Urban infrastructure influences dissolved organic matter quality and bacterial metabolism in an urban stream network

C.P. Arango[1*], J.J. Beaulieu[2], K.M. Fritz[2], B.H. Hill[4], C.M. Elonen[4], M.J. Pennino[5], P.M. Mayer[5], S.S. Kaushal[6], and D.A. Balz[7]

[1]Department of Biological Sciences, Central Washington University, Ellensburg, WA 98926, USA

[2]US EPA, Office of Research and Development, National Risk Management Research Laboratory, Cincinnati, OH 45268, USA

[3]US EPA, Office of Research and Development, National Exposure Research Laboratory, Cincinnati, OH 45268, USA

[4]US EPA, Office of Research and Development, National Health and Environmental Effects Research Laboratory, Duluth, MN 55804

[5]US EPA, Office of Research and Development, National Health and Environmental Effects Research Laboratory, Corvallis, OR 97333, USA

[6]Department of Geology and Earth Systems Interdisciplinary Center, University of Maryland, College Park, MD 20742

[7]Pegasus Technical Services, Cincinnati, OH 45268

*Corresponding author email address, arangoc@cwu.edu

Running Head: urban infrastructure influences dissolved organic matter and bacterial response

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Summary

1. Urban streams are degraded by a suite of factors, including burial beneath urban infrastructure, such as roads or parking lots, which eliminates light and reduces direct organic matter inputs to streams from riparian zones. These changes to stream metabolism and terrestrial carbon contribution will likely have consequences for organic matter metabolism by microbes and dissolved organic matter (DOM) use patterns in streams. Respiration by heterotrophic biofilms drives the nitrogen and phosphorus cycles, but we lack a clear understanding of how stream burial and seasonality affect microbial carbon use.

2. We studied seasonal changes (autumn, spring, summer) in organic matter metabolism by microbial communities in open and buried reaches of three urban streams in Cincinnati, Ohio. We characterized DOM quality using fluorescence spectroscopy and extracellular enzyme profiles, and we measured the respiration response to carbon supplements in nutrient diffusing substrata (NDS). We hypothesized: 1) that algal production would lead to higher quality dissolved organic matter DOM in spring compared to other seasons and in open compared to buried reaches, 2) lower reliance of microbial respiration on recalcitrant carbon sources in spring and in open reaches, and 3) that microbial respiration would increase in response to added carbon in autumn and in buried reaches.

3. Several fluorescence metrics showed higher quality DOM in spring than autumn, but only the metric of recalcitrant humic compounds varied by reach, with more humic DOM in open compared to buried reaches. This likely reflected open reaches as an avenue for direct terrestrial inputs from the riparian zone.

4. Extracellular enzyme assays showed that microbes in buried reaches allocated more effort to degrade recalcitrant carbon sources, consistent with a lack of labile carbon compounds due to limited photosynthesis. Nitrogen acquisition enzymes were highest in autumn coincident with riparian leaf inputs to the streams. Buried and open reaches both responded more strongly to added carbon in autumn when terrestrial leaf inputs dominated compared to the spring when vernal algal blooms were pronounced.

5. Our data show that stream burial affects the quality of the DOM pool with consequences for how microbes use those carbon sources, and that heterotrophic respiration increased on carbon-supplemented NDS in buried and open stream reaches in both seasons. Different carbon quality and use patterns suggest that urban stream infrastructure affects spatio-temporal patterns of bacterial respiration, with likely consequences for nitrogen and/or phosphorus cycling given that carbon use drives other biogeochemical cycles. Management actions that increase light to buried streams could shift the balance between allochthonous and autochthonous DOM in urban streams with consequences for spatio-temporal patterns in bacterial metabolism.

Introduction

As suburban sprawl converts farmland and forests to urban infrastructure, and as the global trend of urbanization continues (Grimm *et al.*, 2008), the biological function of urban streams and its role in water quality has received increased attention (Kaushal *et al.*, 2015). Relatively small increases in impervious surface cover through urbanization can lead to a "flashy" hydrologic regime that reinforces entrenchment and channel incision in streams that are often already channelized to promote storm water drainage (Dunne and Leopold, 1978). These channelized streams retain less particulate organic carbon (Paul and Meyer, 2001) which, in combination with greater nutrient loads (Carpenter *et al.*, 1998) and reduced riparian canopies (Griffiths *et al.*, 2013), can alter the contribution of heterotrophic and autotrophic processes to stream metabolism (Kaushal *et al.*, 2014). For example, canopy opening and nutrient enrichment can increase autotrophy (Bernot *et al.*, 2010; Griffiths *et al.*, 2013; Alberts, Beaulieu & Buffam, 2016), but stream burial can increase the importance of heterotrophy relative to autotrophy (Beaulieu *et al.*, 2014; Pennino *et al.*, 2014). Depending on management, changes in organic matter processing by headwater streams may have an influence on the quantity and quality of organic matter subsidies further downstream along the urban watershed continuum (e.g., Kaushal & Belt, 2012; Pennino *et al*, 2014; Kaushal *et al.,* 2014)

Urban infrastructure expansion frequently results in low order streams being contained in buried pipes (Elmore & Kaushal, 2008). Further, open and buried stream reaches often alternate in an urban hydrological network so that stream metabolism can be vastly different in alternating stream reaches. For example, the severe reduction or absence of photosynthetically active radiation (PAR) fundamentally alters a stream ecosystem by eliminating the contribution of primary production to the food web. Although metabolism in buried streams shifts to net heterotrophic conditions, buried streams support a lower overall rate of ecosystem respiration compared to open reaches (Beaulieu *et al.*, 2014; Pennino *et al.*, 2014). Because buried stream reaches are often optimized to convey water quickly and efficiently for drainage purposes, they have increased water velocity which, in conjunction with net reduction in overall biological demand for nutrients (Beaulieu *et al.*, 2014; Pennino *et al.*, 2014), promotes nutrient export to downstream reaches and ecosystems (Beaulieu *et al.*, 2015). Burial also severely affects standing stocks of organic matter in streams, and buried reaches have lower overall coarse and fine benthic organic matter, periphyton, and chlorophyll *a* standing stocks compared to open reaches (Beaulieu *et al.*, 2014). Organic matter standing stocks in buried reaches also have little seasonality, except for higher coarse benthic organic matter (CBOM) in the fall whereas organic matter standing stocks exhibit pronounced seasonal patterns in open streams (Beaulieu *et al.*, 2014). Although the effect of stream burial on particulate organic matter standing stocks has been investigated, how this effect propagates through the microbial community to determine the abundance and quality of dissolved organic matter (DOM) is unknown.

DOM is an important energy source for ecosystem respiration (Meyer & Edwards, 1990), and microbial assimilation transfers this energy from dissolved sources to higher trophic levels (Meyer, 1994). Streams depend on allochthonous organic carbon inputs from the terrestrial landscape including leaf litter inputs from the riparian zone and DOM exported from soil by groundwater, as well as autochthonous sources from in-stream production of algae and/or macrophytes. These organic matter sources partly determine the quality of the DOM pool available to stream microbial communities. Allochthonous inputs have generally been considered more recalcitrant (i.e., lower quality) than autochthonous sources (McKnight *et al.*, 2001) due to the presence of more structurally complex carbon compounds

(e.g., lignin, tannin). In contrast, autochthonous carbon sources have fewer complex structural compounds and relatively more polysaccharides (e.g., cellulose, hemicellulose), so these carbon sources have been considered more labile (i.e., higher quality), but recent work has shown that terrestrial organic matter can also be a labile carbon source (Guillemette, McCallister & del Giorgio 2013; Attermeyer *et al.,* 2013). Moreover, increased phosphorus concentration enhances the ability of bacteria to consume terrestrial DOM (Guillemetteet al., 2013). Therefore, high nutrient concentrations in urban streams could influence the ability of microbes to respire terrestrial DOM as the allochthonous and autochthonous components of the DOM pool vary seasonally in conjunction with autumn leaf inputs and vernal algal blooms. Urban infrastructure likely also affects the composition of the DOM pool with open reaches having more autochthonous DOM than buried reaches due to greater light availability and associated higher levels of primary production (Kaushal *et al.*, 2014), and greater hyporheic exchange and higher respiration rates in open reaches (Beaulieu *et al.*, 2014) could influence microbial use and transformation of DOM. These seasonal and reach-scale differences in organic matter dynamics in urban streams are likely to influence the quality of the organic carbon pool and associated rates of carbon processing by microbes.

