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ECOSPHERE

Sediment chemistry of urban stormwater ponds and controls on denitrification

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Abstract.   Stormwater ponds and retention basins are ubiquitous features throughout urban landscapes. These ponds are potentially important control points for nitrogen (N) removal from surface water bodies via denitrification. However, there are possible trade-offs to this water quality benefit if high N and contaminant concentrations in stormwater pond sediments decrease the complete reduction of nitrous oxide (N_2O), a potent greenhouse gas, to dinitrogen (N_2) during denitrification. This may occur through decreasing the abundance or efficiency of denitrifiers capable of producing the N_2O reductase enzyme. We predicted that ponds draining increasingly urbanized landscapes would have higher N and metal concentrations in their sediments, and thereby greater N_2O yields. We measured potential denitrification rates, N_2O reductase (*nosZ*) gene frequencies, as well as sediment and porewater chemistry in 64 ponds distributed across eight U.S. cities. We found almost no correlation between the proportion of urban land cover surrounding ponds and the nutrient and contaminant concentrations in the stormwater pond sediments within or across all cities. Regression analysis revealed that the proportion of potential N_2 and N_2O production that could be explained was under different environmental controls. Our survey raises many new questions about why N fluxes and transformations vary so widely both within and across urban environments, but also allays the concern that elevated metal concentrations in urban stormwater ponds will increase N_2O emissions. Urban stormwater ponds are unlikely to be a problematic source of N_2O to the atmosphere, no matter their denitrification potential.

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INTRODUCTION

Urban stormwater runoff is a leading cause of surface water impairment throughout the developed and developing world (US EPA 2004, UNEP GEMS 2008). Constructed stormwater retention and detention ponds are designed to reduce peak stormwater discharge and remove pollutants through passive sedimentation (NRC 2009). The construction of these ponds is a key way that municipalities attempt to reduce the impact of stormwater runoff on downstream water bodies (Bernhardt et al. 2008). Stormwater ponds have consequently become a ubiquitous feature of urban areas in all physiographic regions (Steele et al. 2014).

Stormwater ponds may also act as critical control points (sensu Bernhardt et al. 2017) in watershed-scale nitrogen (N) retention by capturing particulate N and by supporting N removal through denitrification (Zhu et al. 2004, Collins et al. 2010, Bettez and Groffman 2012, Larson and Grimm 2012). Denitrification is a microbially mediated process by which nitrate (NO_3^-) is reduced to gaseous N products (i.e., nitric oxide [NO], nitrous oxide [N_2O], and dinitrogen [N_2]) under low oxygen conditions. It is the primary mechanism by which excess NO_3^- can be permanently removed before entering downstream aquatic ecosystems.

The timing and magnitude of excess NO_3^- delivery to stormwater ponds, and the emissions of N gases from these ponds, can be highly heterogenous within and among cities because of the wide range of residential, commercial, and industrial land uses found in most major U.S. cities. We currently know little about the chemical composition of stormwater pond sediments from these complex urban landscapes and whether stormwater ponds act as important bioreactors to remove NO_3^- as an aquatic pollutant.

The proportions of N gases that are released to the atmosphere by denitrification (i.e., N_2O vs. N_2) in stormwater pond sediments are also unclear. High reactive N concentrations tend to result in the emission of more gaseous N_2O relative to inert N_2 gas, but at the same time, high concentrations of reduced carbon tend to favor complete reduction to N_2 (Firestone et al. 1980, Weier et al. 1993). Understanding whether rates of NO_3^- removal in stormwater ponds are associated with N_2O production is important, because N_2O is a potent greenhouse gas that contributes substantially to both global warming and stratospheric ozone destruction (Ravishankara et al. 2009).

Differences in the community composition and abundance of microbial denitrifiers in pond sediments can also alter the composition of the final gaseous N products released to the atmosphere (Wallenstein et al. 2006). While denitrifiers with genes encoding for nitrite reductase (*nirS* and *nirK*) that lead to the production of N_2O are generally unperturbed by many contaminants (Bissett et al. 2013), populations of denitrifiers capable of producing the nitrous oxide reductase enzyme (via the *nosZ* gene) are sensitive to metal contamination (Holtan-Hartwig et al. 2002). A low abundance or efficiency of these nitrous oxide reducers under stressful conditions can lead to a higher proportion of gaseous N released as N_2O relative to N_2 (the N_2O yield). The abundance of nitrous oxide reducers has been shown to decline in the sediments of increasingly urban environments (Perryman et al. 2011*b*, Wang et al. 2011) which tend to have elevated concentrations of metals (Makepeace 1995). This implies that the combination of elevated NO_3^- and contaminant concentrations within the sediments of ponds receiving runoff from highly urbanized landscapes could significantly increase N_2O yields (Bernhardt et al. 2008).

In this study, we determined whether ponds receiving runoff from urbanized landscapes support high rates of denitrification and N_2O production. We measured potential denitrification rates, denitrification gene frequencies (*nosZ* gene), and sediment and porewater chemistry in 64 permanently wet ponds distributed within and around eight U.S. cities. Our goal was to document and explain the variation in potential rates of the denitrification products N_2 and N_2O from urban surface waters as a function of watershed characteristics and microbial gene abundance. We expected that potential denitrification rates would increase as a function of the amount of adjacent urban land cover and that the proportion of total denitrification products emitted as N_2O would be higher in more heavily urbanized ponds because of higher N and contaminant loading. We compared our denitrification, sediment chemistry, and porewater chemistry measurements with those reported in the literature and to consensus-based ecotoxicological thresholds.

Methods

Study ponds

During the summer of 2014, we collected sediments from 64 ponds within and around the metropolitan statistical areas of Baltimore, Maryland; Boston, Massachusetts; Durham, North Carolina; Miami, Florida; Minneapolis-St. Paul, Minnesota; Phoenix, Arizona; Portland, Oregon; and Salt Lake City, Utah (Fig. 1; Appendix S1: Fig. S1). We selected eight permanently wet ponds per city from a database of urban water bodies compiled by Steele et al. (2014) to span the maximum possible range in adjacent urban land cover. This range included ponds draining a variety of urban land uses (i.e., residential, highways, commercial) to ponds on the outskirts of cities draining primarily unmanaged landscapes. Land cover metrics for both pond watersheds and buffers of increasing distance from the ponds were calculated based on the 2011 National Land Cover dataset (NLCD) and the associated Impervious Surface Cover dataset, both from the United States Geological Survey (USGS) Seamless Server (Fig. 1; Homer et al. 2015). We calculated the proportion of urban land cover in buffers and watersheds as the sum of the percent area covered by NLCD land cover classes 22, 23, and 24 within 250 m of the pond edge. Mean annual precipitation was calculated using PRISM precipitation data for 2010–2014 for each city (PRISM Climate Group 2017).

We delineated pond watersheds using 3 and 10-m digital elevation models, ArcGIS Hydrology tools (ESRI, Redlands, California, USA), and the USGS National Hydrography Dataset. Ponds located in areas with moderate topographic relief, suburbs, and natural areas generally had watersheds delineated accurately with an elevation model-flow accumulation method. However, in urban areas where artificial storm water drainage systems direct runoff into ponds, the watershed delineation process was challenging, particularly when drainage was not visually evident from high-resolution aerial photographs because we lacked access to GIS storm water data in most cities. Due to these many complications in delineating urban watersheds, a confidence rating was assigned to each watershed (Appendix S1: Table S1). In cases of low ratings, knowledge of the storm water drainage system was deemed necessary to accurately delineate the watershed and this led to the exclusion of calculating two pond watersheds in Phoenix, Arizona, USA, where such knowledge was insufficient.

