Dissolved organic carbon dynamics in tallgrass prairie streams

by

Sophie Alexandra Higgs

B.A., Austin College, 2015

A THESIS

submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

Division of Biology
College of Arts and Sciences

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2018

Approved by:

Major Professor
Walter K Dodds

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Abstract

Contrary to the previous notion that a stream acts primarily as the transporter of materials from land to oceans, research has shown that in-stream processing of organic matter and nutrients is significant and relevant at a global scale. Dissolved organic carbon (DOC) is the most abundant form of organic carbon in streams and has been demonstrated as an important source of energy supporting stream food webs. Understanding the dynamics of DOC in streams is, therefore, important in determining the contribution of flowing waters to global carbon storage and release. However, DOC exists as many different compounds, varying in source, composition, and quality. The composition of DOC that ends up in streams is partly controlled by the surrounding watershed, and landscape effects on DOC quality and quantity in streams have been observed. In the North American Tallgrass prairie, woody encroachment has led to changes in riparian vegetation, potentially altering the DOC received by the stream, and making it important to understand rates of DOC transformation as landscape alterations continue. The heterogeneity of the DOC pool makes it difficult to fully describe its components and to measure transformation rates. DOC uptake, or biological use, has been estimated through several methods including in-stream additions of various DOC sources and bottle incubations of stream water and sediments. One problem with addition methods for calculating uptake is that the DOC pool is difficult to replicate and additions of simple compounds or organic leachates do not represent total dissolved organic carbon (TDOC) dynamics. Another potential issue is that additions of a labile compound could potentially alter microbial activity through a priming effect and therefore distort ambient DOC uptake estimates. Finally, uptake parameters are mostly calculated assuming benthic uptake while recent studies have shown that planktonic uptake of DOC can also be significant. We conducted this study with these three considerations in mind.

In the first chapter, we describe our use of *in situ* additions of glucose and bur oak leaf leachate in prairie stream reaches and concentrations of specific components to determine uptake dynamics of various specific DOC components, from a simple sugar to more complex plant compounds. We calculated uptake parameters of glucose and two different oak leaf components. We found that using glucose concentrations rather than TDOC concentrations, as has been done in previous studies, to measure uptake parameters resulted in higher uptake rates, indicating the importance of measuring the specific component added. Through leaf leachate additions, we found that an amino acid like component was consistently taken up faster than a humic-like component. The second chapter addresses the questions of uptake location and priming through a series of recirculating chamber incubations. We found that benthic uptake of leaf leachate was more important than that in the water column. Finally, elevated uptake of one leaf leachate component in the presence of glucose indicated a priming effect on microbial DOC uptake.

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Acknowledgements

Many thanks are due to those who supported me throughout this endeavor. First, I want to thank my advisor, Walter Dodds, for his valuable instruction and guidance. Walter helped me gain confidence as a scientist and encouraged me to, at least partly, overcome my fear of making mistakes. I would also like to thank my committee members, John Blair and Lydia Zeglin, for providing valuable suggestions throughout the formation of my project and in revising this thesis. I would also like to thank the lab technicians who assisted with this project: Maggie "Danger" Spangler, Emily Pender, Emily Samuel, Emma Galitzer, Sammie Grieger, and MacKenzie Cline. I am also thankful for Molly Fischer's assistance in the last round of chamber incubations. It's been a hoot sharing an office and lab with James Guinnip and Anne Schechner. I appreciate their help, advice, sense of humor and willingness to put up with me for this long. I would also like to thank the Kansas Science Communication Initiative and Sarah Hancock, in particular, for providing opportunities in science communication, a vital and enjoyable part of my graduate student training. I am also grateful for the support and friendship I found within the Biology Graduate Student Association. This project was made possible by funding from the National Science Foundation and the Konza Long-Term Ecological Research Program.

Though hundreds of miles away, my family was immensely supportive throughout this process. Mom, Dad, Hayley, and Ashley: I appreciate your advice, encouragement, and love. I undoubtedly could not have done this without you four. Thank you for visiting, for sharing laughs, and for always being there. My final statement of appreciation goes to Ryan Greenway. Thank you for hauling those ridiculously heavy buckets of water, for bringing me green curry during the all-nighters, and for all of the ways you have supported me throughout graduate school. Thank you for believing in me and for steering me to believe in myself.

Chapter 1 - Introduction

Streams were once viewed as channels through which organic carbon and other nutrients are transported from terrestrial ecosystems to oceans. However, many recent studies indicate that DOC can act as a large source of energy to stream food webs (Bott et al. 1984) and that inland waters both transport and transform large quantities of organic carbon (Cole et al. 2007, Tranvik et al. 2009). Thus, streams, lakes, and wetlands should be considered as sinks for organic carbon storage and sources of CO_2 in annual global carbon budgets. This budgeting necessitates estimates of freshwater organic carbon dynamics that can be scaled up from individual streams and lakes to model these processes on a global scale.

In streams, the general degree of heterotrophy (organic carbon use) versus autotrophy (organic carbon production) can depend on the position of a stream along a continuum from headwater to a receiving water body (e.g. Vannote et al. 1980). Specific rates of organic cycling in streams have been estimated in several ways. One example is using stream metabolism metrics and organic carbon concentrations to stoichiometrically estimate rates of organic carbon uptake (e.g. Hall et al. 2015). Uptake rates can also be estimated using a mass balance approach through additions of organic carbon to either stream reaches (e.g. Mineau et al. 2013) or stream water and substrata incubations (e.g. Moody et al. 2013). Added carbon concentrations are then measured over time and the rate at which concentration decreases can be used to make estimates of ambient reach-scale organic carbon uptake. More robust estimates of organic carbon uptake in streams across different biomes are needed to elucidate factors that control organic carbon processing and to constrain estimates of these transformations in streams at the global level.

The majority of the organic carbon within most streams exists in the form of dissolved organic carbon (DOC) (Hobbie and Likens 1973). The DOC pool in a given stream is made up of

many different compounds, varying in biological availability to stream heterotrophs (McDowell and Likens 1988). These various compounds can either be produced within a stream through photosynthesis (autochthonous inputs) or enter a stream from terrestrial ecosystems or upstream export (allochthonous inputs). Several attempts to characterize the ambient DOC pool in streams have resulted in high estimates of both fulvic and humic acids, substances that are formed from the microbial degradation of plant material and are defined through chemical extraction (Larson 1978, Thurman et al. 1982). Others have demonstrated that stream DOC composition changes as water flows downstream through the landscape and is related to factors such as biological and photosynthetic activity both in the stream itself and in adjacent terrestrial ecosystems (McDowell and Likens 1988). The potential fate of DOC compounds depends on chemical structure and can include biological uptake, physical adsorption, chemical interactions, and photooxidation (Dahm 1981, Cory et al. 2014). The complex composition of a stream DOC pool in addition to the many potential fates of its individual components complicates estimations of biological uptake.

A recent review of stream DOC uptake compiled estimates from studies using either reach-scale added DOC mass balance approaches or bioavailability assessments based on laboratory incubations (Mineau et al. 2016). The authors scaled median uptake estimates to stream network carbon budgets and found that reported uptake estimates would require higher than feasible terrestrial DOC input. They therefore concluded that more studies that couple empirical estimates and modeling across multiple scales are needed to refine DOC uptake estimates. However, their models ignored autochthonous inputs, which have been demonstrated to fuel short-term bacterial production in streams through labile algal exudates (Hotchkiss and Hall 2014). Many previous *in situ* studies using an added DOC mass balance approach measure the response of total DOC concentration to additions of simple compounds or leachates (e.g.

Bernhardt and McDowell 2008). These simple additions do not represent the complexity of the ambient DOC pool, potentially leading to the observed overestimates of DOC uptake. In addition, a pulse of a simple DOC compound could cause priming behaviors whereby an increase in highly bioavailable OC alters microbial activity or production (Kuzyakov et al. 2000). Few studies have investigated priming effects in streams but both positive and negative priming effects have been observed previously (Lutz et al. 2012, Hotchkiss et al. 2014), indicating that priming is an important consideration to make when using labile DOC in addition studies. Some recent studies have measured the uptake of specific dissolved organic components rather than total DOC (e.g. Newbold et al. 2006). Following this approach with several DOC sources of varying biological availability and comparing the resulting component-specific uptake estimates to that of total DOC could help elucidate how different components of the DOC pool affect biological use of this important heterotrophic energy source.

Since DOC of terrestrial origin can be a large source of energy to streams (McDowell and Fisher 1976), and DOC quality can affect its biological uptake, terrestrial landscape changes have the potential to indirectly alter stream ecosystem functioning (Berggren and Giorgio 2015). This may be particularly important in ecosystems that are undergoing rapid anthropogenic alterations. One such example is the North American tallgrass prairie, currently intact in less than five percent of its original extant due to anthropogenic degradation by land use change and elimination of critical processes (Samson and Knopf 1994). A previous study at Konza Prairie Biological Station (KPBS), which hosts a tallgrass prairie Long-Term Ecological Research site, found that benthic bacteria isolated from stream reaches with tree canopy cover could metabolize bur oak leaf leachate whereas those isolated from open canopy stream reaches could not use oak leachate as a source of energy (McArthur et al. 1985). This laboratory study indicates functional

differences in bacterial assemblages and their ability to use a DOC source along this prairie stream network, providing rationale for further studies investigating how terrestrial alterations could affect DOC uptake.

This thesis focuses on three major objectives: 1) measuring biological uptake dynamics of two specific DOC sources, one simple sugar and a more complex terrestrial source, in reaches of a tallgrass prairie stream and a nearby urbanized stream, 2) determining the physical location of terrestrial carbon uptake in a prairie stream, and 3) testing for priming of terrestrial DOC uptake by a simple sugar. To accomplish the first objective, we conducted twelve pulse additions of either glucose or bur oak leaf leachate across different seasons in prairie stream reaches with either open grassland or gallery forest riparian vegetation and an urban stream reach. Following the mass balance approach, we calculated uptake parameters for the specific added components as well as for total DOC and compared these results across sites and seasons. For the second objective, we used recirculating chambers amended with either planktonic microbes or substrata biofilm to calculate oxygen consumption and decrease of terrestrial DOC in the water column compared to that on the streambed. Finally, to test for priming we used the same recirculating chamber set up but added glucose as a treatment in addition to leaf leachate, and compared results to leaf leachate alone.

References

Berggren, M., and P. a Giorgio. 2015. Distinct patterns of microbial metabolism associated to riverine dissolved organic carbon of different source and quality. Journal of Geophysical Research: Biogeosciences.

Bernhardt, E. S., and W. H. McDowell. 2008. Twenty years apart: Comparisons of DOM uptake during leaf leachate releases to Hubbard Brook valley streams in 1979 versus 2000. Journal of Geophysical Research: Biogeosciences 113:1–8.

Bott, T., L. Kaplan, and F. Kuserk. 1984. Benthic bacterial biomass supported by streamwater dissolved organic matter. Microbial Ecology 10:335–344.

Cole, J. J., Y. T. Prairie, N. F. Caraco, W. H. McDowell, L. J. Tranvik, R. G. Striegl, C. M. Duarte, P. Kortelainen, J. A. Downing, J. J. Middelburg, and J. Melack. 2007. Plumbing the global carbon cycle: Integrating inland waters into the terrestrial carbon budget. Ecosystems 10:171–184.

Cory, R. M., C. P. Ward, B. C. Crump, and G. W. Kling. 2014. Sunlight controls water column processing of carbon in arctic fresh waters. Science 345:925–928.

Dahm, C. N. 1981. Pathways and Mechanisms for Removal of Dissolved Organic Carbon from Leaf Leachate in Streams. Canadian Journal of Fisheries and Aquatic Sciences 38:68–76.

Hall, R. O., J. L. Tank, M. A. Baker, E. J. Rosi-Marshall, and E. R. Hotchkiss. 2015. Metabolism, Gas Exchange, and Carbon Spiraling in Rivers. Ecosystems.

Hobbie, J. E., and G. E. Likens. 1973. Output of phosphorus, dissolved organic carbon, and fine particulate carbon from Hubbard Brook watersheds. Limnology and Oceanography 18:734–742.

Hotchkiss, E. R., R. O. Hall, M. A. Baker, E. J. Rosi-Marshall, and J. L. Tank. 2014. Modeling

priming effects on microbial consumption of dissolved organic carbon in rivers. Journal of Geophysical Research: Biogeosciences 119:982–995.

Hotchkiss, E. R., and R. O. J. Hall. 2014. Whole-stream 13C tracer addition reveals distinct fates of newly fixed carbon. Ecology.

Kuzyakov, Y., J. K. Friedel, and K. Stahr. 2000. Review of mechanisms and quantification of priming effects. Soil Biology and Biochemistry 32:1485–1498.

Larson, R. A. 1978. Dissolved organic matter of a low-coloured stream. Freshwater Biology 8:91–104.

Lutz, B. D., E. S. Bernhardt, B. J. Roberts, R. M. Cory, and P. J. Mulhollande. 2012. Distinguishing dynamics of dissolved organic matter components in a forested stream using kinetic enrichments. Limnology and Oceanography 57:76–89.

McArthur, J. V., G. R. Marzolf, and J. E. Urban. 1985. Response of bacteria isolated from a pristine prairie stream to concentration and source of soluble organic carbon. Applied and Environmental Microbiology 49:238–241.

Mcdowell, W. H., and S. G. Fisher. 1976. Autumnal processing of dissolved organic matter in a small woodland stream ecosystem. Ecology 57:561–569.

Mcdowell, W. H., and G. E. Likens. 1988. Origin, Composition, and Flux of Dissolved Organic Carbon in the Hubbard Brook Valley. Ecological Monographs 58:177–195.