We used a nutrient diffusing substratum (NDS) approach coupled with extracellular enzyme activity (EEA) assays and characterized DOM via fluorescence to understand how bacterial organic carbon demand and use varies seasonally in buried and open stream reaches of urban streams. EEA assays characterize how microbes allocate energy to acquire different compounds (e.g., labile or recalcitrant carbon, nitrogen, etc.). These assays quantify the microbial demand for and environmental availability of substrates (Sinsabaugh & Follstad Shah, 2012), and they have been used to infer microbial organic nutrient limitation patterns in soils and sediments (e.g., Sinsabaugh, Hill & Follstad Shah, 2009) and within river networks (Hill *et al.*, 2012). DOM fluorescence properties can characterize various fractions of DOM as more or less labile, and allochthonous or autochthonous. This technique has been used to investigate seasonal (Catalan *et al.*, 2013) and landscape (Williams *et al.*, 2016) differences in the composition of the DOM pool in surface waters. We defined three hypotheses based on anticipated seasonal and spatial patterns of organic matter and light availability in urban streams containing open and buried reaches. We hypothesized that spring would have higher quality DOM than other seasons due to higher algal production prior to leaf-out of riparian trees, warming stream temperatures, higher sun angle, and longer day length, and that open reaches would have higher quality DOM than buried reaches due to more algal production in open versus buried reaches. Consequently, we hypothesized that microbes in spring would produce lower extracellular enzyme indicators associated with recalcitrant carbon acquisition, and that microbes in open reaches would exhibit less effort to acquire recalcitrant carbon compared to those in buried reaches. Finally, we hypothesized that microbial respiration would respond more strongly to carbon supplements in NDS in autumn due to the pulse of low quality terrestrial organic matter from the riparian zone and soil pools flushing during autumn rain, and that buried reaches would respond more strongly to carbon supplements in NDS than open reaches due to lower primary production and lower inputs and less retention of allochthonous carbon inputs.

Methods

Study Sites and Experimental Design

We studied three urban streams in or near Cincinnati, Ohio (USA) consisting of paired buried and open study reaches separated by a 30-100 m buffer reach. Two buried reaches flowed through corrugated

pipe and one through a concrete tunnel, and buried stream widths ranged from 0.5-4.5 m. Open reaches were generally incised with restricted riparian zones, contained mobile sandy sediments, and ranged in width from 2.1-3.9 m. In two of the three streams, the buried reach was upstream of the open reach. A more detailed site description can be found in Beaulieu *et al.* (2014).

We collected one water sample from the downstream and upstream end of each buried and open reach of each stream (n=4 in each stream and season) to characterize dissolved organic matter quality in summer and autumn 2011 and in spring 2012. Concurrently, we collected biofilms for EEA analysis from unglazed clay tiles (n=5 in open and n=10 in buried reaches) that had been deployed in the streams for a minimum of 6 weeks. Microbial carbon limitation patterns were measured using NDS. This design allowed us to compare how carbon quality, microbial enzyme activity, and the biofilm response to added carbon varied in space (buried versus open stream reaches) and time (summer, autumn, and spring). We also collected a suite of other environmental data including water chemistry, hydrologic parameters, organic matter standing stocks, and whole stream metabolism and nitrate (NO_3^-) uptake to understand how those factors related to how microbes respond to variation in DOM quality. Nitrate uptake and hydrologic parameters (i.e., transient storage) were measured using whole-stream ^{15}N-NO_3^- and bromide (Br^-) releases. Methods that describe the processing of isotope samples, calculating NO_3^- uptake rate, and modeling transient storage parameters and one- and two-station whole-stream metabolism are beyond the scope of this paper, but are detailed in Beaulieu *et al.* (2014).

DOM Characterization

Dissolved organic matter quality was characterized using fluorescence excitation-emission matrices (EEMs; Coble *et al.*, 1990; Coble 1996; Cory *et al.*, 2010) measured on a Fluoromax-4 spectrofluorometer (Horiba Instruments, Kyoto, Japan). This technique quantifies humic-like, fulvic-like, and protein-like fractions within the bulk DOM pool, which in turn are generally related to the lability or recalcitrance of DOM. EEMs were measured using excitation wavelengths at 10 nm intervals between 240-450 nm at and emission wavelengths at 2 nm intervals from 290-600 nm. Three-dimensional EEMs were then instrument corrected, blank subtracted, and normalized by the water Raman signal (Cory *et al.*, 2010) using Matlab software, but we did not measure absorbance for each sample, so we could not perform the standard inner-filter correction (IFC) on the EEMs on all samples. Based on Kothawala *et al.* (2013), the IFC is needed when absorbance exceeds 0.042. For spring samples only we measured absorbance using a scanning spectrophotometer, and for these samples, absorbance at 600 nm ranged from 0.001 to 0.003, and absorbance at 290 nm ranged from 0.03 to 0.09, indicating that IFC is needed for our samples. Also, using equation 2 (Kothawala *et al.* 2013) and absorbance and EEM data for spring samples, we calculated that the inner filter effect can cause between a 0.7 and 36% reduction in fluorescence, particularly at lower wavelengths where absorbance is highest. However, for the spring samples that were processed with and without the IFC, we found < 5% difference in all the EEM metric results, and we did not correct fluorescence metrics for this study.

The EEMs were used to calculate several DOM quality indices, including the humification index (HIX; Zsolnay *et al.*, 1999; Huguet *et al.*, 2009), the biological freshness index (BIX; Huguet *et al.*, 2009), the fluorescence index (FI; McKnight *et al.*, 2001), and the protein-to-humic ratio (P/H; Coble, 1996; Stolpe *et al.*, 2010). HIX characterizes the humic or autochthonous fractions of DOM (Zsolnay *et al.*, 1999; Ohno, 2002), and it is calculated as the ratio of integrated fluorescence emission intensity between 300-345 nm and between 435-480 nm at 254 nm excitation. Higher HIX values indicate DOM with humic

character whereas lower values indicate either less humic or more autochthonous DOM. BIX was calculated from the ratio of emission at 380 and 430 nm at excitation of 310 nm (Huguet *et al.*, 2009). BIX values <0.7 are associated with allochthonous DOM, values 0.8-1.0 are associated with autochthonous DOM, and values >1.0 are associated with aquatic bacterial sources; higher values indicate greater lability than lower values. FI is calculated from the ratio of the fluorescence intensity at 450 nm and 500 nm at excitation of 370 nm. FI values of about 1.5 indicate fulvic acids from microbes and values of about 1.2 indicate terrestrial-origin fulvic acids (Cory *et al.*, 2010). Finally, P/H was calculated from the EEMs whereby excitation at 275 nm and emission at 340 nm is associated with protein-like organic matter and excitation at 350 and emission at 480 is associated with humic-like organic matter (Coble, 1996; Stolpe *et al.*, 2010).

Extracellular enzyme activities

Biofilm collected from tiles deployed in the buried and open reaches was analyzed for EEA. Microbial assemblages produce extracellular enzymes to degrade organic matter and to acquire nutrients from their environment, and the activity of those enzymes serves as an index of environmental resource availability (Sinsabaugh & Foreman, 2001). Acquisition of labile carbon compounds was measured as β-D-glucosidase (BG) activity, and acquisition of recalcitrant carbon compounds was measured as polyphenol oxidase (POX) activity using the DOPA assay (Sinsabaugh & Foreman 2001; Sinsabaugh & Follstad Shah, 2011). A biofilm carbon quality index (CQI) was estimated as:

$$CQI=\ln POX/(\ln BG+\ln POX)$$

where the natural logs of POX and BG are proxies for the relative abundances of recalcitrant C and labile C, respectively. The CQI is proportional to the amount of recalcitrant C use in stream biofilms and thus characterizes the overall quality of the DOM pool (analogous to the lignocellulose index or LCI) whereby values greater than 0.5 indicate greater effort to acquire recalcitrant carbon and values less than 0.5 indicate greater effort to acquire labile carbon (Sinsabaugh & Follstad Shah, 2011). Nitrogen acquisition was measured as the activity of β-N-acetylglucosaminidase (NACE: EC 3.2.1.50; Sinsabaugh & Foreman 2001).

All EEA assays used microplate protocols (Sinsabaugh *et al.*, 1997; Sinsabaugh & Foreman, 2001) modified by Hill *et al.* (2010). Microplate arrays were run with quadruplicate assays for each tested enzyme and reference standard, which were prepared in sterile deionized water. Fluorescence quenching, or the decrease of emissions caused by interaction between target enzyme substrates and non-reactant chemicals, was measured by comparing fluorescence of standard solutions mixed with sample to that of standard solution mixed with buffer. We measured fluorescence (Model FLX800T, BioTek Instruments, Winooski, VT, USA) at excitation wavelength of 350 nm and emission wavelength of 450 nm.