Sediment collection, characterization, and chemical analysis

We sampled sediment in 2- to 3-d sampling campaigns within each city from June to August 2014 (Appendix S1: Table S2). At each pond, a 2-L composite sample of sediment was taken by scooping the top 5–10 cm of sediment at a 0.5-L m water depth at three distributed edge locations per pond. Surface water samples were taken from a single location and filtered in the field through Whatman GF/F filters (Whatman, Piscataway, New Jersey, USA). Samples were held at 4°C until they were shipped on ice to Durham, North Carolina, USA, arriving within a maximum of 6 d after sampling.

Upon arrival, surface water samples were frozen at −20°C and sediment samples were stored at 4°C. Composite sediment samples from each pond were sieved through a 2-mm brass sieve. Potential copper and zinc contamination by the sieve was assumed to be insignificant relative to the high concentrations in the sediments, and therefore, copper and zinc were still included in further analyses. We subsampled <2 mm diameter sediment into triplicate 1.7-mL plastic centrifuge tubes for DNA analysis and froze the tubes at −80°C. Sieved sediments were then subsampled to determine sediment characteristics such as organic matter content, pH, bulk density, and microbial biomass. We dried sediment samples at 60°C for 48 h and then combusted them at 500°C for 4 h to determine the ash-free dry mass (AFDM). We calculated percent organic matter (% OM) using the equation %OM = ((dry weight − AFDM)/dry weight) × 100. Sediment pH was measured using 3-g air-dried samples in 5 mL of 0.01 mol/L $CaCl_2$, the addition of which can lower sediment pH by ~0.5 pH units compared to water pH (Carter 1993). Ten percent of pH samples were run in triplicate with an average standard deviation of 0.14. The bulk density of sieved sediments was determined by weighing 25 mL of homogenized wet sediment. The maximum potential rate of heterotrophic respiration was estimated using a modified version (Fierer et al. 2003) of the substrate-induced respiration (SIR) method (West
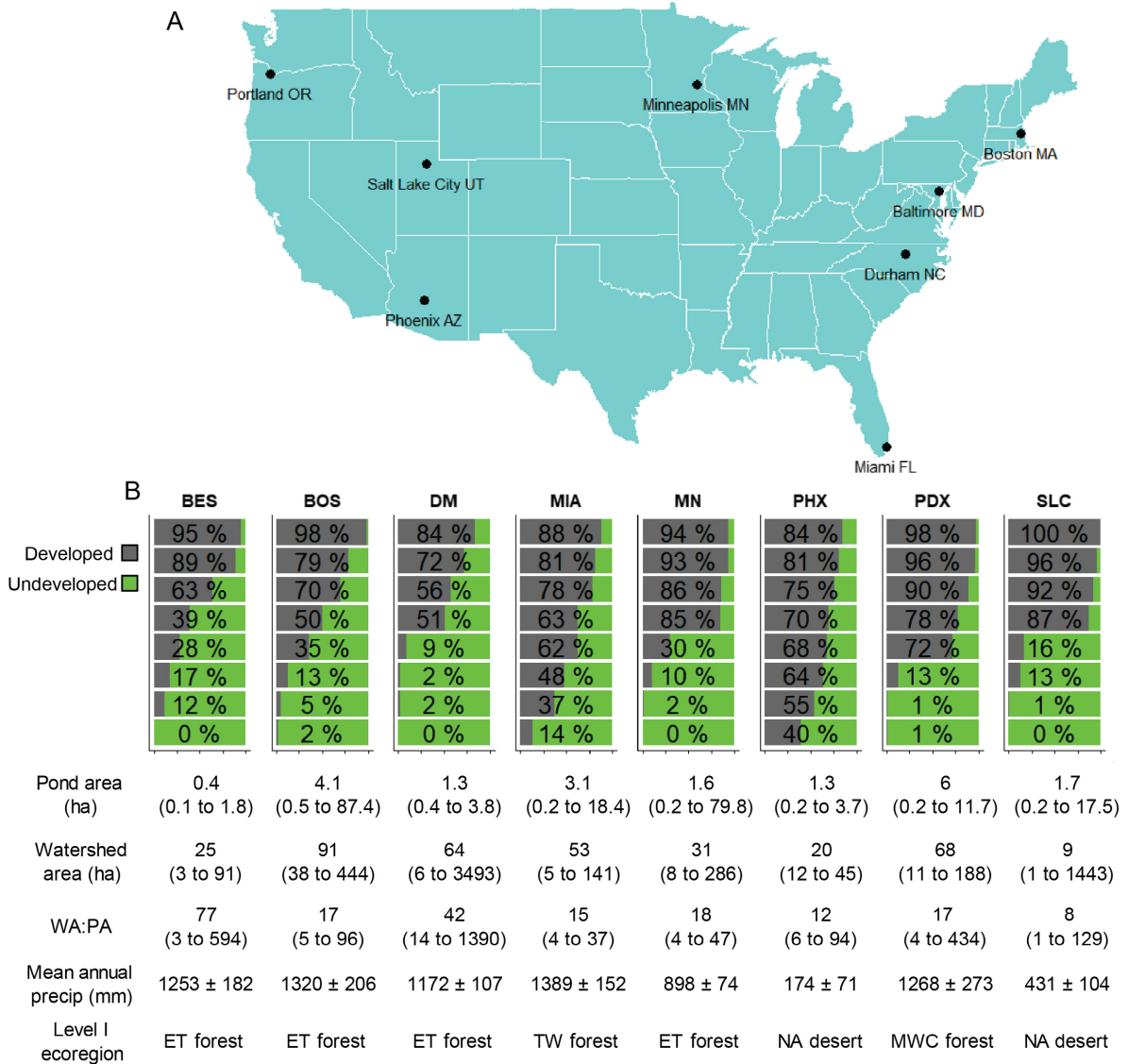
Fig. 1. (A) Map of the eight metropolitan study areas (MSAs). (B) The proportion of urban land cover (NLCD 2011, classes 22–24) within a 250 m buffer around each study pond are shown in gray and written in black over each horizontal bar. Undeveloped areas are shown in green bars. The median area (range in values) of each pond, its watershed, and the ratio of the watershed area to pond area (WA : PA), and the 2010–2014 mean annual precipitation (±SD) are shown below a corresponding boxplot for each MSA. Level I ecoregions include Eastern Temperate Forest (ET Forest), Tropical Wet Forest (TW Forest), North American Desert (NA Desert), and Marine West Coast Forest (MWC Forest; Omernik 1987). Study metropolitan areas include Baltimore, Maryland (BES), Boston, Massachusetts (BOS), Durham, North Carolina (DM), Miami, Florida (MIA), Minneapolis-St. Paul, Minnesota (MN), Phoenix, Arizona (PHX), Portland, Oregon (PDX), and Salt Lake City, Utah (SLC).

and Sparling 1986) as a proxy of microbial biomass. Triplicate sediment samples (5 ± 0.25 g) were amended with 10 mL autolyzed yeast extract solution and sealed in 40-mL acid-washed tubes. Rates of CO_2 production were measured over 4.5 h using an infrared gas analyzer (LI-6265; LI-COR, Lincoln, Nebraska, USA) with N_2 as the carrier gas. Because of the wide range of sediment

types, we calculated the maximum rate (μg C-CO$_2$·[g dry sediment]$^{-1}$·h^{-1}) measured over three time points as the index of SIR-responsive microbial biomass.