Mineau, M. M., C. M. Rigsby, D. T. Ely, I. J. Fernandez, S. A. Norton, T. Ohno, H. M. Valett, and K. S. Simon. 2013. Chronic catchment nitrogen enrichment and stoichiometric constraints on the bioavailability of dissolved organic matter from leaf leachate. Freshwater Biology 58:248–260.

Mineau, M. M., W. M. Wollheim, I. Buffam, S. Findlay, R. Hall, E. Hotchkiss, L. Koenig, W.

McDowell, and T. Parr. 2016. Dissolved organic carbon uptake in streams: A review and assessment of reach-scale measurements. Limnology and Oceanography:1–11.

Moody, C. S., F. Worrall, C. D. Evans, and T. G. Jones. 2013. The rate of loss of dissolved organic carbon (DOC) through a catchment. Journal of Hydrology 492:139–150.

Newbold, J. D., T. L. Bott, L. A. Kaplan, C. L. Dow, K. John, A. K. Aufdenkampe, L. A. Martin, D. J. Van Horn, A. Ade, J. D. Newbold, T. L. Bott, L. A. Kaplan, C. L. Dow, J. K. Jackson, A. K. Aufdenkampe, L. A. Martin, D. J. Van Horn, and A. A. De Long. 2006. Uptake of nutrients and organic C in streams in New York City drinking-water-supply watersheds Published by : The University of Chicago Press on behalf of the Society for Freshwater Science Stable URL : http://www.jstor.org/stable/10.1899/0887-3593(2006)0.

Samson, F., and F. Knopf. 1994. Prairie conservation in north america. BioScience 44:418–421.

Thurman, E. M., R. L. Wershaw, R. L. Malcolm, and D. J. Pinckney. 1982. Molecular size of aquatic humic substances. Organic Geochemistry 4:27–35.

Tranvik, L. J., J. A. Downing, J. B. Cotner, S. A. Loiselle, R. G. Striegl, T. J. Ballatore, P. Dillon, K. Finlay, K. Fortino, L. B. Knoll, P. L. Kortelainen, T. Kutser, S. Larsen, I. Laurion, D. M. Leech, S. L. McCallister, D. M. McKnight, J. M. Melack, E. Overholt, J. A. Porter, Y. Prairie, W. H. Renwick, F. Roland, B. S. Sherman, D. W. Schindler, S. Sobek, A. Tremblay, M. J. Vanni, A. M. Verschoor, E. von Wachenfeldt, and G. A. Weyhenmeyer. 2009. Lakes and reservoirs as regulators of carbon cycling and climate. Limnology and Oceanography 54:2298–2314.

Vannote, R. L., G. W. Minshall, K. W. Cummins, J. R. Sedell, and C. E. Cushing. 1980. The River Continuum Concept. Canadian Journal of Fisheries and Aquatic Sciences 37:130–137.

Chapter 2 - Whole stream uptake of specific dissolved organic carbon components in distinct prairie stream reaches

Abstract

Understanding the rates of organic carbon transformations in streams is important for quantifying global carbon fluxes to and from ecosystems. Dissolved organic carbon (DOC) is the most abundant form of organic carbon in many headwater streams, making the analysis of this pool and its transformations important. Rigorous reach-scale estimates of the biological uptake of DOC are needed to accurately model the impacts of stream processing on global carbon budgets. Complicating these estimation efforts, stream DOC is not well defined in many systems and represents a mixture of many different compounds, each with their own properties and, therefore, physical and chemical interactions. In several previous DOC uptake studies, the authors used pulsed additions of simple carbon compounds into a stream reach and only measured total DOC concentrations in response to the addition. We conducted multiple additions of a labile simple compound (glucose) and a more complex carbon source (leaf leachate) in three stream reaches over different seasons and measured the loss of individual components over time. This allowed us to calculate uptake rates for specific carbon components in addition to the response of total DOC. We found that streams with lower background concentrations of glucose had the highest ambient glucose uptake rates. Calculations made from TDOC concentrations resulted in lower estimates of uptake relative to those determined from glucose only. We measured uptake of leaf leachate components and determined that uptake is measureable for some of them using hours-long *in situ* pulse additions. We also found that specific fluorescent leaf components disappeared at different rates, with an amino acid-like component that was taken up more quickly than its humic counterpart. More uptake measurements in varying stream

ecosystems and across different seasons will contribute to a more complete understanding of the role of small streams in organic carbon processing.

Introduction

Recent studies indicate that inland waters play a significant role in the global carbon cycle, both transporting and transforming large quantities of carbon (Cole et al. 2007, Tranvik et al. 2009). Of specific interest is the flux of carbon dioxide from lakes, streams, and wetlands to the atmosphere because of its implications for global climate change (Butman and Raymond 2011, Raymond et al. 2013). One source of carbon dioxide in freshwater ecosystems is the biological oxidation of organic carbon by respiring heterotrophs (Wetzel 2001). Therefore, a further understanding of the dynamics of organic carbon within various freshwater ecosystems is an important component of accurately modeling the contribution of these ecosystems to carbon storage and release. In streams, carbon can be fixed by autotrophs, but flowing water also picks up terrestrial organic carbon as it moves from headwaters to downstream receiving water bodies, establishing a strong link between terrestrial and aquatic ecosystems (McDowell and Likens 1988). Different sources of organic carbon can vary in quality and biological availability to stream heterotrophs (Hosen et al. 2014). Thus, understanding the rates and drivers of organic carbon transformations under different types of land use and land cover across biomes is important for quantifying stream contributions to the global carbon cycle.

A large percentage of the organic carbon within most streams is in the form of dissolved organic carbon (DOC) (Hobbie and Likens 1973), a pool that has been identified as an important source of energy for stream heterotrophs (Bott et al. 1984). The DOC pool in a stream is a mixture of many different organic compounds varying in chemical composition and spanning a wide range of quality and thus bioavailability (Larson 1978, Thurman et al. 1982, Cory and Kaplan 2012). Additionally, the large effort and expense associated with some compound-specific organics analyses make it difficult to completely characterize or replicate this complex

pool of compounds at any large scale (Volk et al. 1997). Because of the complexity of organic matter, measuring the biological uptake of ambient organic nutrients presents additional challenges over those for inorganic nutrients, like phosphorus and nitrogen, of which only a few different forms dominate biogeochemical fluxes. An important part of measuring biological uptake of a nutrient is to be able to determine the concentration of the given nutrient, and while some components of the DOC pool can be analyzed using simple assays or fluorescence techniques, others are more difficult and expensive to measure (Brack 2003).

Analysis of optical properties through spectrofluorometry is one relatively inexpensive method to trace classes of fluorescent DOC (Coble 1996). In this method, excitation-emission matrices (EEMs) are created for a water sample and the fluorescence intensity at any combination of excitation and emission wavelength indicate the fluorescent signature of the DOC in the sample. Several indices have been developed in order to classify these groups based on bioavailability and also to identify the general sources of the fluorescent DOC, such as whether it is more likely from terrestrial or microbial sources (McKnight et al. 2001, Cory and Kaplan 2012). These indices allow the use of optical properties as a proxy for DOC source, bioavailability, and relative concentration. Fluorescence peaks from EEMs of water samples can be further divided into distinct fluorescent components through parallel factor analysis (PARAFAC), a three-way multivariate data analysis method (Stedmon et al. 2003). This analysis allows a researcher to trace separate distinct components of the DOC pool for a relatively low cost.

Various in situ methods allow estimates of biological uptake of inorganic nitrogen and phosphorus at the reach scale (Stream Solute Workshop 1990, Covino et al. 2010). A recent review showed that these methods have been used less widely to assess DOC uptake (Mineau et

al. 2016). Most of these studies added simple organic compounds like acetate (Johnson et al. 2009) or leachates of soils or plant tissues (Bernhardt and McDowell 2008). Many of these studies measure the response of total DOC concentration rather than following the changes in concentration of the specific added carbon components (Johnson et al. 2009), a possible confounding factor in understanding uptake dynamics of less labile components of DOC. Another potential problem with this method is that adding a simple carbohydrate, that is likely more bioavailable than the ambient DOC pool, could induce some sort of priming effect. Both positive priming effects, whereby microbes increase complex C substrate use as a consequence of the metabolism boost from simple sugar addition (Hotchkiss et al. 2014), and negative priming effects, in which metabolic demand is shifted from an ambient C source to the more bioavailable one (Lutz et al. 2012), have been observed. Measuring uptake rates for specific DOC components spanning the range of ambient DOC bioavailability and comparing that to total DOC uptake during nutrient addition experiments should allow for more accurate estimates of ambient DOC uptake and priming effects.

Land cover can influence terrestrial inputs of DOC to streams leading to differences in the DOC quality and quantity (Balcarczyk et al. 2009, Larson et al. 2014, Bodmer et al. 2016). Furthermore, chemical differences related to different species of leaf litter occur with respect to fluorescent signature and bioavailability to stream microbes (Wymore et al. 2015). Together, these suggest that land use change could indirectly affect stream ecosystem functioning by altering autochthonous and allochthonous DOC inputs and the metabolism of stream heterotrophs. This is particularly important, but not yet studied, in ecosystems that are undergoing widespread land-use changes such as the North American tallgrass prairie (Samson and Knopf 1994). A previous study at Konza Prairie Biological Station (KBPS), which hosts a

National Science Foundation Long-Term Ecological Research (LTER) site, showed functional differences in the ability of bacterial isolates to metabolize different terrestrial carbon sources (McArthur et al. 1985). Specifically, bacteria isolated from upstream reaches, surrounded mostly by grasses and short-statured shrubs, were only able to metabolize grass leachate, whereas those isolated from downstream reaches with greater wooded plant canopy cover could metabolize both grass leachate and bur oak leaf leachate.

Our study aimed to investigate this differential metabolic response of stream microbes to DOC source at the reach scale. Our overarching question was: Can we use in situ nutrient releases to characterize the functional uptake of different DOC sources within prairie streams and use this method to compare uptake across space and time? Our specific objectives were to 1) calculate uptake parameters for a set of organic carbon components that represent a range of bioavailability and 2) compare carbon uptake in different reaches to assess any spatial variation in uptake. We conducted nutrient pulse additions of glucose, a simple carbohydrate, and of bur oak leaf leachate, a complex terrestrial carbon source, to characterize the uptake kinetics of multiple DOC components in three stream reaches: an upstream open prairie reach, a downstream forest-canopied prairie reach, and a reach in an urban watershed. We expected that smaller molecular weight components would be taken up faster relative to higher molecular weight ones in each stream reach. We also expected to see higher uptake rates of leachate components in downstream forest-canopied reaches and the urban stream since they are exposed to tree-derived sources of DOC annually.

Methods

Study sites description and experimental design

Study sites were selected within the Flint Hills ecoregion, which is characterized by tallgrass prairie on rolling hills of soil overlying limestone and shale. Three stream reaches were chosen: a protected prairie reach with gallery forest canopy cover (Gallery), an open upstream protected prairie reach surrounded by shrubs and grasses (Prairie), and one urban stream reach (Urban). Both protected prairie reaches are part of the Kings Creek watershed at KPBS. The chosen gallery and prairie sites at KPBS were similar to the gallery forest and prairie-shrub reaches used by McArthur and others (1985) in the aforementioned leachate study (Figure 2.1). Kings Creek is a well-characterized prairie stream, including its organic matter dynamics (Gray 1997) and is also the site of many past nutrient addition experiments (Dodds et al. 2002, O'Brien et al. 2007, Trentman et al. 2015). Total DOC concentrations in Kings Creek are generally low with a mean of 1.19 mg L^{-1} reported for the period of 1995 to 2009 (Rüegg et al. 2015b). Bulk dissolved organic matter in Kings Creek has been analyzed previously for molecular fractions, fluorescent components, black carbon, and several other organic matter metrics (Jaffé et al. 2012, Ding et al. 2013). One such study used biomarkers to identify and compare organic carbon sources throughout the watershed (Pisani et al. 2016). The urban reach is part of Campus Creek, a stream within an urbanized watershed on the Kansas State University property, which drains an area with a large quantity of impervious surface and is subject to occasional construction and maintenance run-off. This stream has also been the site of prior nutrient uptake studies (Dodds et al. 2002, O'Brien et al. 2007, Trentman et al. 2015). Most site characteristics were measured within each stream reach on the days of pulse additions (Table 2.1). The exception is percent canopy cover estimates, which were made using a densiometer on a single day during the

growing season and were 74.3, 31.2, and 95.8 percent for Gallery, Prairie, and Urban sites respectively.

Nutrient additions

Pulse additions of glucose and leaf leachate were conducted separately according to the Tracer Addition for Spiraling Curve Characterization (TASCC) method (Covino et al. 2010). In this method, a nutrient of interest along with a conservative tracer is introduced into a stream reach as an instantaneous pulse. The tracer is used to account for dilution and dispersion of the solutes, and is traced with conductivity meters or ion electrodes to indicate proper sampling times across the break through curve of the nutrient pulse.

D-Glucose was dissolved into ultra-pure deionized water and then sodium bromide (NaBr) was added as a conservative tracer in the glucose injectate solutions. Leaf leachate injectates were made from bur oak (*Quercus macrocarpa*) leaves, one of the most abundant trees in the gallery forest of Kings Creek (Gray 1997) and the species used in previous DOC uptake experiments at KPBS (McArthur et al. 1985). Leaves were collected shortly after abscission and air dried and stored until use. We ground the leaves just before leaching to maximize concentrated leachate. Ground leaves were leached in room temperature deionized water for twenty-four hours following the procedure of several previous studies analyzing leaf leachate bioavailability (e.g. Wymore et al. 2015). Just before addition, either NaBr or sodium chloride (NaCl), approximately 300 and 500 grams respectively, was dissolved into the leachate as a conservative tracer. Aliquots of glucose and leachate injectates were filtered and stored on ice for later chemical analysis.

