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Jake J. Beaulieu

Paul M. Mayer

Sujay S. Kaushal

Michael J. Pennino

Clay P. Arango

See next page for additional authors

Authors

Jake J. Beaulieu, Paul M. Mayer, Sujay S. Kaushal, Michael J. Pennino, Clay P. Arango, David A. Balz, Timothy J. Canfield, Colleen M. Elonen, Ken M. Fritz, Brian H. Hill, Hodon Ryu, and Jorge W. Santo Domingo

Effects of urban stream burial on organic matter dynamics and reach scale nitrate retention

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Abstract Nitrogen (N) retention in streams is an important ecosystem service that may be affected by the widespread burial of streams in stormwater pipes in urban watersheds. We predicted that stream burial suppresses the capacity of streams to retain nitrate (NO_3^-) by eliminating primary production, reducing respiration rates and organic matter availability, and increasing specific discharge. We tested these predictions by measuring whole-stream NO_3^- removal rates using $^{15}NO_3^-$ isotope tracer releases in paired buried and open reaches in three streams in Cincinnati, Ohio (USA) during four seasons. Nitrate uptake lengths were 29 times greater in buried than open reaches, indicating that buried reaches were less effective at retaining NO_3^- than open reaches. Burial suppressed NO_3^- retention through a combination of hydrological and biological processes. The channel shape of two of the buried reaches increased specific discharge which enhanced NO_3^- transport from the channel, highlighting the relationship between urban infrastructure and ecosystem function. Uptake lengths in the buried reaches were further lengthened by low stream biological NO_3^- demand, as indicated by NO_3^- uptake velocities 17-fold lower than that of the open reaches. We also observed differences in the periphyton enzyme activity between reaches, indicating that the effects of burial cascade from the microbial to the ecosystem scale. Our results suggest that stream

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J. J. Beaulieu (✉) · H. Ryu · J. W. S. Domingo
US EPA, Office of Research and Development, National Risk Management Research Laboratory, Cincinnati, OH 45268, USA
e-mail: beaulieu.jake@epa.gov

P. M. Mayer · T. J. Canfield
US EPA, Office of Research and Development, National Risk Management Research Laboratory, Ada, OK 74821, USA

Present Address:
P. M. Mayer
US EPA, Office of Research and Development, National Health and Environmental Effects Research Laboratory, Corvallis, OR 97333, USA

S. S. Kaushal · M. J. Pennino
Department of Geology and Earth Systems Interdisciplinary Center, University of Maryland, College Park, MD 20742, USA

C. P. Arango
Department of Biological Sciences, Central Washington University, Ellensburg, WA 98926, USA

D. A. Balz
Pegasus Technical Services, Cincinnati, OH 45268, USA

C. M. Elonen · B. H. Hill
US EPA, Office of Research and Development, National Health and Environmental Effects Research Laboratory, Duluth, MN 55804, USA

restoration practices involving "daylighting" buried streams have the potential to increase N retention. Further work is needed to elucidate the impacts of stream burial on ecosystem functions at the larger stream network scale.

Introduction

Streams draining urbanized basins exhibit a suite of ecological degradations including flashy hydrology, elevated concentrations of nutrients and contaminants, and reduced biotic richness (Walsh et al. 2005). These degradations largely result from increased impervious surfaces in the basin and have been the focus of most urban stream ecology research (Wenger et al. 2009). Despite the focus on impervious surfaces, perhaps the most extreme expression of urbanization on stream ecosystems is their burial in drainage pipes to facilitate above-ground construction or stormwater management (Kaushal and Belt 2012). While stream burial has occurred since the Roman empire (Hopkins 2007), ecologists have only recognized stream burial as a common consequence of urbanization in recent decades (Leopold 1968), and only in the last few years has the extent of stream burial been quantified (Elmore and Kaushal 2008).

Stream channels are frequently routed into pipes and buried during the process of urbanization. Over time, the total length of buried stream channels can far exceed that of open stream channels. For example, it is estimated that over half the streams in Baltimore, Maryland (USA) are buried with the probability of stream burial reaching 98 % in the most densely populated areas of the city (Elmore and Kaushal 2008). Similarly, O'Driscoll et al. (2010) reported that stream density in urban catchments was approximately 40 % lower than surrounding rural catchments due to urban stream burial. Although similar data are lacking from other cities, stream burial is likely

ubiquitous throughout large urban centers (Conradin and Buchli 2005; Nam-choon 2005; Wild et al. 2011).

Stream burial fundamentally changes the structure and function of stream ecosystems through several mechanisms including the inhibition of photosynthesis, reduced organic matter inputs, and altered hydrology. Despite the prevalence and obvious ecosystem impact of stream burial, little research has been conducted on the topic, and the existing literature focuses on the effects of short road crossings on macroinvertebrates and fish. For example, Meyer et al. (2005) found that stream burial decreased macroinvertebrate and taxonomic richness in a buried stream in Georgia (USA). Others have reported that stream burial can hinder the in-stream migration of macroinvertebrates and fish (Dedecker et al. 2006; Foster and Keller 2011; Poplar-Jeffers et al. 2009) as well as the longitudinal dispersal of emerging adult insects (Blakely et al. 2006). To our knowledge, however, there are no published reports of the effect of stream burial on other important ecosystem services provided by streams, such as nutrient removal.

Streams draining developed catchments carry elevated nitrogen (N) loads (Howarth et al. 1996; Kaushal et al. 2008a), often in the form of nitrate (NO_3^-), which stimulates algal blooms in N-limited aquatic ecosystems, leading to a cascade of biogeochemical processes that ultimately results in oxygen-depleted waters and eutrophication. Estuarine and coastal zones have been particularly affected by eutrophication where dissolved oxygen routinely falls below levels required to support sensitive aquatic organisms at more than 400 sites across the globe (Díaz and Rosenberg 2008). Management strategies to enhance the capacity of streams and rivers to remove NO_3^- from the water column via biological activity may reduce N delivery to coastal waters (Bukaveckas 2007; Kaushal et al. 2008b). Heterotrophic and autotrophic organisms in streams can assimilate water column NO_3^- into biomass, which may be subject to long term storage in stream beds and floodplains (O'Brien et al. 2012). Some heterotrophic organisms can permanently remove NO_3^- through denitrification, a dissimilatory metabolic process where NO_3^- is converted to dinitrogen (N_2) or nitrous oxide (N_2O) gas. We speculated that stream burial may severely reduce the efficacy of these NO_3^- removal mechanisms by eliminating N assimilation associated with photosynthesis, reducing the quantity and quality of organic matter needed to

K. M. Fritz
US EPA, Office of Research and Development, National Exposure Research Laboratory, Cincinnati, OH 45268, USA

support denitrification and heterotrophic assimilation, and by enhancing hydrologic export from the channel, thus augmenting downstream NO_3^- transport. Stream burial may therefore exacerbate elevated N loading from urban basins to coastal waters.

The biological demand for NO_3^- in temperate zone streams often follows a predictable seasonal cycle (Lutz et al. 2012). High light levels and rising water temperatures during the spring can lead to benthic algal blooms and high assimilative NO_3^- demand, which decreases during the summer when the forest canopy shades streams and alga populations senesce. A secondary peak in NO_3^- demand often occurs during the fall when allochthonous organic matter inputs stimulate heterotrophic metabolism whereas low water temperature and short days tend to suppress winter-time NO_3^- demand. By contrast, light and organic matter availability in buried streams is low throughout the year, which likely dampens seasonal patterns in biological NO_3^- demand. As a consequence, the effect of burial on biological NO_3^- demand may be most pronounced during the spring and fall when the difference in light and organic matter between open and buried streams is most extreme.

Understanding the degree to which ecosystem N retention is impaired is a first step towards setting targets for watershed restoration objectives. In order to determine the effect of burial on NO_3^- removal in streams, we used whole stream $^{15}NO_3^-$ tracer additions to measure NO_3^- uptake in a paired comparison of open and buried reaches in urban streams located in Cincinnati, OH (USA) and Baltimore, MD (USA). Here we report the results of the work in Cincinnati whereas Pennino et al. (2014) report the results from Baltimore in this special issue of Biogeochemistry. We measured NO_3^- uptake seasonally (e.g. summer, fall, winter, spring) over the course of 1 year. To identify the mechanisms controlling NO_3^- uptake, we measured a broad suite of functional and structural stream characteristics. At the patch scale (i.e. 225 cm^2) we estimated bacterial abundance and assessed dissolved organic carbon (DOC) quality using measurements of periphyton extracellular enzyme activity (EEA) rates (Sinsabaugh and Shah 2011). At the ecosystem scale (stream reach) we measured metabolism and hydraulic characteristics. Water chemistry was measured at the top and bottom of the reaches and organic matter standing stocks were estimated by scaling habitat weighted means to the reach.

Methods

Study sites and experimental design

We selected three streams draining urban watersheds within and near the city of Cincinnati, Ohio (USA) (Figs. 1, 2; Table 1). Each stream contained buried and open reaches separated by 30–100 m of open stream channel. The buried reach at Eastgate Creek (EAS) was 740 m long, constructed from interconnected segments of ovoid corrugated metal pipe ranging from 1.5 to 1.8 m in height, and approximately 0.5 m wide at the base. The pipe was relatively clean of debris and sediment. The open reach at EAS had an average wetted width of 2.1 m, was downstream of the buried reach, deeply incised, and lined by a vegetated riparian zone. Sediment in the open reach at EAS was predominantly cobbles, boulders, and exposed bedrock, though restricted portions of the stream bed were composed of pebbles and sand. The buried reach at Amberly (AMB) was 430 m long, 2.5 m in height, 4.5 m wide at the base, constructed from segments of corrugated metal pipe, and filled with sand and pebbles to a depth of ca. 0.15 m. The open reach at AMB had an average wetted width of 3.9 m, was downstream of the buried reach, flowed between parking lots, and the width of the vegetated riparian zone was less than 5 m in many places. The buried reach at Este (EST) was downstream of the open reach, 290 m long, 2.5 m in height, 1.0 m wide at the base, constructed from concrete, and relatively free of sediment. The open reach at EST flowed through a vegetated riparian zone and had a deeply entrenched channel with a wetted width of 2.1 m composed of highly mobile sandy sediments.

