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

C.P. Arango1, J.J. Beaulieu2, K.M. Fritz2, B.H. Hill3, M.J. Pennino4, P.M. Mayer5, S.S. Kaushal4, and D.A. Balz6

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

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

3US EPA, Office of Research and Development, National Health and Environmental Effects Research Laboratory, Duluth, MN 55804

4Department of Geology and Earth Systems Interdisciplinary Center, University of Maryland, College Park, MD 20742

5US EPA, Office of Research and Development, National Risk Management Research Laboratory, Ada, OK 74861, USA

6Pegasus Technical Services, Cincinnati, OH 45268

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 (glucose, arabinose, cellobiose, or a no-carbon control (n=8 each)) to represent differences in bioavailability. The NDS were supplemented with 0.5 M N as NH4Cl and 0.5 M 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 submerging the 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 (g 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 (Shimadzu 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 were 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), a subset of tiles was analyzed for total bacterial counts using qPCR, and a subset of tiles was used for laboratory extracellular enzyme activity assays. Detailed methods for these analyses are described 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 extracellular enzyme 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

Patterns in DOM Variability

We examined differences in dissolved organic matter quality among seasons (summer, autumn, spring) and between reaches (buried, open). HIX, the humification index derived from EEMs, differed by season (GLS, p=0.0005), with autumn having higher HIX than spring or summer, which were not different from each other, and also differed by reach (GLS, p=0.021) with open reaches having higher HIX than buried (Figure 1). Because we did not perform the standard inner-filter corrections on these samples, these values cannot be compared to literature values, and using these data alone, we cannot determine whether our sites or seasons have more allochthonous or autochthonous organic content. Rather they can be used to show relative difference between reach and among seasons whereas other metrics from the EEMs can clarify allochthonous/autochthonous DOM content.

For example, the biological freshness index (BIX) varied by season (GLS, p<<0.0001) but did not differ between buried and open reaches (Figure 2A). Although BIX did not differ between spring and summer, both were significantly higher compared to autumn. Typically BIX values between 0.6-0.7 are associated with DOM having low autochthonous content whereas BIX >0.9 are associated with high autochthonous content. The fluorescence index (FI) characterized whether fulvic acids in the DOM pool are microbially derived (~1.9) or terrestrially derived (~1.4), and FI was higher in summer and spring compared to autumn (GLS, p<<0.0001) but did not differ between buried and open reaches. Summer and spring did not differ from each other.

A fourth index, the protein to humic ratio (P/H), compares autochthonous and labile tryptophan-like and protein-like content (more autochthonous and labile) to more terrestrial and recalcitrant humic-like content. This ratio varied by season (GLS, p<<0.001), with spring and summer having a higher ratio (more protein) compared to fall, and also by reach (GLS, p<<0.0002) with open reaches having lower ratio (more humic-like) than buried reaches.

Patterns in extracellular enzyme activity

We deployed standard tiles for microbes to colonize for 6 weeks prior to collecting all our samples, and we measured extracellular enzyme activity to characterize microbial effort to acquire nutrients and use different carbon sources available in the environment. Extracellular enzymes that degrade L-3,4-dihydroxyphenylalanine (DOPA) + H2O2 (DOPAH2) as a substrate correlate to lignin degradation, so it is a metric of recalcitrant carbon use. While we found no significant differences in DOPAH2 among seasons, we did find that buried reaches had higher DOPAH2 than open reaches (GLS, p=0.024) when we expressed DOPAH2 per unit dry mass (Figure 4a) or per unit carbon (data not shown). We found a similar pattern in the polyphenol oxidase (POX) extracellular enzyme activity. POX is an alternate metric of recalcitrant carbon use, and we found higher POX in buried reaches compared to open reaches (GLS, p=0.0043) (Figure 4b).