Porewater solutes were extracted by shaking 2.5 ± 0.25 g of wet sediment with 25 mL of deionized water for 4 h at room temperature. After settling overnight at 4°C, we centrifuged samples, filtered the supernatant through Whatman GF/F filters, and froze the porewater at −20°C until analysis. We measured chloride (Cl$^-$), nitrate (NO$_3^-$), bromide (Br$^-$), and sulfate (SO$_4^{2-}$) concentrations in porewater samples using an ion chromatograph (ICS-2000) equipped with an AS18 anion column and KOH eluent generator (Dionex Corporation, Sunnyvale, California, USA). Dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) were measured on a TOC analyzer with a TN module (TOC-V CPH; Shidmadzu, Kyoto, Japan). We measured soluble reactive phosphate (SRP) concentrations on a Lachat QuickChem 8500 automated system (Lachat Instruments, Loveland, Colorado, USA) using EPA method 365.1. Ammonium (NH$_4^+$) concentrations were measured using the OPA fluorometric technique (Holmes et al. 1999) and a field fluorometer (10 AU; Turner Designs, Sunnyvale, California, USA). Br$^-$ and SRP were not used in further analyses because 71% of ponds had Br$^-$ concentrations and 61% of ponds had SRP concentrations below the detection limit of 0.01 mg/L.

To determine sediment metal concentrations, dried sediments (48 h at 60°C) were ground using a mortar and pestle and passed through a #80 stainless steel mesh sieve (Method 3051A, US EPA 2007). Sediment samples were microwave digested (SW846-3051, MARS HP500 Plus; CEM Corporation, Matthews, North Carolina, USA) at a ratio of 0.3 g tissue dry weight to 9 mL omnitrace nitric acid (HNO$_3$) and 3 mL omnitrace hydrochloric acid (HCl). Digested samples were analyzed for arsenic (As), aluminum (Al), cadmium (Cd), calcium (Ca), chromium (Cr), cobalt (Co), copper (Cu), iron (Fe), lead (Pb), magnesium (Mg), manganese (Mn), molybdenum (Mo), nickel (Ni), phosphorus (P), potassium (K), silicon (Si), silver (Ag), sodium (Na), strontium (Sr), and zinc (Zn) by inductively coupled plasma-mass spectrometry (ICP-MS, X-Series, Thermo Electron Corporation, Waltham, Massachusetts, USA). For every 18

samples run, we ran one sample in triplicate, two method blanks, and two replicates of fresh water sediment certified reference material (CNS392, RT Corporation, Laramie, Wyoming, USA). Certified reference material recovery was >94% for Zn and Pb ($n = 8$). Values below the minimum limit of detection (LOD = 1 ppb) were replaced with half the minimum detection limit (0.5 ppb) before all measurements were standardized by organic carbon content determined from AFDM.

Denitrification incubations

We simultaneously measured rates of N$_2$ and N$_2$O production during laboratory incubations to estimate potential denitrification in pond sediments. Sediment was weighed into glass containers with air-tight lids (~75 g into 300 mL BOD bottles or ~15 g into 60 mL BOD bottles) following methods used in Fork and Heffernan (2014). Common water for the incubations was spiked with 0.72 g of KNO$_3$ and 0.5 g glucose per liter (Groffman et al. 1999). The goal of the denitrification potential assay is to remove limiting factors to facilitate the maximum activity of existing denitrification enzymes present in the sediment when the sample was taken. However, due to the diversity of the denitrifying community, certain components of the community are not optimized in this assay.

To promote anoxic conditions during incubations, spiked water in each jug was degassed by bubbling compressed helium gas for ~30 min before use. Water was siphoned from the bottom of the jug into glass bottles with sediment until they were overflowing. Then bottles were inverted several times to release any bubbles trapped in the sediment before being refilled again to overflowing. Glass stoppers were pushed into place, and the time was recorded as the start of the incubation. Bottles were kept in the dark at room temperature while incubating and inverted every two hours to minimize diffusion limitation of nitrate supply to the sediment.

Triplicate samples for each time point were run on a membrane-inlet mass spectrometer (MIMS) to determine dissolved N$_2$ and Ar concentrations in the water layer of the sediment incubations (Kana et al. 1994). An in-line copper reduction column heated to 600°C was used to reduce O$_2$ interference with N$_2$ measurements (Eyre et al. 2002). BOD bottles were destructively

sampled at approximately 20 min, 3 h, and 6 h after the start of the incubation. Standards for N_2 concentrations (humid-atmosphere-equilibrated deionized water stirring in high-precision water baths) at 12°C and 22°C were run every six to eight samples. Control BOD bottles filled with only degassed, spiked water were run for each time point and group of samples ($n = 6$). To correct for drift, we interpolated parameter values based on a linear slope and intercept calculated from the change in the N_2:Ar in the standards over time. To correct for the common water being originally degassed, we calculated the Ar concentrations in the controls based on solubility formulas from Hamme and Emerson (2004) and then multiplied the control Ar value by the N_2:Ar of each sample in the group associated with that control to determine the N_2 concentrations in each sample. Following the determination of dissolved N_2 concentrations, we determined the maximum rate of dry-sediment N_2 production measured among the three time points by dividing the average change in N_2 by the incubation time and dry sediment mass. This approach was taken to avoid reporting differences in peak rates that might be attributable to denitrifier response time as a function of sediment type.

To measure N_2O production potential rates, an inverted 60-mL evacuated headspace bottle simultaneously pulled water up through a double-gauged needle near the MIMS intake. Headspace equilibrations were performed by over pressurizing each bottle with 15 mL of N_2 gas and shaking for two minutes. Gas (10 mL) was pulled out of the headspace and injected into 9-mL gas vials. We recorded the volume of water in the headspace bottle. N_2O concentrations were measured within a week using a Teledyne Tekmar 7000 headspace autosampler (Teledyne Tekmar, Mason, Ohio, USA) to inject samples into a Shimadzu GC-17A ver.3 gas chromatograph with a Porapak Q column and electron capture detector (injector temperature = 380°C, column temperature = 80°C, detector temperature = 340°C, with N_2 carrier gas; Shimadzu Corporation, Kyoto, Japan). We used Bunsen coefficients to determine N_2O concentrations in each sample and converted N_2O to N_2O–N. The maximum rate of N_2O production during the incubations was calculated by dividing the average change in N_2O by the incubation time and dry sediment mass in the flask.

Functional gene abundance

We measured the abundance of the *nosZ* gene, which encodes for nitrous oxide reductase, in sediments to compare the abundance of nitrous oxide reducers among study ponds. We extracted DNA from sediments in triplicate for each pond with PowerSoil DNA Isolation kits (MoBio Laboratories, Carlsbad, California, USA). Following extraction, samples were diluted to 3 ng/μL to avoid PCR inhibition. Denitrifier DNA was then amplified using *nosZ* primers (Scala and Kerkhof 1998) and iTaq Universal SYBR mix (Bio-Rad Laboratories, Hercules, California, USA). The average efficiency of the PCR reaction was 79% (minimum: 76%), and all standard curves had R^2 values ≥0.997. Copies were normalized to the amount of extracted sediment. We focused our microbial analysis on dissimilatory reduction via typical denitrifier *nosZ* gene abundance, which encodes for N_2O reductase. We did not try to evaluate the presence or functional influence of atypical non-denitrifier N_2O reduction (Sanford et al. 2012, Van Groenigen et al. 2015) that could have occurred.