We measured NO_3^- uptake rates in the buried and open reaches of all three streams during summer and fall 2011, and winter and spring 2012. This experimental design provided four paired measurements of NO_3^- uptake in buried and open reaches of each stream. Nitrate uptake rate measurements were made during the daylight hours. Water chemistry samples were collected from the top and bottom of each experimental reach immediately prior to the ^{15}N-NO_3^- tracer release and again immediately before the release was terminated. Stream hydrologic parameters, photosynthetically active radiation (PAR), and ecosystem metabolism were measured concurrently with the ^{15}N-NO_3^- tracer release. Samples for benthic organic matter and chlorophyll standing stocks, algal abundance, ecoenzyme
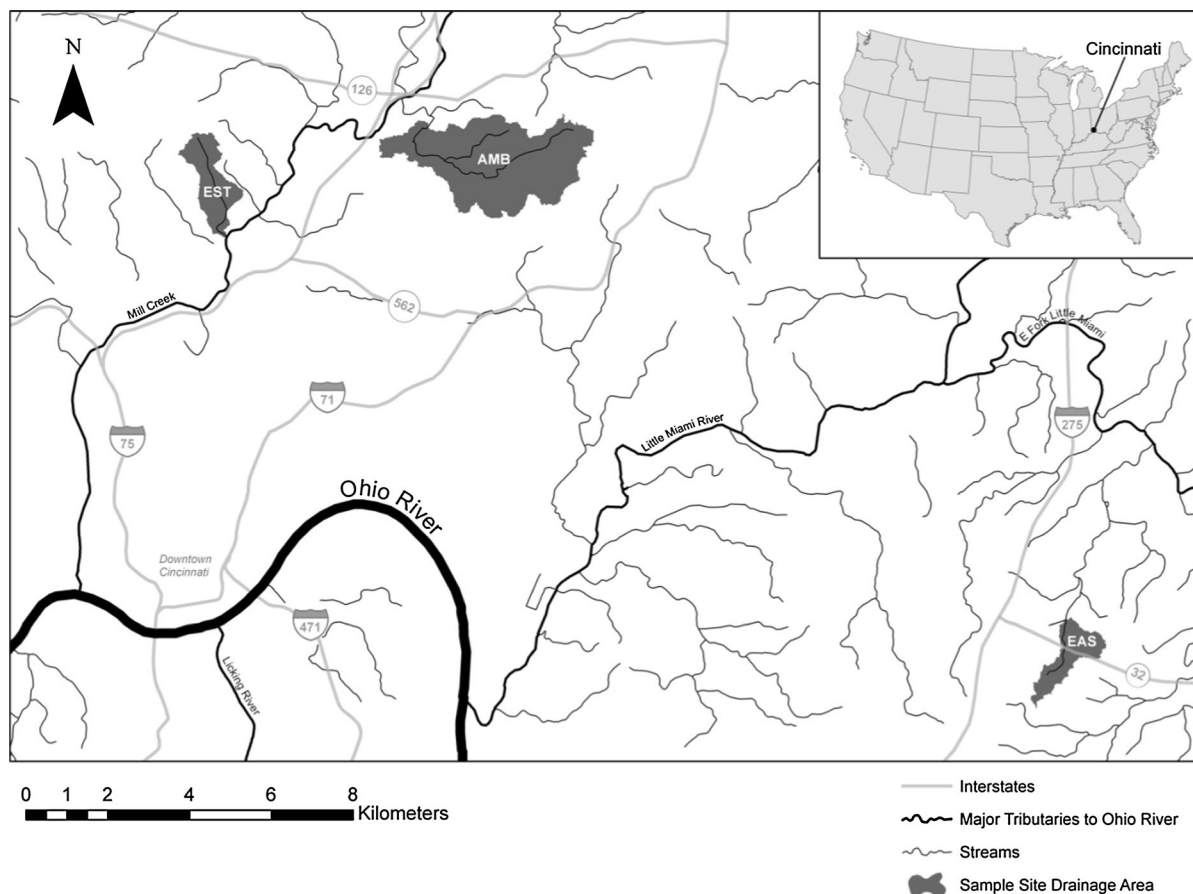
Fig. 1 The three study streams located near Cincinnati, Ohio (USA). The shaded areas represent the drainage area relative to the most downstream sampling location

activity, and DNA extraction were collected within 48 h of the ^{15}N-NO_3^- tracer release.

Hydrology, metabolism, light, and water chemistry

Average stream width was calculated from a minimum of 30 measurements of wetted width distributed evenly along the study reaches. Water velocity was calculated as time to maximum slope of the rhodamine breakthrough curve recorded at the downstream end of each experimental reach using a data sonde and rhodamine sensor (YSI, Yellow Springs, OH, USA). Discharge (Q) was calculated as:

$$Q = \frac{Q_{pump} \times [\ ]_{inj}}{([\ ]_{plt} - [\ ]_{bck})} \quad (1)$$

where Q_{pump} is the pump rate and [ ] is the concentration of rhodamine or Br^- in the Injectate ([ ]$_{inj}$), plateau

([ ]$_{plt}$), and background ([ ]$_{bck}$) samples. Stream depth was calculated as discharge/(width * velocity).

We estimated reach scale rates of gross primary production (GPP) and ecosystem respiration (ER) using temperature and dissolved oxygen measurements made with data sondes (YSI, Yellow Springs, OH, USA) at 5 min intervals for a minimum of 36 h at the top and bottom of each experimental reach. Rates were calculated using the two-station method following Marzolf et al. (1994) with the corrections discussed in Young and Huryn (1998), and the one-station calculations following Roberts et al. (2007). The two-station method was used for the open reach whenever possible (i.e. data from up and downstream sondes were available). If the downstream end of the buried reach exhibited no diel dissolved oxygen trend, then dissolved oxygen dynamics at that site were considered to be decoupled from the

Fig. 2 Pictures of buried streams included in this study. Este (EST) (**a**, **b**) flowed through a concrete tunnel with a narrow wetted width at baseflow. Amberly (AMB) (**c**) flowed through a wide corrugated metal pipe which was filled with sediment to a depth of ca 15 cm. Eastgate (EAS) (**d**) flowed through a corrugated metal pipe that was relatively free of sediment and debris

Table 1 Watershed and study reach characteristics

Stream	Watershed area (km^2)	% impervious area	Open			Buried		
			Reach length (m)	Stream width (m)	Sediment	Reach length (m)	Stream width (m)	Sediment
EAS	1.4	34.0	36–92	1.5–2.4	Cobble/gravel	740	0.6–0.8	Metal
AMB	7.9	16.3	70–177	3.6–4.2	Cobble/gravel/ sand	430	3.7–4.5	15 cm thick layer of pebbles/sand in metal pipe
EST	1.8	23.6	142–209	1.9–2.3	Gravel/sand	290	0.9–1.1	Cement

upstream open reach and the one-station method was used. We measured air–water gas exchange rates using sulfur hexafluoride (SF_6) tracer injections conducted concurrently with the $^{15}NO_3^-$ tracer injections (Marzolf et al. 1994). Photosynthetically active radiation (PAR) was measured 1 m above the water surface in the middle of each reach at 5 min intervals using a cosine corrected photosynthetic irradiance sensor (Odyssey, New Zealand) (Shaffer and Beaulieu 2012).

All water samples were filtered (0.45 μm) in the field, stored on ice during transport to the laboratory,

and either acidifed or frozen, depending on the analyte, prior to analysis. Nitrate + nitrite, hereafter referred to as NO_3^-, dissolved reactive phosphorus (DRP), bromide (Br^-), and ammonium (NH_4^+) were measured using standard colorimetric methods (APHA 2005) and flow injection analysis (Lachat Instruments, Loveland, CO USA). Dissolved organic carbon (DOC) was measured using a total organic carbon (C) analyzer with high-temperature Pt-catalyzed combustion and NDIR detection (Shimadzu TOC-VCPH, Columbia, MD, USA). See below for $^{15}N-NO_3^-$ processing details.

Benthic organic matter

We measured coarse (>1 mm size fraction), fine (<1 mm size fraction), and attached (e.g. periphyton) benthic organic matter standing stocks by collecting material from a known area of the stream bottom at 10–20 locations randomly distributed among habitat units (e.g. pool, riffle, run) according to their relative abundance in a stratified-random sampling design. An open-ended plastic cylinder (0.052 m^2 cross sectional area) was placed up to a maximum depth of 5 cm in the sediment and coarse benthic organic matter (CBOM) was removed by hand. A hand trowel was then used to agitate the sediments, and a subsample (~200 mL) of the resultant slurry was collected and poured over a 1 mm mesh sieve. The material retained on the mesh was composited with the coarse material and the filtrate was collected for fine benthic organic matter (FBOM). Periphyton was collected by scraping a known area of rock surface area (0.006–0.04 m^2) with a toothbrush or stiff-bristled brush operated by a portable drill. The dislodged periphyton was washed into a sample bottle with stream water. Periphyton standing stocks were assumed to be zero on large patches of sand. Dry mass and ash-free dry mass, determined from mass loss after ignition at 500 °C, were measured on all samples. Reach scale organic matter standing stocks were calculated as the sum of the mean value for each habitat unit scaled to the reach. Chlorophyll a was measured on subsamples of the periphyton samples using the trichromatic method (APHA 2005) following extraction with hot ethanol (Sartory and Grobbelaar 1984).

To assess the effect of stream burial on periphyton, while controlling for differences in substrate (e.g. metal pipe vs. natural cobble) and colonization time, we deployed 0.15 m × 0.15 m unglazed clay tiles in each reach and allowed a minimum of 6 weeks for colonization by periphyton. Within 48 h of each tracer release, the tiles were collected and the attached periphyton was dislodged with a toothbrush and razor blade and rinsed into a collection bottle with site water and stored on ice during transport to the laboratory. Three samples were analyzed for algal abundance using a Palmer-Maloney counting cell (Charles et al. 2002), four to ten samples were analyzed for total bacterial counts using quantitative PCR (see below), and five to ten samples were analyzed for EEA (see below).

DNA extraction and Quantitative PCR (qPCR) assays

We extracted DNA from four to ten tiles per reach within 6 h of sample collection. For each sample, biomass was concentrated from a 6 mL subsample via microcentrifugation ($3,000 \times g$ for 15 min). Biofilm pellets were resuspended in 500 μL of lysate buffer and transferred to tubes prior to the bead-beating step. DNA extractions were performed using Mo Bio Power Lyzer/Power Soil kits according to the manufacturer's protocol (MO BIO Laboratories, Carlsbad, CA, USA). DNA extracts were stored at −20 °C until further processing.