We found no evidence of spatio-temporal differences in extracellular enzyme activity (EEA) associated with labile carbon use. However, when we composited metrics of carbon use together to calculate the LCI, an index of carbon lability that compares recalcitrant carbon use to total carbon use, we found that buried reaches had higher use of recalcitrant carbon (GLS, p=0.014), and we also found that summer had greater use of recalcitrant carbon than autumn (GLS, p=0.027). There were no differences between spring and autumn (Figure 5). The LCI was also correlated to the CQI, an alternate carbon quality index derived from EEA on different substrates (data not shown).

Because carbon uptake and use is often linked to the acquisition of nitrogen from the environment, we also analyzed differences in N uptake as activity of -N-acetylglucosaminidase. 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), but there were no differences between reaches (Figure 6).

Carbon limitation

We deployed NDS amended with different carbon sources (glucose, arabinose, cellobiose, and a no-carbon control) to see if patterns in carbon limitation differed between buried and open stream reaches or among seasons. Unfortunately, the NDS we deployed during summer were washed away by thunderstorms that produced very flashy runoff in these urban streams dominated by impervious surface cover. Therefore, we focus our analysis on autumn and spring to contrast the carbon limitation response to a time when leaf inputs dominate compared to when vernal algae blooms dominate.

When respiration on NDS disks was scaled by biomass (mg O2 gAFDM-1 h-1), we found no differences among carbon amendments including the no carbon control. However, when the respiration response was scaled by disk area (g O2 m-2 h-1), all NDS carbon amendments were significantly different than the control in all streams, seasons, and reaches (GLS, p<<0.001), and we found no instances where the respiration response differed among the three carbon amendments during any deployment (GLS, p>0.05). Therefore, we analyzed the nutrient response ratio (NRR) of all carbon types together to detect differences between seasonal and/or reach-scale responses. Furthermore, although we deployed NDS arrays at the top and bottom of the buried reaches, there was no difference in the response, so we categorized them all as “buried.” We found a significant interaction (GLS, p=0.0009) between season (autumn versus spring) and reach (buried versus daylight) whereby the respiration response to added carbon was stronger in autumn compared to spring, but open reaches had the strongest response in the fall and buried reaches had strongest response in the spring (Figure 7).

To see what factors might predict the areal NRR response to added carbon among streams and between seasons and reaches, we analyzed a suite of reach-scale variables including standing stocks (e.g., chl a, periphyton biomass, bacterial cell counts, FBOM, CBOM etc.), water chemistry (e.g., NH4+, NO3-, SRP, DOC, etc.), hydrologic variables (e.g., Q, As/A, travel time, etc.), ecosystem-scale functional attributes (e.g., NO3- uptake, whole-system metabolism), metrics of microbial effort to acquire nutrients using EEA assays, and metrics of dissolved organic matter quality derived from excitation-emission matrices. We found no relationships between the NRR response and water chemistry, hydrology, or ecosystem-scale functional attributes. Although EEA and DOM quality metrics often differed between seasons and reaches, there was no direct relationship between NRR and those metrics. Further, most standing stock metrics were also unrelated to the NRR response, but we did find weak positive relationships between reach-scale standing stocks of CBOM (adonis, p=0.036) and FBOM (adonis, p=0.053).

Discussion (Key points)

Dissolved organic matter analysis

BIX and FI show a clear pattern of more recalcitrant carbon in the autumn, consistent with terrestrial leaf inputs. This is consistent with standing stock data shown in Beaulieu et al. 2014 where autumn had higher CBOM than other seasons and spring had higher chlorophyll a than other seasons. However the standing stock data also show lower overall chlorophyll and CBOM in buried reaches, and reach was not significant for BIX or FI. This suggests that these aspects of the DOM character are more strongly influenced by open reaches than buried reaches, or that DOM character is controlled by particulate and dissolved C inputs at the stream segment or watershed scale rather than the “reach” scale, which in our case means “burial status.” Because buried reaches do not have an avenue for direct riparian inputs and they have nearly no chlorophyll a, this implies that inputs from open reaches impart the BIX and FI characteristics to the DOM pool and that biological activity in buried reaches, which is lower than activity in the open reaches, does little to alter those aspects of the DOM signature. The absolute values of the BIX and FI show that, despite clear seasonal differences, the DOM of these streams has a very weak autotrophic signature. (should we show CBOM, FBOM, Chlorphyll, Periphyton standing stock data by season as Fig 3 in Beaulieu et al. 2014? It would support the DOM interpretation. Maybe it’s OK just to cite the data?)