Statistical analysis

The goal of this study was to span the widest possible range of conditions in urban stormwater ponds across the United States to understand the drivers of denitrification and N_2O yields. We performed all statistical analyses using R version 3.4.1 (R Core Team 2017). To determine whether sediment and porewater characteristics were correlated with adjacent urban land cover, we first calculated the proportion of urban land cover within each delineated watershed and 50, 100, 250, 500, 750, and 1000 m buffers around each pond. We chose the proportion of urban land cover within a 250 m buffer as representative of the intensity of urbanization draining into each pond. We chose to do this because we did not have watershed land cover information for every site, but land cover metrics were available for each buffer distance around every pond. In addition, the 250 m buffer had the strongest correlation with the proportion of urban land cover in delineated watersheds.

Spearman rank correlation analysis was then performed between site characteristics and the proportion of adjacent urban land cover within cities and across all ponds with Bonferroni

corrections within each variable ($n = 9$). This approach was also used to evaluate correlations between adjacent urban land cover and potential rates of denitrification ($n = 24$). We evaluated differences in potential denitrification rates (N_2, N_2O, and N_2O yield) among cities with pairwise comparisons using Tukey and Kramer (Nemenyi) tests with Tukey-Dist approximation for independent samples in the "PMCMR" package (Pohlert 2014). This pairwise comparison was also used for evaluating differences among cities in sediment and porewater characteristics.

We developed multiple linear regression models for potential denitrification rates and denitrifier gene abundance across all surveyed ponds that first separately and then synergistically compared the explanatory power of four different categories of possible predictor variables: substrates, stressors, microbial properties, and sediment and pond properties (Morse et al. 2012). Individual predictor variables were evaluated using log–log linear regressions (except for pH and bulk density which were not transformed) with adjusted P-values for multiple comparisons within each potential denitrification response variable using Bonferroni corrections ($n = 19$).

We built submodels with predictor variables from each categorical group separately for potential N_2 production, potential N_2O–N production, and $nosZ$ gene abundance. TDN, Cu, and Zn were discarded as predictor variables due to Spearman's rho correlations >0.7 with other variables that were better predictors to reduce redundancy within submodels. Candidate predictor variables were put into each multiple linear regression model by order of the adjusted R^2 of their individual relationships with potential denitrification rates. For each submodel, we used the step function in R to perform stepwise multiple linear regression (forward and backward selection) with automated variable selection based on corrected Akaike's information criterion (AIC). This procedure identified the combination of variables within each categorical group that best fit the rates of potential N_2 production, potential N_2O production, and $nosZ$ gene abundance. We then combined the AIC selected predictor variables from each submodel into a single model in the order of their individual relationships and used stepwise regression again to identify the best predictor variables across all groups. To reduce the probability of AIC overfitting, the final AIC was corrected for finite sample sizes.

RESULTS

Urban land cover within a 250 m pond buffer around ponds was not well correlated with either sediment or porewater chemistry (Tables 1, 2). Across all cities, only Zn sediment concentrations were positively correlated with the extent of development in pond buffers ($P = 0.02$, Spearman's rho = 0.40). Within individual cities, we found only one strong relationship between chemical

Table 1. Median (range in values) of sediment properties and porewater and sediment chemical substrates across ponds within different cities.

City	Sediment properties		Porewater and sediment chemical substrates				
	pH ($-\log([H^+])$)	Bulk density (g/mL)	% OM (% mass)	DOC (mg/kg sed)	NH_4^+–N	NO_3^-–N	TDN
BES	6.4 (4.1–7.8)	1.2 (1–1.7)	10.6 (0.3–30.1)	371 (33–1653)	18.9 (0.5–69.8)	1.5 (0.1–5.6)	30.7 (2.2–116)
BOS	4.6 (4.1–5.7)	1.6 (1–1.8)	7.0 (0.4–54.8)	132 (34–370)	1.2 (0–2.9)	0.9 (0.4–1.3)	8.2 (2.5–21.1)
DM	4.1 (3.2–7.2)	1.5 (1.1–1.9)	7.0 (4.9–13.6)	69 (45–325)	2.9 (1.1–35.9)	1 (0.4–2.3)	6.9 (2.7–59.8)
MIA	7 (6.7–7.1)	1.5 (1.3–1.8)	1.5 (0.5–13.8)	238 (108–450)	2 (0.3–7.1)	0.7 (0.3–13.8)	23 (5.1–366.8)
MN	6.8 (5.1–7.2)	1.4 (1–1.8)	3.1 (0.4–36.3)	126 (56–535)	5.9 (1.8–33.9)	0.5 (0.2–4.9)	10 (3.5–93.4)
PHX	7.2 (7.1–7.5)	1.6 (1.1–1.9)	3 (0.7–5.3)	174 (98–337)	3.4 (0.1–8.5)	0.2 (0.1–0.6)	6.3 (3.6–11.9)
PDX	4.8 (4.4–6.3)	1.2 (1.1–1.7)	11 (1.6–23.6)	57 (30–153)	4.5 (0.1–38.9)	0.2 (0.1–0.6)	10.4 (1.2–46.2)
SLC	7.4 (6.1–7.9)	1.2 (1.1–1.6)	3.3 (0.5–15.9)	125 (48–1505)	1.9 (0.1–45.4)	0.5 (0.3–1.4)	4.7 (2.8–63.2)
All	6.7 (3.2–7.9)	1.41 (1–1.9)	4.6 (0.3–54.8)	134 (30–1653)	3.5 (0–69.8)	0.6 (0.1–13.8)	9.0 (1.2–366.8)

Notes: City abbreviations are the same as in Fig. 1. Variables include pH, bulk density (Bulk Dens.), percent organic matter (%OM), dissolved organic carbon (DOC), ammonium (NH_4^+–N), nitrate (NO_3^-–N), and total dissolved nitrogen (TDN). DOC, NH_4^+–N, NO_3^-–N, and TDN were measured in the sediment porewater, while pH and Bulk Dens. were measured in the sediment. There were no significant Spearman correlations ($P < 0.05$ after Bonferroni corrections within variables) between variables and the proportion of urban land cover (NLCD 2011) within a 250 m buffer around each pond.

Table 2. Median (range in values) of microbial properties and porewater and sediment chemical stressors across ponds within different cities.

City	Microbial properties		Porewater and sediment chemical stressors					
	$nosZ$ (10^6 copies/g sed)	SIR (µg C· [g sed]$^{-1}$·h^{-1})	Cl$^-$ (mg/kg sed)	SO$_4^{2-}$ (mg/kg sed)	Zn (mg/g C)	Pb (mg/g C)	Cu (mg/g C)	As (mg/g C)
BES	368 (8–2449)	179 (23–1571)	231 (19–1622)	365 (13–1391)	1.4 (0.5–4)	0.5 (0.1–1.1)	0.6 (0.2–3.6)	40 (12–256)
BOS	45 (2–93)	24 (5–61)	160 (53–2921)	25 (10–166)	2.6 (0.1–14.3)	1.4 (0–6.2)	1.1 (0–4.3)	149 (9–1173)
DM	**173**† **(13–571)**	80 (25–266)	30 (16–62)	34 (2–100)	1.0 (0.5–2.7)	**0.2**‡ **(0.2–0.5)**	0.4 (0.2–0.4)	48 (33–81)
MIA	39 (26–238)	49 (34–91)	61 (30–292)	43 (20–104)	1.0 (0.1–4.1)	0.1 (0–1.1)	0.8 (0.1–4.5)	78 (22–160)
MN	83 (21–4825)	40 (15–264)	63 (29–1783)	99 (41–265)	2.6 (0.2–5.2)	0.3 (0.1–10.1)	0.8 (0.1–2)	82 (16–1193)
PHX	222 (13–1633)	74 (38–206)	236 (34–648)	178 (121–732)	1.9 (1.1–3.4)	0.4 (0.2–0.8)	1.6 (1–3.4)	270 (154–1078)
PDX	85 (3–1087)	225 (135–884)	25 (9–1211)	43 (5–305)	1.6 (0.3–4.3)	0.2 (0.1–0.6)	0.5 (0.2–1.2)	36 (4–166)
SLC	7 (4–42)	88 (52–517)	98 (44–1924)	340 (18–985)	2.6 (0.7–18.6)	0.6 (0.2–49.9)	1.5 (0.4–5.7)	189 (49–15,771)
All	51 (2–4825)	75 (5–1571)	67 (9–2921)	85 (2–1391)	**1.5**§ **(0.1–18.6)**	0.3 (0–49.9)	0.7 (0–5.7)	81 (4–15,771)