Approximately fifty-meter stream reaches with a mixture of riffles and small pools, no tributaries, and minimal groundwater inputs were identified and assessed for travel time using

rhodamine dye the day before each nutrient addition experiment. A twenty- to thirty-minute travel time for the peak of the pulse was targeted. The injectate solution was released at the top of a given reach in an area of significant mixing and either a bromide specific electrode (for NaBr tracer) or a conductivity meter (for NaCl tracer) was used to monitor the progress of the peak at the end of the reach. Samples were collected before the addition and throughout the duration of the tracer peak with more frequent sampling at the top of the peak. Water samples for glucose or total DOC analysis were filtered immediately upon collection with a Whatman GF/F 0.7 µm filter and stored in plastic bottles on ice until returning to the lab at which time they were frozen until analysis. Samples for fluorescent DOC analysis were filtered upon collection, stored in amber vials to prevent photolysis, and analyzed within twenty-four hours of collection. Samples for ion analysis of the tracer salts were kept refrigerated until analysis.

Photolysis control

We assessed the effect of light on fluorescent DOC concentration during each leaf leachate addition. A dilution of the leachate was made in filtered stream water and set in two pans by the streamside; one in full sunlight and one completely shaded. Filtered samples were taken in time series throughout each pulse of leachate and stored in amber vials on ice until analysis. An analysis of covariance (ANCOVA) was used to assess drivers of the proportion of initial fluorescence, the dependent variable, for each individual fluorescent component in all samples across all additions. Time was used as the continuous independent variable and the categorical variables included were light (shade or dark), component, site, and month. The difference between shade and light fluorescence for each time point sample was regressed over time for each fluorescent DOC component to develop individual photolysis corrections for grab samples during the experiment. This correction took the background-corrected decrease in

concentration of the component and corrected for the light degradation expected based on travel time

Water analysis

An ion-selective probe was used to determine concentrations of Br^- in water samples. Standards and samples analyzed at reach room temperature and were stirred throughout analysis. A standard curve was made by fitting an exponential line to the standards and was then used to convert voltage readings to mg L^{-1} concentrations. Previous studies have shown that similar concentrations are obtained with this method as with ion chromatography (Trentman et al. 2015). The Cl^- concentration was determined by ion chromatography using an ICS-1100 ion chromatograph (Thermo-Fisher).

Samples for total DOC analysis were acidified with 100 µL of 2 N HCl to a total volume of 5 mL, purged with air for 5 min to remove inorganic carbon, and subsequently analyzed on a Shimadzu TOC-L analyzer using platinum-catalyzed combustion. We followed the protocol described by Hicks and Cary (1968) for glucose determination in natural waters. Briefly, this analysis is an enzymatic assay that uses the coupled reduction of nicotinamide adenine dinucleotide phosphate (NADP) to reduce resazurin dye that allows for the measurement of low concentrations of glucose (Hicks and Carey 1968). Samples were analyzed on a Turner Fluorometer Model 112 with excitation and emission filters of 546 nm and 589 nm respectively. Fluorescence of a set of standards was fitted to a second order polynomial to make a standard curve during each analysis, converting dye fluorescence to concentration in mg L^{-1}.

Samples for fluorescent DOC analysis were analyzed on a Jobin Yvon Aqualog Fluorometer (Horiba Scientific, Edison, New Jersey) for spectroscopic fluorescence. Samples were excited at a range of wavelengths at 3 nm increments from 240 nm to 450 nm. Integration

time was 0.25 seconds for more concentrated samples and 1.0 minute for more dilute samples, but were later normalized so that all samples could be analyzed together. Emission spectra were collected from 212 nm to 619 nm at increments of 3.28 nm. We processed the resulting EEMs with correction for inner filter effect (Ohno 2002), Raman normalization and blank subtraction with ultra-pure deionized water, and excision of first and second order Rayleigh scattering bands (Stedmon and Bro 2008). We also ran a quinine sulfate standard with a value of one Raman Unit (R.U.) equating to 2.495 quinine sulfate units to make our results comparable to others in the literature. Distinct fluorescent components were identified through parallel factor analysis (PARAFAC) using the DOMFluor toolbox according to Stedmon and Bro (2008). We identified a three-component model through split half analysis and random initialization after running ten three-component models. We uploaded the resulting PARAFAC components to the OpenFluor database to compare them with other components reported in the literature (Murphy et al. 2014). Component 1 was identified as an amino acid-like peak whereas Components 2 and 3 were both identified as humic-like components (Figure 2.2).

To ensure that our conservative tracer did not interfere with carbon analyses, standards for each were run with varying concentrations of NaBr and no effect was found for glucose, fluorescent DOC, or TDOC analysis.

Data analysis

Ambient uptake parameters: uptake length, areal uptake rate, and uptake velocity ($S_{w\text{-}amb}$, U_{amb}, and $V_{f\text{-}amb}$) were calculated as described by Covino et al. 2010. Areal uptake was not calculated for leaf fluorescent components because this requires mass per volume concentrations which were not known. Rather, the relative fluorescence in Raman units was used to calculate

the loss rate constant to estimate S_w and V_f. Linear regression analysis was used to assess potential relationships between ambient glucose and TDOC concentrations and uptake estimates.

Results

Stream characteristics

Stream discharge, estimated in each site at the time of DOC addition using a conservative tracer, ranged from 0.45 to 166 L sec^{-1} with highest discharge measured at Gallery and lowest at Prairie (Table 2.1). Background grab samples were taken at each site prior to nutrient additions to measure ambient levels of specific carbon components (either glucose or PARAFAC components) and total organic carbon (Table 2.1). Ambient TDOC concentrations were highest overall at Urban with lower comparable values in Gallery and Prairie. Ambient glucose concentrations across the three study reaches ranged from 0.046 to 0.22 mg L^{-1} with a median value of 0.07 mg L^{-1}. The highest background glucose concentration was found in the spring at Prairie and the lowest in the summer at Urban.

Fluorescent components within bur oak leaf leachate and stream water were analyzed for each leachate addition. Components 1 and 3 were consistently found in leaf leachate made in ultra-pure water whereas Component 2 was always found in ambient stream water but was sometimes extremely low or undetectable in the leaf leachate. Background levels of PARAFAC components, reported in fluorescence units, were variable (Table 2.1). No correlations were found among fluorescence of any of the components and either discharge or TDOC concentration. However, Component 1, the amino acid like component, appeared to be found in higher concentration in the spring than the late summer or fall, although linear regression of fluorescence versus day of year was not statistically significant ($R^2 = 0.59$, p value = 0.08).

Glucose uptake

Ambient glucose uptake parameters were estimated following the TASCC method (Covino et al. 2010). Ambient uptake lengths ($S_{w\text{-}amb}$) were estimated by regressing dynamic uptake lengths ($S_{w\text{-}dyn}$) versus concentration of glucose carbon (glucose-C) followed by extrapolation back to ambient glucose concentration. The shortest $S_{w\text{-}amb}$ was estimated for Urban and the longest for the Gallery summer site (Table 2.2). Uptake length, however, is simply the average distance over which a molecule of the nutrient of interest remains in the water column before being taken up and is not corrected for stream size (Stream Solute Workshop 1990). We calculated ambient uptake rate (U_{amb})and ambient uptake velocity ($V_{f\text{-}amb}$), both of which take stream discharge into account, making these better parameters for comparison between sites. The most rapid U_{amb} and $V_{f\text{-}amb}$ were estimated at Urban and in the spring at Gallery and the two slowest $V_{f\text{-}amb}$ estimates were both for Prairie, in both the spring and summer (Table 2.2).

Uptake parameters were calculated using both glucose-C concentration and TDOC concentration for all glucose addition experiments, except for the gallery spring site for which TDOC uptake was not calculated due to analytical reasons (Table 2.2). $V_{f\text{-}amb}$ estimates from these separate glucose-C and TDOC methods for each glucose addition experiment were plotted against ambient stream glucose-C and TDOC concentrations respectively to assess relationship (Figure 2.3). A significant positive relationship was found between ambient TDOC concentration and $V_{f\text{-}amb}$ ($p = 0.007$) but this was largely driven by the relatively high Urban values. Although the relationship between glucose concentration and uptake velocity was not significant, the opposite trend was observed in which the site with the highest ambient glucose concentration (Prairie spring) had the lowest $V_{f\text{-}amb}$ estimates and the most rapid $V_{f\text{-}amb}$ estimates were for two

of the sites with lower ambient glucose concentration. A comparison of the $V_{f\text{-}amb}$ estimates made from both methods shows that calculating uptake with TDOC concentration results in lower $V_{f\text{-}amb}$ estimates than when calculations are made using glucose-C concentrations (Figure 2.4).

Leaf leachate uptake

The same uptake metrics were calculated for PARAFAC components except for areal uptake (U_{amb}) since mass per volume concentration is necessary for these calculations and we only had measurements of fluorescence units, not true concentration (Table 2.3). ANCOVA results of proportion initial fluorescence for photolysis correction indicated that all categorical factors considered were significant (Table 2.4). Month and site could not be analyzed concurrently because degrees of freedom were limited by the small sample size. Because effects of site and month were both significant when analyzed separately, light corrections were made separately for each leachate addition experiment. We regressed the proportion of initial fluorescence for each component in both light and dark conditions and used the difference between the slopes of the two regressions to correct each grab sample across the breakthrough curve (Figure 2.5).

In each pulsed leachate addition, PARAFAC Component 1, the amino-acid like component, was consistently taken out of the water column at a faster rate than that of humic-like Component 3 (Table 2.3). $V_{f\text{-}amb}$ estimates for Component 1 ranged from 0.57 to 8.07 mm min^{-1} and were highest during the spring across all sites. A similar pattern was observed for Component 3 $V_{f\text{-}amb}$ which ranged from 0.40 to 5.76 mm min^{-1}.

TDOC uptake is not directly comparable with uptake of PARAFAC components because the latter are reported in fluorescence units rather than mass per volume concentration. However, it is interesting to note that the experiment with the fastest uptake velocity estimate for

Component 1 also had the lowest estimate for TDOC uptake and the opposite, the pulse with the lowest estimate for Component 1 had the highest estimate for TDOC, both of which took place in Gallery.

Because we did not elevate Component 2 sufficiently during our pulsed additions of leachate, we were unable to calculate uptake parameters for Component 2. We did, however, observe an interesting pattern of Component 2 throughout the additions in that the fluorescence of this humic component remained above background concentrations even after our conservative tracer had returned to background levels. In some instances, this component increased over time after the nutrient pulse had already moved past the sampling point (Figure 2.6).

Discussion

Ambient solute concentrations

Ambient TDOC concentrations for the Konza sites were within the range of long term TDOC values for Kings Creek, which shows a trend of decreasing concentration from headwaters to lower gallery reaches (Rüegg et al. 2015b). There are few values of ambient glucose concentrations reported in the literature for headwater streams. Our estimated ambient glucose concentrations were higher than the example estimates (0.0036-0.011 mg L^{-1}) described within the glucose analysis procedure we followed (Hicks and Carey 1968). However, values reported by Hicks and Carey were for samples taken from ponds and brackish waters and may not be comparable to those in headwater streams. Previous sampling of fluorescent components at Kings Creek have also identified three PARAFAC components within background samples, two described as humic-like and one as a protein peak (Jaffé et al. 2012), similar to those identified in the current study.

Glucose uptake

The ambient uptake velocity ($V_{f\text{-}amb}$) estimates we present for glucose are comparable to those reported in previous studies (McDowell 1985, Newbold et al. 2006). A recent review of DOC uptake studies reported a median $V_{f\text{-}amb}$ of 2.91 mm min^{-1} (Mineau et al. 2016), to which most of our estimates fall close. The two high estimates we calculated for Urban and spring Gallery were still within range of values reported globally for simple organic compound uptake. Urban is greatly influenced by human land use and Gallery has some past agricultural influence, however, we did not take any measurements with which to assess the effect of land use on glucose uptake. Bott and Newbold (2013) found higher glucose uptake in a disturbed pasture stream compared to those estimated for undisturbed forest in a comparison of ecosystem parameters in headwater streams with different land use. Another study found no relationship between glucose uptake and human impact but did find that uptake was positively correlated with ecosystem metabolism (Newbold et al. 2006).

Glucose uptake was slowest in Prairie with higher values in both Gallery and Urban (Table 2.2). We have no specific explanation for these differences. Previous studies have found positive relationships between glucose uptake and whole-stream metabolism, community respiration, and primary production (Newbold et al. 2006, Bott and Newbold 2013). Although metabolism was not measured in the current study, whole-stream metabolism estimates have been made previously for KPBS stream reaches similar to our sites. Higher gross primary production has been reported in prairie reaches compared to that in gallery reaches (Riley and Dodds 2013).

Our observation of higher glucose uptake in streams with higher ambient TDOC concentration is opposite to a previously reported negative relationship between glucose-C

removal and TDOC concentration (McDowell 1985). However, McDowell (1985) measured glucose removal in bottle incubations and hypothesized it was mostly by abiotic means. We discuss uptake as if it is a purely biological process but many studies have found that physical properties like adsorption, especially to sediments high in iron and aluminum oxides, can play a large role in removing DOC from stream water (Dahm 1981, McDowell 1985). However, adsorption is still a process that prevents DOC from being exported, and has been hypothesized to be important for retention and subsequent biological use (Dahm 1981). So, our measures of uptake do not necessarily represent immediate biotic use but retention that could allow for such. Also, the pulse addition method could allow for detection of adsorption and desorption during this time of elevated concentrations followed by a decrease.

