

ABSTRACT

From Landscapes to Streams: The Pattern and Function of Labile Dissolved Organic Carbon

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Dissolved organic carbon (DOC) is a broadly important component of stream water chemistry, yet the patterns and the roles of the labile fraction of the DOC pool remain understudied at appropriate scales. I used a whole stream labile DOC addition on the Kenai Peninsula, Alaska, to address how relevant quantities of labile DOC influence stream ecosystem function, from microbes to fish. I additionally quantified the labile DOC pool in streams that spanned a gradient of anthropogenic land covers, during contrasting hydrologic periods. I found that relevant quantities of labile DOC can strongly alter basal ecosystem function. Labile DOC increased ecosystem respiration by ~50%, and generally increased nitrogen uptake. Labile DOC also increased bacterial biomass production, which served as an additional food source for stream consumers. Benthic invertebrate counts were ~9x greater and juvenile salmonid abundances were ~2x greater nearest the labile DOC source. Initially larger juvenile salmonids also grew faster than similar sized reference reach individuals. Labile DOC patterns in the Ozark Highlands were strongly influenced by anthropogenic DOC sources. Sites

receiving municipal wastewater treatment plant (WWTP) effluent had consistently elevated labile DOC concentrations. Developed land use positively influenced labile DOC concentrations in sites without WWTP inputs, but only when there was significant hydrologic connectivity to the landscape, suggesting that anthropogenic patterns of labile DOC are strongly tied to hydrology. Overall, my results suggest that 1) labile DOC can be a critically important component to ecosystem function, and 2) anthropogenic influences on labile DOC can be complex and dependent on meteorological factors. Thus, understanding stream ecosystems, especially in the “anthropocene,” may necessitate adding labile DOC to the currently nutrient-centric paradigm of bottom-up control on stream ecosystem function.

From Landscapes to Streams: The Pattern and Function of Labile Dissolved Organic Carbon

by

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DEDICATION

To my grandfathers, now gone

Thank you for inspiring my love of learning and nature

To my wife, my best friend Ashley

Nothing in this life compares to the joy of being married to you

CHAPTER ONE

Introduction

General Overview

Streams are a function of the landscapes through which they pass and drain (Hynes 1975; Frissell et al. 1986; Ward 1989). Hydrology, topography, geology and vegetation complexly interact to determine the physical and chemical template on which streams are based (Wang et al. 2006; Allan and Castillo 2007; Nelson et al. 2011; Dodds et al. 2014). For example, the “natural flow regime,” the temporal and magnitudinal variability of a stream’s flow, is a product of these interactions with climate (and human influences), and is considered a primary determinant of water quality, physical habitat and resource availability (Poff et al. 1997). As water chemistry, habitat and resources control biotic activity in streams at the spatial and temporal scales that individual organisms directly experience, biota are also dependent on the physical template provided by the landscape (Levin 1992; Thorp et al. 2006). Even under less dynamic paradigms than the natural flow regime, there is a basic understanding that stream water interacts with the terrestrial landscape and then accumulates in stream channels, advecting myriad terrestrial materials on which aquatic organisms base their lives (McClain et al. 2003; Allan and Castillo 2007; Tank et al. 2010).

Resource quantity and quality are critical determinants of stream ecosystem function (Marcarelli et al. 2011). Because of their low place in the landscape, stream ecosystems are frequently donor-controlled systems in terms of resources: the quantity of nutrients and energy that propagate through food webs and drives ecosystem function is

controlled by delivery from terrestrial systems (Polis et al. 1997; Lamberti et al. 2010; Tanentzap et al. 2017). For example, the retention of water on landscapes, influenced largely by topography and soil characteristics, is an opportunity for terrestrial compounds to dissolve into water and be carried to aquatic systems. Landscapes also control the quality of resources available to stream ecosystems, and quality determines the rates and efficiency by which those resources are processed. Terrestrial vegetation is a common resource subsidy to streams, and its quality when it reaches the stream is highly dependent on both its character (e.g., wood vs leaf) and its transport (longer transport time implies greater degradation has occurred to that resource, (Sitters et al. 2015). Because ecological processes are ultimately driven by the evolutionary struggle for resources to use toward reproduction, resource availability is a critical constraint on stream ecosystem processes.

Microbes carry out the bulk of biogeochemical cycling in streams, as well as trophically upgrade resources that are nutritionally poor or possibly unusable for higher trophic levels (e.g., dissolved compounds; Wiegner et al. 2015; Hiltunen et al. 2017). Microbes carry out biogeochemical processes for either catabolic (obtain energy) or anabolic (build biomass) purposes. For instance, reduced (organic) forms of C can be respired for energy or assimilated to form the backbone of biomolecules. These microbial processes ultimately go toward sustaining or producing biomass that becomes available for consumption by higher trophic levels (Hall and Meyer 1998). Biogeochemical cycling and basal biomass production are two fundamental functions of ecosystems to which all other processes will be tied. Understanding the resources that fuel those functions is

therefore paramount to understanding how streams function and predicting how they will respond to human or natural disturbances.

Carbon in Stream Ecosystems

Carbon (C) has been increasingly studied over the past few decades, particularly because of the need to better understand global C cycling as it relates to climate change. Consequently, scientists have recognized that streams are not just ‘pipes’ that passively transport terrestrial C from terrestrial to marine systems (Cole et al. 2007). Older “conventional wisdom” suggested terrestrial C was almost entirely recalcitrant by the time it reached stream systems, and certainly by the time it reached larger order streams in the river network (Battin et al. 2008). However, ignoring the numerous processes governing C flux in stream ecosystems results in erroneous global C budgets, suggesting in-stream processing (Cole et al. 2007). Along with imbalances between terrestrial and marine C fluxes, numerous experiments suggest that terrestrial C is processed and even contributes significantly to stream ecosystem processes (Kaplan and Bott 1982; Wallace et al. 1997; Bernhardt and Likens 2002). Terrestrial C is in fact transformed by biota, implying that streams are also C reactors, not just pipes (Cole et al. 2007; Battin et al. 2008; Battin et al. 2009).

C exists in many forms in all aquatic ecosystems, from a few inorganic molecules (e.g., CO₂) to an enormous variety of different organic compounds, reflecting the numerous terrestrial and aquatic sources and transformations that occur, and spanning a wide size continuum from logs to particles to compounds that easily cross cell membranes (Fisher and Likens 1973; Wetzel 2001; Findlay and Sinsabaugh 2003; Hockaday et al. 2009; Tank et al. 2010). Typically, the most dominant form of organic C

in streams is in dissolved organic matter (DOM), the vast majority of which is (and is usually measured as) dissolved organic C (DOC; (Fisher and Likens 1973; Wetzel 2001)).

Stream DOC derives from numerous sources, including exudates from photosynthesizing macrophytes and algae, but is mostly derived from terrestrial organic matter (Kaplan and Bott 1982; Kaplan and Cory 2016). Terrestrially-derived DOC leaches from dead plant litter and soil organic matter and is often composed of exudates of microbes decomposing the organic matter (Meyer et al. 1998; Marín-Spiotta et al. 2014; Wiegner et al. 2015), so soil DOC can strongly determine stream DOC quantity (Aitkenhead et al. 1999). Wetlands are a particularly notable source of DOC, owing to long retention times and low decomposition rates that maximize the potential for DOC to leach into wetland water (Dillon and Molot 1998). Significant quantities of DOC are also transported into stream systems during rainflow events, especially storms, which shift flowpaths to shallower, more organic soil layers (Lambert et al 2013, Vidon et al 2008, McLaughlin and Kaplan 2013), shifting stream DOC composition, as well (Hu et al 2016, Lambert et al 2013). Stream DOC quantity and composition is therefore tied to hydrologic connectivity to the landscape (Covino 2017).

DOC is a heterogeneous pool of thousands of different types of molecules (Hockaday et al. 2009; Sleighter et al. 2014). The ‘dissolved’ fraction is defined operationally (usually that which passes <0.7 μm pore size filters) rather than strictly dissolved, and many compounds are much larger than microbes can directly use without some processing. The DOC pool therefore spans a wide size continuum, as well as a broad continuum of diagenetic status and susceptibility to biological use and decomposition (lability; (Koehler et al. 2012a)). For example, highly aromatic compounds

are generally resistant to microbial degradation (Cory and Kaplan 2012). However, the stream DOC pool does contain many labile compounds, especially low molecular weight monomeric carbohydrates and organic acids (Berggren et al. 2010).

Labile DOC, at least on short timescales, is used by microbes to fuel myriad aquatic processes (Catalán et al. 2016; Casas-Ruiz et al. 2017). Although terrestrial respiration generally accounts for much more of the CO₂ emitted from stream systems than aquatic respiration, respired DOC is an appreciable fraction of stream CO₂ emissions (Hotchkiss et al. 2015; Winterdahl et al. 2016). Additionally, denitrification is fueled by labile DOC both in that DOC respiration can reduce O₂ concentrations, creating the anoxic environment necessary for denitrifiers to compete for NO₃, and provide the reduced C source to which NO₃ donates electrons. CH₄ emissions can be controlled by labile DOC, as well (Baker et al. 1999; Stanley et al. 2016). Labile DOC is also known to support aquatic food webs. Despite the question remaining as to whether allochthonous C inputs can significantly enhance secondary production (i.e., subsidize food webs) in aquatic ecosystems, labile DOC is transferred from bacteria to higher trophic levels, including predatory insects and fish (Hall and Meyer 1998; Brett et al. 2017; Jones et al. 2018). Labile DOC is therefore a critical basal resource in stream ecosystems, and deserves study to elucidate controls on its quantity across spatial and temporal gradients, as well as further test its role in stream ecosystems.

The roles and patterns of labile DOC are scale dependent, so studies probing these roles and patterns must be conducted at appropriate temporal and spatial scales. For example, the uptake of labile DOC to a single epilithic biofilm in a jar belies the immense heterogeneity throughout a stream reach (i.e., patchiness), including interactions with

multiple types of rock and organic substrates, disturbance regimes and oxygen concentrations (e.g., in the hyporheic zone), which could lead to vastly different patterns of labile DOC use. The most publicly interesting and consequential stream responses (e.g., ecosystem services) to resources or perturbations often occur at reach or larger scales. Therefore, whole-stream experiments that integrate responses over relevant scales are key frameworks for evaluating hypothesized relationships between labile DOC and stream ecosystem structure and function.

Several studies have investigated the role of labile DOC in stream systems using whole-stream additions, but these studies have been either poorly temporally scaled and/or ecologically unrealistic in terms of labile DOC dosing concentrations (Bernhardt and Likens 2002; Wilcox et al. 2005; Johnson et al. 2012; Oviedo-Vargas et al. 2013). Relevant temporal scaling is important if research questions center around the influence of labile DOC increases stemming from chronic changes, such as specific landscape elements (whether natural or anthropogenic). In addition to temporal dependence of responses (e.g., macroinvertebrates will not be subsidized by an hour- long labile DOC enrichment), the role of labile DOC could change seasonally, particularly if seasonal changes alter key ecosystem components such as nutrient availability or light. The fate of labile DOC can be particularly influenced by nutrient availability, as well (Del Giorgio and Cole 1998). In addition, labile DOC is generally not more than 10% of the total stream DOC pool (Kaplan and Newbold 2003; Berggren et al. 2010; McLaughlin and Kaplan 2013), yet enrichment studies often double the amount of DOC present in the study systems, at concentrations relevant only to wastewater spills (Wilcox et al. 2005).

Ecologically relevant experiments are needed to test the roles of labile DOC in stream ecosystems.