Notes: City abbreviations are the same as in Fig. 1. Variables include gene copies of $nosZ$ ($nosZ$), substrate inducible respiration (SIR), chloride (Cl$^-$), sulfate (SO$_4^{2-}$), zinc (Zn), lead (Pb), copper (Cu), and arsenic (As). Cl and SO$_4^{2-}$ were measured in the sediment porewater, while all other variables were measured in the sediment. Significant Spearman correlations ($P < 0.05$ after Bonferroni corrections within variables) between variables and the proportion of urban land cover (NLCD 2011) within a 250 m buffer around each pond are indicated in bold.
  † Spearman's rho = −0.96, $P = 0.027$.
  ‡ Spearman's rho = −0.95, $P = 0.009$.
  § Spearman's rho = 0.40, $P = 0.018$.

parameters and urban development, with a strong negative correlation between sediment Pb concentrations and the extent of buffer development across urban ponds in Durham, North Carolina, USA ($P = 0.009$, Spearman's rho = −0.95).

Although concentrations of inorganic N and carbon in the sediment and porewater spanned a wide range across all ponds, there were few differences in substrate availability among cities (Table 1). Porewater NH$_4^+$–N concentrations were highest and spanned the widest range in Baltimore, Maryland, USA, but median values in cities were otherwise similar. On average, NO$_3^-$–N accounted for <10% of TDN in the porewater. Baltimore, Maryland and Durham, North Carolina had the highest median NO$_3^-$–N concentrations, but two ponds in Miami, Florida had exceptionally high porewater NO$_3^-$–N (10.7 and 13.8 mg N/kg sed). The same two ponds had the highest porewater TDN (325.8 and 366.8 mg N/kg sed), while the rest of ponds had concentrations <120 mg N/kg sed. Sediment organic matter (%OM) was <25% in more than 95% of the ponds. Porewater DOC was exceptionally high in

one pond in Baltimore, Maryland (1653 mg C/kg sed) and another in Salt Lake City, Utah, USA (1505 mg C/kg sed), but otherwise cities had overlapping ranges.

Concentrations of potential stressors for denitrifying microbes that included metals in the sediment (As, Cu, Pb, Zn) and ions in the porewater (Cl$^-$ and SO$_4^{2-}$) spanned a wide range but had limited variation among cities (Table 2). All sediment metals correlated highly ($P < 0.05$, Spearman's rho > 0.5) with each other, and Pb and As were the most variable (coefficient of variation >4). Elevated concentrations of metals did not tend to co-occur within the same cities. Instead, Boston, Massachusetts, USA, had the highest median Pb concentrations, while Phoenix, Arizona, USA, had the highest median Cu and As concentrations. Porewater Cl$^-$ and SO$_4^{2-}$ were highly variable within cities; however, Durham, North Carolina and Portland, Oregon, tended to have lower concentrations.

Rates of potential N$_2$ and N$_2$O production and $nosZ$ gene abundance were highly variable both within and across cities (Table 2, Fig. 2).

Potential N_2 production rates ranged from 0.42 to 35.9 $\mu g \ N \cdot (g \ sed)^{-1} \cdot h^{-1}$, with the highest rates in ponds from Baltimore, Maryland, and Minneapolis, Minnesota. Potential N_2O production rates ranged from 0 to 1.7 $\mu g \ N \cdot (g \ sed)^{-1} \cdot h^{-1}$, with the highest average rates measured in the set of ponds sampled in Baltimore, Maryland. N_2O yield was below 20% (mean = 4%; median = 1%) for all cities except for Portland, Oregon (maximum = 39%), and Minneapolis, Minnesota (maximum = 22%). We observed no consistent relationship between urban land cover adjacent to the ponds and potential N_2 or N_2O production in pond sediments (Appendix S1: Fig. S2). Median copies of the *nosZ* gene in sediments ranged from 7×10^6 copies/g sed in Salt Lake City, Utah, to 368×10^6 copies/g sed in Baltimore, Maryland (Table 2). *nosZ* abundance was highly variable within cities, but Salt Lake City, Utah, tended to have the least denitrifiers with the gene in pond sediments (Appendix S1: Fig. S3). The proportion of adjacent urban land cover was only correlated with reduced *nosZ* abundance in Durham, North Carolina (Table 2).

No single variable explained more than 22% of the observed variation in either N_2 or N_2O production potential and no single variable explained any significant variation in measured N_2O yields (Table 3). The only land cover metric that was individually correlated with a denitrification product was the ratio of watershed area to pond area (WA:PA), which explained a small amount of the variation in N_2 production ($P = 0.045$, $R^2_{adj} = 0.13$). All substrate variables except sediment %OM were weakly positively related to N_2 production rates, yet there were no significant correlations between any measured substrate variable and the observed variation in N_2O production (Table 3). For stressors, only As concentrations were correlated (negatively) with N_2O production ($P = 0.03$, $R^2_{adj} = 0.14$) and only Cu concentrations were correlated (negatively) with N_2 production ($P = 0.04$, $R^2_{adj} = 0.13$). Porewater pH was the strongest negative predictor of N_2O production ($P = 0.02$, $R^2_{adj} = 0.15$). Single variable regression models predicting *nosZ* abundance explained at most 22% of its variance and had predictor variables in common
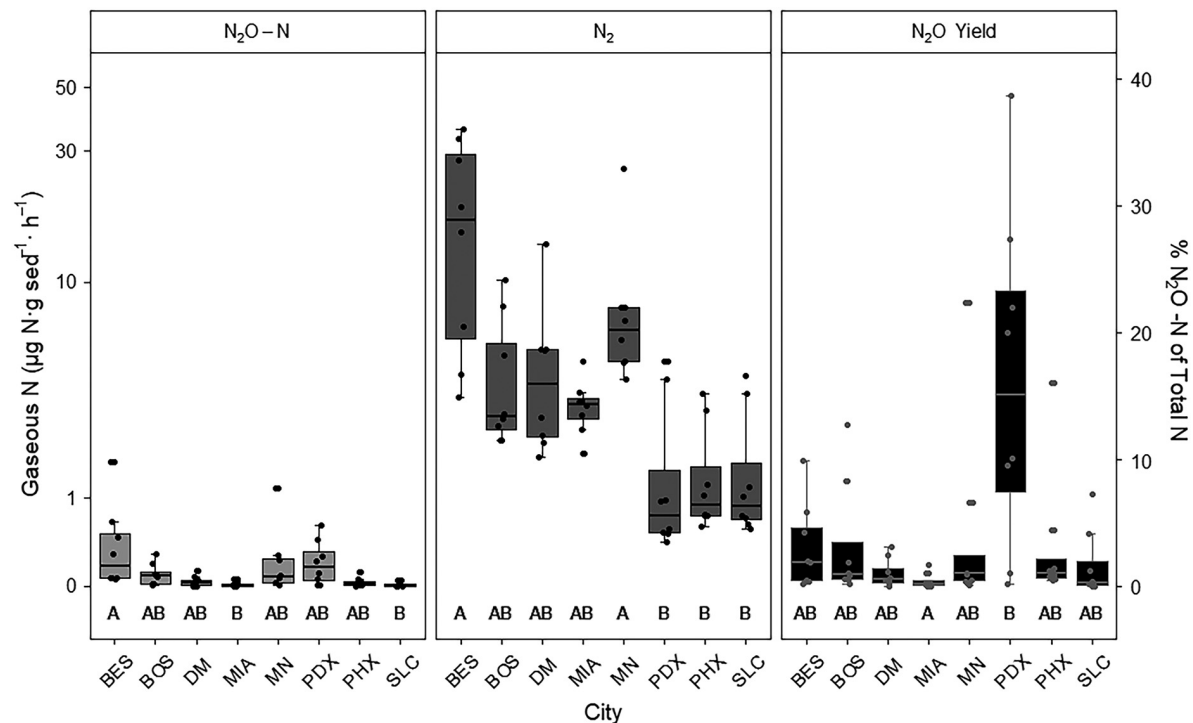

