highest denitrification rates. Periphyton produced intermediate rates across sites. Clipping and blending the grass may have led to larger changes in DOM availability than harvesting and blending of leaves or periphyton. While it might have been preferable to compare fresh leaves with the clippings, we note that clippings are produced and transported to the stream throughout the growing season, while leaves are only produced once per year during autumn. Therefore, comparing fresh clippings and leaves and periphyton resident in the stream channel may actually be more realistic of in situ conditions. Although decaying leaves collected from the stream had diminished DOM availability because rapid loss of
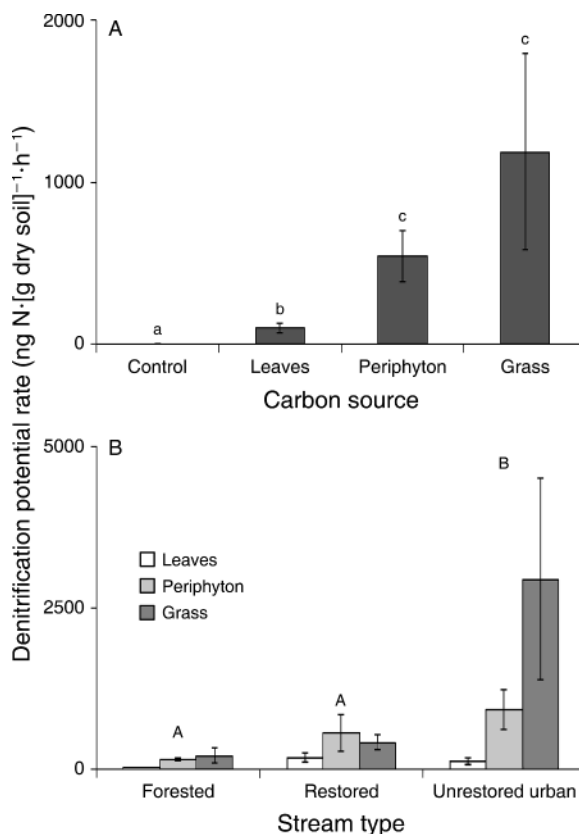

FIG. 9. Comparison of denitrification potential rates associated with different naturally occurring organic carbon sources at two forested (Pond Branch and Baisman Run), two restored (Spring Branch and Minebank), and two unrestored urban (Dead Run and Scotts Level Branch) streams. Values are means ± SE. Letters above the bars represent comparisons (lowercase is carbon source, and uppercase is stream type) that are significant ($\alpha = 0.05$) according to Tukey's studentized range (HSD) test. (A) Results from all six sites are averaged and divided into categories of organic carbon sources, and (B) data are divided by site type and organic carbon source. Some data bars appear to be missing because the potential rates were comparatively low in (A) control and (B) leaves at the forested site.

TABLE 2. Lipid biomarker data from two locations at forested Pond Branch, two locations at restored Spring Branch, and three locations at restored Minebank Run streams (Canuel and Martens 1993, Waterson and Canuel 2008).

		Lipid biomarkers				
			Terrestrial-source indicators			
			Long-chain alcohols		Plant sterols	
Site	Terrestrial : aquatic fatty acid ratio	Long-chain fatty acids (%)	Percentage	Tukey comparison	Percentage	Tukey comparison
Pond Branch				A		A
Forested site 1	0.17 ± 0.11	3.99 ± 2.34	29.40 ± 11.88		47.68 ± 11.29	
Forested site 2	0.36 ± 0.07	7.10 ± 0.29	37.56 ± 8.45		56.44 ± 16.23	
Spring Branch				AB		B
Restored 1994–1997 site 1	0.56 ± 0.27	11.67 ± 3.90	45.68 ± 2.62		76.49 ± 0.85	
Restored 1994–1997 site 2	0.05 ± 0.01	1.36 ± 0.37	16.06 ± 2.24		68.36 ± 1.63	
Minebank Run				B		A
Restored 1998–1999 site 1	0.04 ± 0.02	0.83 ± 0.46	28.98 ± 4.31		38.93 ± 8.22	
Restored 1998–1999 site 2	0.05 ± 0.05	1.16 ± 1.16	19.00 ± 3.23		66.02 ± 3.07	
Restored 2004–2005 site 3	0.01 ± 0.01	0.29 ± 0.29	5.978 ± 0.54		31.27 ± 0.16	

