Carbon limitation patterns are linked to spatio-temporal changes in dissolved organic matter quality in an urban stream

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Abstract

Introduction

Methods

Study Sites and Experimental Design

We studied three urban streams in or near Cincinnati, Ohio (USA), and each stream consisted of paired buried and open study reaches separated by a 30-100 m buffer reach. Two buried reaches flowed through corrugate pipe and one through concrete, 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. A more detailed site description can be found in Beaulieu et al. (2014).

We collected samples to characterize dissolved organic matter quality in summer and autumn 2011 and in spring 2012. Concurrently, we deployed tiles to measure extracellular enzyme activity and nutrient diffusing substrata to measure carbon limitation patterns. 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 (NO3-) uptake to understand how those factors relate to the microbial response to variations in DOM quality. Nitrate uptake was measured with 15N-NO3- release in conjunction with bromide (Br-) as a conservative tracer to calculate and model hydrologic parameters. Methods that describe the processing of isotope samples, calculating NO3- uptake rate, and modeling one- and two-station whole-stream metabolism are beyond the scope of this paper, but they 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 MODEL. This technique allows the differentiation of humic-like, fulvic-like, and protein-like fractions of the bulk DOM pool, which in turn are generally related to the lability or recalcitrance of the DOM available to microbial consumers in the stream. To produce the indices to distinguish among these fractions of DOM, water samples were analyzed on a spectrofluorometer that measured excitation between 240-450 nm at 10 nm intervals and emission from 290-600 nm at 2 nm intervals. The three-dimensional EEMs were then instrument corrected, blank substracted, and normalized by the water Raman signal (Cory et al. 2010), but we did not measure absorbance so we did perform the standard inner-filter correction. Therefore these results will be most useful for relative differences across sites and time rather than for comparison to literature values.

The EEMs we produced allowed us to calculate a variety of indices to characterize the quality of the DOM pool, 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). 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 400 nm at excitation of 370 nm. FI values of about 1.9 indicate fulvic acids from microbes and values of about 1.4 indicate terrestrial fulvic acids. 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 (EEA)

Periphyton cultured on tiles that we deployed in the buried and open reaches was analyzed for extracellular enzyme activities (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 and Foreman 2001). Acquisition of labile carbon compounds was measured as -D-glucosidase activity, acquisition of recalcitrant carbon compounds was measured as polyphenol oxidase (POX) and peroxidase activity. The ratio of recalcitrant carbon acquisition total carbon acquisition (as -D-glucosidase + polyphenol oxidase) characterizes the overall quality of the DOM pool (LCI) whereby larger values represent more recalcitrant carbon (Sinsabaugh and Shah 2011). An alternate metric of recalcitrant carbon acquisition was measured as the activity of L-3,4-dihydroxyphenylalanine (DOPA) + H2O2 as a substrate. Nitrogen acquisition was measured as the activity of 3-N-acetylglucosaminidase (NACE: EC 3.2.1.50).

All EEA assays used microplate protocols developed by Sinsabaugh and colleagues (Sinsabaugh et al. 1997; Sinsabaugh and Foreman 2011) and subsequently modified by Hill et al. (2010). Microplate arrays were run with quadruplicate assays for each tested enzyme and reference standard, where 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)

NDS arrays were deployed in the open reaches, and at the upstream and downstream end of the buried reaches. Each NDS array had one of four 0.5 M carbon amendments to represent differences in bioavailability, glucose, arabinose, cellobiose, or a no-carbon control (n=8 each). The NDS were supplemented with 0.5 N as NH4Cl and 0.5 P as KH2PO4 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 in PVC to shade them and reduce the potential for autotrophic biofilms to colonize the glass disks, and they were deployed for two weeks. Upon collection the samples were sent overnight on ice for laboratory analysis within 24 h.

Laboratory analysis consisted of incubating disks in the dark in water collected from the site, and net oxygen change was calculated 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 (mg O2 cm-2 h-1) and by biomass (mg O2 gAFDM-1 h-1), and in order 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 m) water samples in the field and stored them ice for transport to the laboratory where they were acidified or frozen depending on the analyte. We used standard colorimetric methods to measure nitrate+nitrate (hereafter, NO3-), dissolved reactive phosphorus (DRP), ammonium (NH4+), and bromide (Br-) on a flow injection analyzer (Lachat Instruments, Loveland, CO USA). Dissolved organic carbon (DOC) concentration was measured with a total organic C analyzer with high-temperature Pt-catalyzed combustion and NDIR detection (Shimadzo TOC-VCPH, Columbia, MD, USA).

The breakthrough curve of Br- released in conjunction with the 15N-NO3- 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 (As) and the storage zone exchange coefficient (). From these parameters, we calculated the storage zone residence time (Tsto)

Tsto = As/ \* A

where A is the cross-sectional area of the stream channel calculated from the bromide breakthrough curve and channel measurements. We calculated the storage exchange flux (qs)

qs = \*A

which represents the average water flux through the storage zone per unit length. We also calculated fraction of the median travel time due to transient storage, F200med (Runkel, 2002).

Organic matter standing stocks

We collected 10-20 samples of organic matter from different habitat units in a stratified-random sampling design. Samples for coarse (>1 mm), fine (<1 mm), and attached (i.e., periphyton) organic matter was collected from a 0.052 m2 area isolated by an open-ended plastic cylinder placed no more than 5 cm into the sediment. Coarse benthic organic matter (CBOM) was removed by hand, and the sediments were agitated before taking a fine benthic organic matter (FBOM) sample. We collected periphyton by scraping a known area (0.006-0.04 m2) of a rock with a wire brush. We calculated sample 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 and Grobbelaar 1984).

We deployed unglazed clay tiles for six weeks at all sites to provide a standardized surface for algae and bacteria to colonize in order to minimize any potential among site differences. Tiles were collected with the rest of the samples, and periphyton was removed with a toothbrush and razor blade, rinsed into a bottle with site water, and held on ice until arrival at the laboratory. A subset of tiles was analyzed for algal abundance using a Palmer-Maloney counting cell (Charles et al. 2002), and a subset of tiles was analyzed for total bacterial counts using qPCR, described in detail in Beaulieu et al. (2014).

Statistical Analysis

We used multivariate generalized least squares linear models (GLS) to test how DOM quality (HIX, BIX, FI, P/H) differed among seasons (summer, autumn, spring), between reaches (buried, open), and among streams. We also used GLS to test for differences in ecoenzyme activity (POX, DOPA-H2O2, LCI, NACE) and carbon limitation patterns among seasons, between reaches, and among streams. 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 nitrate (NO3-) uptake. We used permutational multivariate analysis of variance using distance matrices (adonis in the vegan package for R, Oksanen et al. 2016) to test simultaneously how CBOM and FBOM standing stocks affect the response to glucose, arabinose, and cellobiose. All statistical analyses were done using R (R Core Team 2016)

Results

Discussion

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Tables

Figure Captions

Figures