Comparison with TDOC uptake

Uptake calculated using change in TDOC concentrations was lower than estimates made using glucose-C concentration (Figure 2.4). Glucose is a simple sugar that can be used directly by organisms for metabolism whereas TDOC includes more complex compounds that might be less available to stream heterotrophs. Therefore, lower estimates of TDOC uptake than glucose-C might be expected because the uptake parameters for TDOC consider the less bioavailable portions, too (Mineau et al. 2016). Another problem when using TDOC concentrations to measure uptake of a simple compound addition is that the ambient concentration of the compound is not known. A good estimate of ambient nutrient concentration is needed to calculate ambient uptake parameters. An additional consideration is that adding a simple carbohydrate might lead to a priming effect, changing the way in which total organic carbon is used through stimulation of microbial activity (Kuzyakov et al. 2000). We could not assess this with our pulsed releases. Altogether, specific DOC compounds rather than TDOC need to be

measured if one is aiming to determine the dynamics of a given DOC compound and caution should be used in using additions of simple DOC compounds to estimate ambient uptake of TDOC.

Leaf leachate uptake

Both amino acid-like Component 1 and humic-like Component 3 were removed from the water column throughout in situ addition experiments. Component 1 was consistently taken up faster than Component 3, which supports our hypothesis that smaller molecular weight compounds would be taken up more rapidly than their larger counterparts. A previous study that calculated uptake of an amino acid-like component throughout a pulsed addition reported similar uptake velocities (Fellman et al. 2009). However, the other PARAFAC component identified in this study, a humic-like component, did not decrease with downstream distance so it was apparently not retained in the stream. Stream sediment incubation studies have previously shown rapid uptake of amino acids from the water column (Findlay et al. 2003). As stated above, this uptake does not necessarily represent immediate biological use but rather retention (either physical or biological).

The remaining elevated concentrations or increase of Component 2 after conservative tracer had come back down to ambient levels prevented us from estimating uptake of this component (Figure 2.6). One potential explanation for the observed pattern is that Component 2 could be some sort of degradation product or a humic acid of microbial origin. This would be supported by the fact that the leaf leachate solution itself had very low concentrations of Component 2. A previous analysis of DOC in Kings Creek identified one PARAFAC component as a microbial humic component (Jaffé et al. 2012). Using optical properties of DOC, this 2012

study concluded that Kings Creek has high levels of DOC from microbial origin relative to other North American headwaters, making this potential explanation stronger.

Previous studies that have conducted leaf leachate addition experiments and only measured TDOC could have missed patterns delineated when focusing on individual TDOC components. Our potential evidence for in-situ retention of a humic leaf substance on the time scale of a couple of hours complicates use of TDOC uptake further. The unique pattern of Component 2 provides additional support for analyzing the concentration of specific DOC components during nutrient additions to gather more information about the transformations of DOC in stream ecosystems.

Conclusions

Although the dissolved organic carbon pool is a complex mix of many compounds and is therefore difficult to parse out, analyses for specific compounds or components can be used to follow patterns and make uptake estimates for specific components of this pool. Without knowledge of specific component concentrations, it is hard to determine what TDOC uptake measurements from simple compound or leachate additions really indicate. In this study, pulse additions of glucose and oak leaf leachate allowed for the calculation and comparison of uptake across different sites. We found that a humic-like component from leaf leachate was taken up over hours within a fifty-meter stream reach. We also found differences in glucose concentrations and uptake in different stream types, with lower uptake rates in open canopy prairie reaches that had high ambient glucose concentrations. Uncovering DOC uptake patterns such as these can help to improve understanding of DOC retention in headwater streams and their contribution to global carbon cycling.

References

Balcarczyk, K. L., J. B. Jones, R. Jaffé, and N. Maie. 2009. Stream dissolved organic matter bioavailability and composition in watersheds underlain with discontinuous permafrost. Biogeochemistry 94:255–270.

Bernhardt, E. S., and W. H. McDowell. 2008. Twenty years apart: Comparisons of DOM uptake during leaf leachate releases to Hubbard Brook valley streams in 1979 versus 2000. Journal of Geophysical Research: Biogeosciences 113:1–8.

Bodmer, P., M. Heinz, M. Pusch, G. Singer, and K. Premke. 2016. Carbon dynamics and their link to dissolved organic matter quality across contrasting stream ecosystems. Science of the Total Environment 553:574–586.

Bott, T., L. Kaplan, and F. Kuserk. 1984. Benthic bacterial biomass supported by streamwater dissolved organic matter. Microbial Ecology 10:335–344.

Bott, T. L., and J. D. Newbold. 2013. Ecosystem metabolism and nutrient uptake in Peruvian headwater streams. International Review of Hydrobiology 98:117–131.

Brack, W. 2003. Effect-directed analysis: A promising tool for the identification of organic toxicants in complex mixtures? Analytical and Bioanalytical Chemistry 377:397–407.

Butman, D., and P. a. Raymond. 2011. Significant efflux of carbon dioxide from streams and rivers in the United States. Nature Geoscience 4:839–842.

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

Cole, J. J., Y. T. Prairie, N. F. Caraco, W. H. McDowell, L. J. Tranvik, R. G. Striegl, C. M. Duarte, P. Kortelainen, J. A. Downing, J. J. Middelburg, and J. Melack. 2007. Plumbing the global carbon cycle: Integrating inland waters into the terrestrial carbon budget. Ecosystems

10:171–184.

Cory, R. M., and L. a. Kaplan. 2012. Biological lability of streamwater fluorescent dissolved organic matter. Limnology and Oceanography 57:1347–1360.

Covino, T. P., B. L. McGlynn, and R. a. McNamara. 2010. Tracer Additions for Spiraling Curve Characterization (TASCC): Quantifying stream nutrient uptake kinetics from ambient to saturation. Limnology and Oceanography: Methods 8:484–498.

Dahm, C. N. 1981. Pathways and Mechanisms for Removal of Dissolved Organic Carbon from Leaf Leachate in Streams. Canadian Journal of Fisheries and Aquatic Sciences 38:68–76.

Ding, Y., Y. Yamashita, W. K. Dodds, and R. Jaffé. 2013. Dissolved black carbon in grassland streams: Is there an effect of recent fire history? Chemosphere 90:2557–2562.

Dodds, W. K., A. J. López, W. B. Bowden, S. Gregory, N. B. Grimm, S. K. Hamilton, A. E. Hershey, E. Martí, W. H. McDowell, J. L. Meyer, D. Morrall, P. J. Mulholland, B. J. Peterson, J. L. Tank, V. Maurice, and J. R. Webster. 2002. N uptake as a function of concentration in streams. Journal of the North American Benthological Society 21:206–220.

Fellman, J. B., E. Hood, R. T. Edwards, and J. B. Jones. 2009. Uptake of Allochthonous Dissolved Organic Matter from Soil and Salmon in Coastal Temperate Rainforest Streams. Ecosystems 12:747–759.

Findlay, S. E. G., R. L. Sinsabaugh, W. V Sobczak, and M. Hoostal. 2003. Metabolic and structural response of hyporheic microbial communities to variations in supplpy of dissolved organic matter. Limnol. Oceanogr. 48:1608–1617.

Gray, L. J. 1997. Organic matter dynamics in Kings Creek, Konza Prairie, Kansas, USA. Journal of the North American Benthological Society 16:50–54.

Hicks, S. E., and F. G. Carey. 1968. Glucose Determination in Natural Waters. Limnology and

Oceanography 13:361–363.

Hobbie, J. E., and G. E. Likens. 1973. Output of phosphorus, dissolved organic carbon, and fine particulate carbon from Hubbard Brook watersheds. Limnology and Oceanography 18:734–742.

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

Hotchkiss, E. R., R. O. Hall, M. A. Baker, E. J. Rosi-Marshall, and J. L. Tank. 2014. Modeling priming effects on microbial consumption of dissolved organic carbon in rivers. Journal of Geophysical Research: Biogeosciences 119:982–995.

Jaffe, R., D. McKnight, N. Maie, R. Cory, W. H. McDowell, and J. L. Campbell. 2008. Spatial and temporal variations in DOM composition in ecosystems: The importance of long-term monitoring of optical properties. Journal of Geophysical Research: Biogeosciences 113:1–15.

Jaffé, R., Y. Yamashita, N. Maie, W. T. Cooper, T. Dittmar, W. K. Dodds, J. B. Jones, T. Myoshi, J. R. Ortiz-Zayas, D. C. Podgorski, and A. Watanabe. 2012. Dissolved Organic Matter in Headwater Streams: Compositional Variability across Climatic Regions of North America. Geochimica et Cosmochimica Acta 94:95–108.

Johnson, L. T., J. L. Tank, and C. P. Arango. 2009. The effect of land use on dissolved organic carbon and nitrogen uptake in streams. Freshwater Biology 54:2335–2350.

Kuzyakov, Y., J. K. Friedel, and K. Stahr. 2000. Review of mechanisms and quantification of priming effects. Soil Biology and Biochemistry 32:1485–1498.

Larson, J. H., P. C. Frost, M. A. Xenopoulos, C. J. Williams, A. M. Morales-Williams, J. M.

Vallazza, J. C. Nelson, and W. B. Richardson. 2014. Relationships Between Land Cover and Dissolved Organic Matter Change Along the River to Lake Transition. Ecosystems 17:1413–1425.

Larson, R. A. 1978. Dissolved organic matter of a low-coloured stream. Freshwater Biology 8:91–104.

Lutz, B. D., E. S. Bernhardt, B. J. Roberts, R. M. Cory, and P. J. Mulhollande. 2012. Distinguishing dynamics of dissolved organic matter components in a forested stream using kinetic enrichments. Limnology and Oceanography 57:76–89.

McArthur, J. V., G. R. Marzolf, and J. E. Urban. 1985. Response of bacteria isolated from a pristine prairie stream to concentration and source of soluble organic carbon. Applied and Environmental Microbiology 49:238–241.

McDowell, W. H. . 1985. Kinetics and mechanisms of dissolved organic carbon retention in a headwater stream. Biogeochemistry 1:329–352.

Mcdowell, W. H., and G. E. Likens. 1988. Origin, Composition, and Flux of Dissolved Organic Carbon in the Hubbard Brook Valley. Ecological Monographs 58:177–195.

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

Mineau, M. M., W. M. Wollheim, I. Buffam, S. Findlay, R. Hall, E. Hotchkiss, L. Koenig, W. McDowell, and T. Parr. 2016. Dissolved organic carbon uptake in streams: A review and assessment of reach-scale measurements. Limnology and Oceanography:1–11.

Murphy, K. R., C. A. Stedmon, P. Wenig, and R. Bro. 2014. OpenFluor- an online spectral library of auto-fluorescence by organic compounds in the environment. Analytical Methods

6:658–661.

Newbold, J. D., T. L. Bott, L. A. Kaplan, C. L. Dow, K. John, A. K. Aufdenkampe, L. A. Martin, D. J. Van Horn, A. Ade, J. D. Newbold, T. L. Bott, L. A. Kaplan, C. L. Dow, J. K. Jackson, A. K. Aufdenkampe, L. A. Martin, D. J. Van Horn, and A. A. De Long. 2006. Uptake of nutrients and organic C in streams in New York City drinking-water-supply watersheds Published by : The University of Chicago Press on behalf of the Society for Freshwater Science Stable URL : http://www.jstor.org/stable/10.1899/0887-3593(2006)0.

O'Brien, J. M., W. K. Dodds, K. C. Wilson, J. N. Murdock, and J. Eichmiller. 2007. The saturation of N cycling in Central Plains streams: 15N experiments across a broad gradient of nitrate concentrations. Biogeochemistry 84:31–49.

Ohno, T. 2002. Fluorescence inner-filtering correction for determining the humification index of dissolved organic matter. Environmental science & technology 36:742–746.

Pisani, O., W. K. Dodds, and R. Jaffé. 2016. Characterizing organic matter inputs to sediments of small, intermittent, prairie streams: a molecular marker and stable isotope approach. Aquatic Sciences 78:343–354.

Raymond, P. A., J. Hartmann, R. Lauerwald, S. Sobek, C. McDonald, M. Hoover, D. Butman, R. Striegl, E. Mayorga, C. Humborg, P. Kortelainen, H. Durr, M. Meybeck, P. Ciais, and P. Guth. 2013. Global carbon dioxide emissions from inland waters. Nature 503:355–359.

Riley, A. J., and W. K. Dodds. 2013. Whole-stream metabolism: strategies for measuring and modeling diel trends of dissolved oxygen. Freshwater Science 32:56–69.

Rüegg, J., J. J. Eichmiller, N. Mladenov, and W. K. Dodds. 2015. Dissolved organic carbon concentration and flux in a grassland stream: spatial and temporal patterns and processes from long-term data. Biogeochemistry 125:393–408.

Samson, F., and F. Knopf. 1994. Prairie conservation in North America. BioScience 44:418–421.

Stedmon, C. A., and R. Bro. 2008. Characterizing dissolved organic matter fluorescence with parallel factor analysis : a tutorial ... Limnology and Oceanography: Methods 6:572–579.

Stedmon, C. A., S. Markager, and R. Bro. 2003. Tracing dissolved organic matter in aquatic environments using a new approach to fluorescence spectroscopy. Marine Chemistry 82:239–254.

Stream Solute Workshop. 1990. Concepts and Methods for Assessing Solute Dynamics in Stream Ecosystems. Journal of the North American Benthological Society 9:95–119.