Nutrient diffusing substrata

NDS arrays were deployed throughout the open reaches and where light was extinguished at the upstream and downstream ends of the buried reaches. Each NDS deployment site had one of four 0.5 M carbon amendments (a no-carbon control, glucose, arabinose, or cellobiose (n=8 each)) to represent increasing recalcitrance. We initially predicted increased carbon limitation at the downstream end of each buried reach due to microbial processing of DOM through the buried reach, but we found no

difference in carbon-limitation or EEM metrics between the upstream and downstream ends of the buried reaches. Therefore upstream and downstream NDS arrays were both considered "buried" in the statistical analysis. This resulted in n=8 per carbon treatment in open reaches and n=16 per carbon treatment in buried reaches per season and stream. The NDS were supplemented with 0.5 M N as NH_4Cl and 0.5 M P as KH_2PO_4 to alleviate any potential nutrient limitation that could confound interpretation of the heterotrophic response to added carbon, and we used porous glass disks rather than cellulose sponges to eliminate the heterotrophic response to the sponge as a particulate carbon source. NDS arrays were installed within open-ended PVC tubes for shade to reduce the potential for autotrophic biofilms to colonize the glass disks. NDS arrays were collected after a two week deployment and shipped overnight on ice for laboratory analysis within 24 h.

Laboratory analysis of biofilm respiration consisted of submerging the NDS disks in site water, incubating the disks in the dark for 3.5 h, and recording net oxygen change from the start to the end of the incubation. The glass disks were saved for calculation of biomass after weighing oven-dried (60 °C) samples before and after combustion in a muffle furnace (500 °C). The respiration response was scaled by disk area ($\mu g\ O_2\ cm^{-2}\ h^{-1}$) and by biomass ($mg\ O_2\ gAFDM^{-1}\ h^{-1}$), and to compare the respiration response among streams and seasons, we calculated the nutrient response ratio (NRR) as respiration response for an individual NDS cup divided by the mean control response for that particular deployment.

Water chemistry and hydrologic parameters

We collected filtered (0.45 μm) water samples in the field and stored them on ice for transport to the laboratory where they were acidified or frozen depending on the analyte. We used standard colorimetric methods (APHA, 2005) to measure nitrate + nitrite (hereafter, NO_3^-), dissolved reactive phosphorus (DRP), ammonium (NH_4^+), and bromide (Br^-) on a flow injection analyzer (Lachat Instruments, Loveland, CO USA). Dissolved organic carbon (DOC) concentration was measured using high-temperature Pt-catalyzed combustion and NDIR detection (Shimadzu TOC-VCPH, Columbia, MD, USA).

The breakthrough curve of Br^- released in conjunction with the $^{15}N\text{-}NO_3^-$ release was used in OTIS-P (Runkel, 1998), a one-dimensional advection, dispersion and transient storage model, to estimate solute hyporheic exchange parameters such as the cross-sectional area of the transient storage zone (A_s), the storage zone exchange coefficient (α),the storage zone residence time (T_{sto}), and the storage exchange flux (q_s) fraction of the median travel time due to transient storage, F^{200}_{med} (Runkel, 2002). These methods are described in detail in Beaulieu et al. (2014).

Organic matter standing stocks

We collected 10-20 samples of organic matter from different habitat units in a stratified-random design. Samples for coarse (>1 mm), fine (<1 mm), and attached (i.e., periphyton) organic matter were collected from 0.052 m^2 isolated by an open-ended plastic cylinder placed no more than 5 cm into the sediment. CBOM was removed by hand, and the sediments were agitated before taking a fine benthic organic matter (FBOM) subsample. Separately from the benthic organic matter samples, we collected periphyton from 10-20 rocks by scraping a known area (0.006-0.04 m^2, depending on rock size) of a rock with a wire brush. We calculated dry mass and ash-free dry mass of samples by weighing oven-dried (60 °C) samples before and after combustion in a muffle furnace (500 °C). We used a subsample of

periphyton to measure chlorophyll *a* using the trichromatic method (APHA, 2005) following hot ethanol extraction (Sartory & Grobbelaar, 1984).

Statistical Analysis

We used multivariate generalized least squares (GLS) or linear mixed effects (LME) models with alternate variance structures and model optimization (Zuur *et al.*, 2009) to test how DOM quality (HIX, BIX, FI, P/H) differed among seasons (spring, summer, autumn) and between reaches (buried, open). We also used GLS to test for differences in extracellular enzyme activity (POX, NACE, CQI) and response to additional carbon in NDS among seasons and between reaches. Models incorporated stream as a blocking factor. We used linear modeling to test relationships between carbon limitation patterns and water chemistry, hydrologic parameters, organic matter standing stocks, and whole stream metabolism and NO_3^- uptake. We used permutational multivariate analysis of variance using distance matrices (adonis in the vegan package for R; Oksanen *et al.*, 2016) to detect a relationship between the aggregated response of microbial respiration to glucose, arabinose, and cellobiose NDS additions and CBOM and FBOM standing stocks. All statistical analyses were done using R (R Core Team, 2016).

Results

Patterns in DOM Variability

DOM quality differed among seasons (spring, summer, autumn) and between reaches (buried, open). HIX differed by season (GLS, $p=0.0005$), with autumn having higher HIX than spring or summer, which were not different from each other. HIX also differed by reach (GLS, $p=0.021$) with open reaches having higher HIX than buried reaches when compared across all seasons (Figure 1A). P/H was generally < 1 indicating high humics. This ratio varied by season (GLS, $p<<0.001$), with spring and summer having a higher ratio (more protein) compared to fall (Figure 1C), and also by reach (GLS, $p=0.0002$) with open reaches having lower ratio (more humic-like) than buried reaches when all seasons were combined.

BIX and FI varied by season (GLS, $p<<0.0001$) but did not differ between buried and open reaches (Figure 1B and 1D, respectively). Although BIX and FI did not differ between spring and summer, both indices had significantly lower values in autumn compared to spring and summer. The BIX and FI values we measured indicate low autochthonous content and terrestrially-derived fulvic acids respectively in all seasons and reaches.

Patterns in extracellular enzyme activity

POX extracellular enzyme activity within biofilms was higher in buried reaches compared to open reaches (GLS, $p=0.004$) (Figure 2A). Because carbon uptake and use is often linked to the acquisition of N from the environment, we also analyzed differences in NACE. We measured highest values in the autumn, intermediate values in summer, and lowest values in spring with all seasons significantly different from each other (GLS, $p<<0.0001$) (Figure 2B). There were no differences between open and buried reaches.

We found no evidence of spatio-temporal differences in EEA directly associated with labile carbon use (BG). However, biofilm CQI from buried reaches reflected higher use of recalcitrant carbon than open

reaches (GLS, p=0.001), and summer biofilm had greater use of recalcitrant carbon than autumn biofilm (GLS, p=0.027). There were no differences between spring and autumn (Figure 3).

Response to added carbon

We deployed NDS amended with different carbon sources to detect differences in carbon use patterns between buried and open stream reaches, and among seasons. The NDS we deployed during summer were washed away by stormflows. Therefore, we focus our analysis on autumn and spring to contrast the response to added carbon when leaf inputs dominate compared to when vernal algae blooms dominate.

Respiration rates on NDS disks were not different among carbon source treatments when the data were scaled by biomass. However, when the respiration response was scaled by disk area, all NDS carbon amendments were significantly different than the control in all streams, seasons, and reaches (LME, $p<<0.001$). Respiration response was not detectably different among the three carbon amendments during any deployment (LME, $p>0.05$). Generally, autumn had higher NRR compared to spring in both reaches (LME, $p<<0.0001$; Figure 4). We found a significant interaction (LME, p=0.0009) between season (spring versus autumn) and reach (buried versus open) whereby the respiration response to added carbon was stronger for open compared to buried reaches in autumn, but it was stronger for buried compared to open reaches in spring (Figure 4). Further, the difference between the seasonal responses was less pronounced in buried reaches than in open reaches.

No relationships between NRR and water chemistry, hydrology, or ecosystem-scale functional attributes were detected. Although EEA and DOM quality metrics often differed between seasons and reaches, there was no direct linear relationship between NRR and those metrics. Although most standing stock metrics were also unrelated to NRR, there were weak positive relationships between reach-scale standing stocks of CBOM (adonis, p=0.036) and FBOM (adonis, p=0.053) with glucose, arabinose, and cellobiose NRR (Figure 5).