The P/H ratio also shows seasonal differences consistent with BIX and FI with more humic-like components in the autumn, likely due to riparian leaf inputs. In aggregate, the data also show a lower ratio (more humic) in the open compared to the buried reaches. This pattern is consistent with open reaches receiving direct leaf inputs that make the humic character stronger. The HIX data reflects similar patterns to the P/H ratio with autumn having higher humic character than spring or summer, and open reaches having higher humic character than buried reaches. (Could this also suggest that the P/H ratio is more sensitive to different biological processing of the DOM pool in different reaches given that there is more effort to degrade recalcitrant carbon sources in the buried reaches, which would drive this ratio toward protein in the buried reach?). We cannot compare HIX values to the literature, but when BIX and FI are taken into account with P/H ratio, the fluorescence data indicate that DOM in these streams is dominated by terrestrial and/or recalcitrant characteristics despite clear seasonal changes in algal biomass.

Taken together, the DOM data show spatio-temporal differences in the DOM concentration driven by seasonal differences in sources (CBOM versus algal) and the possibility that spatial differences in organic matter inputs or differential processing of DOM can alter the characteristics of the DOM pool.

Extracellular enzyme activity

Carbon degradation enzymes do not show a seasonal pattern, but they are consistent with the idea that biological processing of DOM in the buried reaches can alter the characteristics of the DOM pool. Buried reaches had higher DOPAH2 and POX than open reaches, indicating that the microbial community in the buried reaches allocated more energy toward acquiring recalcitrant carbon sources. This is likely because there was very little labile DOM in the buried reaches due to limited primary productivity, as indicated by low chlorophyll, limited periphyton cover, and an extremely low reach scale GPP estimate. Conversely, the lower values of DOPAH2 and POX in the daylight reach indicate less effort to acquire recalcitrant carbon in parallel with higher levels of chlorophyll a in autumn, winter, and spring. Greater effort to acquire recalcitrant carbon in the buried reaches might suggest rapid use of high quality carbon produced in the open reaches and little export to downstream buried reaches.

We did not find a direct metric that extracellular enzymes favored labile carbon in the open reaches, perhaps because the DOM characteristics suggest an overall dominance of terrestrial/recalcitrant sources. The LCI index aggregates several metrics of labile and recalcitrant carbon use, and it shows greater use of recalcitrant carbon in buried reaches, possibly driven by the significant POX values rather than inclusion of the labile carbon index. However, LCI shows a seasonal effect whereby summer has greater use of recalcitrant carbon than autumn, but that 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. The lack of chlorophyll and reliance on FBOM, a highly processed carbon source, may explain the high use of recalcitrant carbon sources in summer. 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 is dominated by terrestrial sources even in the spring.

These patterns are also evident in N-acquiring enzymes, which have the lowest abundance in the spring, coincident with higher quality algal DOM, and highest values in summer and autumn, when overall chlorophyll is low and the system is dominated by FBOM and CBOM standing stocks respectively

NDS

We assessed overall carbon limitation in autumn and spring, and we found an interaction between season and reach. Biofilms in autumn were always more limited by carbon than biofilms in spring, but the pattern of which reach was more limited in a given season was reversed. Open reaches were more strongly limited by carbon than buried reaches in the autumn, but they were less limited by carbon than the buried in the spring. The overall seasonal effect can be easily explained as autumn having a pulse of DOM from terrestrial leaves whereas the spring has a pulse of higher quality DOM from algal sources. The differential response might be related to the fact that buried reaches always invest more effort in acquiring recalcitrant carbon, so biofilms in the buried reaches might have been better able to use the autumn pulse of terrestrial DOM compared to the open reaches which always invested less in recalcitrant carbon acquisition. Similarly, in the spring, open reaches responded less to the simple carbon sources in the NDS because the system had higher levels of algal DOM overall, but buried reaches appear to get less of this higher quality DOM in general, so they responded more strongly to the NDS.