Human Interactions with the Labile DOC Pool

Anthropogenic landscape disturbances, such as land use change, have significantly increased the global flux of C to freshwater ecosystems, with correspondingly significant changes to the composition of the DOC pool (Regnier et al. 2013; Williams et al. 2016). Descriptive studies have consistently found that urbanization and agriculture either increase bioavailability or increase optical and chemical markers that often correlate with bioavailability (Stanley et al. 2012). Urbanization disconnects streams from their historic landscape sources of DOM, reducing the contribution of complex soil organic matter pools and increasing the contribution of more simple, labile compounds to stream DOM (Petrone et al. 2011; Hosen et al. 2014; Parr et al. 2015). Moreover, significant quantities of labile DOC can be directly piped into aquatic ecosystems from municipal wastewater discharges (Shon et al. 2006; Sickman et al. 2007). Agriculture can similarly alter stream DOM composition, but may lead to selective mobilization of fresher organic matter from shallow soil layers, increasing concentrations of labile DOM but retaining some DOM complexity in comparison to urban watersheds (Wilson and Xenopoulos 2009; Williams et al. 2010; Shang et al. 2018). The actual mechanisms relating human land use to increased stream DOM lability are complex, and involve important interactions with climate (e.g., precipitation), vegetation changes and soil alterations such as compaction (or installation of impervious surfaces, (Giling et al. 2014; Shang et al. 2018). This suggests that patterns could be seasonal (e.g.,(Hosen et al. 2014) and therefore difficult to predict. Yet, under either

urbanized or agricultural land use, the DOC pool is highly likely to be proportionally (i.e., % bioavailable DOC) or totally (i.e., bioavailable DOC concentration) more labile in comparison to natural land use streams (Stanley et al. 2012). More studies are needed that consider the influence of anthropogenic land use on bioavailable DOC in a temporal context associated with changing temperatures or flows.

Problem Statement

DOC has long been considered important to stream ecosystems, but studies quantifying patterns of DOC have largely ignored the combination of two facts: 1) the DOC pool is diverse, and 2) the influence of anthropogenic sources on DOC quality could be dependent on hydrology. Further, experimental tests of the influence of labile DOC on stream systems have often been conducted at irrelevant scales and dosing concentrations, making it difficult and possibly erroneous to scale inferences about the role of labile DOC in whole ecosystem function.

Study Objectives

My overall objective was to elucidate the function and pattern of labile DOC in stream ecosystems in a landscape context. My specific objectives were to 1) Determine how labile DOC influences microbially-driven processes in streams, 2) determine the importance of labile DOC to stream consumers, and 3) identify spatial and temporal patterns of labile DOC in streams with varying levels of anthropogenic land use. Objectives (1) and (2) were explicitly conducted at relevant spatial and temporal scales, and considering both quantity and quality of DOC, to simulate the role of landscape features that are significant sources of labile DOC, specifically wetlands. Objective (3)

considered catchment-scale anthropogenic sources (land use) of bioavailable DOC under varying hydrologic contexts to more completely characterize how humans influence labile DOC concentrations in streams.

Summary of Chapter Contents

This document comprises five chapters, including the current introductory chapter. The second chapter details the effects of a low level (ecologically relevant) labile DOC addition on basal ecosystem components (nitrogen uptake, periphyton production, ecosystem metabolism) of a boreal headwater stream. The third chapter details the consumer (benthic invertebrate and juvenile salmonid) responses to the same low level DOC addition. The fourth chapter describes an observational study measuring labile DOC concentrations across streams with varying levels and types of anthropogenic disturbance and through a winter-to-spring weather transition leading to varying hydrologic scenarios. The fifth chapter synthesizes these works and suggests future directions for work to advance a more complete understanding of labile DOC in stream ecosystems.

Attribution

Chapter two (Low-Level Addition of Dissolved Organic Carbon...) is a published work with coauthoring collaborators, where CJR wrote the manuscript; CJR and RSK analyzed data; JAB analyzed water chemistry; RSK, CMW, RDD and DFW conceived the study, and all authors performed research.

CHAPTER TWO

Low-Level Addition of Dissolved Organic Carbon Increases Basal Ecosystem Function in a Boreal Headwater Stream

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Introduction

Streams are inextricably linked to their catchments. Land use, land cover, topography, geology and climate within a catchment can all figure prominently into determining stream characteristics such as geomorphology, substratum, light availability and nutrient concentrations. Catchment characteristics subsequently affect basal ecosystem processes such as nutrient uptake, decomposition, photosynthesis and overall production of microbial biomass that becomes available for higher trophic levels (Johnson et al. 1997: 199; Allan 2004; Wang et al. 2006; Findlay 2010; Mulholland and Webster 2010; Tank et al. 2010; Nelson et al. 2011). Identifying the effects that landscape features have on stream functioning, such as controls through water chemistry (i.e., via stoichiometric imbalances), is valuable for broadening fundamental understanding of terrestrial-aquatic linkages.

Wetlands are often regarded as catchment features that have the potential to significantly alter water chemistry in streams, leading to changes in community structure and function (King et al. 2012; Walker et al. 2012). Wetlands typically export elevated levels of dissolved organic carbon (DOC; (Dillon and Molot 1997), which can alter conditions such as pH and benthic light availability in addition to providing organic

(Pellerin et al. 2004) and inorganic nutrients (Johnston et al. 1990; Dillon and Molot 1997). Wetland-derived DOC may dominate DOC concentrations in streams draining even moderate levels (>10% catchment) of wetlands (Ågren et al. 2008).

DOC is increasingly recognized as an important resource for streams (Stanley et al. 2012) especially as its oxidation can have important implications for ecosystem carbon budgets (Cole et al. 2007). Functionally, DOC drives bacterial metabolism (Wiegner et al. 2005) and can support a significant portion of ecosystem metabolism (Kaplan et al. 2008) which may transfer to higher trophic levels (Bott et al. 1984; Hall and Meyer 1998). However, the role of DOC in microbial production depends on both DOC quantity and quality (Bott et al. 1984), as well as the availability of necessary nutrients for growth (Stelzer et al. 2003). Labile C often limits bacterial production (Kirchman 1994), and labile dissolved organic matter (DOM) typically stimulates bacterial growth (Findlay 2003); this may be particularly true if inorganic nutrients are available in relatively high concentrations (i.e., if labile C is deficient relative to nutrient availability; (Chrost 1991; Sinsabaugh et al. 1997).

The importance of allochthonous organic matter to stream ecosystem function has long been understood (Tank et al. 2010), but much of what is known about the role of DOC in stream ecosystems comes from micro- or mesocosm incubations. Whole-stream additions of subsidies can be used as simulations of how whole ecosystems may react to a subsidy, integrating the effects of many environmental factors to arrive at a broader-scale response. For studies to be ecologically relevant and informative for decision making in management situations, whole-stream additions must closely mimic reactivity of the

solute in question, at realistic concentrations and for adequately long periods of time to capture seasonal trends in community development and metabolism.

In prior studies, whole-stream additions of labile carbon (C) have increased bacterial biomass (Wilcox et al. 2005) and community respiration (Bernhardt and Likens 2002; Johnson et al. 2012; Oviedo-Vargas et al. 2013), changed bacterial community composition (Bernhardt and Likens 2002; Johnson et al. 2012) and altered inorganic nitrogen (N, Bernhardt and Likens 2002; Johnson et al. 2009; Thouin et al. 2009; Johnson et al. 2012) and phosphorus (P) demand (Oviedo-Vargas et al. 2013). Whole-stream labile DOC additions can also alter the uptake of native DOM pools (Lutz et al. 2012). These labile C additions have been very insightful for studying food webs and biogeochemical cycling, but were applied at unnaturally high concentrations ranging from ~1 to >20 mg C/L corresponding to > 50% of the background DOC. Under most baseflow conditions, very labile C (e.g., some low molecular weight compounds such as carboxylic acids, amino acids or monomeric carbohydrates) compounds are probably not typically more than 10% of total stream water DOC (Kaplan and Newbold 2003; Berggren et al. 2010; McLaughlin and Kaplan 2013). Some short-term releases (<1 d) have utilized much lower labile C concentrations (Newbold et al. 2006; Kaplan et al. 2008; Johnson et al. 2009; Johnson and Tank 2009), but such short-term additions are unable to assess the interaction of DOC and seasonal abiotic factors. In order to determine the role of DOC in whole ecosystems, experimental labile C additions must occur both at realistic concentrations and on seasonal time scales.

We studied the effects of a season-long (Boreal summer, ~3 months) addition of ecologically relevant concentrations of labile DOC on boreal stream metabolism, epilithic

production and nutrient retention. Our objective was to simulate the effect of the highly labile portion of wetland-derived DOC on microbial activity in a system with significant inputs of inorganic N, which was highly available due to catchment-scale alder (*Alnus* spp.) stands. Wetland and alder cover are strong predictors of DOC and DIN, respectively, in Kenai lowland streams (Shaftel et al. 2012; Walker et al. 2012). The catchment of the study stream exhibited relatively low wetland influence (low DOC), but relatively high alder cover (high DIN); thus, we predicted that microbial activity would be strongly limited by labile C availability. Specifically, we hypothesized that labile DOC addition would 1) increase demand for inorganic nitrogen, 2) increase bacterial biomass production (BBP) and 3) increase ecosystem respiration (ER). Labile DOC-induced demand for inorganic N with increased BBP and ER would help clarify the role of wetlands in boreal stream production and illustrate the importance of alder to stream heterotrophs. Moreover, an increase in heterotrophic production could result in greater photosynthetic rates in periphytic autotrophs, because bacterial-algal dynamics in periphyton are often tightly coupled (Scott et al. 2008). This study helps to elucidate the combined role of two dominant landscape elements: wetlands, which are common in boreal landscapes, and alder, which are either dominant or becoming increasingly common with range expansion in boreal zones (Hiltbrunner et al. 2014). More broadly, our study furthers the increasingly recognized importance of DOC within stream ecosystems (Stanley et al. 2012) in addition to the well-established linkage of wetlands, and catchments in general, to streams.

Methods

Study Area and Experimental Design

The study stream was a first-order tributary of the South Anchor River on the lower Kenai Peninsula of south-central Alaska (59.77974 °N, 151.55518 °W). Comprehensive descriptions of the study site, previously identified as ANC-1203, and region can be found in Shaftel et al. (2011; 2012), King et al.(2012), Walker et al. (2012) and Whigham et al. (2012). Briefly, the dominant vegetation in the catchment was Lutz spruce (*Picea lutzii*), paper birch (*Betula papyrifera*), and willow (*Salix* spp.) with riparian vegetation dominated by bluejoint grass (*Calamagrostis canadensis*; Fig. 2.1). Alder (*Alnus* spp.) covers 12.6% of the study catchment, which leads to significant inorganic N inputs (Shaftel et al. 2012). Wetland cover (35.2%) is dominated by discharge slope wetlands, which have low retention times and lower DOC export than other wetland classifications (Walker et al. 2012, <http://www.cookinletwetlands.info>).

We established a 75 m upstream reference reach and a 75 m downstream treatment reach, separated by an 80 m intermediate reach excluded from the study. Within each experimental reach, we established sampling locations at 10, 37.5, and 75 m downstream from the top of each reach (designated with either R (Reference) or T (Treatment)). An additional sampling location was established immediately upstream of the 0 m mark of the T. We validated the similarity of the reaches by measuring several physicochemical variables. Wetted width (R: 1.49 ± 0.26 m; T: 1.30 ± 0.30 m (mean \pm SD)), mean depth (R: 0.12 ± 0.07 m; T: 0.12 ± 0.07 m), channel slope (R: 5.3%; T: 4.9%) and sinuosity (R: 1.07; T: 1.06) were very similar between reaches. When measured in both reaches, discharge (Q) differed by no more than 4% (0.6 L/s). Substrate in both

reaches was predominantly gravel and small cobble. Both reaches were dominated by riffle and run habitat. Likewise, pre-dose (days -21, -14 and -6) measurements of DIC,



Figure 2.1. Photos of the reference reach of ANC1203, facing upstream, showing encroaching herbaceous vegetation in late June (A), July (B) and August (C) of 2013.

We also ensured similarity of riparian topography and vegetation (i.e., hillslope was not directly adjacent to reaches and riparia were dominated by *C. canadensis*).