Total bacterial densities were estimated using a eubacterial universal 16S rRNA gene qPCR assay developed by Suzuki et al. (2000) with the BACT1369F (5′-CGGTGAATACGTTCYCGG-3′) and PROK1492R (5′-GGWTACCTTGTTACGACTT-3′) primers and TM1389F hydrolysis probe (5′-6FAM-CTTGTACA CACCGCCCGTC-TAMRA-3′). The qPCR assay was performed in 25 μL reaction volumes containing 1× TaqMan universal PCR master mix with AmpErase uracil-N-glycosylase (Applied Biosystems, Foster City, CA, USA), 0.2 μg μL^{-1} bovine serum albumin, the corresponding primers at 1 μM and the TaqMan reporter probe at 0.5 μM. Ten- and 50-fold dilutions of each DNA extract were used to test for PCR inhibition (Ryu et al. 2011; Toledo-Hernandez et al. 2013). The amplification protocol involved an initial incubation at 50 °C for 2 min to activate uracil-N-glycosylase, a 10 min incubation at 95 °C, followed by 40 cycles of 95 °C for 15 s and 56 °C for 1 min, and a 10 min incubation at 72 °C. The qPCR assay was performed using a 7900 HT Fast Real-Time Sequence Detector (Applied Biosystems, Foster City, CA, USA) in duplicate MicroAmp Optical 96-well reaction plates with MicroAmp Optical Caps (Applied Biosystems, Foster City, CA, USA). PCR data were analyzed using the instrument's Sequence Detector software (version 2.2.2, Applied Biosystems). qPCR standard curves were generated in duplicate for each 96 well plate by plotting threshold cycle (CT) values against the number of target copies corresponding to serially diluted plasmid standards (Integrated DNA Technologies, Coralville, IA, USA). The target copy numbers (T) were estimated by the following equation

$$T = [D/(PL660)] \times 6.022 \times 10^{23} \qquad (2)$$

where D (g μL^{-1}) is plasmid DNA concentration, and PL (bp) is plasmid length in base pairs. The standard

curve was generated from five 10-fold plasmid dilutions. Two no-template controls per PCR plate were used to check for cross-contamination.

Extracellular enzymes activities (EEA)

Periphyton collected from five and ten tiles deployed in the open and buried reaches, respectively, was analyzed for extracellular enzyme activities (EEA). Extracellular enzymes are produced by microbial assemblages to aid in the degradation of organic matter and the acquisition of limiting nutrients and can serve as an index of resource availability (Sinsabaugh and Foreman 2001). Labile C acquisition was measured as the activity of β-D-glucosidase. Two oxidases (polyphenol oxidase and peroxidase) were analyzed to assess recalcitrant C acquisition and the ratio of recalcitrant C acquisition (phenol oxidase) to total C acquisition (β-D-glucosidase + phenol oxidase) represents an index of the quality of the dissolved organic C pool (LCI; Sinsabaugh and Shah 2011).

All EEA assays used the microplate protocols originally developed by Sinsabaugh and colleagues (Sinsabaugh et al. 1997; Sinsabaugh and Foreman 2001) and modified according to Hill et al. (2010). Each microplate array included quadruplicate assays for each enzyme and each reference standard. All substrate and reference solutions were prepared in sterile deionized water. Quenching, the decrease of fluorescent emissions caused by the interactions of enzyme substrates with non-reactant chemicals in the assays, was estimated by comparing the fluorescence of the supernatant of standard solutions mixed with sample to that of the standard solution mixed with buffer. Substrate and sample controls (each mixed with buffer) were assayed in quadruplicate on the same microplate. Fluorescence was measured using a fluorometer (Model FLX800T, BioTek Instruments, Winooski, VT, USA) with an excitation wavelength of 350 nm and an emission wavelength of 450 nm.

$^{15}NO_3^-$ tracer injections, sampling and analysis

We measured NO_3^- uptake rates in each reach by conducting whole stream $^{15}NO_3^-$ tracer additions. We used a reciprocating piston pump (Fluid Metering Inc, Syosset, NY, USA) to meter a solution of 99.99 % ^{15}N potassium nitrate ($K^{15}NO_3^-$), sodium bromide (NaBr, conservative tracer), and rhodamine (conservative tracer) into each reach. The target tracer enrichments in the stream water were +5,000 ‰ $^{15}NO_3^-$, +500 μg L^{-1} Br^-, and +15 μg L^{-1} rhodamine. The tracer additions were maintained until the tracer attained steady state (i.e. plateau) for 2–4 h as determined from in situ rhodamine concentration measurements made at the downstream end of each experimental reach.

We collected filtered water samples (0.45 μm pore size) for $^{15}NO_3^-$, NO_3^- concentration, and Br^- at the upstream and downstream ends of the reach before (background samples) and immediately prior to terminating the tracer releases (plateau samples). Background and plateau samples were collected in triplicate and quintuplicate, respectively. Rhodamine was measured continuously at the upstream and downstream ends of the reaches using a data sonde (YSI, Yellow Springs, OH, USA). We located the upstream sampling station at the furthest upstream point where the tracers were well mixed across the stream width. We assumed the site was well mixed if the rhodamine concentration differed <5 % across the width of the stream channel during the tracer release. The downstream sampling site was located at the end of the pipe for the buried reaches, resulting in a 20–209 min water travel time (mean = 81 min), and for the open reaches the downstream station was located a distance equivalent to 60 min of water travel time from the upstream station.

Filtered $^{15}NO_3^-$ samples were stored on ice during transport to the laboratory and frozen at −20 °C until analysis. Tracer $^{15}NO_3^-$ was measured at Colorado Plateau Laboratory (NAU, Flagstaff, AZ, USA) using the denitrifier method (Coplen et al. 2012; Sigman et al. 2001). Briefly, the method utilizes *P. aureofaciens*, a denitrifier lacking the enzyme to reduce N_2O to N_2, to convert NO_3^- and nitrite (NO_2^-) to N_2O which is subsequently analyzed for N isotopic composition using an isotope ratio mass spectrometer (Thermo-Finnigan Delta Plus). The instrument was calibrated using USGS and IAEA NO_3^- isotope standards.

Isotope values were reported as $\delta^{15}N$ (‰) = $[(R_{sample}/R_{std}) - 1]1000$ where R_{sample} is the $^{15}N/^{14}N$ of the sample and R_{std} is the $^{15}N/^{14}N$ of the standard, atmospheric N_2. All $\delta^{15}N$ values were converted to mole fraction (MF) of ^{15}N $[^{15}N/(^{14}N + ^{15}N)]$, and the tracer ^{15}N flux at each station, corrected for natural abundance, was calculated as the product of the $^{15}NO_3^-$ MF, NO_3^- concentration, and discharge (Q).

NO$_3^-$ uptake rate calculations

We calculated NO$_3^-$ uptake rates at the whole stream scale using the nutrient spiraling framework (Newbold et al. [1981]; Stream Solute Workshop [1990]). Nitrate uptake was determined from the downstream decrease in ^{15}NO$_3^-$ flux according to the first order model:

$$\ln {}^{15}\mathrm{NO}_{3\,downstream}^- = \ln {}^{15}\mathrm{NO}_{3\,upstream}^- - k(\text{upstream} - \text{downstream})$$

(3)

where ^{15}NO$_3^-$ is the tracer ^{15}NO$_3^-$ flux at the upstream and downstream ends of the reach, k is the first order rate constant for NO$_3^-$ uptake, and (upstream − downstream) is the length of the measurement reach (m). We calculated NO$_3^-$ uptake length (S$_w$), the average distance traveled by a NO$_3^-$ ion prior to removal from the water column, as the inverse of k. We calculated NO$_3^-$ uptake velocity (v$_f$, mm min^{-1}), a metric which accounts for the influence of water depth and velocity on S$_w$, as:

$$v_\mathrm{f} = (\mathrm{Q/w}) * k$$

(4)

where Q is discharge and w is stream width.

For reaches where NO$_3^-$ uptake was below detection (i.e. the ^{15}NO$_3^-$ tracer flux did not decrease across the length of the experimental reach) we calculated the maximum possible k value the stream could have supported without resulting in a measurable decrease in tracer ^{15}NO$_3^-$ flux (e.g. method detection limit). The method detection limit was calculated using simulation modeling based on the observed variability in the replicate NO$_3^-$ concentration, ^{15}NO$_3^-$, and Br$^-$ measurements. Following the approach of Hanafi et al. ([2007]), we defined a distribution of possible values for each analyte during the background and plateau sampling using the mean and standard deviations of the 3–5 replicate measurements. We then randomly picked values from the defined distributions for each analyte and calculated k as described above. This process was repeated 10,000 times and the mean and 95 % confidence interval was calculated from these 10,000 k estimates. We then decreased the mean ^{15}NO$_3^-$ tracer flux value for the downstream station during the plateau by increments of 0.1 % until the 95 % confidence interval no longer included 0. We defined the minimum detection limit as the smallest

k value with a 95 % confidence interval that did not include 0.

Statistical analysis

We used paired t-tests to test for differences in NO$_3^-$ uptake rates (e.g. S$_w$ and v$_f$) and ER between open and buried reaches. Paired t-tests block for spatial (e.g. stream to stream) and temporal (e.g. season) variability that may otherwise obscure the effect of reach. We substituted the minimum detection limit for the true NO$_3^-$ uptake rate in the 3 buried and 1 open reach where the ^{15}NO$_3^-$ tracer flux did not decline across the reach. The true uptake rate lies somewhere between 0 and the minimum detection limit, therefore this approach will overestimate the uptake rates. Since three of the four reaches where the NO$_3^-$ uptake was below detection were in buried reaches, this statistical analysis will underestimate the effect of stream burial on NO$_3^-$ uptake.

Stream burial could influence NO$_3^-$ uptake via hydrologic and/or biologic mechanisms. If the effect is purely hydrologic, S$_w$ should scale linearly with specific discharge (Q$_s$), defined as the ratio of discharge and width (Hall et al. [2009]; Stream Solute Workshop [1990]). Any deviation from this relationship indicates that burial has affected the biological demand for NO$_3^-$. To assess the relative importance of these two mechanisms we plotted the ratio of S$_w$ in the buried and open reaches of each stream versus the ratio of Q$_s$ in the buried and open reaches of each stream. Deviation from the 1:1 line indicates that burial has affected the biological demand for NO$_3^-$.

We calculated the ratio of NO$_3^-$ v$_f$ in the open and buried reaches of each stream and season as an indicator of the magnitude of the burial effect on biological NO$_3^-$ demand. To determine the factors controlling the burial effect, we used simple linear regression to relate this metric to the length of the buried reach, water travel time through the buried reach, and the ratio of ER in open to buried reaches.

We used general linear models to test for an effect of reach (e.g. buried or open), season, and a reach X season interaction on fine, coarse, and attached benthic organic matter standing stocks. The 10–20 replicate samples of each organic matter type collected from each reach-season combination were used in the model. The reach scale organic matter standing stock values (i.e. one value per reach) were used to explain variation in ecosystem process rates (see below).

When ecosystem process rates (e.g. NO_3^- uptake or ER) were found to differ between open and buried reaches, we used general linear models to determine which variables were responsible for the differences. We used a backward selection procedure starting with a model containing all candidate independent variables and their interaction with reach (e.g. buried or open). A significant interaction would indicate that a specific variable is controlling the process, but is doing so differently in the buried and open reaches. We first removed all non-significant two-way interactions from the model. If no interactions were retained, we also removed reach as a main effect because it yielded no additional insight into the factors responsible for the stream burial effect. We repeated this procedure using data from only the buried reaches to determine which factors control variation in uptake rates among buried streams. We used one-way analysis of variance, with season as the main effect, to determine if the temporal component of the experimental design (i.e. seasonal sampling) generated variability in NO_3^- cycling rates and other stream characteristics.