Discussion

Seasonal patterns of DOM characteristics

An earlier study showed that these urban streams had higher CBOM biomass in autumn compared to other seasons and higher chlorophyll *a* biomass in spring than in other seasons (Beaulieu et al. 2014). These seasonal changes in CBOM and chlorophyll biomass should result in corresponding changes in DOM sources and characteristics in autumn and spring. As we hypothesized, BIX and FI, metrics of labile DOM, showed a clear pattern of less labile carbon during autumn and more labile carbon during spring, likely due to riparian leaf fall producing a large influx of terrestrial DOM in the fall and vernal algal blooms producing a large influx of autochthonous DOM in the spring. HIX was similar to BIX and FI with autumn having higher humic character than spring or summer. This pattern was also seen in the P/H ratio, which showed more humic-like components in the autumn compared to the spring whereas summer was not distinctly different. Collectively, these patterns reflect the reach-scale standing stock data collected during this study. This seasonal pattern is seen in temperate forested mountain streams (Villanueva, Navarro & Albarino, 2016), ephemeral Mediterranean streams that flow during the autumn-spring wet season (Catalan *et al.*, 2013), and in other urbanized streams (Hosen *et al.*, 2014). Therefore,

temperate zone seasonality of autumn riparian leaf inputs and spring algal blooms imparts the dominant seasonal signature to the DOM pool of these temperate urban streams even though they have limited riparian zones due to channelization.

Despite the strong and consistent seasonal differences measured across multiple DOM optical properties in this study and higher primary production and algal standing stocks in the spring in a previous study (Beaulieu 2014), the low absolute values of BIX and FI showed that the DOM pool in the seasons we studied had a weak autochthonous component and a strong signature of terrestrially-derived fulvic acids. Dominance of terrestrial- or humic-derived carbon in the DOM pool may be a general pattern in streams draining urbanized basins. For example, terrestrial DOM sources include upwelling ground water, leaking stormwater infrastructure (Kaushal & Belt, 2012), and runoff from impervious surfaces (Hope *et al.*, 2004). DOM derived from these sources may overwhelm any autochthonous signature in streams. Alternatively, the year-round, stronger, more recalcitrant terrestrial characteristics could indicate that heterotrophic biofilms, which are typical in urban streams (Johnson, Tank & Dodds, 2009), rapidly remove high quality DOM from the water column. For example, labile autochthonous carbon stimulates water column carbon use for energy metabolism and/or assimilation (Franke, Bonnell & Ziegler, 2013). Furthermore, the presence of algal biofilms enhanced the EEA of heterotrophic biofilms, suggesting the rapid use of labile DOM in the presence of autochthonous production (Rier, Shirvinski & Kinek, 2014).

Spatial patterns of DOM characteristics

Our hypothesis that open reaches would have more labile carbon than buried reaches was not supported by the optical properties of the DOM pool. Although there were lower overall chlorophyll and CBOM biomass in buried compared to open reaches, reach type was not a significant predictor of BIX or FI, metrics that indicate labile DOM. One explanation for the lack of a burial effect on BIX and FI is that these optical properties of the DOM pool are determined by processes at the larger stream segment or catchment scale, rather than the reach scale. For example, in a previous study at the stream network scale and across a range of discharges in urbanized catchments, BIX never had a strongly autochthonous character despite many instances of net ecosystem productivity in the spring across 30 months of continuous sampling (Smith & Kaushal, 2015). Further, a cross-system study found that catchment scale land use was a good predictor of DOM composition (Williams *et al.*, 2016), which implies that catchment urbanization could have overwhelmed reach-scale differences in organic matter dynamics in our highly urbanized streams (16-34% impervious surface cover; Beaulieu *et al.*, 2014).

In contrast to BIX and FI, which were not affected by burial, HIX was higher in open reaches compared to buried reaches, which was contrary to our hypothesis that buried reaches would have lower quality DOM. This pattern was also reflected in the P/H ratio, which was likely driven by the relative abundance of humic-like compounds (denominator of the ratio) rather than patterns in aquatic production that affected low molecular weight fractions of the DOM pool (numerator of the ratio), consistent with the year-round humic nature of DOM in these urban streams. The pattern of higher HIX in open reaches was largely driven by high HIX in autumn (Figure 1A) possibly due to microbial processing of leached leaf litter that could increase humic compounds (Hur, Park & Schlautman 2009) or autumn rains that flushed humic compounds from the soil organic matter pool (Marín-Spiotta *et al.*, 2014; Singh et al. 2014). That buried reaches neither received direct inputs of riparian leaf litter nor retained litter exported from upstream due to higher velocities and fewer retention structures (Beaulieu *et al.*, 2014) could partially

explain the lower HIX values in buried reaches. Alternatively, dilution of the DOM pool by lower HIX sewage sources that leak into the buried reaches (Smith & Kaushal, 2015) could reduce HIX in the buried reaches. Sorption of humic compounds has been observed in other studies (Ohno, 2002; Zsolnay *et al.*, 1999), but we collected water for EEM metrics from the top and bottom of the buried reaches, and there was no significant difference in HIX collected at either end of a buried reach at a given sample time (data not shown). Although the EEA data indicated greater use of recalcitrant carbon in buried reaches compared to open reaches (see below), the lack of change in HIX as water flows through the buried reaches implies that microbial processing of humic compounds was not enough to reduce the HIX of the DOM pool. Higher HIX in the open reaches in spring is counter-intuitive given the presence of large algal standing stocks and high GPP, which would be expected to produce labile DOM. It is possible that the high HIX values resulted from DOM leached from greenfall inputs during leaf out and/or flower or seed production (Lewis & Likens, 2007), but we did not observe a CBOM peak in spring. However, in the overall context of this study, the median spring HIX values are lower than autumn in open and buried reaches, which is still consistent with an overarching seasonal affect driven by terrestrial sources in the autumn and aquatic sources in the spring.

Despite spatio-temporal differences in the DOM composition driven by seasonal differences in CBOM and algae, the DOM data showed that these urban streams were dominated by terrestrial humic sources, likely from constant seepage of DOM from soils to streams throughout the watershed. These data also showed secondary control over DOM quality due to spatial differences in organic matter inputs that alter the characteristics of the DOM pool in buried versus open reaches. Therefore urban infrastructure can influence the characteristics of the DOM pool in the urban stream network.

Patterns in Carbon Use – EEA

EEA reflects the composition of the DOM pool, as perceived by the microbial community. Although P/H and HIX indicated more humic and recalcitrant DOM in open reaches, buried reaches had higher POX activity than open reaches. This supported our hypothesis that the microbial community in buried reaches would allocate more energy toward acquiring recalcitrant carbon sources than in the open reach, regardless of season. This pattern was consistent with experiments showing greater POX activity in low light conditions (Wagner *et al.*, 2015). Lower values of POX in the open reaches indicate less effort to acquire recalcitrant carbon, likely because DOM leached from primary producers supplies an alternative, high quality carbon source. Conversely, the greater effort to acquire recalcitrant carbon in buried reaches is consistent with low chlorophyll, limited periphyton cover, and an extremely low reach-scale GPP (reported in Beaulieu *et al.*, 2014). This implies rapid use of high quality carbon produced in the open reaches and little export to downstream buried reaches, and is consistent with generally greater EEA in the presence of algal biofilms (Rier *et al.*, 2014).

The CQI, which aggregates EEA measures into a composite index of carbon use, also showed greater use of recalcitrant carbon in buried reaches. However, CQI showed a seasonal effect whereby summer had greater use of recalcitrant carbon than autumn, but autumn and spring were not different. This pattern may be driven by low CBOM, low chlorophyll *a*, and high FBOM in open reaches during the summer, and it suggests that the autumn pulse of terrestrial CBOM leaches a labile fraction of DOM (Attermeyer *et al.*, 2013) that microbes can use despite being dominated by low BIX and FI compounds. Furthermore, the lack of difference in enzyme activity between spring and autumn despite the major differences in

CBOM and chlorophyll may reflect the overall terrestrial signature of the DOM pool, which was dominated by terrestrial sources even in the spring.