We continuously dosed the treatment reach with labile DOC for 62 days, starting on 25 June 2013 (day 1 of dosing) and terminating on 25 August 2013. The dosing solution consisted of sodium acetate ($C_2H_3NaO_2$) as a labile DOC source. Acetic acid (dissociated acetate in H_2O) is an important component of the labile C pool in other boreal streams, at least 1 – 8% of the total DOC measured during spring freshet (Berggren et al. 2010). We targeted raising concentrations by 0.150 mg/L acetate-C, assuming a baseflow discharge of 30 L/s (King et al. 2012; Shaftel et al. 2012). The level of DOC enrichment was chosen to represent the concentration of labile DOC typically found in streams in this region that have high wetland cover. Of the total DOC in such streams (10-13 mg/L), about 10% was found to be bioavailable (i.e., 1.2-1.5 mg/L; RDD, unpublished data); thus, our dosing concentration was conservative, although acetate is highly labile and similarly labile compounds likely represented only a portion of that labile DOC fraction.

A FMI model QBG pump (Fluid Metering Inc., Syosset, NY) delivered dosing stock to point T0 of the stream at 3.6 mL/min from a 100 L covered stock tank via tubing housed in a PVC conduit. The first 5 m of the reach below T0 were shallow and turbulent, so solutes were mixed by the sampling point at T10. To be clear, we did not adjust the dosing rate to changes in discharge, and instead let the dosing concentration be a function of discharge, which varied modestly throughout the growing season (see Results).

Table 2.1 DOC concentrations (mg/L), presented as mean (SE), averaged over the reference reach (n=6) and at treatment reach locations T0, T10 and T75 (each n=2), with dosing calculations, mean daily temperature (°C) and discharge (Q; L/s) for each date. Cumulative dosed acetate is based on the confirmed dosing rate of 375 g acetate-C per day.

Day of Dosing	DOC (mg/L)				Cumulative Dosed Acetate-C (g)	Q (L/s)	Temp (°C)
	Reference Reach (n=6)	T0 (n=2)	T10 (n=2)	T75 (n=2)			
-21	4.96 (0.059)	4.824 (0.048)	-	-	-	38.9	-
-14	3.88 (0.041)	3.774 (0.049)	-	-	-	36.6	-
-6	2.21 (0.017)	2.263 (0.040)	-	-	-	28.0	-
1	2.44 (0.055)	2.634 (0.026)	2.632 (0.012)	2.718 (0.123)	8	22.4	7.27
8	3.35 (0.019)	3.339 (0.003)	3.429 (0.018)	3.315 (0.019)	2625	31.7	6.69
14	2.85 (0.015)	2.701 (0.02)	2.973 (0.047)	2.760 (0.093)	4875	22.9	5.83
22	2.22 (0.017)	2.188 (0.026)	2.490 (0.014)	2.182 (0.044)	7875	17.1	8.37
28	2.08 (0.056)	1.982 (0.068)	2.124 (0.009)	1.919 (0.018)	10125	13.9	8.64
36	2.81 (0.086)	2.639 (0.137)	2.990 (0.062)	2.367 (0.008)	13125	14.6	8.65
42	1.92 (0.049)	1.912 (0.062)	2.257 (0.001)	1.986 (0.012)	15375	14.6	7.53
50	3.19 (0.042)	3.195 (0.053)	3.367 (0.049)	3.105 (0.044)	18375	20.0	7.42
56	1.85 (0.013)	1.857 (0.033)	2.116 (0.0325)	1.899 (0.025)	20625	15.9	7.25

Table 2.2. Summary of water chemistry for each collection event. Data are mean (SE) concentrations from R10 and T0 (for NH₄-N, NO_x-N, DIN and PO₄-P, n=2 for days -21 to 22 , n=9 for days 28 to 56; for all DIC n=2). “<MDL” (below method detection limit) indicates inability to detect analyte due to low concentrations.

Day of Dosing	NH ₄ -N (µg/L)		NO _x -N (µg/L)		DIN (µg /L)		PO ₄ -P (µg /L)		DIC (mg/L)	
	R10	T0	R10	T0	R10	T0	R10	T0	R10	T0
-21	22.8 (2)	20.0 (0.75)	432 (2.5)	428 (1)	455 (0.5)	448 (2)	41.1 (0.69)	40.2 (1.75)	4.292 (0.16)	4.38 (0.10)
-14	<MDL	<MDL	441 (0.5)	439 (1)	441 (0.5)	439 (1)	53.2 (1)	53.0 (0.10)	4.6465 (0.08)	4.67 (0.02)
-6	<MDL	<MDL	432 (0.5)	422 (3)	432 (0.5)	422 (3)	59.2 (0.90)	58.5 (0.65)	5.90 (0.03)	5.93 (0.005)
1	<MDL	<MDL	216 (0.5)	185 (0)	216 (0.5)	185 (0)	59.45 (0.15)	59.0 (2.25)	5.68 (0.03)	5.80 (0.08)
8	<MDL	<MDL	291 (0.5)	283 (1)	291 (0.5)	283 (1)	61.9 (0.05)	60.9 (0.25)	5.17 (0.07)	5.46 (0.01)
14	<MDL	<MDL	275 (0)	262 (1.5)	275 (0)	262 (1.50)	60.7 (1)	63.3 (0.5)	5.91 (0.04)	6.00 (0.04)
22	<MDL	<MDL	339 (0.5)	322 (1.5)	339 (0.5)	322 (1.50)	67.4 (0.5)	68.5 (0.5)	5.96 (0.01)	5.90 (0.16)
28	<MDL	<MDL	427 (1.53)	425 (2.08)	427 (1.50)	425 (2.08)	75.1 (0.93)	72.4 (1.56)	6.01 (0.08)	6.37 (0.13)
36	22.2 (0.73)	20.8 (0.95)	438 (0.58)	441 (0.58)	460 (1.20)	462 (1.30)	52.9 (0.19)	53.8 (0.80)	6.11 (0.25)	6.02 (0.07)
42	26.7 (1.1)	20.1 (0.20)	423 (1.2)	431 (1.53)	450 (2.33)	451 (1.45)	54.4 (0.99)	54.0 (1.19)	6.8835 (0.07)	6.09 (0.13)
50	23.3 (0.17)	22.9 (0.6)	356 (0.33)	363 (0.33)	380 (0.33)	386 (0.58)	51.5 (1.24)	50.3 (0.79)	6.2025 (0.13)	6.30 (0.03)
56	23.13 (1.7)	21.7 (1.29)	418 (0.58)	422 (0.88)	441 (2)	444 (2)	55.1 (1.04)	53.5 (0.43)	7.113 (0.13)	7.08 (0.02)

Water Chemistry

We collected water samples from each sampling location in 1 L dark bottles on either the first or second day of every week from June 4 (day -21) to August 19 (day 56). We immediately placed samples on ice. Coincident with water sampling, we calculated discharge by measuring change in conductivity with the metered addition of a known solution of NaCl (Webster and Valett 2006).

We returned to the lab and filtered water in a Geotech 2.4 L barrel filter apparatus (Geotech Environmental Equipment, Colorado) through a pre-rinsed cellulose acetate membrane filter (0.45 μm). Aliquots for analysis of dissolved N and P were immediately frozen. DOC and total dissolved carbon (TDC) were determined using water filtered with a nylon membrane filter.

All N and P constituents were analyzed on a Lachat Quik Chem 8500 series 2 continuous flow injection analyzer. DOC and TDC were analyzed using a Shimadzu TOC-Vcsh. Dissolved inorganic carbon (DIC) was calculated as $\text{TDC} - \text{DOC} = \text{DIC}$. All analyses followed standard methods (APHA 1998).

Net Nutrient Uptake

Net nutrient uptake was calculated by measuring ambient longitudinal declines in nutrient concentrations (e.g., Marti et al. 2004) for DIN and $\text{PO}_4\text{-P}$. Uptake length (S_w) was calculated as the inverse slope of the regression line of log-transformed nutrient concentrations against distance. In order to standardize uptake to reach characteristics, we converted uptake length to uptake velocity (V_f) using the equation $V_f = \frac{uz}{S_w}$, where u = velocity and z = depth (Webster and Valett 2006). We approximated velocity and depth

each as a power function of discharge (Leopold et al. 1964) to estimate velocity and depth on sampling dates where only discharge was measured.

Bacterial Biomass Production (BBP) and Photosynthesis

We used the dual-label radioassay method to measure bacterial biomass production (BBP) and photosynthesis of epilithic periphyton (Neely and Wetzel 1995; Scott et al. 2008; Taylor et al. 2012). On days 12, 30 and 56, we collected 7 small rocks and site water from R10 and T10. Rocks were placed in plastic bags and transported to the lab in the dark on ice. Rocks were placed in unfiltered site water (taken from the appropriate reach), in 60 mL glass jars with silicon-septa lids. For each reach, 3 jars were covered in foil as dark treatments, 3 jars were left uncovered and 1 jar was immediately injected with buffered formalin to a final concentration of 4% as a killed control. Using a 50 μ L syringe, we injected each jar with 25 μ L of ^{14}C -labeled $\text{NaH}^{14}\text{CO}_3$ solution (45 $\mu\text{Ci/mL}$). We then placed the jars in a 10 - 12°C water bath upside-down (periphyton facing up) under grow lights (305-350 μE). After 1.5 h, we injected 25 μ L of ^3H -labeled L-leucine (75 $\mu\text{Ci/mL}$) into each jar and continued incubations for 30 min. We stopped incubations by injecting buffered formalin to a final concentration of 4%. Jars were stored at 4°C until processing.

We combined incubation water and scraped periphyton from each rock into a slurry and poured the slurry into 1-3 (depending on slurry volume) 50 mL centrifuge tubes. We centrifuged slurries for 45 minutes and decanted all but 5 or 10 mL of supernatant to avoid disturbing or removing settled sample at the bottom of the tube. Then, the contents of the centrifuge tubes for each individual rock slurry were combined into one tube and 10% trichloroacetic acid (TCA) was added to a final concentration of

5% (v/v) TCA. We vigorously shook the samples and placed the tubes on ice for 1 h. We then followed the methods in Taylor et al. (2012) exactly for isolating and measuring biological incorporation of radio-substrates and Wetzel and Likens (Wetzel and Likens 2000) for converting radioactivity to biological activity. Briefly, we filtered slurry onto a polycarbonate filter (0.2 μm pore size) and washed the filter with acid, ethanol and DI. Then we dissolved material attached to the filters in an alkaline solution for 1 h at 85 °C and measured an aliquot of this solution for radioactivity on a Beckman LS 6500 liquid scintillation counter (Beckman Coulter, Fullerton, California). We measured rock surface area using a foil mass-to-area relationship and rock volumes by weighing the volume of water that filled a jar containing the rock.

Ecosystem Metabolism

We measured ecosystem metabolism using the single-station method corrected for reaeration (K_{O_2}) using propane following Bott (2006). K_{O_2} was measured by bubbling propane at the top of the reaches and collecting 5 water samples each at the 10 m and 75 m locations after coincident NaCl additions had reached plateau. To estimate K_{O_2} for periods of metabolism in which we did not perform propane injections, we regressed measured K_{O_2} against Q for each reach, resulting in linear equations used to predict K_{O_2} based on Q measured on the first and final days of sonde deployment. Dissolved O_2 was logged at 20 min intervals using two calibrated YSI Exo1 sondes (YSI, Inc., Yellow Springs, OH) deployed at T75 and R75 such that full diel curves were generated for dosing days -4 to 0 (pre-dose), 9 to 13, 23 to 27 and 37 to 41. Gross primary production (GPP) was estimated by calculating the area under the corrected rate of oxygen change

curve above the mean nightly respiration rate, and daily ecosystem respiration (ER) was calculated by multiplying the mean hourly nighttime respiration rate by 24.

Data Analysis

Effects of the acetate addition on nutrient uptake were analyzed by propagating the 95% confidence intervals (CIs) of the slopes from the regression models with the calculations for V_f and comparing the CIs between the reference and treatment reaches on each date (Hanafi et al. 2007; Johnson et al. 2012; Oviedo-Vargas et al. 2013). We deemed V_f s to be significantly different if the CIs did not overlap.

Labile C effects on BBP and photosynthesis were analyzed using the R package *nlme* (version 3.1.1, (R Core Team 2015), Vienna, Austria) to produce a fully-nested, fixed-effect generalized least squares model (*gls* function). We modeled heteroscedasticity by reach using the *varIdent* function to create a weighting object that was used in the *gls* function. When appropriate, we examined post-hoc multiple comparisons using the *pairs* function within the R package *lsmeans* (version 3.1.1, R Core Team, Vienna, Austria). Any effects were considered significant at $\alpha=0.05$. Means are presented ± 1 standard error.