When necessary, we applied a natural log transformation to the dependent variable to ensure model residuals conformed to the assumptions of normality and homogeneity. The data set was composed of 24 observations of 32 independent variables, therefore not all independent variables could be included as candidates in the statistical models without overfitting. We reduced the number of candidate variables by choosing representative variables from groups of related measures. We further reduced the pool of candidate variables by using expert opinion to remove intercorrelated variables. No two variables in final candidate set had Pearson correlation coefficients greater than 0.5. We conducted all statistical analyses in R (R Development Core Team 2011).

Results

Physicochemical characteristics

Water temperature ranged from 3.0 °C in winter to 23.8 °C during the summer (Table 2). Stream discharge (Q) ranged from 0.9 to 80 L s^{-1} during the study, was highest during the winter, lowest during the summer, and greater in AMB than EST or EAS. Water velocities in the buried reaches (range:

1.9–14.2 m min^{-1}) exceeded those in the open reaches (range: 0.8–4.0 m min^{-1}) of the same stream by a factor of 3.8, on average ($p < 0.001$, Table 2). Stream width was greater in the open reaches than in the buried reaches at EST and EAS ($p < 0.001$), though not in AMB. Specific discharge ($Q_s = Q/w$), an index for the potential hydrologic control of S_w, was 2.0 and 3.0 times greater in the buried than open reaches at EAS and EST, respectively. At AMB, however, Q_s in the buried reaches was only 0.76 of that in the open reach.

Photosynthetically active radiation (PAR) in the open reaches was lowest during the summer (mean = 2.0 mol photon m^{-2} day^{-1}) and equally high during the fall and winter (mean = 3.6 mol photon m^{-2} d^{-1}; Table 2). No PAR penetrated into the middle of the buried reaches. Due to instrument malfunctions, PAR data are not available for the open reaches during the spring season.

Stream nutrient concentrations were moderately high throughout the study (Table 2). Nitrate ranged from 164 to 822 µg N L^{-1} (mean = 476 µg N L^{-1}) and did not differ by stream or reach ($p > 0.07$). Ammonium averaged 9 µg N L^{-1} and composed less than 2 % of the dissolved inorganic N pool among all reaches. Dissolved reactive phosphorus was lowest in EAS (mean = 25 µg P L^{-1}) and averaged 65 µg P L^{-1} in EST and AMB. DOC averaged 3.0 mg L^{-1}, ranged from 1.7 to 4.5 mg L^{-1}, and did not differ by reach ($p = 0.54$).

Benthic organic matter, bacteria abundance, and extracellular enzyme activity

At the per sample scale, benthic organic matter and chlorophyll *a* standing stocks were greater in the open than buried reaches ($p < 0.001$; Fig. 3a–d). CBOM standing stocks varied seasonally in both open and buried reaches, with the highest values occurring during the fall ($p < 0.001$). Both periphyton and chlorophyll *a* standing stocks were greatest during the spring and winter in the open reaches ($p < 0.001$), but did not vary seasonally in the buried reaches ($p > 0.05$). Periphyton was more abundant in the buried reaches lined with concrete (EST) and metal (EAS) than the reach that had filled in with sediment ($p < 0.001$). Reach-scale standing stocks are presented in Table 2 and follow the patterns described above, with the exception of periphyton at EST. At

Table 2 Study mean and range values for physicochemical variables including hydrology, water chemistry, organic matter standing stocks, and ecosystem metabolism

	EAS				AMB				EST			
	Open		Buried		Open		Buried		Open		Buried	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Temperature (°C)	11.3	7.4–17.8	11.9	8.1–16.6	12.6	3.8–23.0	11.6	3.0–21.5	14.3	3.8–23.8	13.8	3.0–22.1
Q (L s^{-1})	3.3	1.7–4.9	2.2	0.9–3.2	31.0	8.0–60.5	27.1	5.0–52.8	7.9	5.2–11.7	10.3	6.1–13.1
Velocity (m min^{-1})	1.4	0.9–1.7	6.9	4.3–9.3	2.4	0.8–4.0	5.9	1.9–9.2	2.5	2.0–3.0	9.2	6.7–14.2
Chemistry/light												
NO_3^--N (µg L^{-1})	375	259–592	310	226–428	499	314–692	535	258–804	510	164–822	629	378–811
NH_4^+-N (µg L^{-1})	4	1–6	5	1–6	13	1–35	20	6–48	7	1–16	8	3–19
DRP (µg L^{-1})	26	17–36	24	18–30	62	32–91	69	32–104	64	43–86	54	41–66
DOC (mg L^{-1})	3.8	2.8–4.3	3.6	2.6–4.2	2.8	1.8–3.5	3.0	1.7–4.5	2.6	1.9–3.3	2.5	1.9–2.8
PAR[a] (mol m^{-2} day^{-1})	2.1	[b]	0	0–0	2.8	1.5–4.3	0	0–0	3.2	2.4–4.3	0	0–0
Reach scale organic matter												
FBOM (g AFDM m^{-2})	6.3	2.8–11.5	0.5	0–1.0	12.5	4.8–18.0	9.2	4.0–16.6	20.2	12.5–40.4	2.0	0.7–3.7
CBOM (g AFDM m^{-2})	8.1	2.2–21.7	1.4	0–2.9	32.9	19.0–47.5	4.5	1.4–7.9	30.5	13.3–70.5	4.5	0.7–9.9
Periphyton (g AFDM m^{-2})	24.0	8.1–56.4	7.0	3.5–10.2	5.9	2.5–10.4	0.3	0.1–0.4	3.2	1.0–8.4	6.0	3.9–8.3
Metabolism												
GPP[c] (g O$_2$ m^{-2} day^{-1})	1.6	0.3–2.9			0.7	0.1–2.2			1.5	0.1–5.3		
ER (g O$_2$ m^{-2} day^{-1})	6.6	2.2–13.2	0.4	0.1–1.0	1.1	0.5–1.8	1.3	0.6–2.4	4.5	1.4–8.4	0.9	0.02–2.1
NEP[d]	−5.0	−13–−1			−0.4	−1.4–−0.4			−3.0	−7.8–−0.3		

Q discharge, NO_3^- nitrate, NH_4^+ ammonium, CBOM coarse benthic organic matter, GPP gross primary production, ER ecosystem respiration, NEP net ecosystem production, DRP dissolved reactive phosphorus, DOC dissolved organic carbon, PAR photosynthetically active radiation, FBOM fine benthic organic matter

[a] PAR: No measurements were made in the open reaches during the spring season due to instrument malfunctions

[b] Only one measurement made due to instrument malfunctions

[c] GPP: Zero in buried reaches

[d] NEP: Only calculated in open reaches. Equal to ER in buried reaches

Fig. 3 **a** Coarse benthic organic matter (CBOM) standing stocks, **b** fine benthic organic matter standing stocks, **c** benthic chlorophyll a, and **d** periphyton standing stocks in the buried and open reaches during each sampling season. *Error bars* are standard errors of the mean
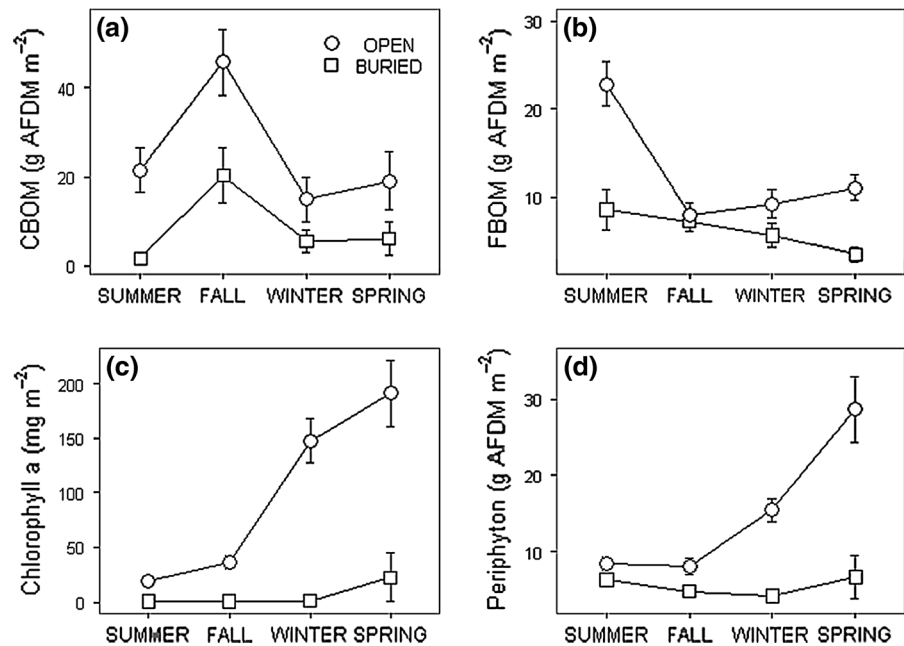
EST, areal periphyton standing stocks on individual rocks in the open reach were greater than on the concrete bed of the buried reach, but reach scale standing stocks were greater in the buried channel due to the preponderance of sand in the open reach (e.g. ≥ 35 % of the benthic surface area) which did not support periphyton (Table 2).

Algal cell density on tiles was three orders of magnitude greater in the open (mean = 2.1×10^6 ind cm^{-2}) than buried (mean = 1.0×10^3 ind cm^{-2}) reaches ($p < 0.001$; Fig. 4a). The number of 16S rRNA gene copies per cm^2 of colonized tile surface ranged from 6.5×10^3 to 2.6×10^7 and was related to stream and season ($p < 0.001$), but did not differ by reach ($p = 0.59$; Fig. 4b). The LCI calculated from the EEA levels associated with periphyton collected from the tiles ranged from 0.43 to 0.99 (mean = 0.84) and was 20 % greater in the buried than open reaches ($p = 0.002$; Fig. 4c).

Stream metabolism

Dissolved oxygen (DO), expressed as percent saturation, exhibited strong diurnal variation in the open reaches that was greatly attenuated, or completely absent, in the buried reaches. GPP ranged from 0.05 to 5.25 g O_2 m^{-2} day^{-1} (mean = 1.28) in the open reaches and the highest values were observed during

winter or spring (Table 2). The buried reaches were isolated from PAR and supported no GPP. ER was lower in the buried (mean = -0.86 g O_2 m^{-2} day^{-1}) than open (mean = -1.28 g O_2 m^{-2} day^{-1}) reaches ($p = 0.02$). Log transformed ER in the open and buried reaches was positively correlated with total organic matter standing stocks (AFDM m^{-2}) ($p = 0.01$, $r^2 = 0.24$). Net ecosystem production in the open reaches was negative, with the exception of AMB during the spring (NEP = 0.4 g O_2 m^{-2} h^{-1}; Table 2).