The EEA data support our hypothesis that microbes would invest more effort in recalcitrant carbon acquisition in buried reaches. However, the optical properties of the DOM pool indicate that open reaches had higher HIX and lower P/H compared to buried reaches. This discrepancy may be due to differences in the composition of the DOM pool in the water column, where the EEM samples were collected, and at the sediment-water boundary layer where the microbial community was sampled for EEA. For example, labile carbon produced in the benthos of the open reaches may be rapidly and selectively processed by microbes with little being transported to the water column. Alternatively, the discrepancy between the optical and microbial indicators may be due to a temporal mismatch. The microbial EEA indicators likely reflect the integrated response of the microbial community to a DOM pool that varies on a diurnal basis with primary production, whereas the optical indices reflect the composition of the DOM pool at the moment the grab sample was collected. The mismatch between EEA and EEMs might also be related to specific substrates used in the EEA assays not corresponding to the compounds that determine the optical properties of DOM.

Some EEA metrics did not conform to the DOM characteristics; for example, we found no seasonal or reach scale differences in labile carbon acquisition effort measured as BG. However, N-acquiring enzymes (NACE) had the lowest abundance in the spring, coincident with higher quality algal DOM, and had the highest values in summer and autumn when overall chlorophyll was low and the system was dominated by lower quality FBOM and CBOM standing stocks respectively. Greater N-acquiring activity is associated with increasing C recalcitrance (Sinsabaugh & Follstad Shah, 2012), so this finding is consistent with a more labile pool of carbon in spring and a more recalcitrant pool in other seasons. For example, organic matter C:N ratio was lower during spring in forested Mediterranean streams (Villanueva *et al.*, 2016), and higher quality spring DOM in temperate rainforest streams was likely used as a source of labile C and N (Fellman *et al.*, 2009). Beaulieu *et al.* (2014) found no significant seasonal differences between NO_3^- or NH_4^+ concentrations, suggesting that higher quality spring DOM acted as a nitrogen source as well as a carbon source. Previous work has found seasonal changes in microbial demand for organic N in response to changes in C:N ratio and composition of organic matter (Kaushal & Lewis, 2005), and more work needs to be done to understand the role of organic matter as an energy source vs. a nitrogen source in some urban streams. The combined approach of using EEA and EEMs provided distinct and complementary information about the characteristics of, and microbial use of, the DOM pool. While some patterns in the EEMs were not confirmed by EEA, the combined approach showed that stream burial and seasonality influence the DOM pool, and although we did not see corresponding seasonal differences in EEA, we did find spatial differences in how microbes use carbon sources in buried versus open reaches of the urban stream network.

Patterns in Carbon Use – NDS

Biofilms in autumn responded more strongly to labile carbon in NDS than in spring, which supported our hypothesis that terrestrial leaf fall would depress DOM quality in autumn. However, the reach-scale response to added carbon varied between seasons. Open reaches responded more strongly to carbon than buried reaches in autumn, but responded less strongly to added carbon than buried reaches in the spring. Stronger response to added carbon in open reaches during autumn may be a result of the pulse of DOM from terrestrial leaves that entered the open reaches during leaf-fall whereas the weaker

response in open reaches during spring may be a result of the pulse of labile DOM derived from algal sources. These explanations are corroborated by the DOM optical properties. DOC concentration did not vary between seasons (data not shown), suggesting that the pulse of autumn leaves and spring algae blooms changed DOM composition rather than quantity.

Alternatively, differences in response to added C between reaches might be related to secondary reach-scale factors. For example, EEA assays confirmed that biofilms in buried reaches always invested more effort to acquire recalcitrant carbon, so they might have been better able to utilize the autumn pulse of terrestrial DOM compared to the open reaches. In contrast, biofilms in open reaches always invested less effort toward recalcitrant carbon acquisition which, when compounded by the fact that the pulse of autumn leaves was delivered directly to the open reaches, could have led to a stronger response to labile carbon supplements. Similarly, in the spring, open reaches responded less to the simple carbon sources in the NDS because the system had higher levels of high quality algal DOM. Some of this high quality DOM must be exported to buried reaches given the higher P/H ratio there, and the lower P/H ratio in open reaches is consistent with the potential for rapid use of algal DOM *in situ* by heterotrophic biofilms (Franke *et al.*, 2013; Rier *et al.*, 2014).

We found different results when we expressed respiration by area or biomass. When expressed by area, the temporal and spatial patterns were highly significant, but no patterns were evident when expressed by biomass. Therefore, the biofilm response to added carbon is not to increase the per cell carbon use rate, but simply to accumulate greater biomass. Given the fact that we relieved N and P limitation to focus on the carbon amendment response, these results might be most applicable to agricultural and urban streams which tend to have chronically high background nutrient concentrations (Carpenter *et al.*, 1998). The rapid processing of added carbon could also be a function of generally high inorganic nutrient concentrations in these urban streams in combination with the nutrients added to the NDS (Rosemond *et al.*, 2015).

Although we hypothesized that responses to the different carbon types in the NDS arrays would vary, biofilms responded similarly to all carbon sources (glucose, arabinose, cellobiose). Arabinose has been used as a less labile form of carbon in some studies (e.g., Kaplan *et al.*, 2006), but our results suggest that it is just as bioavailable as glucose in this study system. Similarly, we used cellobiose as a breakdown product of cellulose that we predicted would be less bioavailable than glucose or arabinose, yet it was equally bioavailable to those more simple carbon sources. It is unclear if arabinose and cellobiose bioavailability is equally high as glucose in most streams or if it was high in these urban streams because of the systemic dominance of recalcitrant carbon and/or the presence of N and P in the NDS agars.

Overall, these results indicate spatio-temporal variation in biofilm carbon use patterns related primarily to seasonal changes in the DOM pool and secondarily to reach scale patterns, such as stream burial. We found that the pulse of labile autochthonous carbon in the spring might have acted as a nutrient source as well as an energy source, but more work is needed to resolve this conclusively. Additionally, we documented strong responses to added carbon in NDS in these urban streams which could have been partly induced by the dominance of humic DOM sources from the watershed, limited *in situ* production of labile DOM due to stream burial, high background nutrient concentration leading to rapid CBOM consumption (e.g., Rosemond *et al.*, 2015), or some combination of those factors. Together, differences in carbon use patterns among buried and open reaches likely have implications at the river network

scale, particularly in drainages dominated by urban infrastructure that alternate between buried and open stream reaches. In particular, differential carbon use along the urban stream continuum is likely to have consequences for biogeochemical cycling of other nutrients and for downstream export of DOM, nutrients, and inorganic carbon.

Our work also suggests important considerations for management and restoration of urban streams. Stream daylighting is an engineering approach to urban stream restoration whereby buried streams are redesigned to be open to light (Pinkham, 2000). Daylighting is increasingly seen as an effective management approach to improve stream water quality and ecosystem function with respect to nutrient cycling in urban ecosystems (Beaulieu *et al.*, 2014; Pennino et al. 2014). Our results show that an additional mechanism of change may be to increase the amount and influence of autochthonous organic matter which has been shown to stimulate denitrification, a nitrogen removal process that can improve water quality (Newcomer *et al.*, 2012). Moreover, changing the balance of allochthonous and autochthonous DOM could also have consequences for the composition of the bacterial community (Attermeyer *et al.*, 2014), so stream daylighting might alter functional ecosystem attributes driven by which microbes are present in the community. Few studies have examined the biogeochemical impacts of daylighting streams (Newcomer Johnson *et al.*, 2016) and how the ecosystem changes over time after daylighting. Future research on carbon use in buried streams should elucidate how daylighting affects stream ecosystem function and provisioning of ecosystem services like nutrient reduction through *in situ* labile carbon production, especially in the broader context of compositional differences in dissolved carbon from watershed, riparian, and floodplain or groundwater sources.

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References

Alberts J.M., Beaulieu J.J., & Buffam I. (2016) Watershed land-use and seasonal variation constrain the influence of riparian canopy cover on stream ecosystem metabolism. *Ecosystems,* DOI: 10.1007/s10021-016-0040-9.

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

Attermeyer, K., Premke K., Hornick T., Hilt S. & Grossart H.P. (2013) Ecosystem-level studies of terrestrial carbon reveal contrasting bacterial metabolism in different aquatic habitats. *Ecology*, **94**, 2754-2766.

Attermeyer, K., Hornick T., Kayler Z.E., Bahr A., Zwirnmann E., Grossart H.P. & Premke K. (2014) Enhanced bacterial decomposition with increasing addition of autochthonous to allochthonous carbon without any effect on bacterial community composition. *Biogeosciences*, **11**, 1479-1489.

Beaulieu J.J., Mayer P.M., Kaushal S.S., Pennino M.J., Arango C.P., Balz D.A., Canfield T.J., Elonen C.M., Fritz K.M., Hill B.H., Ryu H. & Santo Domingo J.W. (2014) Effects of urban stream burial on organic matter dynamics and reach scale nitrate retention. *Biogeochemistry,* **121**, 107-126.