Results

Mean daily water temperature on sampling dates ranged from 5.83 °C to 8.65 °C. Discharge measured on sampling dates ranged from 13.9 L/s to 31.7 L/s during dosing, but exhibited a decline from 38.9 L/s on our first, pre-dosing collection date. Mean discharge during the dosing period (n=14) was 15.6 ± 1.9 L/s.

Water Chemistry

DOC concentrations during our study ranged from 1.85 mg/L to 4.96 mg/L, with higher concentrations during pre-dosing corresponding to higher discharge from spring freshet. During the dosing period, mean ambient DOC concentrations were relatively stable (R: 2.52 ± 0.184 mg/L, T0: 2.49 ± 0.182 mg/L; Table 2.1). Due to the relatively small fraction of dosed acetate-C in the total stream DOC, we deemed it too imprecise to calculate uptake velocities from aggregate declines in DOC; however, mean DOC concentration at T75 was less than the mean concentration at T10 on all but the first dosing date (Table 2.1).

Based on a constant dosing rate and discharge (i.e., variability solely a function of discharge measured during water sampling events and other dates when median velocity was calculated) throughout the 62 d of the study, dosing concentration was 0.254 ± 0.017 mg/L acetate-C, ~10% above ambient DOC.

Nutrient concentrations measured at R10 and T0 are given as background for a reach, as longitudinal declines through each reach are incorporated into uptake metrics (see *Net Nutrient Uptake*). Therefore, the variability in nutrient concentrations presented for each reach can be viewed as a function of temporal change (i.e., weekly). $\text{NH}_4\text{-N}$ concentrations were below detection limits for most of the study (days -14 to 28), and low when detectable (R10: 23.6 ± 0.79 $\mu\text{g/L}$; T0: 21.1 ± 0.54 $\mu\text{g/L}$; Table 2.2). $\text{NO}_x\text{-N}$ was the dominant component of DIN (R10: 374 ± 22.3 $\mu\text{g/L}$, T0: 369 ± 24.8 $\mu\text{g/L}$) and ranged from 185 $\mu\text{g/L}$ to 441 $\mu\text{g/L}$ (Table 2.2). $\text{PO}_4\text{-P}$ concentrations were relatively high and stable during our study (R10: 57.6 ± 2.47 $\mu\text{g/L}$, T0: 57.3 ± 2.47 $\mu\text{g/L}$; Table 2.2).

Net Nutrient Uptake

DIN V_f largely followed the same trend in both reaches, peaking on days 1 and 8, decreasing to day 28, and then relatively unchanging in magnitude through the rest of the study (Fig. 2.2). DIN V_f was undetectable in both reaches during the pre-dosing period. During the dosing period, DIN V_f was detectable (lower CIs greater than 0) in both reaches on each measurement day, except CI's in the reference reach overlapped 0 slightly on days 28 and 50 (both lower CIs $\sim 0.025 \text{ mm s}^{-1}$). DIN V_f in the treatment reach was significantly greater than R V_f on days 1, 8, 22, 28, 50 and 56 (Fig. 2.2). The greatest difference between reaches was on days 1 and 8, when treatment V_f reached 2.1 and 3.0 mm s^{-1} , respectively, compared to 1.31 and 1.17 mm s^{-1} on days 1 and 8, respectively, in the reference reach.

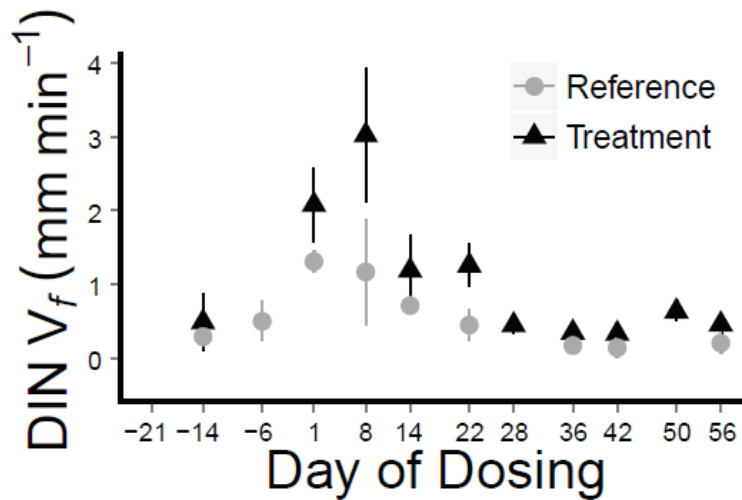


Figure 2.2. DIN uptake velocities (V_f , mm s^{-1}) calculated from longitudinal declines in DIN ($\text{NH}_4\text{-N} + \text{NO}_x\text{-N}$) in the reference reach (grey circles) and treatment reach (black triangles). Error bars are 95% confidence intervals propagated from the slopes of the regression models. DIN V_f was greater in the treatment reach relative to reference reach V_f on days 1, 8, 22, 28, 50 and 56. Any V_f that was not measurable (i.e., no significant longitudinal decline) was excluded from the figure.

PO₄-P V_f was non-significant (CIs overlapped 0) for both reaches on each day of measurement, regardless of dosing period, and CIs calculated for each reach always overlapped.

Bacterial Biomass Production (BBP) and Photosynthesis

Mean BBP (Fig. 2.3) ranged from $0.009 \pm 0.001 \mu\text{g C cm}^{-2} \text{ h}^{-1}$ to $0.010 \pm 0.002 \mu\text{g C cm}^{-2} \text{ h}^{-1}$ in the reference reach, and $0.015 \pm 0.003 \mu\text{g C cm}^{-2} \text{ h}^{-1}$ to $0.026 \pm 0.008 \mu\text{g C cm}^{-2} \text{ h}^{-1}$ in the treatment reach. The magnitude of BBP did not significantly differ across dates within either reach (Fig. 2.3). BBP was affected by the labile C addition ($F=6.49$, $P=0.002$). BBP was consistently higher in the treatment than the reference reach, and approximately 2.5x greater on days 16 and 56, reaching $0.026 \mu\text{g C cm}^{-2} \text{ h}^{-1}$ on both dates (Fig. 2.3). Although the P-value was close to alpha, light incubation (i.e., dark vs light) had no effect on BBP ($F=2.25$, $P=0.073$).

We observed no response to acetate addition in photosynthesis ($F=1.52$, $P=0.260$), although photosynthesis was ~ 30% higher in treatment than reference incubations on day 30 (Fig. 2.4). Photosynthesis was very low in both reaches on day 56 (R: $0.046 \pm 0.065 \mu\text{g C cm}^{-2} \text{ h}^{-1}$, T: $0.010 \pm 0.008 \mu\text{g C cm}^{-2} \text{ h}^{-1}$; Fig. 2.4).

Ecosystem Metabolism

Measured K_{O2} ranged from 0.0552 min^{-1} to 0.0896 min^{-1} . Because estimated K_{O2} was a function of Q and the average Q during each deployment was within the range of Q observed during propane evasions, estimated K_{O2} was always within the measured values. Across both reaches, ER was greatest during the first dosing period measurement (days 9

- 13) and steadily decreased as the study progressed. During pre-dosing, ER was always slightly greater in the treatment than the reference reach, with reach differences ranging

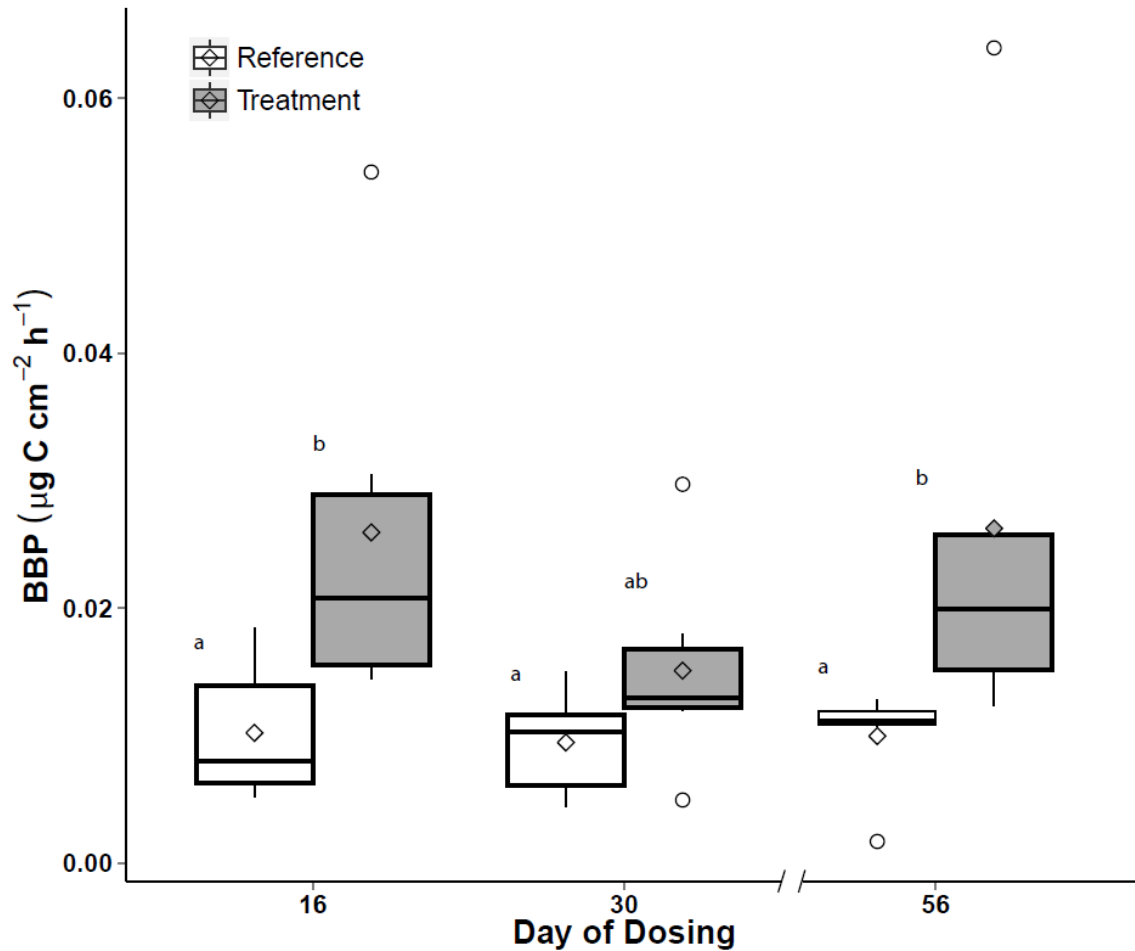


Figure 2.3. Bacterial biomass production (BBP) in reference (white) and treatment (grey) reaches standardized to surface area of the rock ($\mu\text{g C cm}^{-2} \text{ h}^{-1}$). Different letters indicate significantly different means ($P < 0.05$). Boxes represent the interquartile range, containing the median (black line) and mean (open diamond). 'Whiskers' extend to furthest data point within 1.5 times the interquartile range, while dots are outliers. $n=6$.

from $0.59 - 0.78 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1}$. Acetate addition increased ER in the treatment reach following onset of dosing (Fig. 2.5). ER was generally $>25\%$ higher ($>2 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1}$ difference) in the treatment than in the reference reach, except day 23 when it was only