Fig. 2. Potential N_2O–N and N_2 production (left y-axis) and N_2O yield (right y-axis) within each study metropolitan area (same abbreviations as Fig. 1).

Table 3. Summary of log-transformed linear regression models among potential denitrification rates, nitrous oxide reducer abundance, and explanatory variables.

Category	Variable	Source	Units	df	N₂ +/−	N₂ R^2_{adj}	N₂O–N +/−	N₂O–N R^2_{adj}	N₂O Yield +/−	N₂O Yield R^2_{adj}	nosZ +/−	nosZ R^2_{adj}
Potential Denitrif.	N₂	I	μg N·g sed^{-1}·h^{-1}	62	.	.	.	.	.	.	.	.
	N₂O–N	I	μg N·g sed^{-1}·h^{-1}	62	+	0.09	.	.	.	.	.	.
	N₂O Yield	I	% Total N	62	−	0.08	+	0.49***	.	.	.	.
	nosZ	S	10^6 copies/g sed	58	+	0.18**	+	0.12	+	0.00	.	.
Substrates	% OM	S	% mass	62	+	0.06	+	0.10	+	0.00	+	0.06
	DOC	PW	mg C/kg sed	62	+	0.19**	+	0.00	−	0.05	+	0.08
	TDN	PW	mg N/kg sed	62	+	0.22**	+	0.01	−	0.01	+	0.12
	NO₃⁻–N	PW	mg N/kg sed	62	+	0.19**	+	0.02	−	0.00	+	0.01
	NH₄⁺–N	PW	mg N/kg sed	59	+	0.19**	+	0.07	−	0.00	+	0.22**
Stressors	Cl	PW	mg/kg sed	62	+	0.06	+	0.00	−	0.03	+	0.00
	SO₄	PW	mg/kg sed	62	+	0.02	−	0.00	−	0.04	+	0.05
	Zn	S	mg/g C	61	−	0.02	−	0.01	−	0.00	−	0.06
	Pb	S	mg/g C	61	−	0.00	−	0.00	−	0.00	−	0.07
	Cu	S	mg/g C	61	−	0.13*	−	0.04	−	0.00	−	0.08
	As	S	μg/g C	61	−	0.12	−	0.14*	−	0.01	−	0.14*
Microbial	SIR	S	μg C−CO₂·(g sed)$^{-1}$·h^{-1}	62	+	0.00	+	0.08	+	0.07	+	0.17*
Sediment and pond properties	pH	S	−log([H+])	61	−	0.00	−	0.15*	−	0.10	+	0.00
	BD	S	g/mL	62	−	0.01	−	0.05	−	0.01	−	0.00
	WA:PA	A	ratio	60	+	0.13*	+	0.01	−	0.00	+	0.22**

Notes: Variable abbreviations include gaseous nitrogen (N₂), nitrous oxide (N₂O–N), N₂O Yield (calculated as N₂O–N/(N₂+N₂O–N)), bulk density (BD), the ratio of watershed area to pond area (WA:PA), and those listed in Table 1. The source of the measurement is listed as I, incubation; S, sediment; PW, porewater, and A, area. All simple linear regression results are based on log-log relationships, except for pH and bulk density. Any adjusted R² (R^2_{adj}) values less than zero are reported as 0.00.

Significant correlations with Bonferroni corrections for multiple comparisons within each denitrification parameter are indicated with $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

with both potential N₂ and N₂O production (Table 3). NH₄⁺–N and WA:PA were the strongest individual predictor variables ($P = 0.003$ and 0.002, $R^2_{adj} = 0.22$ and 0.22, respectively). Unlike either denitrification potential product, SIR rates were positively related to nosZ abundance ($P = 0.01$, $R^2_{adj} = 0.17$). nosZ abundance was correlated (positively) with N₂ ($P = 0.008$, $R^2_{adj} = 0.18$) but not N₂O potential production ($P = 0.07$, $R^2_{adj} = 0.12$).

Bi-directional stepwise multiple regression with automated variable selection by AIC on subsets of explanatory variables revealed different multivariate controls on potential N₂ and N₂O production (Table 4). The substrate submodel of NO₃⁻–N and NH₄⁺–N accounted for 33% of the variation in N₂ production, which performed better than both the stressor ($P = 0.0003$, $R^2_{adj} = 0.23$) and sediment and pond properties ($P = 0.002$, $R^2_{adj} = 0.13$) submodels. After the combination of the four submodels and another round of bi-directional stepwise AIC

variable selection, 46% of the variation was accounted for and NO₃⁻–N was the most important predictor variable (Table 4). In contrast to the best predictor submodels of N₂, the stressor and sediment and pond properties submodels accounted for the most variability in potential N₂O production ($P = 0.001$ and 0.0003, $R^2_{adj} = 0.18$ and 0.21, respectively; Table 4). The combined submodels performed worse at predicting N₂O potential production than N₂, and only explained 25% of the variation. In order of relative importance, pH, bulk density, and sediment As concentrations emerged as the best predictors in the combined model.

Only the stressor and sediment and pond properties multiple linear regression submodels explained more of the variation in nosZ abundance than any single variable model after a bi-directional stepwise procedure ($P = 0.0002$ and <0.0001, $R^2_{adj} = 0.24$ and 0.29, respectively; Table 4). Land cover represented by WA:PA was the strongest predictor variable in the final combined model

Table 4. Multiple linear regression model comparisons.