Stream $^{15}NO_3^-$ uptake

We detected a decline in $^{15}NO_3^-$ tracer flux across the reach in 19 of 24 tracer experiments (Table S1 in Supplementary material). Three buried reach and one open reach tracer injection did not yield a decrease in $^{15}NO_3^-$ flux and we calculated the method detection limit for these measurements. One measurement was not executed properly (i.e. poor mixing at the upstream station) and yielded no information on NO_3^- uptake rates.

Nitrate uptake length (S_w) was greater in the buried reach of every stream during all seasons, with the possible exception of the summer measurement in EST where NO_3^- uptake in the open reach was below detection (Fig. 5a–d; Table S1 in Supplementary material). The detection limit for k in this reach is
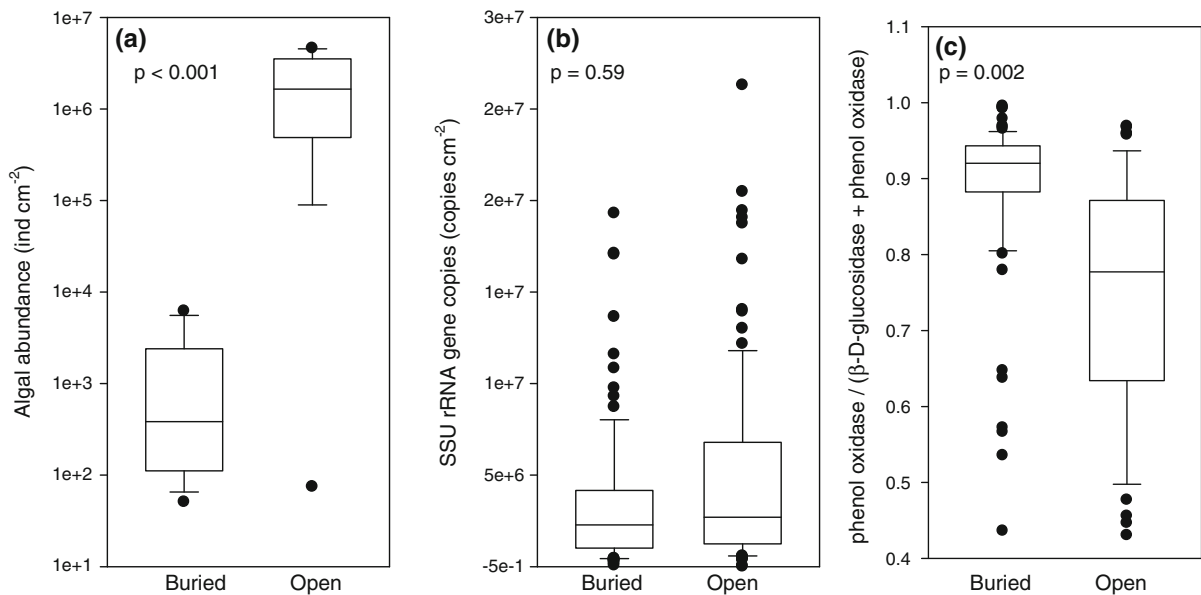
Fig. 4 Algal cell abundance (**a**), small-subunit rRNA gene copies (index of eubacterial abundance) (**b**), and carbon quality index (LCI) (**c**) in the buried and open reaches. *P* values are derived from paired *t*-tests

2.04×10^{-3} m^{-1}, indicating that the true value for the uptake length was ≥ 490 m (e.g. $1/k$), whereas S_w in the buried reach was 2,016 m. Nitrate uptake lengths in the buried reaches exceeded those in the open reaches of the same stream by a factor of 29 ($p < 0.001$), on average. Nitrate uptake length shortened with increasing ER ($p = 0.002$, $r^2 = 0.33$) and was not significantly related to season ($p = 0.53$).

In all cases the effect of burial on S_w was disproportionate to the effect on Q_s, indicating that burial suppressed biological N demand (Fig. 6). This was also evident in the NO_3^- ν_f measurements which were greater in the open reaches of all streams during all seasons, with the possible exception of the summer measurement in EST where NO_3^- uptake in the open reach was below detection (Fig. 5e; Table S1 in Supplementary material). The true value of ν_f in this reach was between 0 and 0.46 mm min^{-1}, whereas ν_f in the buried reach was 0.32 mm min^{-1}. Nitrate uptake velocities in the open reaches (range: 0.05–1.98 mm min^{-1}) exceeded those in the buried reaches (range: 0.003– 0.74 mm min^{-1}) of the same stream by a factor of 17.1 ($p < 0.001$). Uptake velocity was not significantly related to season ($p = 0.79$). The only factor retained in the full model was ER (positive correlation, $p = 0.046$, $r^2 = 0.14$), but in pair-wise comparison, ν_f was weakly predicted

by algal abundance (positive correlation, $p = 0.04$, $r^2 = 0.15$) and was marginally correlated with reach-scale GPP (positive correlation, $p = 0.052$). Nitrate uptake velocity was not related to the standing stock of any benthic organic matter compartment.

Nitrate uptake velocity in the buried reaches varied considerably (range: 0.003–0.74 mm min^{-1}), but was unrelated to stream, season, or any other measured variable (e.g. water chemistry, temperature, organic matter, etc.). Similarly, the difference in NO_3^- ν_f between pairs of open and buried reaches was highly variable, but unrelated to stream, season, buried reach length, or water travel time through the buried reach.

Discussion

Our data clearly indicate that stream burial reduces NO_3^- retention and fundamentally alters the flow of energy through streams. We found that NO_3^- uptake length (S_w) was 29 times greater in buried than open reaches, reflecting differences in hydrology and biological N demand between reaches. Biological N demand in buried reaches was lower than that of open reaches due to reduced organic matter quantity and quality, as well as the absence of assimilatory NO_3^- demand by autotrophs. Stream burial also increased specific
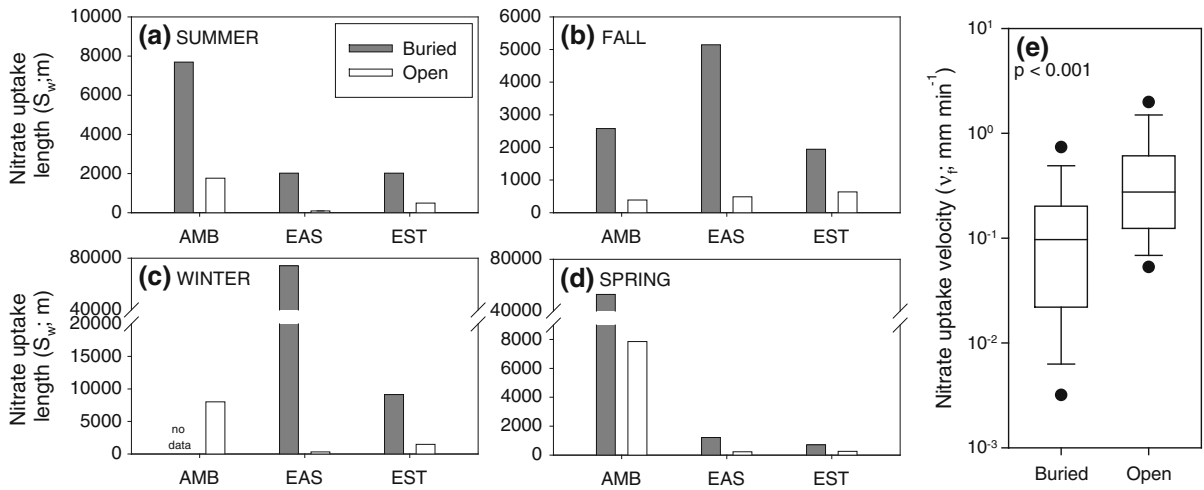
Fig. 5 a–d Paired barplots of nitrate uptake length (S_w) by reach. Each panel represents one season and each pair of bars represents the buried and open reaches within one of the three study sites. **e** Nitrate uptake velocity (v_f) for the open and buried reaches. P value derived from a paired t test
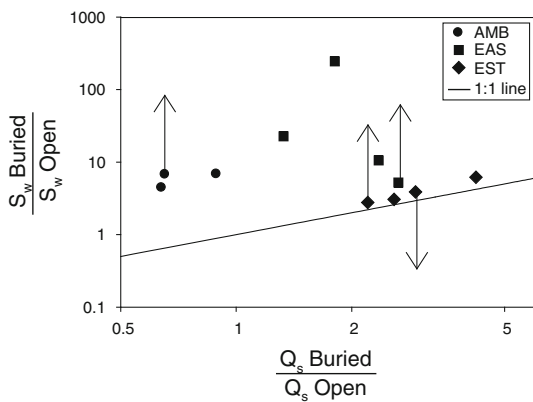

Fig. 6 Effect of stream burial on NO_3^- uptake length (S_w) versus the effect of burial on specific discharge (Q_s). Each point represents a unique stream-season combination (n = [3 streams * 4 seasons] − 1 missing data point = 11). Data points calculated with minimum detection limit (mdl) estimates are flagged with arrows indicating the direction the points would shift if S_w was greater than the mdl

discharge (Q_s) in two of the three buried reaches, which further enhanced the hydrologic transport of NO_3^- from the reach contributing to greater downstream loads of NO_3^- to N-sensitive water bodies.

While stream burial reduced biological NO_3^- demand, buried streams are not sterile ecosystems devoid of biological activity. The buried reaches in this study supported periphyton communities, ER, and NO_3^- uptake, but at much lower levels than in open

reaches. These extensively engineered systems are typically not considered as functional units involved in material transformations (Kaushal and Belt 2012), but our data suggest they should be considered an integral component of urban stream networks and included in assessments of stream ecosystem function at the river network scale, particularly in systems where the headwaters are dominated by buried streams. Buried streams located in the extreme headwaters (i.e. storm drain inlets) are well connected to the watershed and may facilitate the rapid transport of organic matter and nutrients to stream networks. The effect of urban stormwater infrastructure on downstream ecosystem function should be investigated in future research.

Effect of stream burial on NO_3^- uptake: hydrologic mechanisms

We found that stream burial increased NO_3^- S_w, indicating that burial substantially reduces the N-removal capacity of streams. This effect could have occurred through biotic or abiotic mechanisms, or a combination of both. An abiotic factor that affects S_w is stream water NO_3^- concentration. As NO_3^- concentration increases, the pool of NO_3^- molecules that biological activity could remove increases, making it less likely that any one molecule will be removed per unit time, causing S_w to lengthen, even under constant biological N demand. However, NO_3^- concentration

can also influence biologic N demand through Michaelis–Menten kinetics (Mulholland et al. 2002) which predicts that NO_3^- concentration can increase more rapidly than biological N demand, resulting in lengthening S_w with increasing NO_3^- concentration. We intentionally minimized the effect of NO_3^- concentration in our analysis by comparing uptake length in buried and open reaches that were nested within streams and separated by only 30–100 m, which ensured that NO_3^- concentrations did not differ substantially between reaches. Specific discharge (Q_s), calculated as discharge divided by width, is another abiotic factor that has a direct effect on S_w. Specific discharge is reported in units of area per time and expresses the benthic surface area over which stream water passes per unit time. Large Q_s values indicate that water column nutrients are rapidly transported over large expanses of stream sediment with little opportunity for biological uptake.