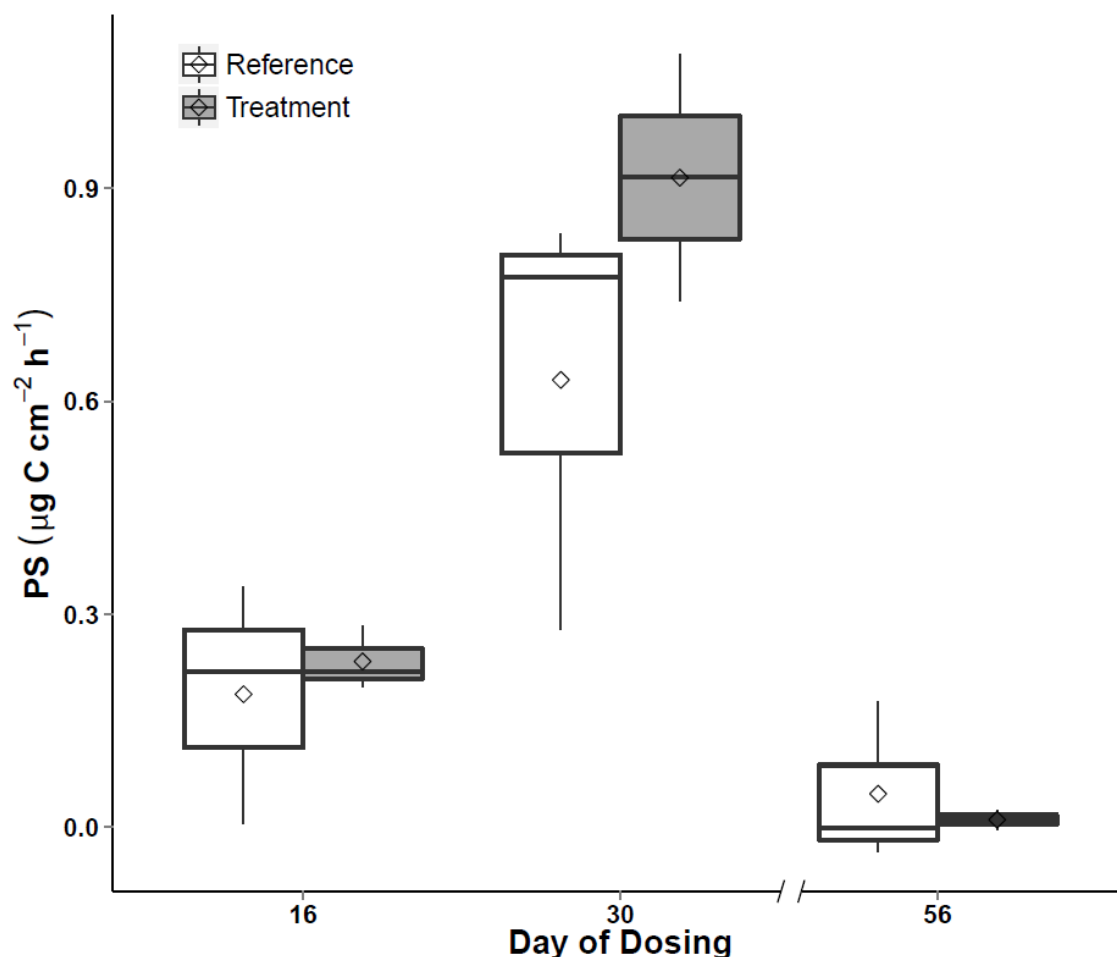


Figure 2.4. Photosynthesis in reference (white) and treatment (grey) reaches standardized to surface area of the rock ($\mu\text{g C cm}^{-2} \text{ h}^{-1}$). Boxes represent the interquartile range, containing the median (black line) and mean (open diamond). 'Whiskers' extend to furthest data point within 1.5 times the interquartile range, while dots are outliers. $n=3$.

11% greater, and reached a maximum of 52% greater on day 27, corresponding to a $\sim 4.6 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1}$ difference.

Gross primary production (GPP) in both reaches decreased throughout the dosing period, peaking on the first day (9) of the first dosing period measurements ($\sim 8.1 - 9.75 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1}$, compared to $< 1 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1}$ during the last measurement period). GPP showed a positive response to the carbon enrichment during the first post-dosing measurement days (days 9 - 11), reaching 16 - 20% higher rates (differences of 1.1 - 1.6 g

$\text{O}_2 \text{ m}^{-2} \text{ d}^{-1}$) compared to the reference reach, though the difference decreased to slightly negative responses on the last days of that period (days 13 and 14; Fig. 2.6) when it was raining (RSK, personal observation). A slight negative response was consistently observed during the second dosing period measurements (days 23 - 27), while there was no response during the final measurement period (day 37 - 41; Fig. 2.6).

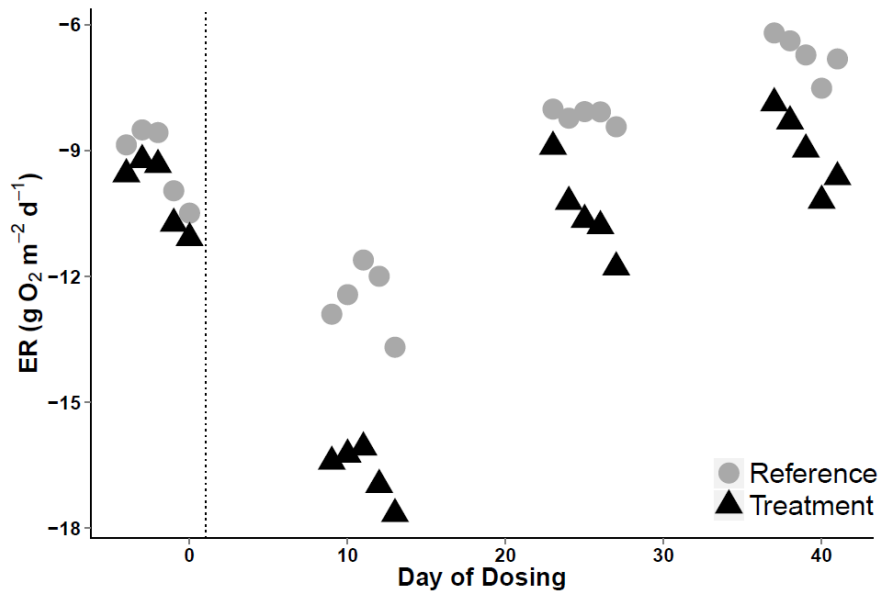


Figure 2.5. Ecosystem respiration (ER, $\text{g O}_2 \text{ m}^{-2} \text{ d}^{-1}$) in the dosed treatment reach (black triangles) and the reference reach (grey circles) on each date of ecosystem metabolism monitoring. Fine dashed vertical line is the date on which dosing commenced.

Discussion

Labile DOC enrichments have stimulated numerous basal processes in other experiments, but additions have generally been applied at high concentrations ($>1 \text{ mg/L}$) and/or for short time periods (on the order of days). In contrast, our enrichment concentration was based on actual measurements of wetland-derived labile DOC and was added throughout a boreal growing season. While the intensity of whole-stream manipulations hinders replication, the realism that comes with appropriate scaling is a

beneficial tradeoff that has been both advocated (Carpenter 1998; Schindler 1998; Oksanen 2001) and successfully implemented (Bernhardt and Likens 2002; Cross et al. 2006; Johnson et al. 2012; Zwart et al. 2016) to further understanding of ecosystem function and accurately guide management decisions. Regardless, strong inferences from studies lacking replication require multiple lines of evidence. Our study was limited to one stream with an upstream reference and downstream treatment reach, but diel oxygen changes, radioisotope-labelled substrate uptake and nutrient declines pointed to a real effect of our DOC addition on basal ecosystem processes. The results of our enrichment agree with the growing literature base that DOC plays a fundamental role in driving numerous basal ecosystem processes (Stanley et al. 2012).

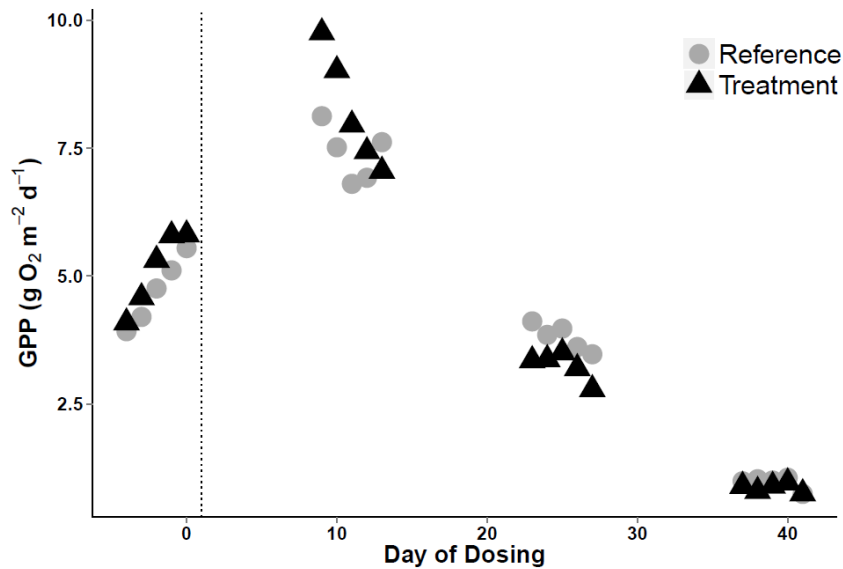


Figure 2.6. Gross primary production (GPP, $\text{g O}_2 \text{ m}^{-2} \text{ d}^{-1}$) in the dosed treatment reach (black triangles) and the upstream reference reach (grey circles) on each date of ecosystem metabolism monitoring. Fine dashed vertical line is the date on which dosing commenced.

DOC enrichment increased ER, generally eliciting at least 25% higher rates than reference respiration rates throughout the entire study, and approaching 52% on day 27.

Other C additions have shown significant increases in respiration. Bernhardt and Likens (2002) observed a 7x increase in CO₂ production when adding 5-7 mg/L of acetate-C to a stream. Johnson et al. (2012) elicited a 2x increase in ER with a 6 mg/L acetate addition. Oviedo-Vargas et al. (2013) dosed a stream with 1 mg/L of acetate and observed 4-23% increases in ER. At a maximum dosing concentration of ~0.30 mg/L acetate-C, our dosing concentration was between ~5% (Bernhardt and Likens 2002; Johnson et al. 2012) and ~30% (Oviedo-Vargas et al. 2013) of other dosed concentrations in studies that examined whole-stream respiration. Therefore, our manipulation elicited ER increases that were comparable to (Oviedo-Vargas et al. 2013), although we added acetate at a more environmentally realistic concentration than all of the previously mentioned studies that examined ER responses to labile C additions. Because the magnitude of ER did not necessarily correspond to labile DOC concentrations across these studies, more whole-stream experiments should be done to examine factors that predict how ER responds to changes in DOC availability.

While ER was stimulated by DOC enrichment throughout the study, BBP benefited from added acetate mostly when photosynthesis was relatively low. Bacteria existing on inorganic substrata (e.g., rocks) are largely dependent on water column C and nutrient availability in the absence of coexisting algae (e.g., in the dark or after scouring events that restrict algal biomass; (Romani et al. 2004; Olapade and Leff 2005). C exuded by algae is labile and can alleviate C-limitation of tightly associated (e.g., periphytic) microbial heterotrophs (Wyatt and Turetsky 2015), resulting in preferential utilization of autochthonous C over allochthonous C (Olapade and Leff 2005; Franke et al. 2013). Moreover, even though acetate is a generally labile form of DOC, algal exudates contain

nutrients, such as N, making the exudates sources of both C and N that can be used preferentially over strict DOC (Lutz et al. 2011; Ghosh and Leff 2013). Indeed, water-column DOC quality may be inconsequential to production of periphytic bacteria when associated algal biomass is sufficient to provide C resources within the biofilm (Kamjunke et al. 2015). Therefore, periphytic bacteria may have been utilizing algal exudates rather than DOC from the incubation water, resulting in no reach-level difference in BBP rates when photosynthesis was high. Increases in BBP suggest that bacteria were not only consuming acetate for energy (i.e., respiration), but were also growing, possibly serving as a resource for higher trophic levels.