Rate and abundance models	AIC selected models	R^2_{adj}	P	K	AIC	AIC$_c$	Rejected variables
Potential N$_2$ production							
Submodels							
Substrates	$0.43 \times NO_3^- + 0.23 \times NH_4^+ + 1.04$	0.33	<0.0001	2	−6.7	−6.5	DOC, %OM
Stressors	$-0.46 \times As + 0.23 \times Cl^- + 0.22 \times$ Pb + 2.36	0.23	0.0003	3	−1.3	−0.9	SO$_4^{2-}$
Microbial	NA	NA	NA	0	NA	NA	SIR
Soil & pond properties	$0.28 \times WA{:}PA + 0.21$	0.13	0.0024	1	7.8	7.9	pH, BD
Combined submodel	$0.45 \times NO_3^- + 0.20 \times NH_4^+ + 0.12 \times$ WA:PA $- 0.25 \times As + 0.22 \times$ Pb + 1.93	0.46	<0.0001	5	−14.7	−13.6	Cl$^-$
Potential N$_2$O production							
Submodels							
Substrates	$0.57 \times \%OM - 4.40$	0.10	0.0058	1	95.7	95.8	NH$_4^+$, NO$_3^-$, DOC
Stressors	$-0.94 \times As + 0.48 \times Pb + 1.12$	0.18	0.0012	2	89.4	89.6	Cl$^-$, SO$_4^{2-}$
Microbial	$0.63 \times SIR - 3.87$	0.08	0.0120	1	97.0	97.1	NA
Soil & pond properties	$-0.66 \times pH - 2.32 \times BD + 3.68$	0.21	0.0003	2	86.3	86.5	WA:PA
Combined submodel	$-0.53 \times pH - 1.94 \times BD - 0.39 \times$ As + 4.07	0.25	0.0002	3	84.2	84.6	%OM, SIR, Pb
***nosZ* gene abundance**							
Submodels							
Substrates	$0.51 \times NH_4^+ + 3.57$	0.22	0.0002	1	61.2	61.3	NO$_3^-$, %OM, DOC
Stressors	$-0.60 \times As + 0.41 \times SO_4^{2-} + 5.09$	0.24	0.0002	2	61.3	61.5	Cl$^-$, Pb
Microbial	$0.72 \times SIR + 3.85$	0.17	0.0006	1	66.7	66.8	
Soil & Pond Properties	$0.73 \times WA{:}PA + 0.32 \times pH + 0.03$	0.29	<0.0001	2	57.1	57.3	BD
Combined submodel	$0.59 \times WA{:}PA + 0.31 \times SIR - 0.43 \times$ As + 0.41 $\times$ pH + 1.67	0.42	<0.0001	4	46.4	47.2	NH$_4^+$, SO$_4^{2-}$

Notes: Variables and statistical metrics include Akaike's Information Criterion (AIC). Only the coefficient estimates (coef.) for individual variables in the final 'Combined Submodels' model, and the same abbreviations as those reported in Table 1 and Table 2. All variables have been log transformed except for pH and bulk density.

which accounted for 42% of the variance in *nosZ* gene abundance in pond sediments (Table 4).

Discussion

Contrary to our expectations, urban stormwater ponds did not support substantially higher potential rates of denitrification or N$_2$O production relative to ponds draining less urbanized landscapes. This suggests that despite receiving potentially elevated nutrient loads, the capacity of these hydrologic control structures to remove excess N was still constrained to the same extent by environmental controls as ponds in less developed watersheds. It also suggests that these stormwater ponds are not important sources of the potent greenhouse gas N$_2$O. We found that emissions of N$_2$ made up >90% of potential gaseous N emissions from most ponds and that

approximately one-third of the variation in N$_2$ production across all ponds could be explained by differences in the availability of inorganic nitrogen. While N$_2$O emissions were highly variable within and across cities, no combination of potential explanatory variables could account for more than 25% of this variation.

Prior work had led us to expect that most urban ponds would have a combination of high nitrogen inputs (Collins et al. 2010) and metal contaminated sediments (Campbell 1994, Mayer et al. 1996, Liebens 2001, Nowell et al. 2013) that might promote high rates of incomplete denitrification. Because we found no strong relationship between the intensity of urban land use surrounding ponds and the ratio of denitrification products (N$_2$O yield) produced by the sediment microbial community, our work calls both of these starting assumptions into question. Neither the availability

Table 5. Ranges in sediment copper (Cu), zinc (Zn), and lead (Pb) concentrations in various stormwater controls measures reported in a non-exhaustive review of published literature.

Study	Location	Type of stormwater control measure reported	Cu (mg/kg)	Zn (mg/kg)	Pb (mg/kg)
This study	8 USA cities	Natural and stormwater ponds	4–246	5–643	bdl–298
El-Mufleh et al. (2014)	France	Infiltration/detention–infiltration basins	84–317	683–2070	30–819
Gallagher et al. (2011)	Maryland, USA	Stormwater ponds	19–90	40–580	10–55
Camponelli et al. (2010)	Maryland, USA	Stormwater retention pond	33–79	61–243	NA
Karlsson et al. (2010)	Sweden	Stormwater ponds	60–225†	300–900†	55–100†
Clozel et al. (2006)	France	Retention or infiltration ponds	95–343	731–2070	178–670
Casey et al. (2005)	Maryland, USA	Stormwater ponds	18–341	53–1155	9–116
Sparling et al. (2004)	Maryland, USA	Stormwater retention ponds and wetlands	bdl–19	bdl–59	bdl–18
Scher et al. (2004)	France	Stormwater retention ponds	40–270	49–730	1–160
Dechesne et al. (2004)	France	Stormwater infiltration basin	3–176	19–1827	5–225
Liebens (2001)	Florida, USA	Stormwater retention ponds	bdl–55	0.3–622	5–777
Karouna-Renier and Sparling (2001)	Maryland, USA	Stormwater ponds	8–12	21–42	10–12
Bishop et al. (2000)	Ontario, Canada	Stormwater detention ponds	11–63	29–535	2–68
Mayer et al. (1996)	Ontario, Canada	Stormwater detention ponds	30–151	98–610	46–202
Yousef et al. (1990)	Florida, USA	Retention/detention basins	1–73	1–538	4–1025
Nightingale (1987)	California, USA	Retention and recharge basins	10–49	NA	8–107
Wigington et al. (1983)	Washington, D.C., USA	Detention basins	7–31	26–434	103–241
Nightingale (1975)	California, USA	Stormwater retention basin	4–45	21–387	57–764

Note: bdl, below detection limit.
† Values approximated from Fig. 3 in Karlsson et al. (2010).

of substrates (inorganic N and DOC) nor the concentrations of stressors (metals) were consistently related to surrounding land use across ponds. Instead, we measured a great deal of variation across all of the ponds and found, similar to Baalousha et al. (2016), that even ponds surrounded by significant buffers of undeveloped land were receiving measurable quantities of urban pollutants via direct deposition.

The range of sediment Cu, Zn, and Pb concentrations reported in this study fell within the range of those found in other stormwater ponds (Table 5; Nightingale 1975, 1987, Wigington et al. 1983, Yousef et al. 1990, Mayer et al. 1996, Bishop et al. 2000, Liebens 2001, Karouna-Renier and Sparling 2001, Dechesne et al. 2004, Scher et al. 2004, Sparling et al. 2004, Casey et al. 2005, Clozel et al. 2006, Camponelli et al. 2010, Karlsson et al. 2010, Gallagher et al. 2011, El-Mufleh et al. 2014). The majority of ponds (56%) had sediment metal concentrations that were below the freshwater sediment threshold effect concentrations (TECs) believed to be the lower limit of toxicity to benthic macroinvertebrates and other sediment-dwelling organisms (Table 6, MacDonald et al. 2000). However, five of the 64 ponds exceeded the Probable Effect Concentrations (PECs) for at least one urban

Table 6. A comparison of sediment metal concentrations reported in this study to consensus-based threshold effects concentrations (TEC) and probable effects concentrations (PEC) for sediment-dwelling organisms established by MacDonald et al. (2000).

Parameters	Cu (mg/kg)	Zn (mg/kg)	Pb (mg/kg)
TEC	32	121	36
% exceedance of TECs in this study	44%	22%	25%
PEC	149	459	128
% exceedance of PECs in this study	2%	5%	8%

metal, and 27 were between the recommended TEC and PEC levels (Table 6). Pollution in this small subset of stormwater ponds likely reduces their potential value as suitable habitat that promotes aquatic biodiversity within cities (Oertli 2018), turning them instead into ecological traps for pollution intolerant species (Clevenot et al. 2018).