Thurman, E. M., R. L. Wershaw, R. L. Malcolm, and D. J. Pinckney. 1982. Molecular size of aquatic humic substances. Organic Geochemistry 4:27–35.

Tranvik, L. J., J. A. Downing, J. B. Cotner, S. A. Loiselle, R. G. Striegl, T. J. Ballatore, P. Dillon, K. Finlay, K. Fortino, L. B. Knoll, P. L. Kortelainen, T. Kutser, S. Larsen, I. Laurion, D. M. Leech, S. L. McCallister, D. M. McKnight, J. M. Melack, E. Overholt, J. A. Porter, Y. Prairie, W. H. Renwick, F. Roland, B. S. Sherman, D. W. Schindler, S. Sobek, A. Tremblay, M. J. Vanni, A. M. Verschoor, E. von Wachenfeldt, and G. A. Weyhenmeyer. 2009. Lakes and reservoirs as regulators of carbon cycling and climate. Limnology and Oceanography 54:2298–2314.

Trentman, M. T., W. K. Dodds, J. S. Fencl, K. Gerber, J. Guarneri, S. M. Hitchman, Z. Peterson, and J. Rüegg. 2015. Quantifying ambient nitrogen uptake and functional relationships of uptake versus concentration in streams: a comparison of stable isotope, pulse, and plateau approaches. Biogeochemistry 125:65–79.

Volk, C. J., C. B. Volk, and L. A. Kaplan. 1997. Chemical composition of biodegradable dissolved organic matter in streamwater. Limnology and Oceanography 42:39–44.

Wetzel, R. G. 2001. Limnology: lake and river ecosystems. 3rd edition. Gulf Professional Publishing.

Wymore, A. S., Z. G. Compson, W. H. McDowell, J. D. Potter, B. A. Hungate, T. G. Whitham, and J. C. Marks. 2015. Leaf-litter leachate is distinct in optical properties and bioavailability to stream heterotrophs. Freshwater Science 34:857–866.

TABLES AND FIGURES

Table 2.1 Site characteristics for study reaches. Values represent characteristics measured on the day of an individual nutrient release. PARAFAC components (C1, C2, and C3) are presented in fluorescence units. Data not determined in cells with "-."

Site	Date	Discharge (L sec^{-1})	Reach length (m)	Average wetted width (m)	Ambient [TDOC] (mg L^{-1})	Ambient [Glucose] (mg L^{-1})	Ambient PARAFAC C1, C2, C3
Prairie	04/10/16	1.10	55.0	1.11	1.80	0.217	-
	07/08/16	1.68	57.0	1.26	1.08	0.081	-
	08/09/16	0.42	56.0	1.05	-	-	0, 0.21, 0.13
	04/20/17	23.6	98.0	1.97	1.85	-	1.7, 0.35, 0.27
Gallery	11/12/15	8.73	32.5	2.19	1.58	0.151	-
	04/23/16	63.2	54.0	5.23	1.43	0.048	-
	07/19/16	37.9	63.5	4.85	0.92	0.060	-
	05/25/17	106	112	4.84	0.95	-	0.39, 0.26, 0.20
	10/18/17	7.79	36.0	2.13	1.16	-	0.06, 0.29, 0.36
Urban	08/02/16	9.01	52.6	3.17	3.98	0.046	-
	06/22/17	1.42	31.5	2.55	3.06	-	0.42, 0.59, 1.2

Table 2.2 Uptake parameters for Glucose pulse addition experiments. Both sets of parameter estimates are displayed: those made using specific glucose concentrations and those made using TDOC concentrations.

Site	Date	Glucose-C $S_{w\text{-}amb}$ (m)	Glucose-C U_{amb} (µg m^{-2} min^{-1})	Glucose-C $V_{f\text{-}amb}$ (mm min^{-1})	TDOC $S_{w\text{-}amb}$ (m)	TDOC U_{amb} (µg m^{-2} min^{-1})	TDOC $V_{f\text{-}amb}$ (mm min^{-1})
Prairie	04/10/16	35.0	0.00015	1.7	38.1	0.00186	1.0
	07/08/16	50.8	0.00005	1.6	72.6	0.00119	1.1
Gallery	11/12/15	64.0	0.00023	3.7	82.8	0.00305	2.9
	04/23/16	28.3	0.00050	25.6	-	-	-
	07/19/16	111.0	0.00010	4.2	716.8	0.00060	0.7
Urban	08/02/16	8.2	0.00038	20.9	13.6	0.05277	12.6

Table 2.3 Uptake parameters for leaf leachate pulse addition experiments. Both sets of parameter estimates are displayed: those made using relative fluorescence of leaf components and those made using TDOC concentrations. Areal uptake (U) is omitted here due to inability to determine mass per volume for fluorescent components.

Site	Date	C1 $S_{w\text{-}amb}$ (m)	C3 $S_{w\text{-}amb}$ (m)	TDOC $S_{w\text{-}amb}$ (m)	C1 $V_{f\text{-}amb}$ (mm min^{-1})	C3 $V_{f\text{-}amb}$ (mm min^{-1})	TDOC $V_{f\text{-}amb}$ (mm min^{-1})
Prairie	08/09/16	41.9	59.9	-	0.57	0.40	-
	04/20/17	124	259.9	384	5.65	2.69	1.82
Gallery	05/25/17	170	238.0	983	8.07	5.76	1.39
	10/18/17	135	-	30.7	1.63	-	7.13
Urban	06/22/17	11.1	12.2	9.2	3.01	2.73	3.62

Table 2.4 Analysis of covariance (ANCOVA) for photolysis correction. Site and month had to be analyzed separately because of loss of degrees of freedom when including both.

Effect	Analysis including site				
	SS	Degr. of Freedom	MS	F	p
Intercept	0.000540	1	0.000540	0.09729	0.755685
time	0.116879	1	0.116879	21.06725	0.000012
site	0.065847	2	0.032923	5.93436	0.003542
shade/light	0.157255	1	0.157255	28.34487	0.000001
component	0.107207	2	0.053603	9.66191	0.000134
Error	0.626913	113	0.005548		

Effect	Analysis including month				
	SS	Degr. of Freedom	MS	F	p
Intercept	0.000000	1	0.000000	0.00000	1.000000
time	0.100913	1	0.100913	19.14907	0.000027
month	0.107804	4	0.026951	5.11416	0.000807
shade/light	0.157187	1	0.157187	29.82756	0.000000
component	0.107207	2	0.053603	10.17166	0.000088
Error	0.584956	111	0.005270		

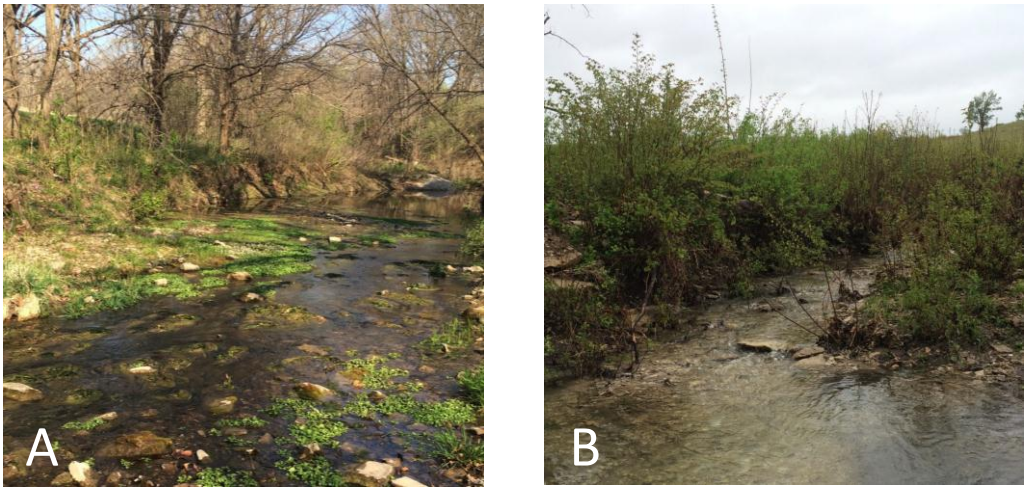

Figure 2.1 Gallery reach (A) and prairie reach (B) used for pulse releases at Kings Creek at Konza Prairie Biological Station.

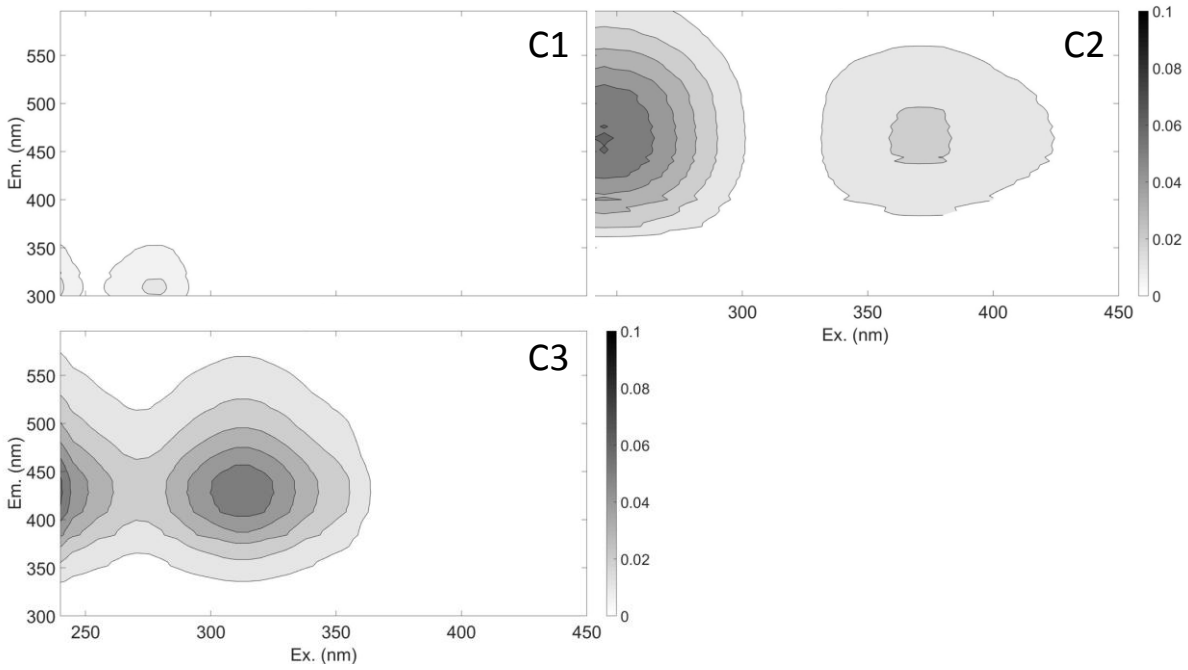

Figure 2.2 Excitation-emission matrices of the three PARAFAC components identified within leaf leachate and stream water.

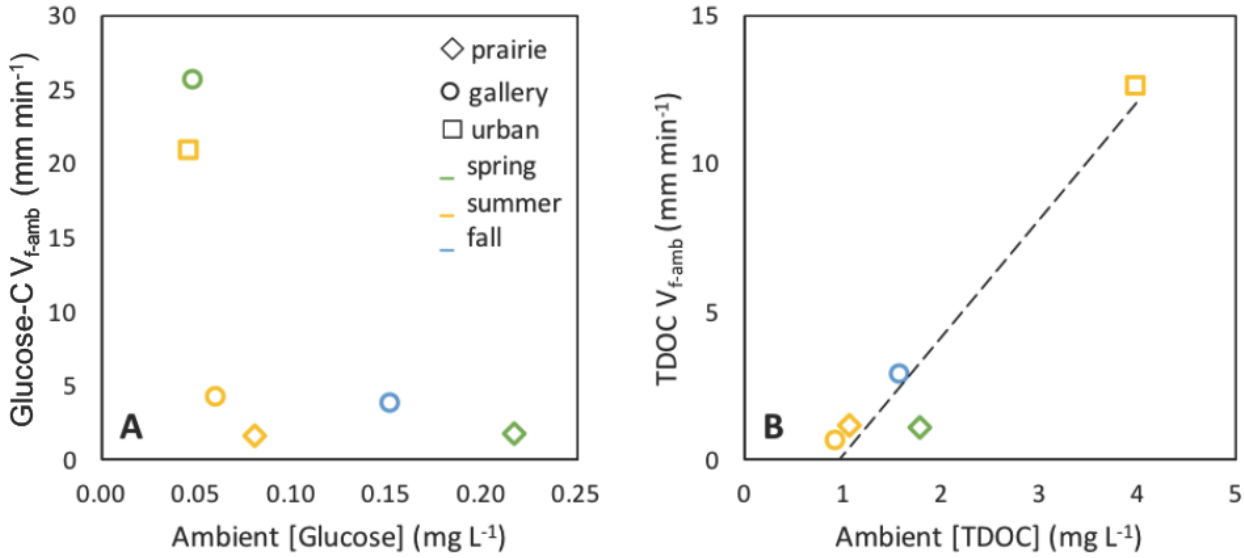

Figure 2.3 Relationship between ambient solute concentration and Vf-amb estimates from (A) glucose concentrations and (B) TDOC concentrations (p = 0.007, r2 = 0.93) for each pulse addition. Color coding is to indicate season and shapes indicate study reach.