The effect of stream burial on Q_s varied across streams and was related to pipe design. The buried reach at AMB flowed through an oversized corrugated metal pipe (i.e. 4.5 m wide at base) with a Q_s about 20 % lower than the open reach. In EST and EAS, however, the buried reaches were constructed of narrow pipes that restricted the stream width to less than 1 m and the Q_s values ranged from 130 to 420 % of that in the open reaches. If the biological demand for NO_3^- did not differ between open and buried reaches, then the effect of stream burial on uptake length should scale linearly with the effect of burial on Q_s. It follows that if the effect of stream burial on uptake length was strictly due to hydrology, all observations should fall along the 1:1 line of a plot of the effect of burial on S_w versus the effect of burial on Q_s, where the 'effect size' is expressed as the ratio of the parameter value in the buried and open reaches (Fig. 6). All observations fell above the 1:1 line, indicating that differences in hydrology were not sufficient to account for differences in S_w between open and buried reaches in these streams. The unexplained effect, represented by the residuals between the observations and the 1:1 line, reflects reduced biological demand for NO_3^- in the buried reaches.

The relative importance of biological NO_3^- demand and Q_s in determining the effect of stream burial on S_w differed by stream. In AMB, stream burial enhanced hydrologic NO_3^- retention, as indicated by the lower Q_s, yet S_w was longer in the buried reach, a clear indication that burial increased S_w through purely biological mechanisms. On the other extreme, the data points from EST fell near the 1:1 line, indicating that the effect was predominantly hydrological. EAS represents an intermediate case where both hydrologic and biological effects were important, but the biological effect in EAS outweighed that of the hydrologic effect by a factor of 39 (median = 10).

Effect of stream burial on NO_3^- uptake: biological mechanisms

An alternative measure of NO_3^- uptake that accounts for Q_s is uptake velocity (v_f) which is expressed in units of distance per time and can be thought of as the depth of the water column from which NO_3^- can be removed via biological activity per unit time (Stream Solute Workshop 1990). Uptake velocity is independent of hydrology and represents an index of biological NO_3^- demand within the reach. Stream burial reduced v_f by a factor of 17, on average, representing the net effect of burial on biological NO_3^- removal processes. Our study included numerous indices of autotrophic and heterotrophic removal processes (e.g. GPP, ER, algal abundance) that explained variation in v_f nearly as well. However, all these indices were affected by stream burial and as a result were correlated, complicating our efforts to resolve the relative importance of these variables in controlling v_f.

Nitrate uptake could be directly and indirectly affected by the absence of light in buried streams. Without sunlight, the reach is incapable of supporting GPP and is completely dependent on energy subsidies from outside the local channel to support metabolic activities. The absence of autochthonous production in buried streams can cascade throughout the ecosystem, ultimately affecting organic matter quality, quantity, and the metabolic rates of heterotrophic organisms. In addition to the indirect effects of GPP on NO_3^- uptake, GPP directly affects NO_3^- uptake through the assimilative demand of primary producers, which can be the dominant N uptake mechanism in streams (Hall and Tank 2003; Hall et al. 2009). Although algae in the buried reaches were not contributing to NO_3^- uptake via autotrophic processes, it is possible that they contributed to NO_3^- retention through heterotrophic mechanisms. Some algal taxa have been documented to survive prolonged darkness through facultative heterotrophy, increasing the oxidation rates and the

variety of organic substrates utilized in the dark (Tuchman et al. 2006). The contribution of algal respiration to carbon cycling in the buried reaches is unlikely to be large, however, given their extremely low abundance (Figs. 3c, 4a).

Although the effect of burial on the abundance of autotrophs was expected, the effect on total periphyton biomass was not as easily predicted. Periphyton consists of a mixed community of autotrophs and heterotrophs which may interact antagonistically or mutalistically (Findlay et al. 1993). Elimination of autotrophs could benefit heterotophs if the two groups were competing for space or nutrients. If heterotrophs are relying on algae as a source of labile carbon, however, the loss of autotrophs could result in reduced heterotrophic biomass. While burial clearly reduced the abundance of the autotrophic community, periphyton abundance (g AFDM m^{-2}) was not affected to the same extent (Figs. 3c, d, 4a) and bacterial density was unaffected (Fig. 4b). These data indicate that the trophic link between bacteria and algae was not strong in these streams and that bacteria density is relatively resistant to stream burial. Therefore, the net effect of stream burial on periphyton was to produce a less dense assemblage dominated by heterotrophs. The shift in the composition of the periphyton assemblage is supported by the ratio of periphyton to chlorophyll a, an index of the relative abundance of heterotrophs in the periphyton assemblage, which was two orders of magnitude greater in the buried (mean = 68) than open reaches (mean = 0.7). Future studies need to take into consideration the identity, diversity, and functional (denitrifying) potential of the bacterial fraction to further understand how the bacterial network is impacted by stream burial.

Nitrate uptake velocity correlated positively with ER, which was greater in the open reaches. ER can be an important assimilative mechanism for water column NO_3^- removal in streams (Fellows et al. 2006; Hoellein et al. 2007; Newbold et al. 2006) and low ER in the buried reaches likely contributed to their reduced biological NO_3^- demand. In addition to functioning as a direct NO_3^- sink, ER can indirectly promote NO_3^- removal by creating the anoxic conditions necessary for denitrification, a type of anaerobic respiration in which NO_3^- is reduced to N_2 or N_2O gas and permanently removed from the ecosystem (Mulholland et al. 2009).

An important energy source for aerobic and anaerobic respiration is particulate organic carbon (Hedin 1990). Particulate organic carbon standing stocks were substantially greater in open reaches, partially explaining differences in ER between reaches. Low organic matter standing stocks in the buried reaches likely resulted from a combination of reduced allochthonous and autochthonous inputs and limited retention. The buried reaches had greatly simplified geomorphology with few in-stream devices to physically retain organic matter for subsequent microbial processing (Hoover et al. 2006).

Dissolved organic carbon is another important energy source for microbial respiration (Wiegner et al. 2005). While we found no differences in DOC concentration between reaches, the microbial LCI indicated a strong reduction in DOC quality in the buried reaches. This is most likely explained by the absence of photosynthesis and photodegradation in the buried reaches, which can be important sources of labile DOC in aquatic ecosystems (Bertilsson and Jones 2003; Moran and Covert 2003). Reduced DOC quality may have further limited heterotrophic metabolic processes, including NO_3^- uptake, in the buried reaches. Carbon limitation and low heterotrophic metabolic rates suggest that buried streams are functionally analogous to cave streams where C limitation greatly depresses biological N demand (Simon and Benfield 2002).

We measured the abundance of the bacterial component of the heterotrophic community, but our method did not quantify fungal abundance. Fungi can be important consumers in streams and the relative abundance of bacterial and fungal biomass is largely a function of particle size and composition, where fungi tend to dominate coarse particulate organic matter while bacteria dominate inorganic substrates (Findlay et al. 2002). The buried reaches in this study had little coarse particulate organic matter (Fig. 3a) and predominantly inorganic substrates, which suggests that they had lower fungal biomass than the open reaches. Differences in fungal biomass between open and buried reaches, determined by substrate characteristics at the mm to cm scale, may contribute to differences in ER and NO_3^- uptake at the reach scale.

Although our experimental design captured seasonal patterns in organic matter standing stocks and stream metabolism (Fig. 3a–d; Table 2), there were no statistically significant seasonal patterns in NO_3^- S_w, v_f, or the magnitude of the burial effect on these uptake

parameters. This may be due to counteracting seasonal drivers. For example, the stimulation of algal NO_3^- uptake by high light levels in the spring may be balanced by the inhibitory effect of cool water temperatures (Piña-Ochoa and Àlvarez-Cobelas 2006). Similarly, the effects of elevated water temperature during the summer may be balanced by low light. These offsetting effects were likely compounded by a relatively small sample size within each season (i.e. 3 streams per season). Nevertheless, the seasonal component of the study demonstrates that stream burial consistently increases NO_3^- spiraling length and reduces v_f, regardless of water temperature, stream discharge, organic matter availability, or any of the other controlling factors that vary seasonally.

Nitrate uptake velocity in the buried reaches are among the lowest reported in the literature for daylighted streams and two of the buried-reach v_f values are an order of magnitude lower than the minimum value reported from a survey of 72 streams using similar measurement methods (Mulholland et al. 2008). Although the buried-reach v_f values spanned a wide range (0.003–0.74 mm min^{-1}), the variability was not attributed to season, stream, or any of the other variables measured in this study. Similarly, the magnitude of the burial effect on NO_3^- v_f spanned a broad range among streams and seasons, but was unrelated to the length of the buried reach, water travel time through the buried reach, or differences in ER between open and buried reaches. Future studies should employ experimental designs better suited to explaining variation in NO_3^- v_f among buried reaches, possibly by examining a larger number and greater variety of buried reaches. This information could be used to inform management actions designed to minimize the effect of stream burial on NO_3^- removal.

Stream restoration implications

Stream burial reduces the NO_3^- removal capacity of streams, but this capacity may be restored through 'stream daylighting' (Pinkham 2000), a stream management practice where buried streams are removed from underground pipes and placed in new stream channels on the land surface. Numerous streams have been daylighted around the world, ranging from small headwaters to non-wadeable rivers (Buchholz and Younos 2007; Conradin and Buchli 2005; Pinkham 2000). The motivation for these projects is typically economic, including the need to eliminate the costly maintenance of aging infrastructure, or to create an aesthetic centerpiece for a revitalized commercial district. Although many of these projects claim a water quality benefit (Wild et al. 2011), none have collected the data to demonstrate it. Demonstrating the efficacy of stream daylighting to restoring a stream's NO_3^- removal capacity remains an important research need that our dataset has begun to address.

While daylighting may restore the NO_3^- removal capacity of buried reaches, it should be recognized that the NO_3^- removal capacity of most open reaches in urban areas is also impaired. The mean v_f value for the open reaches (0.50 mm min^{-1}) in this study was a factor of 3 lower than the mean v_f reported from a much larger study of NO_3^- uptake rates in streams using similar isotope tracer methods (Hall et al. 2009). Urban streams are subject to a suite of stressors that are likely to inhibit stream ecosystem functioning including flashy flows, emerging contaminants, and degraded riparian zones (Rosi-Marshall & Royer 2012; Walsh et al. 2005). It should be recognized, therefore, that stream daylighting is unlikely to restore the NO_3^- removal capacity of streams to pre-development levels, rather daylighting may increase NO_3^- removal capacity to a level equivalent to that of adjacent open reaches. The best results will likely be realized when the daylighted channels include elements specifically designed to promote NO_3^- retention such as a reduction of stream bank incision to reconnect floodplains or physical mechanisms to reduce stream velocity and retain organic matter (Groffman et al. 2005; Kaushal et al. 2008b; Mayer et al. 2010; Mayer et al. 2013; Passeport et al. 2013; Sivirichi et al. 2011).