Although we expected current rates of both nutrient and pollutant loading to be related to land cover, these loads might not have translated into predictable shifts in sediment chemistry because of differences in the age of ponds and nearby urban development, retention time, groundwater

exchange (Hall et al. 2016, Gabor et al. 2017), and the imprint of legacy pollutants from agricultural or industrial activity. In addition, differences in current management strategies (such as lawn fertilization around ponds or the addition of Cu as an algaecide) among commercial, residential, and industrial urban land uses may have resulted in variable chemical loading. What is clear is that the chemistry of urban pond sediments was not as consistently altered by urbanization as has been observed for streams. The land cover metric of the ratio of watershed area to pond area (WA:PA) played a surprisingly important role in predicting both potential N_2 production and *nosZ* gene abundance. This ratio may be a better indicator of the delivery of limiting resources to the ponds in this study than the concentrations we measured in the snapshot sediment samples.

Variation in N_2

Because we did not find the expected relationship between the extent of urbanization and pond porewater and sediment chemistry, we abandoned our initial plan to compare highly urbanized and green space protected ponds to one another and instead examined the potential for substrate, stressor, and land cover factors to explain the observed variation across all ponds in the production of N_2 and N_2O and in the abundance of *nosZ* genes. As anticipated, porewater NO_3^-–N was the single strongest predictor of potential N_2 production. Increasing NO_3^-–N availability in the permanently anaerobic sediments of stormwater ponds has been previously shown to stimulate denitrifier activity in Baltimore (Groffman et al. 2005, Bettez and Groffman 2012), and our regression analysis indicated that this holds across many cities. We were therefore surprised that NO_3^-–N was unrelated to potential N_2O production, N_2O yield, or *nosZ* gene abundance.

We found limited evidence for our hypothesis that sediment metal contamination might reduce denitrification efficiency. Only sediment Cu concentrations were negatively correlated with potential N_2 production in single factor regression analysis, yet this interaction was a weaker predictor than the highly correlated sediment As and Pb concentrations and therefore not included in the multiple regression analysis. Indeed, our best model to predict N_2 variation uncovered a positive association between Pb and potential

rates of N_2 production. While this is certainly not a strong test of the role of metal contaminants in affecting denitrification, it does suggest that metals are not a primary constraint on denitrification rates. Similarly, while high Cl^- concentrations have been shown to inhibit denitrification in urban stream sediments (Hale and Groffman 2006), we found no evidence that Cl^- levels were an important constraint on denitrification potential or denitrifier abundance. It is possible that denitrifier communities in most of the ponds in this study were resilient to stressors such as Cl^- and metals (Perryman et al. 2011*a*) because of a history of previous exposure and adaptation.

Variation in N_2O

A survey of streams throughout the continental United States found the highest N_2O emissions from streams draining urbanized landscapes (Beaulieu et al. 2011). We did not find any direct evidence of the same trend in potential N_2O emissions from urban pond sediments (Appendix S1: Fig. S2). Porewater pH was the strongest predictor of potential N_2O production, with higher pH associated with declining N_2O production (Table 3). Sediment pH can act as a distal control on rates of denitrification by altering denitrifier community composition and diversity (Wallenstein et al. 2006). The majority of the ponds we sampled had N_2O yields <10%, which was comparable to the yields previously reported for streams by Beaulieu et al. (2011). We found only eight ponds (13% of 64 ponds) that had N_2O yields that exceeded 10%. These eight ponds tended to have lower median DOC (59 mg C/kg sed) and Cl^- (29 mg/kg sed) concentrations in their porewater as compared to the entire dataset, and four of these high yield ponds were in Portland, Oregon.

Variation in nosZ gene abundance

Denitrifiers can only produce the nitrous oxide reductase enzyme to reduce gaseous N_2O to N_2 if they have the necessary *nosZ* gene. The relative abundance of this gene was clearly not just a function of microbial biomass because we found a relatively weak relationship between SIR (a proxy for microbial biomass) and the abundance of the *nosZ* gene (Table 3). Instead, the relative abundance of the *nosZ* gene was also responsive to WA:PA, sediment As concentrations, and pH (Table 4). The relatively weak correlation between *nosZ* gene

abundance and N_2 rates (Table 3) suggested that environmental controls play an important role constraining synthesis of the nitrous oxide reductase enzyme as well. The limited strength of this correlation could also be the result of an incomplete characterization of all possible N_2O reducers. We did not measure atypical non-denitrifier N_2O reducers that have been shown to play a significant functional role in N_2 production (Sanford et al. 2012, Van Groenigen et al. 2015). Our best model to predict variation in typical *nosZ* abundance shared predictor variables with those of both the best N_2 and N_2O models. While each model was distinct, none of them explained more than half of the variation in the response variable. This raises the question whether this unexplained variability in potential denitrification rates and *nosZ* gene abundance was controlled by common or distinct factors not measured in this study, which could affect niche differentiation among different clades of denitrifiers (Juhanson et al. 2017).

Variation across all ponds

When compared to rates measured using the denitrification enzyme assay (DEA) method (Groffman et al. 1999) reported by Bettez and Groffman (2012) from Baltimore, Maryland, the rate of mean potential N_2O–N production in Baltimore, Maryland (0.41 ± 0.55 mg N·kg^{-1}·h^{-1}), was lower and more variable than previously found in wet stormwater management control structures (1.07 ± 0.25 mg N·kg^{-1}·h^{-1}) in the same city. The mean potential N_2O–N production rate in Phoenix, Arizona ponds (0.03 ± 0.04 mg N·kg^{-1}·h^{-1}), was also lower than that reported previously for retention basins in Phoenix, Arizona (0.1–2.8 mg N·kg^{-1}·h^{-1}) by Larson and Grimm (2012). This might be expected because the acetylene in DEA assays blocks the reduction of N_2O to N_2 and causes a greater accumulation of N_2O, but it is unclear whether acetylene more broadly inhibits denitrification. By measuring N_2 and N_2O simultaneously without acetylene inhibition and by using the same technique for all ponds, we compared the variability in pond sediment denitrification potential within and across U.S. cities without the methodological artifacts that arise from comparing denitrification rates among studies.

Our results suggest that physiographic and climatic factors could have an influence on the potential denitrification rates of stormwater ponds.

Western cities (Phoenix, Arizona, Portland, Oregon, and Salt Lake City, Utah) had lower potential N_2 production rates relative to eastern cities (Fig. 2). However, the three western cities did not have any unique measured characteristics shared among all three relative to eastern cities that might explain this difference. Only Salt Lake City, Utah, had distinctly lower *nosZ* abundance that might explain the reduction in potential N_2 production (Appendix S1: Fig. S3). While broad survey studies such as this study give us a better understanding of the range of possible potential denitrification rates, they are limited in their ability to measure and predict the complex set of factors that drive denitrification.

Conclusions

In the most geographically distributed survey to date of denitrification potential in urban ponds in the United States, we found enormous chemical and biophysical variation (over two orders of magnitude) and a limited ability to explain denitrification potential as a function of land use or sediment or porewater chemistry. There was no single and simple link between the intensity of urban land cover and potential denitrification rates. The N_2O yield from most ponds was within the range of those documented in freshwater bodies draining undeveloped landscapes, leading us to conclude that urban stormwater ponds are not likely to be important sources of N_2O to the atmosphere, no matter their potential rates of denitrification.

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