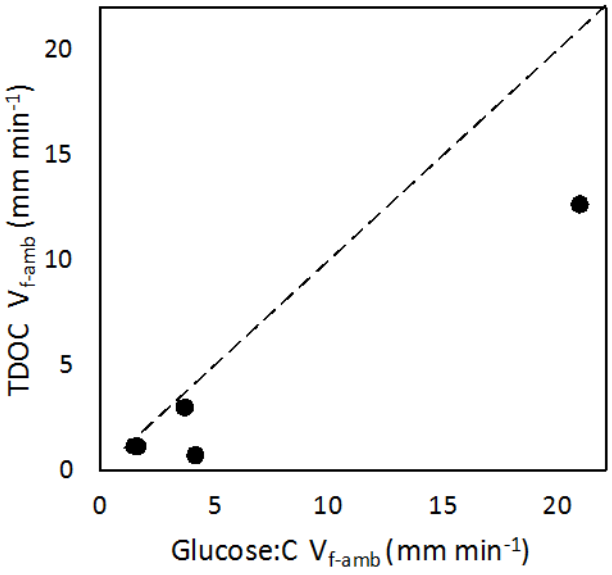

Figure 2.4 Comparison of ambient uptake velocity (Vf-amb) calculated using glucose concentration to that using TDOC. Plotted line shows a 1:1 ratio.

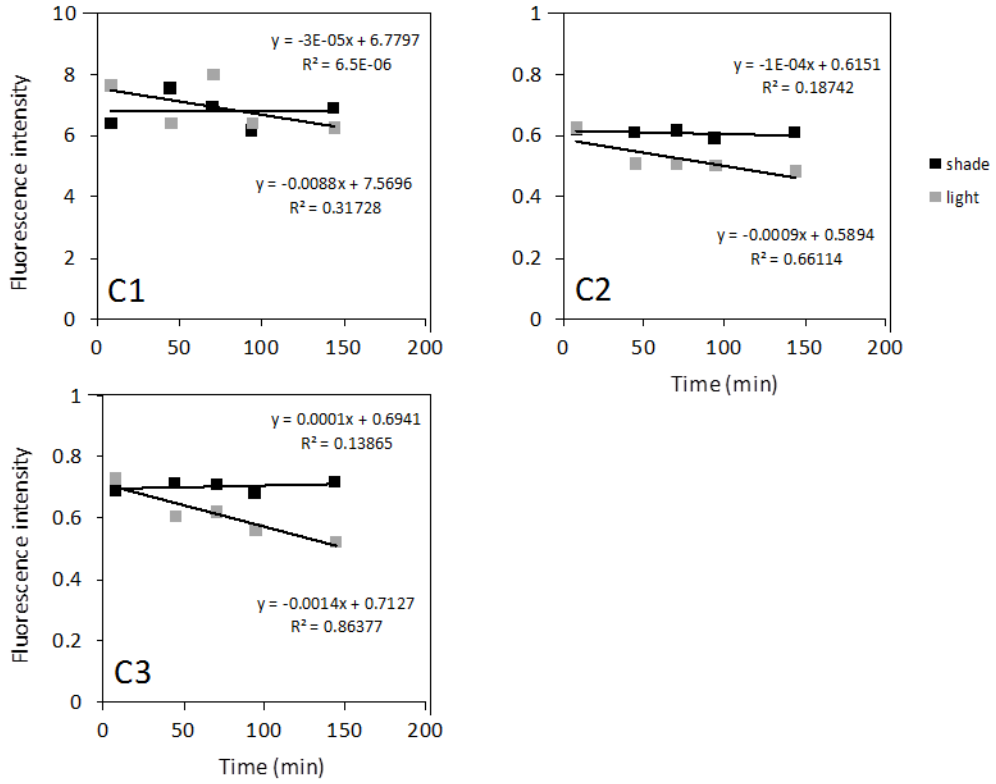

Figure 2.5 Examples of light correction regressions for calculation of fluorescent component uptake.

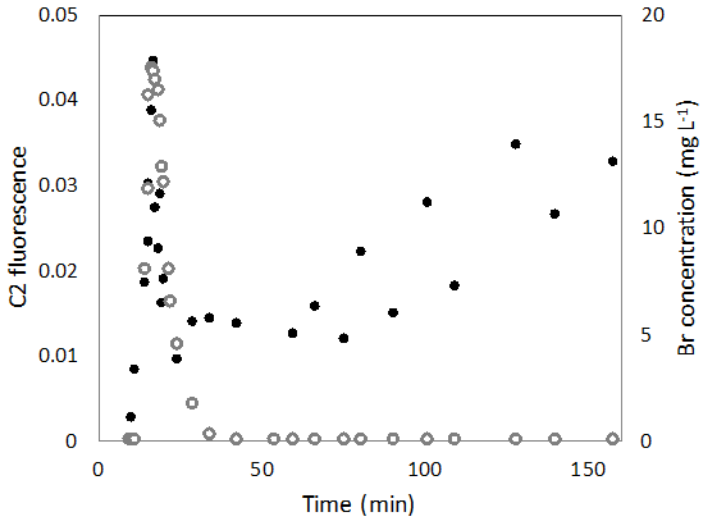

Figure 2.6 Example of the breakthrough curve of C2 (filled circles, the second component of fluorescence analysis, see text) and Br (unfilled circles) during the spring Prairie leachate addition. C2 fluorescence is shown in Raman units.

Chapter 3 - Using recirculating chambers to investigate terrestrial dissolved organic carbon uptake location and priming

Introduction

Terrestrially derived dissolved organic carbon (t-DOC) can be a significant source of energy to streams (Mcdowell and Fisher 1976). Different sources of t-DOC can result in different metabolic responses by stream heterotrophs, suggesting that anthropogenic landscape alterations can indirectly affect stream ecosystem functioning (Berggren and Giorgio 2015). One well documented landscape change in the North American tallgrass prairie is riparian woody expansion (Veach et al. 2014), potentially altering the source of t-DOC received by prairie streams through runoff, groundwater, and litter fall. A previous study at Konza Prairie Biological Station (KPBS) found that benthic bacteria isolated from stream reaches with extensive tree canopy cover could metabolize bur oak leaf leachate whereas those from open canopy stream reaches could not use oak leachate as a source of energy, indicating functional differences in bacterial assemblages along the stream network (McArthur et al. 1985). Another study analyzing the contributions and sources of organic matter (OM) to stream sediments found that upper reaches of the Konza watershed receive most of their (OM) input from C_4 grasses while lower reaches with woody riparian vegetation have more inputs from tree and algae sources (Pisani et al. 2016). So, sources of OM can differ throughout this single drainage basin and microbial assemblages are responsive to OM source. Establishing a more detailed understanding about the biological use and transformation of varying OM source is important in predicting effects of ecosystem function on land-use change and other anthropogenic drivers.

Uptake of various sources of dissolved organic carbon (DOC) has been estimated for streams by adding varying types of DOC and analyzing its loss from the stream water column, a

subject which was recently reviewed (Mineau et al. 2016). This review concluded that the median estimates reported in the literature for stream DOC uptake would require an unrealistically high amount of terrestrial OM inputs. Both *in situ* and mesocosm methods have been applied and one consistency is that these studies use uptake parameter calculations that assume uptake to be a mostly benthic or hyporheic process (e.g. Stream Solute Workshop 1990). However, a recent study has indicated that planktonic microbes can also play a significant role in the processing of t-DOC, especially in smaller order streams (Graeber et al. 2018). This raises the question of whether planktonic bacterial uptake is significant enough to warrant inclusion when calculating reach-scale uptake parameters and scaling estimates up to the network level. If so, leaving out this water column processing could be partially driving apparent overestimates of per area uptake found by Mineau et al. (2016).

Another recognized problem with the current suite of DOC uptake studies is that when making uptake measurements by elevating the concentration of a single source of DOC, as explained above, microbes react differently to different sources of OM meaning these uptake parameters probably don't fully represent ambient total DOC (TDOC) uptake (Mineau et al. 2016). This is because uptake of substrates depends upon enzyme kinetics which have increased rates of activity with greater substrate concentration. Furthermore, there is potential for a single source of DOC to influence how other carbon constituents are transformed (Kuzyakov et al. 2000, Lutz et al. 2012). For example, several DOC uptake studies use simple compounds like glucose or acetate, which could lead to stimulation of microbial metabolism, extracellular enzyme production, or biomass, resulting in inaccurate ambient TDOC uptake parameter calculations.

Our study focused on these two considerations of in-stream DOC uptake: uptake location and the potential for microbial priming. Specifically, we wanted to know: 1) the relative contribution of planktonic bacteria to DOC uptake estimates and 2) the degree to which microbial priming could influence DOC uptake studies using simple sugars. We used leaf leachate additions in recirculating chambers to assess 1) the relative contribution of planktonic and benthic microbial assemblages to t-DOC transformations and 2) the potential for priming effects on t-DOC with addition of a simple sugar. First, since previous studies have found that streambed biota dominate nutrient retention (e.g. Reisinger et al. 2015), we predict that a larger percentage of the leachate will be taken up by benthic rather than planktonic processes. Second, we expect to see some degree of t-DOC uptake priming with the addition of a simple sugar.

Methods

Recirculating chamber and site description

We used recirculating chambers described by Rüegg et al. (2015) to test our hypotheses. Briefly, these are rectangular acrylic boxes of approximately 1 L volume with a stage for holding stream sediments or rocks. Water velocity, created by a motor and propeller, can be controlled with regulators to achieve target rotations per minute, which has been related to measured velocities in the working section of the chambers through linear regression. Another important characteristic of these chambers is that they can be effectively sealed off from atmosphere, allowing us to monitor dissolved oxygen concentrations to make estimates of respiration.

The same site from Kings Creek at Konza Prairie Biological Station that was used for Gallery in Chapter 1 was used to collect water and biofilm for this study. Descriptions of Kings Creek can be found in the text of Chapter 1 and characteristics for the Gallery stream section are found in Table 2.1. Leaf leachate was also made in the same way as described in Chapter 1.

Experimental design

Three sets of chamber incubations were conducted in order to explore our study questions (Table 3.1). All three experiments were conducted over twelve hours in the dark to prevent photosynthesis from occurring during incubation. Chambers were incubated in a lab with a temperature held at 18ºC, roughly the average temperature of the stream water at the time of the experiments and velocity was set to approximately 0.03 m sec^{-1}.

The first two rounds of incubations, run on March 19th and 21st 2018, were used to assess the question of uptake location. Incubation round 1 tested for planktonic microbial respiration and DOC uptake. This setup consisted of three types of replicates: a treatment with unfiltered stream water and added leaf leachate; a control with unfiltered stream water only; and a respiration control with filtered stream water only. Incubation round 2 was used to assess benthic microbial respiration and DOC uptake. For this incubation, water was filtered through a glass fiber filter (Whatman GF/F nominal retention size 0.7 µm) to exclude planktonic microbes and stream biofilm grown on clay tiles in the stream for approximately two weeks was added to the chamber platform. This setup consisted of two types of replicates: a treatment with filtered stream water and added leaf leachate; and a control with filtered stream water only. Within each of these two incubations, comparison of means between control and treatment was used to test whether leaf leachate addition stimulated respiration or led to faster uptake of DOC. Rate comparisons between the first two incubations allowed us to assess the relative contribution of planktonic and benthic microbes to DOC uptake. Since we were directly comparing the rates of Incubations 1 and 2, these experiments were run within two days and water for the two incubations was collected at the same time. Stream water was collected on the day of Incubation 1 and half of the water was filtered for use in the respiration control and for Incubation 2. All

water, filtered and unfiltered, was kept in the dark at 18ºC until use. Biofilm tiles were collected immediately before Incubation 2 and kept in the same conditions as described above until placed inside recirculating chambers.

Incubation 3 was used to test for priming of t-DOC by glucose addition. Each chamber in this incubation was fitted with unfiltered stream water and stream biofilm tiles. There were two types of replicates in this incubation: a treatment with unfiltered water and leaf leachate; and a control with unfiltered water, leaf leachate, and added glucose. Comparisons between the mean dissolved oxygen consumption and the proportion decrease of DOC of treatment and control were used to test for stimulation of respiration and DOC uptake from glucose addition.

Dissolved oxygen and organic carbon analyses

Dissolved oxygen concentration (mg L^{-1}), water temperature, and barometric pressure were measured in each chamber for the duration of the incubations with YSI Professional Optical Dissolved Oxygen (ProODO) instruments (Yellow Springs Instruments, Yellow Springs, OH). Probes were calibrated with air saturated water prior to chamber incubations. Measurements were logged every ten minutes, although several of the ProODO meters lost connection with their probes during the last incubation and so manual readings were recorded at times that logging was not occurring.

Samples were taken from each chamber at the start and end of incubations in order to analyze initial and final concentrations or relative fluorescence of TDOC and components from Parallel Factor Analysis (PARAFAC) respectively. TDOC and PARAFAC component analysis was conducted as described above in the Chapter 2 methods. The same three PARAFAC components identified in Chapter 2, one amino acid and two humic-like components, were also found in the leachate and stream water for this study (Figure 2.2).

Data analysis

Dissolved oxygen concentration was regressed with time for each individual chamber and the resulting slopes were used to calculate oxygen consumption (g m^{-2} day^{-1}) for each chamber. Proportion decrease of TDOC and PARAFAC components was calculated as follows: $Proportion\ decrease = \frac{[initial]-[final]}{[initial]}$ where [initial] and [final] are the concentrations of initial and final concentrations in mg L^{-1} for TDOC and in fluorescence units for PARAFAC components. Means of replicate oxygen consumption and DOC proportion decrease were calculated, separately, and unpaired t-Tests were run in R (R Core Team 2017) to detect difference between treatment and control means for each incubation.

Results

Respiration

There was no difference detected in oxygen (DO) consumption between treatment and control for Incubations 1 and 2, indicating that leaf leachate addition did not affect respiration rates for either planktonic or benthic microbes (Figure 3.1). Mean DO consumption calculated for Incubation 2 (0.83 g m^{-2} day^{-1}) was more than twice the rate estimated for Incubation 1 (0.40 g m^{-2} day^{-1}), reflecting higher rates of benthic respiration than planktonic respiration.