Stream daylighting may be impractical in some space-limited urban environments. A possible alternative may be 'skylighting' where large grates, or other screened openings, are installed directly above the buried stream channel. Even if these devices are only a few meters in length, the patches of light would likely create localized areas of primary production that could stimulate biological NO_3^- removal. A related concept is to minimize the length of continuous stream burial whenever possible. Even short patches of the stream channel exposed to sunlight could stimulate NO_3^- retention for some distance downstream.

Nitrate uptake length is directly affected by Q_s, which is largely dictated by the design of the

underground stream channel. Wide pipes may reduce Q_s, enhancing NO_3^- retention, while narrow pipes tend to increase Q_s and promote hydrologic NO_3^- export. Wider pipes with a lower slope may also promote the retention of alluvium which will increase bed roughness and transient storage and further enhance organic matter and nutrient retention. A potential management intervention to promote NO_3^- retention in buried streams is to adopt a policy of promoting wider pipes. This should be tempered, however, with the recognition that extremely shallow buried reaches can be a dispersal barrier for aquatic organisms (Jungwirth et al. 1998), though this may not be a large concern in concentrated urban environments where the biological communities are already impaired. Another consideration is that disruption to the stream during pipe construction, which may be proportional to the size of the pipe, should be minimized according to best management practices. An alternate management action is to adopt channel designs that increase water travel time during periods of low flow, but do not impede water at high flows when flood risks are greater. One such design may include a small meandering channel within the pipe that effectively increased the length of the flow path at low flows, but at high flows the water would over flow the channel walls within the pipe and fill the entire pipe diameter. Another approach may be to increase bed roughness and/or install structures in pipes that slow water velocity and retain sediment, thereby creating a shallow benthic zone that may function to support microbial activity.

Urban stream managers can also control the bed material in buried streams, which may have implications for organic matter standing stocks and NO_3^- uptake. The buried streams with exposed metal (Fig. 2d) or cement stream bottoms (Fig. 2a, b) supported larger periphyton standing stocks than the buried stream that had filled in with a shallow layer of pebbles and sand (Fig. 2c; Table 2), likely because the more stable substrate provided better growing conditions (Cattaneo et al. 1997). This effect was somewhat offset, however, by the greater particle trapping ability of the sand and pebbles which retained substantially more FBOM than the metal or cement stream bottoms (Table 2). Whether FBOM or periphyton provides a stronger NO_3^- sink is not well known, though measurements in forested streams suggest FBOM supports higher NO_3^- uptake rates (Hoellein et al.

2009). Despite the differences in organic matter composition between buried streams, NO_3^- v_f did not differ between these reaches. Furthermore, our experimental design lacked replication within the three buried stream types (i.e. pebble, metal, concrete) making it difficult to associate any differences in NO_3^- v_f with streambed composition. Our study did demonstrate, however, that regardless of streambed composition, stream burial suppresses NO_3^- uptake. Future work should investigate whether this effect can be minimized by designing buried streams that support periphyton, trap particulate organic matter, and support hyporheic flow paths (Lawrence et al. 2013).

Effect of stream burial on NO_3^- transport at the watershed scale

Our data demonstrate that burial substantially reduces NO_3^- retention in streams. What may be of more interest to watershed managers, however, is the cumulative impact of stream burial on NO_3^- transport at the watershed scale. A related question is how much additional NO_3^- retention may be realized by daylighting currently buried reaches. In the next phase of this research, we will design a watershed model to explore these questions as a first step toward setting targets for watershed restoration. We anticipate the approach will be particularly useful for investigating the relationship between the spatial distribution of buried stream reaches and watershed scale NO_3^- retention along stream networks.

Summary and Conclusions

We found that stream burial increased NO_3^- uptake length through a combination of mechanisms. Burial eliminated primary production in the buried reaches which reduced biological NO_3^- demand, autochthonous organic matter inputs, and DOC quality. Burial also reduced allochthonous particulate organic matter availability by isolating the channel from the riparian zone and reducing in-stream organic matter retention. Low organic matter availability and quality translated to depressed ER rates and reduced biological NO_3^- demand. In some instances, stream burial increased Q_s, which further reduced the capacity of the stream channel to retain NO_3^-. The effect of stream burial on Q_s was not uniform across sites, however, and

oversized pipes can reduce Q_s and enhance hydrologic NO_3^- retention. Designing buried reaches with low Q_s values is one management action that may minimize the effect of stream burial on NO_3^- uptake. Further research will be necessary to elucidate the factors that control variation in N uptake among buried streams and to quantify the mechanisms of N transformation (e.g. denitrification).

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References

APHA (2005) Standard methods for the examination of water and wastewater. American Public Health Association, Washington DC

Bertilsson S, Jones JB Jr (2003) Supply of dissolved organic matter to aquatic ecosystems: autochthonous sources. In: Findlay SEG, Sinsabaugh RL (eds) Aquatic ecosystems. Interactivity of dissolved organic matter. Elsevier Science, San Diego, pp 3–24

Blakely TJ, Harding JS, McIntosh AR, Winterbourn MJ (2006) Barriers to the recovery of aquatic insect communities in urban streams. Freshw Biol 51(9):1634–1645

Buchholz T, Younos T (2007) Urban stream daylighting. Case study evaluations. In: VWRRC special report SR35-2007. Virginia Polytechnical Institute, Blacksburg

Bukaveckas PA (2007) Effects of channel restoration on water velocity, transient storage, and nutrient uptake in a channelized stream. Environ Sci Technol 41(5):1570–1576

Cattaneo A, Kerimian T, Roberge M, Marty J (1997) Periphyton distribution and abundance on substrata of different size along a gradient of stream trophy de Montréal. Hydrobiologia 354(1–3):101–110

Charles DF, Knowles C, Davis RC (2002) Protocols for the analysis of algal samples collected as part of the U.S. Geological Survey National Water-Quality Assessment Program. In: Report No. 02-06. Patrick Center for Environmental Research, The Academy of Natural Sciences, Philadelphia, p 124

Conradin F, Buchli R (2005) The Zurich stream day-lighting program: enhancing urban environment by environmental upgrading and restoration. In: Marsalek J, Sztruhar D, Giulianelli M, Urbonas B (eds) NATO science series: IV: earth and environmental sciences. Springer, Netherlands, pp 277–288

Coplen TB, Qi H, Révész K, Casciotti KL, Hannon JE (2012) Determination of the $\delta^{15}N$ and $\delta^{18}O$ of nitrate in water; RSIL lab code 2900. In: Révész K, Coplen TB (eds) Methods of the Reston Stable Isotope Laboratory. U.S. Geological Survey Techniques and Methods. http://pubs.usgs.gov/tm/2006/tm10c17/

Dedecker AP, Goethals PLM, D'Heygere T, De Pauw N (2006) Development of an in-stream migration model for *Gammarus pulex* L. (Crustacea, Amphipoda) as a tool in river restoration management. Aquat Ecol 40(2):249–261

Díaz RJ, Rosenberg R (2008) Spreading dead zones and consequences for marine ecosystems. Science 321(5891):926–929

Elmore AJ, Kaushal SS (2008) Disappearing headwaters: patterns of stream burial due to urbanization. Front Ecol Environ 6(6):308–312

Fellows CS, Valett HM, Dahm CN, Mulholland PJ, Thomas SA (2006) Coupling nutrient uptake and energy flow in headwater streams. Ecosystems 9(5):788–804

Findlay S, Howe K, Fontvielle D (1993) Bacterial-algal relationships in streams of the hubbard brook experimental forest. Ecology 74(8):2326–2336

Findlay S, Tank J, Dye S, Valett HM, Mulholland PJ, McDowell WH, Johnson SL, Hamilton SK, Edmonds J, Dodds WK, Bowden WB (2002) A cross-system comparison of bacterial and fungal biomass in detritus pools of headwater streams. Microb Ecol 43(1):55–66

Foster HR, Keller TA (2011) Flow in culverts as a potential mechanism of stream fragmentation for native and nonindigenous crayfish species. J N Am Benthol Soc 30(4):1129–1137

Groffman PM, Dorsey AM, Mayer PM (2005) N processing within geomorphic structures in urban streams. J N Am Benthol Soc 24(3):613–625

Hall RO, Tank JL (2003) Ecosystem metabolism controls nitrogen uptake in streams in Grand Teton National Park. Limnol Oceanogr 48(3):1120–1128

Hall RO, Tank JL, Sobota DJ, Mulholland PJ, O'Brien JM, Dodds WK, Webster JR, Valett HM, Poole GC, Peterson BJ, Meyer JL, McDowell WH, Johnson SL, Hamilton SK, Grimm NB, Gregory SV, Dahm CN, Cooper LW, Ashkenas LR, Thomas SM, Sheibley RW, Potter JD, Niederlehner BR, Johnson LT, Helton AM, Crenshaw CM, Burgin AJ, Bernot MJ, Beaulieu JJ, Arango CP (2009) Nitrate removal in stream ecosystems measured by ^{15}N addition experiments: total uptake. Limnol Oceanogr 54(3):653–665

Hanafi S, Grace M, Webb JA, Hart B (2007) Uncertainty in nutrient spiraling: sensitivity of spiraling indices to small

errors in measured nutrient concentration. Ecosystems 10(3):477–487

Hedin LO (1990) Factors controlling sediment community respiration in woodland stream ecosystems. Oikos 57(1):94–105

Hill BH, McCormick FH, Harvey BC, Johnson SL, Warren ML, Elonen CM (2010) Microbial enzyme activity, nutrient uptake and nutrient limitation in forested streams. Freshw Biol 55(5):1005–1019

Hoellein TJ, Tank JL, Rosi-Marshall EJ, Entrekin SA, Lamberti GA (2007) Controls on spatial and temporal variation of nutrient uptake in three Michigan headwater streams. Limnol Oceanogr 52(5):1964–1977

Hoellein TJ, Tank JL, Rosi-Marshall EJ, Entrekin SA (2009) Temporal variation in substratum-specific rates of N uptake and metabolism and their contribution at the stream-reach scale. J N Am Benthol Soc 28(2):305–318

Hoover TM, Richardson JS, Yonemitsu N (2006) Flow-substrate interactions create and mediate leaf litter resource patches in streams. Freshw Biol 51(3):435–447

Hopkins JNN (2007) The Cloaca Maxima and the monumental manipulation of water in archaic Rome. Waters Rome 4:1–15

Howarth RW, Billen G, Swaney D, Townsend A, Jaworski N, Lajtha K, Downing JA, Elmgren R, Caraco N, Jordan T, Berendse F, Freney J, Kudeyarov V, Murdoch P, Zhu ZL (1996) Regional nitrogen budgets and riverine N&P fluxes for the drainages to the North Atlantic Ocean: natural and human influences. Biogeochemistry 35(1):75–139