Beaulieu J.J., Golden H.E., Knightes C.D., Mayer P.M., Kaushal S.S., Pennino M.J., Arango C.P., Balz D.A., Elonen C.M., Fritz K.M. & Hill B.H. (2015) Urban stream burial increases watershed-scale nitrate export. *PLOS One,* DOI:10.1371/journal.pone.0132256.

Bernot M.J., Sobota D.J., Hall Jr R.O., Mullholland P.J., Dodds W.K., Webster J.R., Tank J.L., Ashkenas L.R., Cooper L.W., Dahm C.N., Gregory S.V., Grimm N.B., Hamilton S.K., Johnson S.L., McDowell W.H., Meyer J.L., Peterson B., Poole G.C., Valett H.M., Arango C., Beaulieu J.J., Burgin A.J., Crenshaw C., Helton A.M., Johnson L., Merriam J., Niederlehner B.R., O'Brien J.M., Potter J.D., Sheibley R.W., Thomas S.M., & Wilson K. (2010) Inter-regional comparison of land-use effects on stream metabolism. *Freshwater Biology*, **55**, 1874-1890.

Carpenter S.R., Caraco N.F., Correll D.L., Howarth R.W., Sharpley A.N. & Smith V.H. (1998) Nonpoint pollution of surface waters with phosphorus and nitrogen. *Ecological Applications* **8**, 559-568.

Catalan N., Obrador B., Alomar C. & Pretus J.L. (2013) Seasonal and landscape factors drive dissolved organic matter properties in Mediterranean ephemeral washes. *Biogeochemistry* **112**, 261-274.

Charles D.F., Knowles C. & Davis R.C. (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 .

Coble P.G. (1996) Characterization of marine and terrestrial DOM in seawater using excitation emission matrix spectroscopy. *Marine Chemistry*, **51**, 325–346.

Coble P.G., Green S.A., Blough N.V. & Gagosian R.B. (1990) Characterization of dissolved organic-matter in the black-sea by fluorescence spectroscopy. *Nature*, **348**, 432–435

Cory R.M., Miller M.P., McKnight D.M., Guerard J.J. & Miller P.L. (2010) Effect of instrument-specific response on the analysis of fulvic acid fluorescence spectra. *Limnology and Oceanography Methods*, **8**, 67–78.

Dunne T, & Leopold L.B. (1978) *Water in Environmental Planning*. Freeman, New York.

Elmore A.J. & Kaushal S.S. (2008) Disappearing headwaters: patterns of stream burial due to urbanization. *Frontiers in Ecology and Environment*, **6**, 308-312.

Fellman J.B., Hood E., D'Amore D.V., Edwards R.T. & White D. (2009) Seasonal changes in the chemical quality and biodegradability of dissolved organic matter exported from soils to streams in coastal temperate rainforest watersheds. *Biogeochemistry* **95**, 277-293.

Franke D., Bonnell E.J. & Ziegler S.E. (2013) Mineralisation of dissolved organic matter by heterotrophic stream biofilm communities in a large boreal catchment. *Freshwater Biology*, **58**, 2007-2026.

Griffiths N.A., Tank J.L., Royer T.V., Roley S.S., Rosi-Marshall E.J., Whiles M.R., Beaulieu J.J. & Johnson L.T. (2013) Agricultural land use alters the seasonality and magnitude of stream metabolism. *Limnology and Oceanography* **58**, 1513-1529.

Grimm N.B., Faeth S.H., Golubiewksi N.E., Redman C.L., Wu J., Bai X. & Briggs J.M. (2008) Global change and the ecology of cities. *Science*, **319**, 756-760.

Guillemette F., McCallister S.L. & del Giorgio P.A. (2013) Differentiating the degradation dynamics of algal and terrestrial carbon within complex natural dissolved organic carbon in temperate lakes. *Journal of Geophysical Research – Biogeosciences*, **118**, 963-973.

Hill B.H., McCormick F.H., Harvey B.C., Johnson S.L., Warren M.L. & Elonen C.M. (2010) Microbial enzyme activity, nutrient uptake and nutrient limitation in forested streams. *Freshwater Biology*, **55**, 1005–1019.

Hill B.H., Elonen C.M., Seifert L.R., May A.A. & Tarquinio E. (2012) Microbial enzyme stoichiometry and nutrient limitation in US streams and rivers. *Ecological Indicators*, **18**, 540-551.

Hope D., Naegeli M.W., Chan A.H. & Grimm N.B. (2004). Nutrients on asphalt parking surfaces in an urban environment. *Water, Air, and Soil Pollution*, **4**, 371-390.

Hosen J.D., McDonough O.T., Febria C.M. & Palmer M.A. (2014) Dissolved organic matter quality and bioavailability changes across an urbanization gradient in headwater streams. *Environmental Science and Technology*, **48**, 7817-7824.

Huguet A., Vacher L., Relexans S., Saubusse S., Froidefond J.M. & Parlanti E. (2009) Properties of fluorescent dissolved organic matter in the Gironde Estuary. Organic Geochemistry, **40**, 706–719.

Hur J., Park M. & Schlautman M.A. (2009) Microbial transformation of dissolved leaf litter organic matter and its effects on selected organic matter operational descriptors. Environmental Science and Technology, **43**, 2315-2321.

Johnson L.T., Tank J.L. & Dodds W.K. (2009) The influence of land use on stream biofilm nutrient limitation across eight North American ecoregions. *Canadian Journal of Fisheries and Aquatic Sciences*, **66**, 1081-1094.

Kaplan L.A., Newbold J.D., Van Horn D.J., Dow C.L., Aufdenkampe A.K. & Jackson J.K. (2006) Organic matter transport in New York City drinking-water-supply watersheds. *Journal of the North American Benthological Society*, **25**, 912-927.

Kaushal S.S. & Lewis W.M. (2005) Fate and transport of organic nitrogen in minimally disturbed montane streams of Colorado, USA. *Biogeochemistry*, **74**, 303-321.

Kaushal S.S. & Belt K.T. (2012) The urban watershed continuum: evolving spatial and temporal dimensions. *Urban Ecosystems*, **15**, 409-435.

Kaushal S.S., Delaney-Newcomb K., Findlay S.E.G., Newcomer T.A., Duan S.W., Pennino M.J., Sivirichi G.M., Sides-Raley A.M., Walbridge M.R. & Belt K.T. (2014) Longitudinal patterns in carbon and nitrogen fluxes and stream metabolism along an urban watershed continuum. *Biogeochemistry*, **121**, 23–44.

Kaushal S.S., McDowell W.H., Wollheim W.M., Newcomer Johnson T.A., Mayer P.M., Belt K.T. & Pennino M.J. (2015) Urban evolution: the role of water. *Water*, **7**, 4063-4087.

Kothawala D.N., Murphy K.R., Stedmon C.A., Weyhenmeyer G.A. & Tranvik L.J. (2013) Inner filter correction of dissolved organic matter fluorescence. *Limnology and Oceanography: Methods*, **11**, 616-630.

Lewis G.P. & Likens G.E. (2007) Changes in stream chemistry associated with insect defoliation in a Pennsylvania hemlock-hardwoods forest. *Forest Ecology and Management*, **238**, 199-211

Marin-Spíotta E., Gruley K.E., Crawford J., Atkinson E.E., Miesel J.R., Greene S., Cardona-Correa C. & Spencer R.G.M. (2014) Paradigm shifts in soil organic matter research affect interpretations of aquatic carbon cycling: transcending disciplinary and ecosystem boundaries. *Biogeochemistry*, **117**, 279-297.

McKnight D.M., Boyer E.W., Westerhoff P.K., Doran P.T., Kulbe T. & Andersen D.T. (2001) Spectrofluorometric characterization of dissolved organic matter for indication of precursor organic material and aromaticity. *Limnology and Oceanography*, **46**, 38–48.

Meyer J.L. (1994) The microbial loop in flowing waters. *Microbial Ecology*, **28**, 195-199.

Meyer J.L. & Edwards R.T. (1990) Ecosystem metabolism and turnover of organic carbon along a blackwater river continuum. *Ecology*, **71**, 668-677.

Newcomer T.A., Kaushal S.S., Mayer P.M., Shields A.R., Canuel E.A., Groffman P.M. & Gold A.J. (2012) Influence of natural & novel organic carbon sources on denitrification in forested, degraded-urban, & restored streams. *Ecological Monographs*, **82**, 449–466.