In Incubation 3, there was a significant difference between mean oxygen consumption in treatment and control chambers with rates of 3.0 and 2.1 g m^{-2} day^{-1} in treatment and control chambers respectively. Oxygen consumption from Incubation 3 is not directly comparable to those rates of the first two Incubations because they were not conducted at the same time and therefore used water collected on a different day and biofilm grown at a different time. However, it is interesting to point out that respiration of the control from Incubation 3 was more than twice the rate found in the treatment of Incubation 2 (the most comparable treatment).

Organic carbon components

Differences in the mean TDOC proportional decrease of treatment and control of Incubations 2 and 3 were both significant (Figure 3.2). In the case of Incubation 2, a higher proportion of TDOC was removed from the water column when leaf leachate was added than when there was no leaf leachate addition. However, since no decrease in dissolved oxygen was observed in the treatment of this incubation, the decrease in TDOC observed here is likely due to physical processes rather than metabolism. In Incubation 3, a higher proportion of TDOC was removed from the water column when glucose was added.

Difference in PARAFAC components was only found for Component 3 (C3) between treatment control in Incubation 3 (Figure 3.3). So C3 fluorescence decreased more from initial to final when glucose was added to the chamber.

Discussion

Location of DOC uptake

The higher rates of oxygen consumption estimated for Incubation 2 compared to Incubation 1 indicates that benthic communities have a stronger influence on community respiration than planktonic communities. However, using tile area and chamber volume to scale up to reach dimensions, an unrealistic average stream depth of 0.54 m is calculated whereas mean stream depths of below 0.25 m have been reported for this site (Rüegg et al. 2016). So, the water column to streambed ratio in the chambers is higher than what is found in the stream, meaning that this higher respiration found in the benthic communities would likely be amplified further if properly scaled to the accurate stream proportions.

Respiration rate increase with leaf leachate addition has been previously documented in bottle incubation studies (McDowell 1985). Such a pattern was not observed in our study as there

was no difference in oxygen consumption between treatment and control in either Incubation 1 or 2. However, the study by McDowell (1985) used 24 hour incubations compared to our 12 hour incubations and it could be the case that we did not run them long enough to observe this pattern.

Proportional decrease of TDOC over time was statistically greater with leaf leachate addition compared to the control in Incubation round 2. No significant decrease in PARAFAC components was determined for either Incubation 1 or 2. The fact that a higher mean proportion difference from initial to final was found in the treatment of Incubation 3 but was not accompanied with a decrease in respiration could indicate that most of the TDOC decrease was due to physical processes like adsorption rather than microbial usage (Dahm 1981, McDowell 1985). We only analyzed the chromophoric DOC and so even though we did not see a decrease in PARAFAC components over time, there could be some other leaf leachate components that are being removed from the column through adsorption.

Priming

Both respiration and proportional decrease of TDOC were elevated with glucose addition in Incubation 3. PARAFAC Component 3 was the only individually traced DOC component for which uptake was stimulated by glucose addition. This priming behavior could have implications for *in situ* estimates of reach scale DOC uptake using simple carbohydrate additions. Hotchkiss and others (2014) modeled the effect of priming by glucose in rivers and estimated an 87% increase in DOC uptake. On the other hand, one study found a negative priming effect from a large addition of labile DOC to a stream reach (Lutz et al. 2011). There are several potential mechanisms for priming including alterations of metabolic rates, extracellular enzyme production, and biomass (Kuzyakov et al. 2000). Given the short time of our chamber incubations (twelve hours), it is important to note that each of these mechanisms are likely to

occur on different time-scales. Studies in soils, which typically measure priming over units of days, have found that increases in microbial activity occur on shorter time-scales than changes in biomass (Kuzyakov et al. 2000). However, relatively fast rates of microbial biomass increase have been reported in some freshwater ecosystems and therefore could still be considered as a mechanism (Pollard and Ducklow 2011). These priming effects should be taken into consideration when using simple carbohydrate additions to estimate ambient DOC uptake.

Conclusions

We found that benthic respiration and uptake of DOC was higher magnitude than that of planktonic communities. Although the increase of TDOC with leaf addition in the biofilm incubation was likely due to physical processes, adsorption is still a form of removal from the water column and can have implications for downstream export (Dahm 1981). Priming of one component of terrestrial DOC was observed in chamber Incubation 3 and provides further evidence for this phenomenon in stream ecosystems.

References

Balcarczyk, K. L., J. B. Jones, R. Jaffé, and N. Maie. 2009. Stream dissolved organic matter bioavailability and composition in watersheds underlain with discontinuous permafrost. Biogeochemistry 94:255–270.

Berggren, M., and P. a Giorgio. 2015. Distinct patterns of microbial metabolism associated to riverine dissolved organic carbon of different source and quality. Journal of Geophysical Research: Biogeosciences.

Bernhardt, E. S., and W. H. McDowell. 2008. Twenty years apart: Comparisons of DOM uptake during leaf leachate releases to Hubbard Brook valley streams in 1979 versus 2000. Journal of Geophysical Research: Biogeosciences 113:1–8.

Bodmer, P., M. Heinz, M. Pusch, G. Singer, and K. Premke. 2016. Carbon dynamics and their link to dissolved organic matter quality across contrasting stream ecosystems. Science of the Total Environment 553:574–586.

Bott, T., L. Kaplan, and F. Kuserk. 1984. Benthic bacterial biomass supported by streamwater dissolved organic matter. Microbial Ecology 10:335–344.

Bott, T. L., and J. D. Newbold. 2013. Ecosystem metabolism and nutrient uptake in Peruvian headwater streams. International Review of Hydrobiology 98:117–131.

Brack, W. 2003. Effect-directed analysis: A promising tool for the identification of organic toxicants in complex mixtures? Analytical and Bioanalytical Chemistry 377:397–407.

Butman, D., and P. a. Raymond. 2011. Significant efflux of carbon dioxide from streams and rivers in the United States. Nature Geoscience 4:839–842.

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

Cole, J. J., Y. T. Prairie, N. F. Caraco, W. H. McDowell, L. J. Tranvik, R. G. Striegl, C. M. Duarte, P. Kortelainen, J. A. Downing, J. J. Middelburg, and J. Melack. 2007. Plumbing the global carbon cycle: Integrating inland waters into the terrestrial carbon budget. Ecosystems 10:171–184.

Cory, R. M., and L. a. Kaplan. 2012. Biological lability of streamwater fluorescent dissolved organic matter. Limnology and Oceanography 57:1347–1360.

Cory, R. M., C. P. Ward, B. C. Crump, and G. W. Kling. 2014. Sunlight controls water column processing of carbon in arctic fresh waters. Science 345:925–928.

Covino, T. P., B. L. McGlynn, and R. a. McNamara. 2010. Tracer Additions for Spiraling Curve Characterization (TASCC): Quantifying stream nutrient uptake kinetics from ambient to saturation. Limnology and Oceanography: Methods 8:484–498.

Dahm, C. N. 1981. Pathways and Mechanisms for Removal of Dissolved Organic Carbon from Leaf Leachate in Streams. Canadian Journal of Fisheries and Aquatic Sciences 38:68–76.

Ding, Y., Y. Yamashita, W. K. Dodds, and R. Jaffé. 2013. Dissolved black carbon in grassland streams: Is there an effect of recent fire history? Chemosphere 90:2557–2562.

Dodds, W. K., A. J. López, W. B. Bowden, S. Gregory, N. B. Grimm, S. K. Hamilton, A. E. Hershey, E. Martí, W. H. McDowell, J. L. Meyer, D. Morrall, P. J. Mulholland, B. J. Peterson, J. L. Tank, V. Maurice, and J. R. Webster. 2002. N uptake as a function of concentration in streams. Journal of the North American Benthological Society 21:206–220.

Fellman, J. B., E. Hood, R. T. Edwards, and J. B. Jones. 2009. Uptake of Allochthonous Dissolved Organic Matter from Soil and Salmon in Coastal Temperate Rainforest Streams. Ecosystems 12:747–759.

Findlay, S. E. G., R. L. Sinsabaugh, W. V Sobczak, and M. Hoostal. 2003. Metabolic and

structural response of hyporheic microbial communities to variations in supplpy of dissolved organic matter. Limnol. Oceanogr. 48:1608–1617.

Graeber, D., J. Rosenstand, M. Heinz, J. J. Rasmussen, D. Zak, B. Gücker, B. Kronvang, and N. Kamjunke. 2018. Going with the flow: Planktonic processing of dissolved organic carbon in streams. Science of the Total Environment 625:519–530.

Gray, L. J. 1997. Organic matter dynamics in Kings Creek, Konza Prairie, Kansas, USA. Journal of the North American Benthological Society 16:50–54.

Hall, R. O., J. L. Tank, M. A. Baker, E. J. Rosi-Marshall, and E. R. Hotchkiss. 2015. Metabolism, Gas Exchange, and Carbon Spiraling in Rivers. Ecosystems.

Hicks, S. E., and F. G. Carey. 1968. Glucose Determination in Natural Waters. Limnology and Oceanography 13:361–363.

Hobbie, J. E., and G. E. Likens. 1973. Output of phosphorus, dissolved organic carbon, and fine particulate carbon from Hubbard Brook watersheds. Limnology and Oceanography 18:734–742.

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

Hotchkiss, E. R., R. O. Hall, M. A. Baker, E. J. Rosi-Marshall, and J. L. Tank. 2014. Modeling priming effects on microbial consumption of dissolved organic carbon in rivers. Journal of Geophysical Research: Biogeosciences 119:982–995.

Hotchkiss, E. R., and R. O. J. Hall. 2014. Whole-stream 13C tracer addition reveals distinct fates of newly fixed carbon. Ecology.

Jaffé, R., Y. Yamashita, N. Maie, W. T. Cooper, T. Dittmar, W. K. Dodds, J. B. Jones, T.

Myoshi, J. R. Ortiz-Zayas, D. C. Podgorski, and A. Watanabe. 2012. Dissolved Organic Matter in Headwater Streams: Compositional Variability across Climatic Regions of North America. Geochimica et Cosmochimica Acta 94:95–108.

Johnson, L. T., J. L. Tank, and C. P. Arango. 2009. The effect of land use on dissolved organic carbon and nitrogen uptake in streams. Freshwater Biology 54:2335–2350.

Kuzyakov, Y., J. K. Friedel, and K. Stahr. 2000. Review of mechanisms and quantification of priming effects. Soil Biology and Biochemistry 32:1485–1498.

Larson, J. H., P. C. Frost, M. A. Xenopoulos, C. J. Williams, A. M. Morales-Williams, J. M. Vallazza, J. C. Nelson, and W. B. Richardson. 2014. Relationships Between Land Cover and Dissolved Organic Matter Change Along the River to Lake Transition. Ecosystems 17:1413–1425.

Larson, R. A. 1978. Dissolved organic matter of a low-coloured stream. Freshwater Biology 8:91–104.

Lutz, B. D., E. S. Bernhardt, B. J. Roberts, R. M. Cory, and P. J. Mulhollande. 2012. Distinguishing dynamics of dissolved organic matter components in a forested stream using kinetic enrichments. Limnology and Oceanography 57:76–89.

Lutz, B. D., E. S. Bernhardt, B. J. Roberts, and P. J. Mulholland. 2011. Examining the coupling of carbon and nitrogen cycles in Appalachian streams: The role of dissolved organic nitrogen. Ecology 92:720–732.

McArthur, J. V., G. R. Marzolf, and J. E. Urban. 1985. Response of bacteria isolated from a pristine prairie stream to concentration and source of soluble organic carbon. Applied and Environmental Microbiology 49:238–241.

McDowell, W. H. . 1985. Kinetics and mechanisms of dissolved organic carbon retention in a

headwater stream. Biogeochemistry 1:329–352.

Mcdowell, W. H., and S. G. Fisher. 1976. Autumnal processing of dissolved organic matter in a small woodland stream ecosystem. Ecology 57:561–569.

Mcdowell, W. H., and G. E. Likens. 1988. Origin, Composition, and Flux of Dissolved Organic Carbon in the Hubbard Brook Valley. Ecological Monographs 58:177–195.

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

Mineau, M. M., C. M. Rigsby, D. T. Ely, I. J. Fernandez, S. A. Norton, T. Ohno, H. M. Valett, and K. S. Simon. 2013. Chronic catchment nitrogen enrichment and stoichiometric constraints on the bioavailability of dissolved organic matter from leaf leachate. Freshwater Biology 58:248–260.

Mineau, M. M., W. M. Wollheim, I. Buffam, S. Findlay, R. Hall, E. Hotchkiss, L. Koenig, W. McDowell, and T. Parr. 2016. Dissolved organic carbon uptake in streams: A review and assessment of reach-scale measurements. Limnology and Oceanography:1–11.

Moody, C. S., F. Worrall, C. D. Evans, and T. G. Jones. 2013. The rate of loss of dissolved organic carbon (DOC) through a catchment. Journal of Hydrology 492:139–150.

Murphy, K. R., C. A. Stedmon, P. Wenig, and R. Bro. 2014. OpenFluor- an online spectral library of auto-fluorescence by organic compounds in the environment. Analytical Methods 6:658–661.