Only negligible or slight negative effects of whole-stream DOC enrichments on GPP have been observed in previous studies (Johnson et al. 2012; Oviedo-Vargas et al. 2013). In contrast, our results show a possibly positive effect of DOC enrichment on GPP, at least when light appeared to be readily available. Chamber measurements of GPP on small cobbles (larger than the small gravel substrate used for radiolabeling presented here) suggested that the acetate addition may have facilitated increased GPP by maintaining algal biomass (Yeager et al., unpublished data). Several, non-mutually exclusive possible explanations exist for benefits of labile DOC on algae. First, many algae are capable to some degree of facultative heterotrophy, but this metabolic pathway is mostly used by algae existing in dark conditions (Tuchman et al. 2006). This process could have allowed algae to utilize more C at night, increasing total growth. In this case, increased GPP during the day would be reflective of increased algal biomass produced overnight. Second, respiration of added DOC would have increased would have increased CO₂, possibly benefiting algal photosynthesis (Hasler et al. 2016), especially if

heterotrophs utilizing acetate were tightly associated to algae in biofilms. Last, DOC enrichment could have spurred heterotrophic microbes to increase mineralization of organic nutrients, increasing nutrient availability for autotroph growth. We also observed some slightly negative GPP responses to acetate addition. Oviedo-Vargas et al. (2013) observed up to 30% decreases in GPP due to a ~ 1 mg/L addition of acetate and reasonably attributed that result to microbial competition for nutrients. We did not observe any effects of acetate on photosynthesis, but measurements were temporally separated from GPP measurements and photosynthesis was measured on easily scoured substrate (small gravel) that was probably not representative of the whole ecosystem. Overall, any effects of labile DOC on GPP are likely small, at least in comparison to effects on ER, but the combination of possible positive and negative effects observed in the present study warrants further research.

Even though acetate addition appeared to substantially increase ER, and appeared to have some influence on GPP, we caution the interpretation of these results due to the length of the reaches and the use of a single-station approach to measuring O_2 changes, as well as uncertainty in K_{O_2} measurement. The upstream O_2 footprint of the treatment reach (length = ~ 0.5 v/ K_s , $\sim 40\%$ measured O_2 turnover within reach; (Chapra and Di Toro 1991; Reichert et al. 2009) likely included the reference reach and, especially, the intermediate reach. Minimal reach differences in pre-dose ER and GPP, in addition to agreeing chamber metabolism measurements (Yeager et al., unpublished data), are evidence that our metabolism estimates were representative and useful for evaluating the influence of acetate addition, even if the rates are not explicitly limited to the designated reaches. Analysis using the range of possible predicted K_{O_2} throughout each period (i.e., K_{O_2}

based on Q at the beginning and end of sonde deployment) showed that while the magnitude of overall measurements could vary up to $\sim 2 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1}$, the pattern (relative differences) between reference and treatment reach values did not change, and it is unlikely that our observed changes in K could alter the conclusion that acetate addition significantly increased ER. Detecting whole-stream metabolic effects of labile C dosing could be better facilitated with a two-station approach and analysis that simultaneously estimates metabolic parameters and their uncertainty without propagating errors from gas evasion techniques (*see* Van de Bogert et al. 2007; Holtgrieve et al. 2010; Hotchkiss and Hall 2014).

Decreases in overall DIN uptake are likely attributable to decreases in GPP due to light limitation from a regenerating canopy of herbaceous vegetation, which became very dense toward the middle and end of the study (Fig. 2.1). Autotrophs often dominate inorganic nutrient uptake (Fellows et al. 2006), and this decrease through the dosing period cannot be attributed to temperature, because temperatures were lowest from days 6 to 13 and then higher and relatively stable through the rest of the study. The positive response of GPP to acetate addition early in the study may then suggest that the greatest increases in N uptake are also partly due to an autotrophic response: the greatest reach differences in N uptake occurred near when GPP was highest. However, slightly negative GPP responses were observed directly after we measured a positive DIN uptake response (day 22). Elevated DIN uptake with increased C availability has been noted by several other studies (e.g., Bernhardt and Likens 2002; Johnson et al. 2009; Johnson et al. 2012), but has been attributed to heterotrophic demand. C addition probably spurred microbial heterotrophs to utilize at least some inorganic N from the water column, as evidenced by

the positive DIN uptake in the treatment reach when GPP was particularly low in both reaches. Because net nutrient uptake measurements were temporally separated from whole-stream metabolism measurements, it would be difficult for us to positively attribute increases or decreases in DIN uptake to changes in GPP.

The limited response of DIN uptake to acetate enrichment later in the study may suggest that the C addition did not alleviate heterotrophic C demand (relative to nutrient availability) and microbes were mineralizing organic N to preferentially utilize associated organic C (Lutz et al. 2011; Kirchman 2012; Ghosh and Leff 2013), releasing some inorganic N. Indeed, $\text{NH}_4\text{-N}$ was undetectable until day 36 when reach differences in N uptake became particularly low, which may have allowed algal uptake of NH_4 to slow enough for heterotrophic release of NH_4 within the whole stream to exceed uptake. As labile C from relatively recalcitrant sources such as leaf litter are used up, subsidized labile C may become more important to aid heterotrophs in breakdown of leaf litter, a process that may cause leaching of more inorganic N.

We did not observe any significant $\text{PO}_4\text{-P}$ uptake, which could be due to a few factors. Phosphate buffering, the process of abiotic sorption to and release from particles, can maintain steady water column $\text{PO}_4\text{-P}$ concentrations (Froelich 1988; Lottig and Stanley 2007). Additionally, heterotrophic P demand could have been satisfied by sediment-bound P (Oviedo-Vargas et al. 2013). Our method for measuring nutrient uptake did not distinguish between uptake and remineralization processes; therefore, any biological uptake could have been masked by release processes.

Our acetate addition was meant to simulate the highly labile, bioavailable fraction of wetland-derived DOC, but natural DOC is a highly complex, heterogeneous pool of

many different organic molecules lying on a wide spectrum of lability and C:Nutrient ratios. Natural DOC sources are likely to be utilized differently than simple organic C compounds since nutrient availability can interact with DOM characteristics in affecting DOM mineralization (Franke et al. 2013). Nevertheless, we elicited microbial responses that point to high demand for and utilization of DOC. Despite being a highly heterotrophic stream prior to dosing, our low-level acetate addition substantially increased ER. The majority of ER was probably derived from hyporheic (Mulholland et al. 2001) or organic substrates such as leaf litter (e.g., *Calamagrostis canadensis*, Shafiel et al. 2011). Indeed, labile C can be limiting to mineralization rates of relatively recalcitrant leaf litter, especially when nutrients are highly available (Ardon and Pringle 2007; Pastor et al. 2014). Additionally, DOM is often viewed as inhibitory to photosynthesis, because some DOM is colored and attenuates light, limiting algal growth (but see Frost et al. 2007). Our research may indicate that DOC can have a positive or negative effect on primary production on a whole-ecosystem scale. More studies are needed to examine how different DOM sources interacting with nutrient availability may affect all microbial processes, but particularly primary production, directly or indirectly.

The response of ER to relatively low levels of labile C inputs may have only been possible due to high DIN deriving from alder, as nutrient availability can strongly influence organic matter mineralization (Franke et al. 2013). Thus, our study suggests that, through coupled metabolism and nutrient uptake (Fellows et al. 2006; Schlesinger et al. 2011), landscape features that disproportionately influence water chemistry can also be ‘coupled’. Alder-derived N and wetland-derived labile C may complementarily drive ecosystem function synergistically or temporally separated. Increased rates of

biogeochemical cycling, microbial activity and biomass accrual could arise where flow paths converge (McClain et al. 2003). Altering wetland subsidies of C to streams could change potential utilization of alder-derived N within streams, and *vice versa*.

Alternatively, wetland-derived C and alder-derived N could more individually drive ecosystem function at different times of the year. Burrows et al. (2017) found that C limitation in boreal streams was much greater during the winter, and observed some nutrient (N or P) limitation only during the summer, indicating relative demands of C:Nutrients shifted seasonally. Burrows et al. (2017) also found that this C limitation was consistent with landscape-flowpath dominated delivery and bioavailability in boreal streams (Laudon et al. 2011). This suggests that complementary landscape features may stabilize ecosystem function throughout the year. Alder cover is already known to be a determining factor of litter breakdown on the Kenai Peninsula (Shaftel et al. 2011).

Wetlands are well recognized for their roles in headwater stream functioning (Meyer et al. 2003; Stanley et al. 2012) and have been shown to support food webs that maintain juvenile salmonids in this region (Dekar et al. 2012). Thus, landscape elements such as wetlands and alder, as well as other catchment features that exert high influence on basal resources, should be viewed as having a high potential to control ecosystem processes in a possibly complementary interaction.

CHAPTER THREE

Dramatic Consumer Response to Low-Level Additions of Dissolved Organic Carbon: A Whole-Stream Ecosystem Experiment

Introduction

Ecologists have long recognized that stream ecosystems use and transport significant quantities of terrestrial organic matter, the dominant form typically being dissolved organic carbon (DOC; (Warren et al. 1964; Fisher and Likens 1973; Dahm 1981). In comparison to lakes, relatively little work has been conducted at relevant temporal and spatial scales to determine the role terrestrial DOC plays in stream food webs. Much focus has instead been placed on stream ecosystem dependencies on inputs of the particulate fraction of this organic matter, such as leaf litter, which can indeed be a particularly significant resource (Wallace et al. 1999). Instead of being a key resource fueling ecosystem function, DOC is often relegated to the role of a “modulator” of ecosystem properties because it alters conditions such as light and pH for processes including primary production (Prairie 2008; Stanley et al. 2012). In contrast, nutrients (e.g., nitrogen and phosphorus) are viewed as the “volume knob” directly controlling the magnitude of ecosystem processes and dominating how biotic ecosystem processes operate (Prairie 2008). Part of this view stems from a large percentage of the terrestrial DOC pool being resistant to biological degradation on short timescales (Wiegner et al. 2005; Koehler et al. 2012). However, a considerable portion of the DOC pool is in fact labile and readily used by microbial osmotrophs, especially bacteria (Dahm 1981; Wiegner et al. 2005; Koehler et al. 2012).

Stream consumer energy and biomass are frequently supported by allochthonous DOC (Hall and Meyer 1998; Collins, Kohler, et al. 2016; Collins, Sparks, et al. 2016; Neres-Lima et al. 2017), but limited evidence exists to suggest that DOC is an important subsidy to stream consumers (i.e., increases production rather than just serves as a resource) such as macroinvertebrates or fish (Polis et al. 1997; Brett et al. 2017). Bacteria and fungi, the microbial users of DOC, are nutritionally poor in terms of essential lipids and may not strongly contribute to consumer growth, leading to a general consumer preference for high quality algal sources (Marcarelli et al. 2011; Collins et al. 2016; Guo et al. 2016). Allochthonous resources can subsidize secondary production, but likely only when autochthonous resources (i.e., algae) are adequately abundant (Tanentzap et al. 2014; Guo et al. 2016; Crenier et al. 2017). Additionally, DOC incorporation into food webs is subject to heavy respiratory losses, such as low bacterial growth efficiency and bacterivorous grazing, and a large proportion of labile DOC assimilated by bacteria is likely to never be available for metazoan consumers (Del Giorgio and Cole 1998; Hall et al. 2000; Berglund et al. 2007; Lischke et al. 2017). Allochthonous DOC may contribute little to stream production.

On the other hand, terrestrial DOC-consuming bacteria can be “upgraded” by bacterivorous protists, better supporting growth of higher trophic levels (Wiegner et al. 2015; Hiltunen et al. 2017), although this increase in quality incurs respiratory losses (Anderson et al. 2017). Additionally, benthic primary production in all but the most closed canopy streams may be adequate to satisfy macroinvertebrate demands for autochthonous energy (Neres-Lima et al. 2017), allowing allochthonous inputs to supplement algal portions of diets. Whether DOC subsidizes metazoan consumers may

depend on the quantity and quality of DOC available to microbes (Faithfull et al. 2011; Hitchcock et al. 2016; Hiltunen et al. 2017), but spatially and temporally appropriate experiments are needed to provide evidence that environmentally relevant concentrations of DOC can subsidize stream metazoans.