Newcomer Johnson T.A., Kaushal S.S., Mayer P.M, Smith R.M. & Sivirichi G.M. (2016) Nutrient retention in restored streams and rivers: a global review and synthesis. *Water*, **8**, doi:10.3390/w8040116.

Ohno T. (2002) Fluorescence inner-filtering correction for determining the humification index of dissolved organic matter. *Environmental Science and Technology*, **36**, 742–746.

Oksanen J., Guillaume Blanchet F., Friendly M., Kindt R., Legendre P., McGlinn D., Minchin P.R., O'Hara R. B., Simpson G.L., Solymos P., Stevens M.H.H., Szoecs E. & Wagner H. (2016). *vegan: Community Ecology Package*. R package version 2.3-5. https://CRAN.R-project.org/package=vegan.

Paul M.J. & Meyer J.L. (2001) Streams in the urban landscape. *Annual Review of Ecology and Systematics*, **32**, 333-365.

Pennino M.J., Kaushal S.S., Beaulieu J.J., Mayer P.M. & Arango C.P. (2014) Effects of urban stream burial on nitrogen uptake and ecosystem metabolism: Implications for watershed nitrogen and carbon fluxes. *Biogeochemistry*, **121**, 247–269.

Pinkham R. (2000) *Daylighting: New Life for Buried Streams*. Rocky Mountain Institute, Snowmass, CO.

R Core Team (2016). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna. URL https://www.R-project.org/.

Rier S.T., Shirvinski J.M. & Kinek K.C. (2014) In situ light and phosphorus manipulations reveal potential role of biofilm algae in enhancing enzyme-mediated decomposition of organic matter in streams. *Freshwater Biology*, **59**, 1039-1051.

Rosemond A.D., Benstead J.P., Bumpers P.M., Gulis V., Kominoski J.S., Manning D.W.P., Suberkropp K. & Wallace J.B. (2015) Experimental nutrient additions accelerate terrestrial carbon loss from stream ecosystems. *Science*, **347**, 1142-1145.

Runkel R.L. (1998) *One-Dimensional Transport with Inflow and Storage (OTIS): A Solute Transport Model for Streams and Rivers*. U.S. Geological Society, Water Resources Investigations Report 98-4018.

Runkel R.L. (2002) A new metric for determining the importance of transient storage. *Journal of the North American Benthological Society*, **21**, 529–543.

Sartory D.P. & Grobbelaar J.U. (1984) Extraction of chlorophyll a from freshwater phytoplankton for spectrophotometric analysis. *Hydrobiologia*, 114, 177–187.

Singh S., Inamdar S., Mitchell M. & McHale P. (2014) Seasonal pattern of dissolved organic matter (DOM) in watershed sources: influence of hydrologic flow paths and autumn leaf fall. *Biogeochemistry*, **118**, 321-337.

Sinsabaugh R.L., Findlay S., Franchini P. & Fisher D. (1997) Enzymatic analysis of riverine bacterioplankton production. *Limnology and Oceanography* **42**, 29–38.

Sinsabaugh R.L. & Foreman C.M. (2001) Activity profiles of bacterioplankton in a eutrophic river. *Freshwater Biology*, **46**, 1239–1249

Sinsabaugh R.L., Hill B.H. & Follstad Shah J.J. (2009) Ecoenzymatic stoichiometry of microbial organic nutrient acquisition in soil and sediment. *Nature*, **462**, 795-798.

Sinsabaugh R.L. & Follstad Shah J.J. (2011) Ecoenzymatic stoichiometry of recalcitrant organic matter decomposition: the growth rate hypothesis in reverse. *Biogeochemistry*, **102**, 31–43

Sinsabaugh R.L. & Follstad Shah J.J. (2012) Ecoenzymatic stoichiometry and ecological theory. *Annual Review of Ecology, Evolution, and Systematics*, **43**, 313-343.

Smith R.M. & Kaushal S.S. (2015) Carbon cycle of an urban watershed: exports, sources, and metabolism. *Biogeochemistry*, **126**, 173-195.

Stolpe B., Guo L., Shiller A.M. & Hassellov M. (2010) Size and composition of colloidal organic matter and trace elements in the Mississippi River, Pearl River and the northern Gulf of Mexico, as characterized by flow field-flow fractionation. *Marine Chemistry* **118**, 119–128

Villanueva V.D., Navarro M.B. & Albarino R. (2016) Seasonal patterns of organic matter stoichiometry along a mountain catchment. *Hydrobiologia*, **771**, 227-238.

Wagner K., Besemer K., Burns N.R., Battin T.J. & Bengtsson M.M. (2015) Light availability affects stream biofilm bacterial community composition and function, but not diversity. *Environmental Microbiology*, **17**, 5036-5047.

Williams C.J., Frost P.C., Morales-Williams A.M., Larson J.H., Richardson W.B., Chiandet A.S. & Xenopoulos M.A. (2016) Human activities cause distinct dissolved organic matter composition across freshwater ecosystems. *Global Change Biology*, **22**, 613-626

Zuur A.F., Ieno E.N., Walker N.J., Saveliev A.A. & Smith GM. (2009) *Mixed effects models and extension in ecology with R*. Springer, New York.

Zsolnay A., Baigar E., Jimenez M., Steinweg B. & Saccomandi F. (1999) Differentiating with fluorescence spectroscopy the sources of dissolved organic matter in soils subjected to drying. *Chemosphere*, **38**, 45–50

Figure Legends

Figure 1. Spatio-temporal variation in the (A) humification index (HIX), (B) biological freshness index (BIX), (C) protein to humic ratio (P/H), and (D) fluorescence index values derived from excitation-emission matrices. Lines within boxes are medians, box ends are 1st and 3rd quartiles, whiskers are 1.5 times the interquartile range. Different letters indicate seasonal means that are different from each other.

Figure 2. Spatio-temporal variation in the (A) polyphenol oxidase (POX), and (B) β-N-acetylglucosaminidase (NACE) activities. Different letters indicate seasonal means that are different from each other.

Figure 3. Spatio-temporal variation in the carbon quality index (CQI), where larger values indicate more recalcitrant carbon in the dissolved organic matter pool. Different letters indicate seasonal means that are different from each other.

Figure 4. Spatio-temporal variation in the nutrient response ratio (NRR: respiration/mean control) to added carbon when measured on an areal basis ($\mu g\ O_2\ cm^{-2}\ h^{-1}$).

Figure 5. Nutrient response ratio to added carbon increases with CBOM standing stock. Line of best fit shows the aggregate response of a multivariate permutational analysis where all carbon types were analyzed simultaneously.

Figures

Figure 1.