Newbold, J. D., T. L. Bott, L. A. Kaplan, C. L. Dow, K. John, A. K. Aufdenkampe, L. A. Martin, D. J. Van Horn, A. Ade, J. D. Newbold, T. L. Bott, L. A. Kaplan, C. L. Dow, J. K. Jackson, A. K. Aufdenkampe, L. A. Martin, D. J. Van Horn, and A. A. De Long. 2006. Uptake of

nutrients and organic C in streams in New York City drinking-water-supply watersheds Published by : The University of Chicago Press on behalf of the Society for Freshwater Science Stable URL : http://www.jstor.org/stable/10.1899/0887-3593(2006)0.

O'Brien, J. M., W. K. Dodds, K. C. Wilson, J. N. Murdock, and J. Eichmiller. 2007. The saturation of N cycling in Central Plains streams: 15N experiments across a broad gradient of nitrate concentrations. Biogeochemistry 84:31–49.

Ohno, T. 2002. Fluorescence inner-filtering correction for determining the humification index of dissolved organic matter. Environmental science & technology 36:742–746.

Pisani, O., W. K. Dodds, and R. Jaffé. 2016. Characterizing organic matter inputs to sediments of small, intermittent, prairie streams: a molecular marker and stable isotope approach. Aquatic Sciences 78:343–354.

Raymond, P. A., J. Hartmann, R. Lauerwald, S. Sobek, C. McDonald, M. Hoover, D. Butman, R. Striegl, E. Mayorga, C. Humborg, P. Kortelainen, H. Durr, M. Meybeck, P. Ciais, and P. Guth. 2013. Global carbon dioxide emissions from inland waters. Nature 503:355–359.

Reisinger, A. J., J. L. Tank, E. J. Rosi-Marshall, R. O. Hall, and M. A. Baker. 2015. The varying role of water column nutrient uptake along river continua in contrasting landscapes. Biogeochemistry 125:115–131.

Riley, A. J., and W. K. Dodds. 2013. Whole-stream metabolism: strategies for measuring and modeling diel trends of dissolved oxygen. Freshwater Science 32:56–69.

Rüegg, J., J. D. Brant, D. M. Larson, M. T. Trentman, and W. K. Dodds. 2015a. A portable, modular, self-contained recirculating chamber to measure benthic processes under controlled water velocity. Freshwater Science 34:831–844.

Rüegg, J., W. K. Dodds, M. D. Daniels, K. R. Sheehan, C. L. Baker, W. B. Bowden, K. J.

Farrell, M. B. Flinn, T. K. Harms, J. B. Jones, L. E. Koenig, J. S. Kominoski, W. H. McDowell, S. P. Parker, A. D. Rosemond, M. T. Trentman, M. Whiles, and W. M. Wollheim. 2016. Baseflow physical characteristics differ at multiple spatial scales in stream networks across diverse biomes. Landscape Ecology 31:119–136.

Rüegg, J., J. J. Eichmiller, N. Mladenov, and W. K. Dodds. 2015b. Dissolved organic carbon concentration and flux in a grassland stream: spatial and temporal patterns and processes from long-term data. Biogeochemistry 125:393–408.

Samson, F., and F. Knopf. 1994. Prairie conservation in north america. BioScience 44:418–421.

Stedmon, C. A., and R. Bro. 2008. Characterizing dissolved organic matter fluorescence with parallel factor analysis : a tutorial ... Limnology and Oceanography: Methods 6:572–579.

Stedmon, C. A., S. Markager, and R. Bro. 2003. Tracing dissolved organic matter in aquatic environments using a new approach to fluorescence spectroscopy. Marine Chemistry 82:239–254.

Stream Solute Workshop. 1990. Concepts and Methods for Assessing Solute Dynamics in Stream Ecosystems. Journal of the North American Benthological Society 9:95–119.

Thurman, E. M., R. L. Wershaw, R. L. Malcolm, and D. J. Pinckney. 1982. Molecular size of aquatic humic substances. Organic Geochemistry 4:27–35.

Tranvik, L. J., J. A. Downing, J. B. Cotner, S. A. Loiselle, R. G. Striegl, T. J. Ballatore, P. Dillon, K. Finlay, K. Fortino, L. B. Knoll, P. L. Kortelainen, T. Kutser, S. Larsen, I. Laurion, D. M. Leech, S. L. McCallister, D. M. McKnight, J. M. Melack, E. Overholt, J. A. Porter, Y. Prairie, W. H. Renwick, F. Roland, B. S. Sherman, D. W. Schindler, S. Sobek, A. Tremblay, M. J. Vanni, A. M. Verschoor, E. von Wachenfeldt, and G. A. Weyhenmeyer. 2009. Lakes and reservoirs as regulators of carbon cycling and climate. Limnology and

Oceanography 54:2298–2314.

Trentman, M. T., W. K. Dodds, J. S. Fencl, K. Gerber, J. Guarneri, S. M. Hitchman, Z. Peterson, and J. Rüegg. 2015. Quantifying ambient nitrogen uptake and functional relationships of uptake versus concentration in streams: a comparison of stable isotope, pulse, and plateau approaches. Biogeochemistry 125:65–79.

Vannote, R. L., G. W. Minshall, K. W. Cummins, J. R. Sedell, and C. E. Cushing. 1980. The River Continuum Concept. Canadian Journal of Fisheries and Aquatic Sciences 37:130–137.

Veach, A. M., W. K. Dodds, and A. Skibbe. 2014. Fire and grazing influences on rates of riparian woody plant expansion along grassland streams. PLoS ONE 9.

Volk, C. J., C. B. Volk, and L. A. Kaplan. 1997. Chemical composition of biodegradable dissolved organic matter in streamwater. Limnology and Oceanography 42:39–44.

Wetzel, R. G. 2001. Limnology: lake and river ecosystems. 3rd edition. Gulf Professional Publishing.

Wymore, A. S., Z. G. Compson, W. H. McDowell, J. D. Potter, B. A. Hungate, T. G. Whitham, and J. C. Marks. 2015. Leaf-litter leachate is distinct in optical properties and bioavailability to stream heterotrophs. Freshwater Science 34:857–866.

TABLES AND FIGURES

Table 3.1 Experimental design of chamber incubations.

Incubation	Date	Replicate	Tiles	Water	Amendment
1	03/19/18	Treatment	blank	unfiltered	leachate
		Control	blank	unfiltered	-
		Respiration control	blank	filtered	-
2	03/21/18	Treatment	biofilm	filtered	leachate
		Control	biofilm	filtered	-
3	06/12/18	Treatment	biofilm	unfiltered	leachate, glucose
		Control	biofilm	unfiltered	leachate

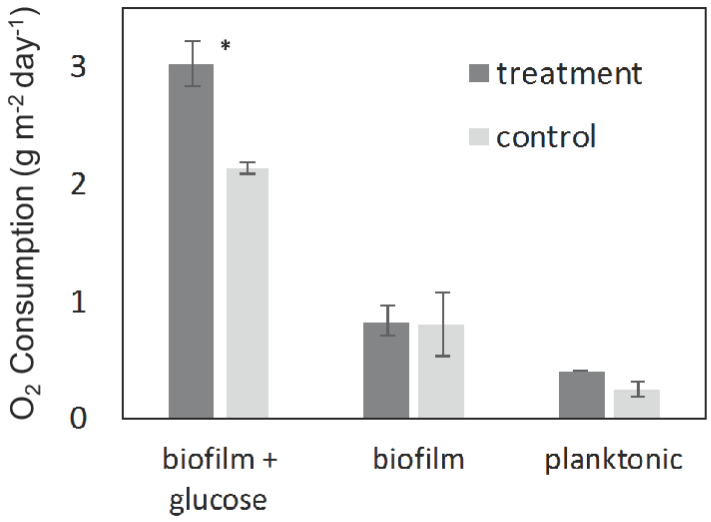

Figure 3.1 Mean oxygen consumption (and standard error) for each incubation in the order: 3, 2, 1. Statistically significant difference between treatment and control is indicated by * (p = 0.05).

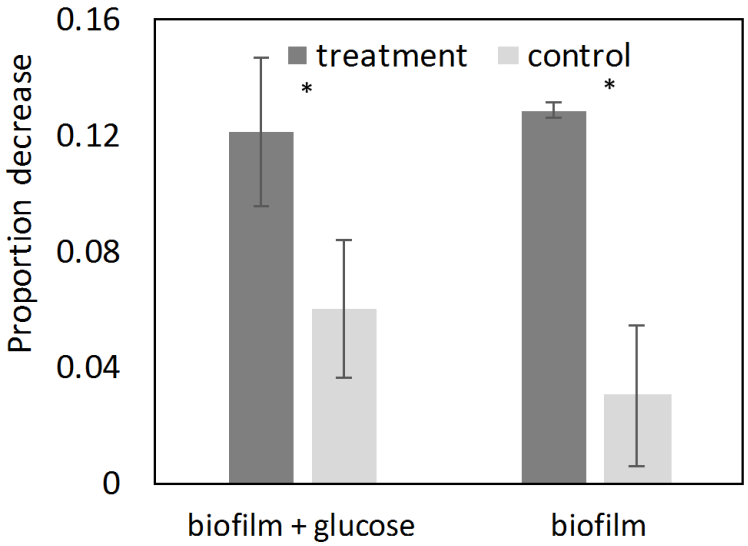

Figure 3.2 Mean proportion (with standard error) decrease of total dissolved organic carbon for Incubations 2 and 3. Statistically significant difference between treatment and control is indicated by * (p = 0.04, 0.007).

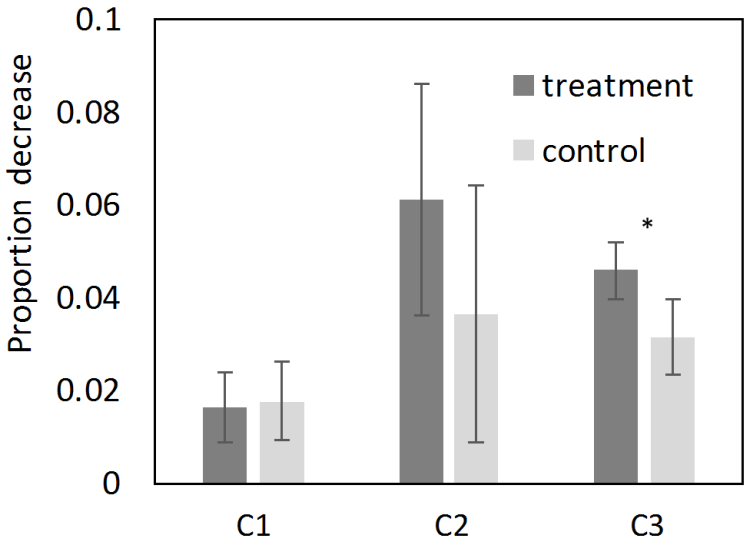

Figure 3.3 Mean proportion decrease of PARAFAC components (C1, C2, C3 described in the text) within incubation 3. Statistically significant difference between treatment and control is indicated by * (p = 0.1).

Chapter 4 - Conclusions

Conclusions

Studies aiming to characterize the uptake and bioavailability of dissolved organic carbon (DOC) have increased in past years (Mineau et al. 2016). We add to this growing body of literature with reach scale uptake estimates from glucose and bur oak leaf leachate additions in a tallgrass prairie biome and urban streams (Chapter 2); assessment of relative contributions of benthic and water column processes to DOC retention (Chapter 3); and an investigation of priming effects from the addition of a simple carbohydrate (Chapter 3). Our findings collectively indicate that refined estimates that consider solute-specific uptake, autochthonous inputs, and priming effects could be necessary to accurately scale DOC retention rates up to network rates across biomes.

Our results highlight areas that need more investigation in order to refine DOC uptake estimates in streams. Firstly, we found that some components of leaf leachate are subject to photolysis during the time scale of reach scale addition experiments. We corrected grab samples for light effects during each experiment, something that, to the best of our knowledge, has not been done previously. Future studies using leachates containing chromophoric DOC should follow a similar approach to correct for loss due to photolysis to avoid making overestimates of retention. We made uptake estimates from an addition of a specific simple carbohydrate and found that uptake parameters calculated from compound-specific concentrations resulted in faster uptake rates than when we used total dissolved organic carbon (TDOC) concentrations to estimate the same parameters. This indicates the importance of having accurate background concentrations of specific components during such studies. We also found a potential priming effect of one leaf leachate component through our recirculating chamber incubation. However,

this should be considered as an initial investigation supporting the possibility of priming and more work is needed to assess priming mechanisms and time-scales in freshwater ecosystems.

We refer to leaf leachate fluorescent components as "DOC" throughout the text because we are interested in heterotrophic use and other retention mechanisms of reduced carbon. However, we recognize that these organic components likely also contain nitrogen and phosphorous. Ratios of carbon to both nitrogen and phosphorus within organic matter have been previously correlated with degradation and could affect uptake rates (Wiegner and Seitzinger 2004).

Future directions

Although we were able to detect the uptake of specific DOC components of different bioavailability, we did this at separate times during elevated levels of a single DOC source. We recommend future studies that measure compound-specific uptake, as we did, but that use concurrent additions of a suite of various DOC sources spanning levels of bioavailability. Such uptake estimates compared to estimates made from additions of single sources could allow for a primary *in situ* investigation of priming effects. Also, more advanced methods of analyses giving more specific identification of organic carbon compounds (e.g. GC mass spectrometry) could yield more detailed information.

References

Mineau, M. M., W. M. Wollheim, I. Buffam, S. Findlay, R. Hall, E. Hotchkiss, L. Koenig, W. McDowell, and T. Parr. 2016. Dissolved organic carbon uptake in streams: A review and assessment of reach-scale measurements. Limnology and Oceanography:1–11.

Wiegner, T. N., and S. P. Seitzinger. 2004. Seasonal biovailability of dissolved organic carbon and nitrogen from pristine and polluted freshwater wetlands. Limnol. Oceangr. 45:1703–1712.