Labile DOC additions have been used to examine the role of DOC in streams at spatial scales representative of whole-stream ecosystem responses. Past continuous enrichments lasting longer than one day consistently spurred respiration and nitrogen demand (Bernhardt and Likens 2002; Johnson et al. 2012; Oviedo-Vargas et al. 2013), and have even increased macroinvertebrate and fish abundances and production (Warren et al. 1964; Wilcox et al. 2005). While informative, these additions have been extreme in terms of dosing concentrations. Labile DOC is generally not more than 10% of the total stream DOC pool (Kaplan and Newbold 2003; Berggren et al. 2010; McLaughlin and Kaplan 2013). Yet, past additions have raised DOC concentrations by >50%, at dosing concentrations up to 20 mg C/L (e.g., Wilcox et al. 2005). These concentrations more reflect labile DOC inputs from wastewater spills rather than concentrations typically observed across gradients of, e.g., natural or anthropogenic land cover (Stanley et al. 2012), as evidenced by frequently observed blooms of the “sewage bacterium” *Sphaerotilus* spp. (Warren et al. 1964; Bernhardt and Likens 2002; Johnson et al. 2012). Similarly, Fuller et al. (2004) reported that their labile DOC enrichment of ~2 mg C/L (~50% increase above ambient) may have been detrimental to some macroinvertebrate taxa due to gill fouling by bacteria.

Extreme labile DOC enrichments also ensure consumer subsidization by unrealistically overpowering energetic inefficiencies inherent to microbial pathways.

They may load ecosystems toward complete bacterial dominance, detrimentally altering conditions or drowning out possible microbial interactions that may occur from more relevant concentrations. For example, algal responses to increased labile DOC availability are often negative, likely because bacteria are excellent competitors for nutrients when they are not dependent on algal C (Blomqvist et al. 2001; Bechtold et al. 2012). Responses to extreme enrichments may therefore not be indicative of how streams respond to increases in DOC (except in extreme cases), and may oversell how streams depend on allochthonous labile DOC. A whole-stream experiment using environmentally relevant concentrations is necessary to answer questions about the role of labile DOC in stream ecosystems and how streams might respond to environmental changes such as browning or land use change (Carpenter 1996; Carpenter 1998; Monteith et al. 2007; Stanley et al. 2012; Solomon et al. 2015; Weyhenmeyer et al. 2016).

Relevant scale in whole ecosystem experiments requires a tradeoff with replication (Carpenter 1998; Schindler 1998). Thus, scientific rigor in such experiments is often achieved through avenues other than true replication. Unreplicated, whole ecosystem experiments can lead to strong inferences but only when several conditions are met. First, pre-manipulation measurements of responses in a continuously monitored reference and experimental units are comparable. Second, responses are ecologically meaningful in magnitude. Third, the direction of response is consistent with *a priori* expectations. Last, gradient responses are strong evidence of manipulation where there is an expectation of recovery or depletion of effect away from the source of the manipulation (Barley and Meeuwig 2017). When these conditions are met, unreplicated, whole ecosystem experiments are strong tools for inferring the roles of myriad

environmental factors or perturbations at relatively large scales. We added ecologically relevant concentrations of a highly labile form of DOC to a boreal headwater stream to estimate how stream consumers depend on and respond to allochthonous DOC. Because this same labile DOC addition subsidized stream microbes, increasing respiration, biofilm production, and whole-stream N demand (Table 3.1; Robbins et al. 2017), we expected a “trophic upsurge” of energy to invertebrate and fish consumers (Table 3.1; Tanentzap et al. 2014). A trophic upsurge is a bottom-up food web effect, where additional inputs of

Table 3.1. Basal ecosystem responses (Dissolved inorganic N (DIN) uptake, bacterial biomass production (BBP) and photosynthesis (PS) of epilithic periphyton using radiolabeling, and open reach ecosystem respiration (ER) and gross primary production (GPP) measurements) quantified in Robbins et al (2017) during this labile DOC addition, and hypothesized responses for measurements in this study. Labile DOC effects in other whole-stream enrichments occurred at much higher dosing concentrations.

Basal Ecosystem Component	Response to this DOC addition (Robbins et al. 2017) or hypothesized response (this paper)	Responses to other whole-stream labile DOC additions
DIN uptake	Positive	Positive effects; (Bernhardt and Likens 2002; Johnson et al. 2009; Johnson et al. 2012; Blaen et al. 2014)
Periphyton BBP	Positive	NA
Periphyton PS	No effect	NA
ER	Positive	Positive effects; (Bernhardt and Likens 2002; Johnson et al. 2012; Oviedo-Vargas et al. 2013)
GPP	Positive and Negative	Negative or no effect; (Johnson et al. 2012; Oviedo-Vargas et al. 2013)
Benthic invertebrate densities	Hypothesized: Positive	Positive and negative; (Warren et al. 1964; Fuller et al. 2004; Wilcox et al. 2005)
Fish densities	Hypothesized: Positive	Positive; (Warren et al. 1964)
Fish growth	Hypothesized: Positive	Positive; (Warren et al. 1964)

allochthonous carbon increase heterotrophic carbon mobilization and production at the base of the food web (e.g., bacteria), resulting in greater food availability for higher

trophic levels (Tanentzap et al. 2014). A trophic upsurge is therefore subsidization of a food chain (Polis et al. 1997). We hypothesized that added labile DOC would increase densities of fast-growing, multivoltine benthic invertebrate taxa (e.g., chironomids). We also hypothesized that fish densities and growth would increase due to higher benthic invertebrate densities. Because of inefficiencies of DOC-to-consumer trophic pathways, and the low dosing concentration employed (see *Methods*), we expected low effects of DOC on higher trophic levels, but we explicitly did not expect negative effects on consumers.

Methods

Site Information

We conducted our experiment on the western Kenai Peninsula in a headwater tributary of the South Fork Anchor River, previously identified as SANC 1203 (King et al. 2012; Shaftel et al. 2012; Walker et al. 2012); 59.77974° N, 151.55518° W; Fig. 3.1 A&B) during summer 2013. We selected SANC 1203 for this experiment for several reasons: (1) well-characterized catchment and water chemistry from previous studies, including wetland (32 %, largely discharge slope wetlands that export limited quantities of DOC; (Shaftel et al. 2012; Walker et al. 2012; Gracz 2013 Dec) and N₂-fixing alder cover (12.6%; (Shaftel et al. 2011; Shaftel et al. 2012); (2) elevated NO₃-N (~200-500 µg/L) directly related to the alder and (3) relatively low levels of ambient DOC (1.8 – 3.3 mg/L at baseflow) when compared to peat-rich catchments nearby (10 – 20 mg/L, (Walker et al. 2012); (4) similar channel width (1.4 m), depth (0.12 m), substrate (gravel-cobble, woody debris), riparian vegetation (Bluejoint grass: *Calamagrostis canadensis*)

and gradient (5.3%) over a distance of sufficient length to delineate reaches that would be comparable prior to any experimental manipulation; (5) representative of headwater streams throughout the Kenai lowlands and other boreal regions; and (6) moderate to high densities of juvenile Dolly Varden (*Salvelinus malma*, Fig. 3.1C), the most widespread and abundant salmonid in headwater streams in this region (King et al. 2012).

Experimental Design

We used upstream reference and downstream treatment reaches to assess the effect of labile DOC on. We chose a reach length of 75 m to include sufficient length to represent reach heterogeneity (e.g., multiple riffle-pool sequences), but which also approximated the length over which the labile DOC (acetate) addition might be removed based on median acetate uptake velocities from a whole-stream DOC uptake synthesis (Mineau et al. 2016). The paired experimental reaches were identified based on similarity in width, depth, slope, sinuosity, dominant substrate, riparian topography and vegetation, and water chemistry (Table S1), resulting in an 80 m intermediate reach that was not part of the study. Sampling locations within each reach were designated by meters from the top of the reach.

DOC was dosed as a solution of sodium acetate ($C_2H_3NaO_2$) from June 25th, 2013 (dosing day 1) through August 25th, 2013 (day 62). Acetic acid (dissociated acetate in H_2O) is a product of anaerobic wetland processes and so represents a common DOC substrate for microbes in boreal streams (Berggren et al 2010). We used a model QBG pump (Fluid Metering Inc., Syosset, New York, USA) to deliver dosing stock from a



Figure 3.1. Photos of the study stream, SANC 1203. A) The upper portion of the treatment reach pre-labile DOC dosing in early June; B) Labile DOC dosing station, with protective tarp removed from stock container, at the top of the treatment reach in early August, showing extensive vegetation growth; C) large Dolly Varden (*Salvelinus malma*) parr (age 1+) captured while electrofishing.

100-L covered stock tank to the top of the treatment reach (Fig. 3.1B). Acetate was dosed at a rate of 3.6 mL/min, resulting in a 62-d mean concentration of 250 µg/L acetate-C.

We chose this acetate dosing concentration to mimic labile DOC concentrations found in peatland streams of the western Kenai (~1-1.5 mg/L labile DOC out of 8-13 mg/L total DOC, RDD *unpublished data*). Acetate is part of the most bioavailable fraction of the labile DOC pool, but 250 µg/L is representative of acetic acid-C concentrations measured in other boreal streams (Berggren et al. 2010). This concentration also represented about 10% of background DOC in the study stream (mean 62-d DOC, reference reach = 2.52 mg/L).

Weekly estimates of acetate-C based on discharge fluctuation and confirmed dosing rates were 142 to 324 µg/L (median= 264 µg/L, mean=250 µg/L). Concentrations fluctuated proportionately with discharge, which ranged from 13.9 L/s to 31.7 L/s during the dosing period. We did not attempt to maintain a constant dosing concentration of acetate-C because DOC naturally fluctuates with discharge, typically being diluted by surface-water runoff in these headwater streams (RSK, unpublished data).

Benthic Invertebrate Sampling

We sampled benthic invertebrate assemblages by placing five Hester-Dendy (HD) artificial substrate samplers (0.16 m² sampling area; Wildco, Buffalo, New York, USA) at sampling locations 5-10 m, 35-40 m, and 70-75 m downstream from the top of each reach (15 samplers per reach). We placed samplers at 5-10 m, rather than 0-5 m, to ensure adequate solute mixing had occurred before water reached the samplers. HDs allowed us to estimate invertebrate response to DOC additions on a standardized substrate similar to the woody debris and submerged riparian roots found throughout both

stream reaches without disturbing the benthos. We considered alternative quantitative methods (e.g., Surber, Hess samplers), but we deemed them excessively disruptive to other key benthic measurements given the relatively small size of the stream and the fact that we already had disrupted the substrate on day -17 during backpack electrofishing (see next).

We secured HDs to cobbles with zip-ties longitudinally (~1 m apart) through the thalweg of each sampling location. We deployed HDs on day 29 and collected on day 56. We did not attempt to deploy samplers pre-dosing because 1) we had disturbed substrate by wading in the stream during electrofishing and 2) there was not sufficient time between spring runoff and the first day of dosing for colonization of HDs, as 28 d is the standard deployment time for HDs (King and Richardson 2008). We only deployed samplers for the last 28 d of the dosing period because we hypothesized that if any effects on macroinvertebrates would be evident, it would be during the latter half of the study because generation times for even the fastest of taxa in streams with ambient temperatures between 5 and 10° C, as found in our study stream, would make earlier deployments of limited additional value. HD samples were collected by gently lifting the HD off the stream bottom and simultaneously placing a 250 µm mesh sieve under the submerged sampler prior to removal from the water. HDs were then put into a large storage bag and immediately stored on ice. Upon return to the laboratory, invertebrates were removed from disassembled HD samplers with a toothbrush while rinsing with tap water in a 250 µm sieve and stored in 5% buffered formalin (v/v) for later identification. Individual invertebrates retained in the HD sample were counted and identified to the lowest practical taxonomic unit, typically genus. Chironomidae (non-biting midges),

Limnephilidae and Naididae (small Oligochaeta) were identified at the family level, whereas Amphipoda, Hydrachnidia (water mites), Nemata (nematodes), Ostracoda (seed shrimp) and Turbellaria (flatworms) were identified at these higher levels of taxonomic classification.