We got different results when we expressed carbon limitation by area or biomass (i.e., gAFDM-1). When expressed by area, results were highly significant, but when expressed by biomass, there were no differences. This implies that 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 response to added carbon, these results might not apply to carbon additions in low nutrient streams, but they may be reasonably applicable to agricultural and urban streams which tend to have higher background nutrient concentrations.

Biofilms responded the same to all carbon sources (glucose, arabinose, cellobiose). Although arabinose has been used as a less labile form of carbon in some studies (e.g., Newbold et al. 2006), our results show 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 as glucose. It’s unclear if arabinose and cellobiose bioavailability is equally high as glucose in most streams or if it was high in this stream because of the systemic dominance of recalcitrant carbon and/or the presence of N and P in the NDS agars.

Interestingly, although we measured distinct differences in the DOM pool and microbial use of different carbon and nitrogen sources, none of those metrics were related to the NDS response to added carbon. The only relationship we found was a positive response to CBOM and FBOM, and although this relationship is sensible, it is very weak and probably driven by the fall NDS response to significantly higher CBOM stocks. The lack of a relationship with nearly all of the environmental data collected despite the NDS response being consistent with the spatio-temporal patterns of DOM and EEA may reflect a significant difference in the carbon sources we used for the NDS. We used very simple carbon sources with respect to the sources in the overall bulk DOM pool, so the biofilm response to these highly labile sources would be different than the environmental conditions biofilms were exposed to on the tiles used for EEA. The NDS could also have exerted high selection pressure for a particular subset of microbes that responded differently than the natural community as a whole. Alternatively, the fact that the NDS had added N and P that was unavailable to the environmental biofilms could have induced a different response. Despite not being able to link DOM characteristics or EEA metrics in a linear relationship with the NDS response, the NDS response was still consistent with those metrics suggesting this can be a good tool to explore patterns of carbon use streams.

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Tables (part of me wants to have another table with site descriptions, but then again we can just refer to your 2014 paper)

Table 1. Coefficients from adonis, a permutational multivariate analysis of variance using distance matrices, show weak relationships between nutrient response and particulate carbon standing stocks

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Glucose NRR | Arabinose NRR | Cellobiose NRR | P value |
| CBOM | 0.072 | 0.060 | 0.064 | 0.036 |
| FBOM | 0.014 | 0.011 | 0.01 | 0.053 |

Figure Captions

Figure 1. Spatio-temporal variation in the humification index (HIX) derived from excitation-emission matrices.

Figure 2. Seasonal variation in the (A) biological freshness index (BIX) and (B) fluorescence index (FI) derived from excitation-emission matrices.

Figure 3. Spatio-temporal variation in the protein-to-humic ratio (P/H) derived from excitation-emission matrices.

Figure 4. Reach-scale variation in ecoenzyme activity on (A) L-3,4-dihydroxyphenylalanine (DOPA) + H2O2 (DOPAH2) and (B) polyphenol oxidase (POX), both metrics of recalcitrant carbon use.

Figure 5. Spatio-temporal variation in the LCI, and index of carbon quality where larger values indicate more recalcitrant carbon in the dissolved organic matter pool.

Figure 6. Seasonal variation in -N-acetylglucosaminidase (NACE) activity of stream biofilms.

Figure 7. Spatio-temporal variation in the nutrient response ratio (NRR: respiration/mean control) to added carbon when measured on an areal basis (g O2 cm-2 h-1).

Figures

Figure 1.

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Figure 2. (Make a 2 panel graphic eventually)



Figure 3.

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Figure 4.



Figure 5.

C:\Users\ArangoC\Documents\R Files\Cinn R dev\Cincy-Carbon-Limitation\output\figures\lciByReachSeason.tiff

Figure 6.



Figure 7.

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