Notes: Values are means ± SE. Letters represent comparisons between the three streams that are significant ($\alpha = 0.05$) according to Tukey's studentized range (HSD) test.

soluble leaf constituents occurs after immersion, decaying leaves are still an important source of DOM because, as they sit in streams, they are colonized by fungi and other microorganisms that continue to break down the leaf matrix and release DOC while building an organic carbon-rich biofilm (Gessner et al. 1999). Thus, our results show that fresh lawn clippings may have substantial short-term effects on N removal via denitrification, while leaves and periphyton may have more sustained N removal over time at a lower instantaneous denitrification rate. Overall, higher denitrification rates occurred in the urban sites, intermediate denitrification in the restored sites, and relatively lower denitrification in the forested sites. Possible reasons for higher denitrification potential rates in urban areas are elevated nitrate concentrations and organic carbon quality (Groffman et al. 2005, Harrison et al. 2011).

In order to understand how changes in organic carbon sources might influence the mass balance of nitrate in a stream, we multiplied measured DOC loads by published bioavailability values to estimate the amount of denitrification that could be potentially supported at each site.

Reported ranges for DOC bioavailability in forested streams included 0–40% in the Rocky Mountains of Colorado, USA (Kaushal and Lewis 2005), and 21 ± 7% in New Jersey, USA (Wiegner and Seitzinger 2004), and ~2% in Swan-Canning catchments in Australia (Petrone et al. 2009).

In urban streams, DOC bioavailability ranged from 16% to 17% with a mode of 16% (Petrone et al. 2009). We used a value of 16% DOC bioavailability for the urban streams, as well as for the restored streams because we could not find literature values of DOC bioavailability in restored streams. Bioavailability of organic carbon varies across geography and studies, so we opted to use values from a common study that examined both urban and forested land uses (Petrone et al. 2009). We estimated the percentage of daily nitrate load reduction (kg/d) that may be possible with bioavailable DOC from each stream using the following formula:

$$NO_3^- \text{ load reduction} = 100 \times \frac{\text{bioavailability} \times \text{DOC load}}{NO_3^- \text{ load} \times 4}$$

where bioavailability is the percentage of DOC that is assumed to be bioavailable, DOC load is the mean daily DOC load (kg/d), and NO_3^- load is the mean daily nitrate load (kg/d). The equation is divided by 4 because 4 mg of CBOD are needed for each mg of nitrate

TABLE 3. Estimation of potential nitrate load reduction through denitrification based upon available DOC and literature ranges for DOC bioavailability (from Petrone et al. 2009).

Stream	Discharge (L/d)	Mean DOC (kg/d)	Estimated bioavailability (%)	Bioavailable DOC (kg/d)	Bioavailable DOC (kg/d) ÷ 4	Mean nitrate (kg/d)	Potential nitrate load removal (%)
Pond Branch (forested)	345 279	108	2	2.16	0.54	1	54
Baisman Run (forested)	4 653 736	874	2	17.48	4.37	747	1
Spring Branch (restored)	23 608	5	16	0.80	0.22	9	2
Minebank Run (restored)	5 261 577	1 319	16	211.04	52.76	859	6
Dead Run (unrestored urban)	18 192 911	7 430	16	1 188.80	297.19	2 497	12
Scotts Level Branch (unrestored urban)	8 103 227	3 415	16	546.40	136.60	1 029	13

TABLE 2. Extended.