Jungwirth MS, Schmutz S, Weiss S (eds) (1998) Fish migration and fish bypasses. Fishing New Books, Vienna

Kaushal S, Belt K (2012) The urban watershed continuum: evolving spatial and temporal dimensions. Urban Ecosyst 15(2):409–435

Kaushal SS, Groffman PM, Band LE, Shields CA, Morgan RP, Palmer MA, Belt KT, Swan CM, Findlay SEG, Fisher GT (2008a) Interaction between urbanization and climate variability amplifies watershed nitrate export in Maryland. Environ Sci Technol 42(16):5872–5878

Kaushal SS, Groffman PM, Mayer PM, Striz E, Gold AJ (2008b) Effects of stream restoration on denitrification in an urbanizing watershed. Ecol Appl 18(3):789–804

Lawrence JE, Skold ME, Hussain FA, Silverman DR, Resh VH, Sedlak DL, Luthy RG, McCray JE (2013) Hyporheic zone in urban streams: a review and opportunities for enhancing water quality and improving aquatic habitat by active management. Environ Eng Sci 30(8):480–501

Leopold LB (1968) Hydrology for urban land planning—a guidebook on the hydrologic effects of urban land use. In: Geological Survey Circular 554. Geological Survey Circular 554

Lutz BD, Mulholland PJ, Bernhardt ES (2012) Long-term data reveal patterns and controls on stream water chemistry in a forested stream: walker Branch, Tennessee. Ecol Monogr 82(3):367–387

Marzolf ER, Mulholland PJ, Steinman AD (1994) Improvements to the diurnal upstream-downstream dissolved-oxygen change technique for determining whole-stream metabolism in small streams. Can J Fish Aquat Sci 51(7):1591–1599

Mayer PM, Groffman PM, Striz EA, Kaushal SS (2010) Nitrogen dynamics at the groundwater-surface water interface of a degraded urban stream. J Environ Qual 39(3):810–823

Mayer PM, Schechter SP, Kaushal SS, Groffman PM (2013) Effects of stream restoration on nitrogen removal and transformation in urban watersheds: Lessons from Minebank Run, Baltimore, Maryland. Watershed Sci Bull 4(1). http://www.cwp.org/effects-of-stream-restoration-on-nitrogen

Meyer JL, Poole GC, Jones KL (2005) Buried alive: potential consequences of burying headwater streams in drainage pipes. In: Proceedings of the Georgia Water Resources Conference

Moran MA, Covert JS (2003) Photochemically mediated linkages between dissolved organic matter and bacterioplankton. In: Findlay SEG, Sinsabaugh RL (eds) Aquatic ecosystems: interactivity of dissolved organic matter. Academic Press, Amsterdam, pp 243–262

Mulholland PJ, Tank JL, Webster JR, Bowden WB, Dodds WK, Gregory SV, Grimm NB, Hamilton SK, Johnson SL, Marti E, McDowell WH, Merriam JL, Meyer JL, Peterson BJ, Valett HM, Wollheim WM (2002) Can uptake length in streams be determined by nutrient addition experiments? Results from an interbiome comparison study. J N Am Benthol Soc 21(4):544–560

Mulholland PJ, Helton AM, Poole GC, Hall RO, Hamilton SK, Peterson BJ, Tank JL, Ashkenas LR, Cooper LW, Dahm CN, Dodds WK, Findlay SEG, Gregory SV, Grimm NB, Johnson SL, McDowell WH, Meyer JL, Valett HM, Webster JR, Arango CP, Beaulieu JJ, Bernot MJ, Burgin AJ, Crenshaw CL, Johnson LT, Niederlehner BR, O'Brien JM, Potter JD, Sheibley RW, Sobota DJ, Thomas SM (2008) Stream denitrification across biomes and its response to anthropogenic nitrate loading. Nature 452(7184):202–206

Mulholland PJ, Robert O. Hall J, Sobota DJ, Dodds WK, Findlay SEG, Grimm NB, Hamilton SK, McDowell WH (2009) Nitrate removal in stream ecosystems measured by ^{15}N addition experiments: denitrification. Limnol Oceanogr 54(3):666–680

Nam-choon K (2005) Ecological restoration and revegetation works in Korea. Landsc Ecol Eng 1(1):77–83

Newbold JD, Elwood JW, Oneill RV, Vanwinkle W (1981) Measuring nutrient spiralling in streams. Can J Fish Aquat Sci 38(7):860–863

Newbold JD, Bott TL, Kaplan LA, Dow CL, Jackson JK, Aufdenkampe AK, Martin LA, Van Horn DJ, de Long AA (2006) Uptake of nutrients and organic C in streams in New York City drinking-water-supply watersheds. J N Am Benthol Soc 25(4):998–1017

O'Brien JM, Hamilton SK, Podzikowski L, Ostrom N (2012) The fate of assimilated nitrogen in streams: an in situ benthic chamber study. Freshw Biol 57(6):1113–1125

O'Driscoll M, Clinton S, Jefferson A, Manda A, McMillan S (2010) Urbanization effects on watershed hydrology and in-stream processes in the Southern United States. Water 2(3):605–648

Passeport E, Vidon P, Forshay K, Harris L, Kaushal S, Kellogg D, Lazar J, Mayer P, Stander E (2013) Ecological engineering practices for the reduction of excess nitrogen in

human-influenced landscapes: a guide for watershed managers. Environ Manage 51(2):392–413

Pennino MJ, Kaushal SS, Beaulieu JJ, Mayer PM, Arango CP (2014) Effects of urban stream burial on nitrogen uptake and ecosystem metabolism: implications for watershed nitrogen and carbon fluxes. Biogeochemistry. doi:10.1007/s10533-014-9958-1

Piña-Ochoa E, Àlvarez-Cobelas M (2006) Denitrification in aquatic environments: a cross-system analysis. Biogeochemistry 81(1):111–130

Pinkham R (2000) Daylighting: new life for buried streams. In. Rocky Mountain Institute, Old Snowmass, Colorado

Poplar-Jeffers IO, Petty JT, Anderson JT, Kite SJ, Strager MP, Fortney RH (2009) Culvert replacement and stream habitat restoration: implications from brook trout management in an Appalachian Watershed, U.S.A. Restor Ecol 17(3):404–413

R Development Core Team (2011) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria

Roberts BJ, Mulholland PJ, Hill WR (2007) Multiple scales of temporal variability in ecosystem metabolism rates: results from 2 years of continuous monitoring in a forested headwater stream. Ecosystems 10(4):588–606

Rosi-Marshall EJ, Royer TV (2012) Pharmaceutical compounds and ecosystem function: an emerging research challenge for aquatic ecologists. Ecosystems 15(6):867–880

Ryu H, Tran H, Ware MW, Iker B, Griffin S, Egorov A, Edge TA, Newmann N, Villegas EN, Domingo JWS (2011) Application of leftover sample material from waterborne protozoa monitoring for the molecular detection of Bacteroidales and fecal source tracking markers. J Microbiol Methods 86(3):337–343

Sartory DP, Grobbelaar JU (1984) Extraction of chlorophyll a from freshwater phytoplankton for spectrophotometric analysis. Hydrobiologia 114(3):177–187

Shaffer JM, Beaulieu JJ (2012) Calibration of the Odyssey™ Photosynthetic Irradiance Recorder™ for absolute irradiance measures. J Freshw Ecol 27(4):599–605

Sigman DM, Casciotti KL, Andreani M, Barford C, Galanter M, Bohlke JK (2001) A bacterial method for the nitrogen isotopic analysis of nitrate in seawater and freshwater. Anal Chem 73(17):4145–4153

Simon KS, Benfield EF (2002) Ammonium retention and whole-stream metabolism in cave streams. Hydrobiologia 482(1–3):31–39

Sinsabaugh RL, Foreman CM (2001) Activity profiles of bacterioplankton in a eutrophic river. Freshwater Biol 46(9):1239–1249

Sinsabaugh RL, Shah JJF (2011) Ecoenzymatic stoichiometry of recalcitrant organic matter decomposition: the growth rate hypothesis in reverse. Biogeochemistry 102(1–3):31–43

Sinsabaugh RL, Findlay S, Franchini P, Fisher D (1997) Enzymatic analysis of riverine bacterioplankton production. Limnol Oceanogr 42(1):29–38

Sivirichi GM, Kaushal SS, Mayer PM, Welty C, Belt KT, Newcomer TA, Newcomb KD, Grese MM (2011) Longitudinal variability in streamwater chemistry and carbon and nitrogen fluxes in restored and degraded urban stream networks. J Environ Monit 13(2):288–303

Stream Solute Workshop (1990) Concepts and methods for assessing solute dynamics in stream ecosystems. J N Am Benthol Soc 9(2):95–119

Suzuki MT, Taylor LT, DeLong EF (2000) Quantitative analysis of small-subunit rRNA genes in mixed microbial populations via 5 '-nuclease assays. Appl Environ Microbiol 66(11):4605–4614

Toledo-Hernandez C, Ryu H, Gonzalez-Nieves J, Huertas E, Toranzos GA, Santo Domingo J (2013) Tracking the primary sources of fecal pollution in a tropical watershed: a one year study. Appl Environ Microbiol 79(5):1689–1696

Tuchman NC, Schollett MA, Rier ST, Geddes P (2006) Differential heterotrophic utilization of organic compounds by diatoms and bacteria under light and dark conditions. Hydrobiologia 561:167–177

Walsh CJ, Roy AH, Feminella JW, Cottingham PD, Groffman PM, Morgan RP (2005) The urban stream syndrome: current knowledge and the search for a cure. J N Am Benthol Soc 24(3):706–723

Wenger SJ, Roy AH, Jackson CR, Bernhardt ES, Carter TL, Filoso S, Gibson CA, Hession WC, Kaushal SS, Marti E, Meyer JL, Palmer MA, Paul MJ, Purcell AH, Ramirez A, Rosemond AD, Schofield KA, Sudduth EB, Walsh CJ (2009) Twenty-six key research questions in urban stream ecology: an assessment of the state of the science. J N Am Benthol Soc 28(4):1080–1098

Wiegner TN, Kaplan LA, Newbold JD, Ostrom PH (2005) Contribution of dissolved organic C to stream metabolism: a mesocosm study using ^{13}C enriched tree-tissue leachate. J N Am Benthol Soc 24(1):48–67

Wild TC, Bernet JF, Westling EL, Lerner DN (2011) Deculverting: reviewing the evidence on the 'daylighting' and restoration of culverted rivers. Water Environ J 25(3):412–421

Young RG, Huryn AD (1998) Comment: improvements to the diurnal upstream-downstream dissolved oxygen change technique for determining whole-stream metabolism in small streams. Can J Fish Aquat Sci 55(7):1784–1785