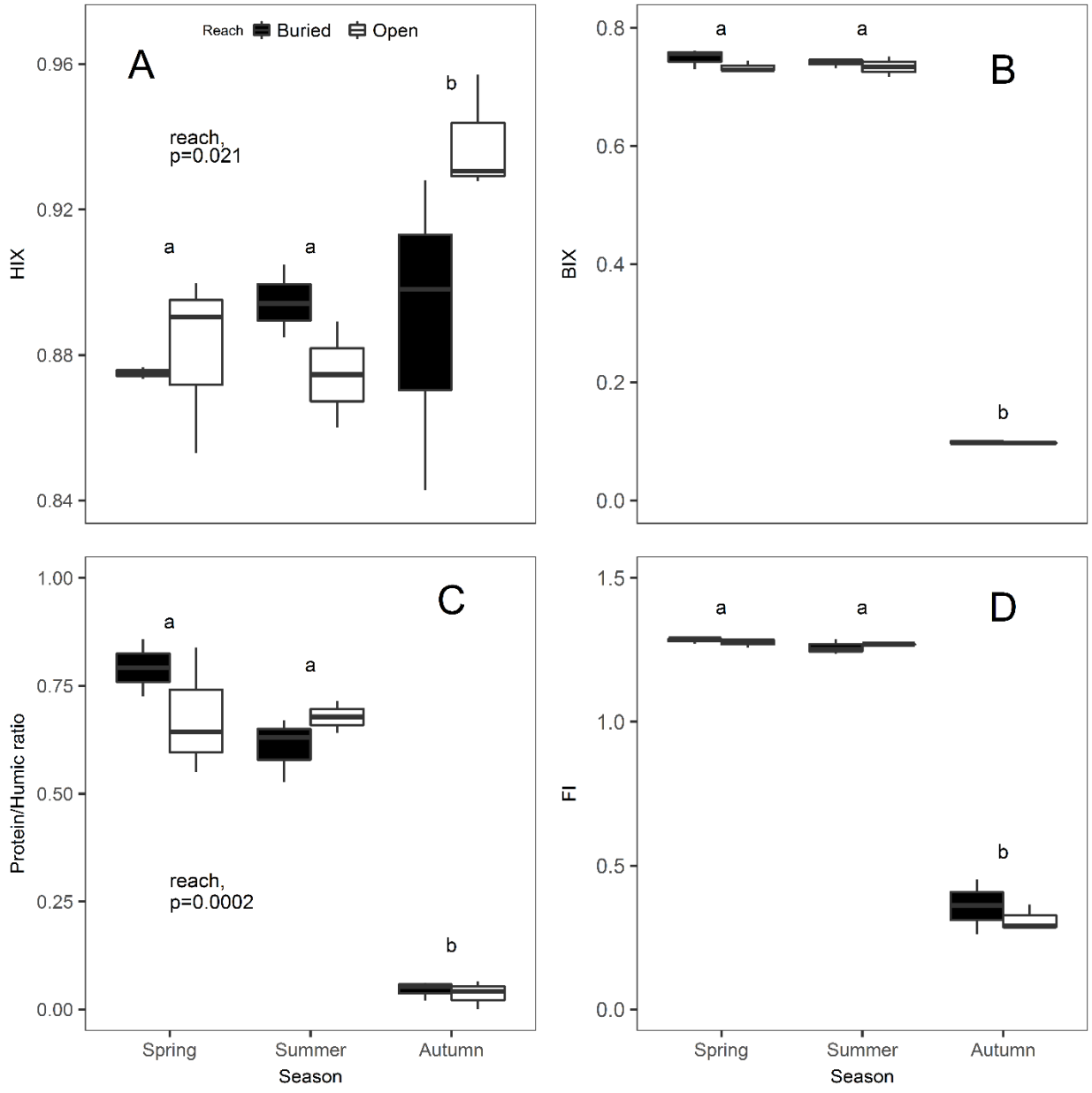
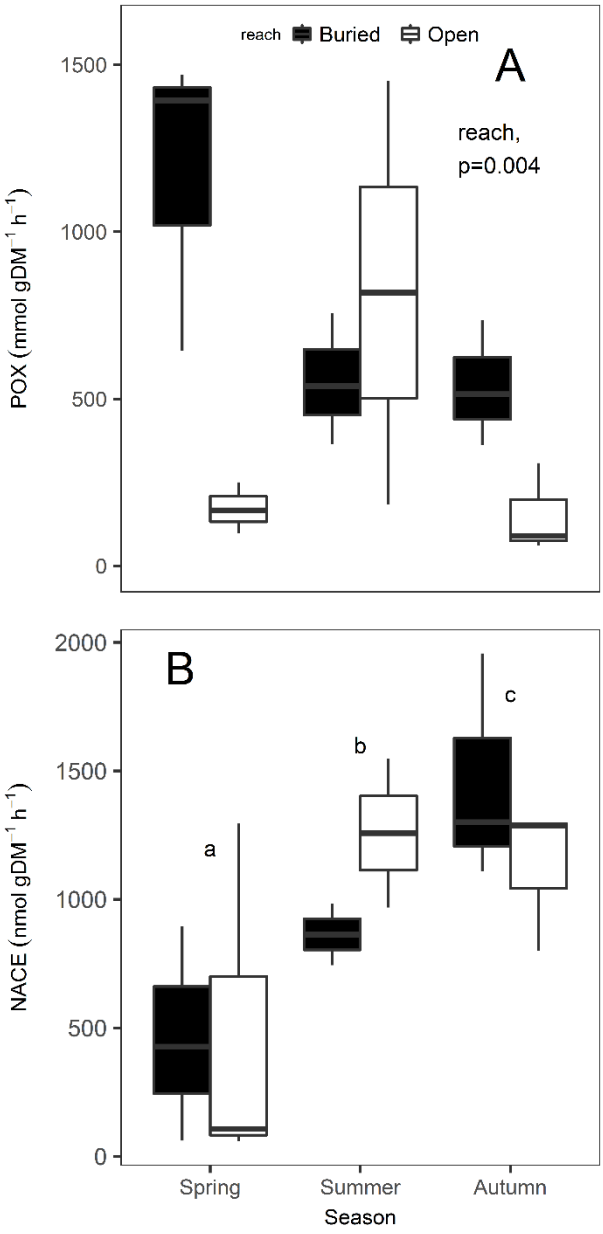


Figure 2.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NACE (nmol gDM-1 h-1)
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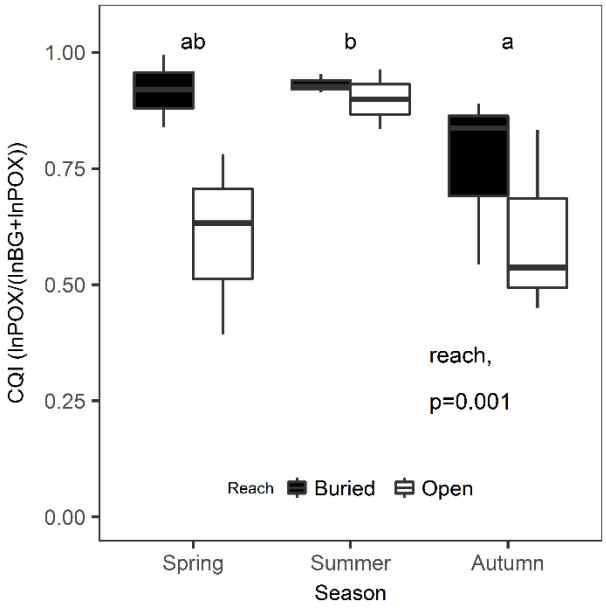
1500
1000
500
0
Spring
Summer
Autumn
Season

Figure 3.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CQI (lnPOX/(lnBG+lnPOX))
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Reach
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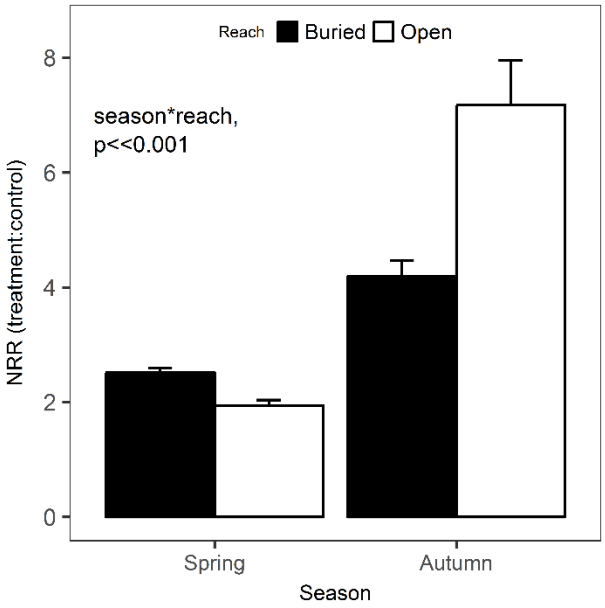
Open
Spring
Summer
Autumn
Season

Figure 4.


Reach
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season*reach,
p<<0.001
NRR (treatment:control)
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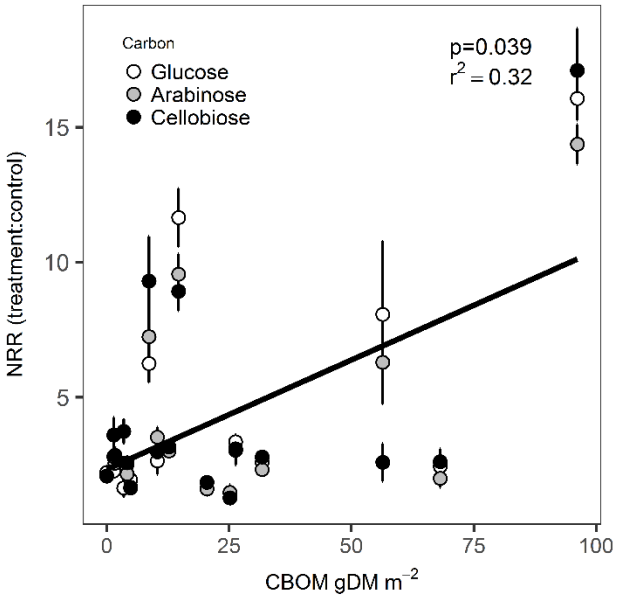
Spring
Autumn
Season

Figure 5.


Carbon
Glucose
Arabinose
Cellobiose
p=0.039
r2 = 0.32
NRR (treatment:control)
15
10
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25
50
75
100
CBOM gDM m−2