	Lipid biomarkers	
	Aquatic-source indicators	
	Diatom sterols	
Short-chain alcohols (%)	Percentage	Tukey comparison
		A
32.62 ± 13.27	0.89 ± 0.89	
17.59 ± 8.48	6.30 ± 1.76	
		AB
11.92 ± 1.24	1.92 ± 0.18	
9.84 ± 0.97	9.69 ± 0.35	
		B
17.31 ± 3.60	6.68 ± 0.52	
25.34 ± 6.05	7.30 ± 0.25	
9.51 ± 0.59	40.41 ± 3.36	

removed (U.S. EPA 1993). Results show that Pond Branch (forested) has ample carbon for 54% removal of nitrate by denitrification, while Baisman Run (forested) is more limited because it has high nitrate loading from septic systems and less bioavailable DOC. At the urban sites, bioavailable DOC load is highly limiting to denitrification despite higher bioavailability (Table 3). Even if the DOC load were 100% bioavailable, there would not be enough for complete denitrification at Spring Branch (restored); instead, it would only be enough for denitrification of 14% of the nitrate load. Thus, according to this estimate, bioavailable DOC limits N removal through denitrification at all sites besides Pond Branch (forested), where nitrate concentrations are relatively low.

Implications for riparian management and restoration

Studies of stream restoration effects on ecosystem functions such as N cycling are limited but growing (Bukaveckas 2007, Roberts et al. 2007, Kaushal et al. 2008b, Klocker et al. 2009, Sivirichi et al. 2011). Previous work has shown linkages between DOC and nitrate (Munn and Meyer 1990, Mayer et al. 2010, Sivirichi et al. 2011) and indicate that management efforts to increase groundwater residence time and increase DOC availability may improve N removal capacity (Striz and Mayer 2008, Mayer et al. 2010). Integrated stormwater management and stream restoration may be a means to foster N removal capacity by enhancing denitrification in some cases by increasing availability of organic carbon, stream–floodplain interaction, and increasing residence time (Collins et al. 2010, Mayer et al. 2010, Sivirichi et al. 2011). Denitrification rate potentials in restored streams have been shown to increase with increasing amounts of organic carbon in riparian-zone sediments (Gift et al. 2010) and debris dams (Groffman et al. 2005, Harrison et al. 2012). Surprisingly, organic carbon from residential lawns was

shown to impact the N cycle of streams more than background sources. Given that C:N stoichiometry can be an important influence on denitrification, management strategies that increase organic carbon relative to N may increase N removal (Park et al. 2008, Taylor and Townsend 2010). Therefore, an improved understanding of coupled carbon and nitrogen biogeochemical cycles in urban watersheds is critical for enhancing denitrification and N removal along larger managed stream networks (Sivirichi et al. 2011, Kaushal and Belt 2012).

CONCLUSIONS

We found that concentrations and loads of nitrate and DOC varied with runoff and there were elevated loads of organic carbon at urban sites. Stable-isotope and lipid biomarker data demonstrate that urbanization alters the amount and source of organic carbon delivered to streams. Management of riparian vegetation influences denitrification rates at the riparian–stream interface. Managing amounts, sources, and quality of organic carbon is critical for managing nitrogen flux in stormwater management systems and urban restoration stream projects. Future work should investigate how specific restoration and stormwater management strategies impact the coupling of carbon and nitrogen cycles, and assess the implications for managing denitrification in human-dominated watersheds.

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SUPPLEMENTAL MATERIAL

Appendix A

Table of studies from a broad range of land uses and contexts that examine how different organic carbon sources affect denitrification rates (*Ecological Archives* M082-016-A1).

Appendix B

Aerial photographs of sampling locations at the forested Pond Branch and Baisman Run, the restored Minebank Run and Spring Branch, and the unrestored urban Scotts Level Branch and Dead Run streams (*Ecological Archives* M082-016-A2).

Data Availability

Data associated with this paper have been deposited in Dryad: http://dx.doi.org/10.5061/dryad.4gk00