Salmonid Sampling

We sampled juvenile Dolly Varden (Salmonidae: *Salvelinus malma*) by three-pass electrofishing with a Smith-Root LR-24 (Smith-Root, Inc., Vancouver, WA, USA) in three subsections of each reach (0 - 25 m, 25 - 50 m, 50 - 75 m) on dosing days -17 and 62. Each subsection was separated at the top and bottom with a block net (4 mm mesh) that was secured tightly to the benthos and stream bank with stakes, and weighted down with large cobbles to eliminate any gaps. We checked block nets for trapped fish at the end of each of the 3 passes. Captured fish were placed in an aerated bucket, anesthetized with 70 mg/L tricaine methane sulfonate (MS-222), measured for fork length (FL) and mass (g), and released. We expressed fish abundance as total observed counts and not as densities (no./m²) because reaches were identical in length and very similar in mean width (reference: 1.49 ± 0.26 m; treatment: 1.30 ± 0.30 m). Further, we did not use depletion models to extrapolate observed counts per pass to total fish counts because these models are biased and unreliable (Rosenberger and Dunham 2005).

Parr (year 1+ or older) captured on day -17 were tagged with 8.4 mm passive integrated transponder (PIT) tags (Biomark MiniHPT8, Boise, ID, USA; (Bailey et al. 1998; Chittenden et al. 2008). We tagged 39 parr from each reach (78 total), recapturing 39 of the total tagged parr (see Results). PIT tags were used for mark-recapture estimation of growth rate.

Data Analysis

We tested for treatment and gradient effects on counts of total invertebrates and the top four taxa (representing 95% of the total abundance) by fitting generalized linear models (GLMs) with counts on each HD being predicted by an interaction between reach and distance from the top of the reach. We hypothesized that there would be no effect of distance in the reference reach whereas, if added DOC had any effect on abundance, we might also observe the highest values at 5-10 m and a decline in abundance down the length of the treatment reach (evidenced by a significant ($P < 0.05$) Reach*Distance interaction). Such a gradient response is considered strong evidence for attributing effects of experimental manipulations in unreplicated experiments (Barley and Meeuwig 2017). Based on the distribution of taxon counts, overdispersion, residual deviance and AIC for each model (compared to simpler models), we specified GLMs with negative binomial error families and log link functions (Zuur et al. 2009). We chose not to employ multivariate analyses (e.g., mvabund; (Wang et al. 2012)) because we were most interested in detecting increases in abundance of dominant taxa that might fuel growth and abundance of Dolly Varden rather than changes in species composition, which are confounded by abundance in multivariate analyses. Hester-Dendy samples were used as replicates within the 5 m, 37 m and 75 m locations in each reach. Invertebrate GLMs were fit using the glm.nb function in the MASS package (Venables and Ripley 2013) in R (version 3.4.3, R Core Team, Vienna, Austria).

We compared Dolly Varden fry (age 0-1) and parr (age 1+) qualitatively using size-frequency histograms, stratified by age class, between reaches. We lacked any reasonable form of within-reach replication to perform statistical tests, but we argue that

relative differences pre-dosing (day -17), when fry were just starting to emerge (see Results), clearly showed that both reaches hosted nearly identical patterns of abundance by age class. Further, neither reach had detectable gradient patterns in the distribution of juvenile Dolly Varden prior to dosing. Thus, large absolute differences between reaches on day 62, the end of the experiment, should be compelling evidence for a DOC treatment effect. Further, a decline in abundance in the treatment reach with distance from the dosing station would also provide additional support for a DOC treatment effect.

Instantaneous growth rates (IGR) were used to quantify the rate of change of mass for each Dolly Varden that was recaptured in the same reach it was initially tagged prior to dosing. Only one recaptured individual migrated between reaches (reference to treatment). IGRs were calculated as $[\ln(W_f) - \ln(W_i)]/t$, where W_f is weight (g) at recapture (Hopkins 1992), W_i is weight (g) at initial capture and t is growth period (d). Because we expected IGR to be greater for smaller individuals and individual responses could respond could be size-dependent (e.g., gape can influence prey size), we used $\ln(W_i)$ as a covariate in ANCOVA (IGR predicted by Reach* $\ln(W_i)$; lm function in R) to control for differences in initial weight (Elliott 1975; Hopkins 1992). We interpreted a significant Reach factor and/or Reach* $\ln(W_i)$ interaction as evidence for differences in salmonid growth between reference and treatment reaches.

Results

Invertebrate Response

Total invertebrate counts per HD sampler at the upstream (T5) locations of the treatment reach contained ~ 9x as many individuals as any of the three reference reach

locations (R5, R37.5, R75), with the effect of DOC addition declining away from the dosing station (gradient response; Reach*Distance $P=0.013$, Fig. 3.2). We individually modelled the taxa comprising 95% of benthic invertebrate abundance. *Baetis* spp. (Ephemeroptera: Baetidae; a small, multivoltine mayfly nymph) was the most abundant taxa, accounting for 52% of benthic invertebrates, while Chironomidae larvae (Diptera; non-biting midges) were 33% of benthic invertebrates (Fig. 3.2). Naididae (Oligochaeta, freshwater worm) and *Zapada* nymphs (Plecoptera: Nemouridae; a uni- or semi-voltine stonefly) comprised 6% and 4% of the total benthic invertebrates, respectively. *Baetis* nymphs were 10x more abundant in the treatment reach than the reference reach (Table 3.2, Reach effect $P<0.001$), and Chironomidae larvae were ~4x more abundant in the treatment reach (Table 3.2, Reach effect $P<0.001$). Chironomidae and *Baetis* abundances declined downstream through the treatment reach, although the Reach*Distance interaction was significant only for Chironomidae ($P=0.002$; *Baetis* Reach*Distance $P=0.099$; Table 3.2). Naididae and *Zapada* trended toward greater abundance in the treatment reach (Table 3.2). Naididae exhibited a marginal gradient effect (Reach*Distance interaction $P<0.10$, Table 3.2, Table 3.3) and *Zapada* nymphs were marginally more abundant in the treatment reach than the reference reach (Reach effect $P<0.10$, Table 3.2, Table 3.3). Other identified taxa were generally more abundant in the treatment reach, and no identified taxon had considerably lower abundances in the treatment reach compared to the reference reach (Table 3.3).

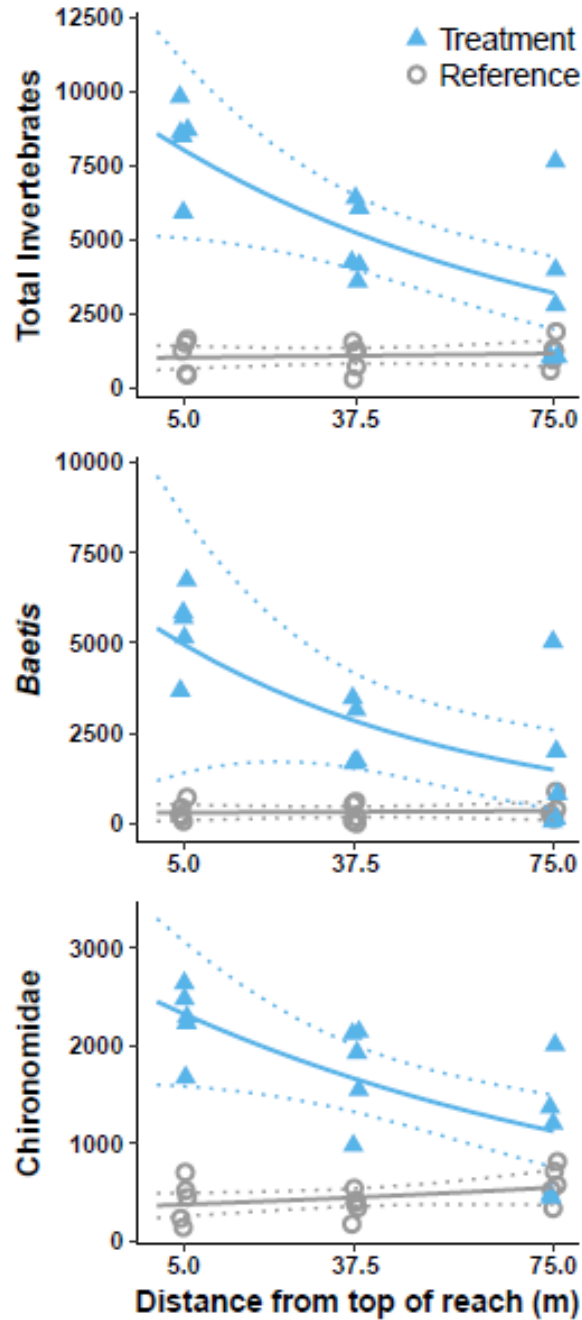


Figure 3.2. Counts per sample for total benthic invertebrates, *Baetis* spp. and Chironomidae (two taxa accounting for 85% of total benthic invertebrates counted, Table 3.2) from Hester-Dendy samplers from the reference (grey circles) or treatment (blue triangles) reach. Samplers were incubated in the stream reaches from days 29 - 56. Samplers were deployed at ~1 m intervals at points 5, 37.5 and 75 m downstream from the top of each reach – each sampler was treated as a replicate from those locations, so points are jittered on x-axis. Solid lines are predicted fits for negative binomial regressions and dotted lines are 95% confidence intervals for the predicted fits.

Table 3.2. Summary of all four modelled taxa, accounting for 95% of individuals counted, and total benthic invertebrate responses. Total Invertebrates includes all individual macroinvertebrates counted, not just modelled taxa. Counts are sums of individuals from each taxon across all 15 Hester-Dendy samplers in the reference and treatment reach. P-values are from negative binomial generalized linear models predicting counts per Hester-Dendy sampler with Reach and Distance from the top of each reach. Distance alone was never a significant ($P \leq 0.05$) predictor. Significant Reach*Distance interactions were a decrease in abundance in the treatment reach with distance away from the acetate dosing station (Fig. 3.2, Table 3.3).

Taxon	Counts (Reference)	Counts (Treatment)	P (Reach)	P (Reach*Distance)	Deviance Explained (%)
<i>Baetis</i> spp.	4878	46863	<0.001	0.099	56.1
Chironomidae	6800	25475	<0.001	0.002	73.2
Naididae	1737	4420	0.360	0.078	35.0
<i>Zapada</i> spp.	1687	2425	0.088	0.386	14.3
Total Invertebrates	16553	82620	<0.001	0.013	74.3

Table 3.3. Counts for all benthic invertebrate taxa identified at each sampling location in reference (R) and treatment (T) reaches, 5, 37.5 and 75 m downstream from the top of each reach. Counts are totals from five replicate Hester-Dendy samplers at each sampling location.

Taxon	R 5	R 37.5	R 75	T 5	T 37.5	T 75
<i>Baetis</i> spp.	1636	1382	1860	27092	11725	8046
Chironomidae	2022	1839	2939	11301	8698	5476
Naididae	711	688	338	1116	1776	1528
<i>Zapada</i> spp.	548	620	519	1037	754	634
Oligochaeta	27	21	18	148	416	227
Ostracoda	53	114	23	160	416	65
<i>Cinygmula</i> spp.	113	129	99	134	169	103
Amphipoda	54	67	62	84	180	92
Acarina	40	59	23	83	62	69
Turbellaria	33	67	28	67	50	42
Nemata	9	14	24	64	96	77
Simuliidae	60	43	22	76	40	31
<i>Neoplasta</i> spp.	9	10	46	110	60	22
<i>Rhyacophila</i> spp.	25	25	13	17	18	19
<i>Ecclisomyia</i> spp.	6	14	0	8	14	75
<i>Isoperla</i> spp.	9	20	8	32	6	13
Limnephilidae	16	14	5	6	18	3
Chloroperlidae	1	0	0	3	8	4
Collembola	1	1	0	6	4	3
<i>Epeorus</i> spp.	0	1	0	12	0	0
Tardigrada	0	0	4	8	0	0
<i>Ameletus</i> spp.	0	4	0	2	0	5
Tipulidae	5	1	0	0	0	5
Copepoda	0	0	4	0	0	1
Perlodidae	0	0	3	0	0	0
Psychodidae	2	0	1	0	0	0

Fish Response

Dolly Varden abundance for both parr and fry age classes were highly similar between reaches pre-dosing (Fig. 3.3). There were 39 parr in each reach during pre-dosing, and 25 and 21 fry in the reference and treatment reaches, respectively (Table 3.3). On day 62, both reaches had considerably higher parr and fry abundances due to immigration and fry emergence through the growing season; however, both fry and parr abundances were 1.7x greater in the treatment reach compared to the reference reach (Fig. 3.3).

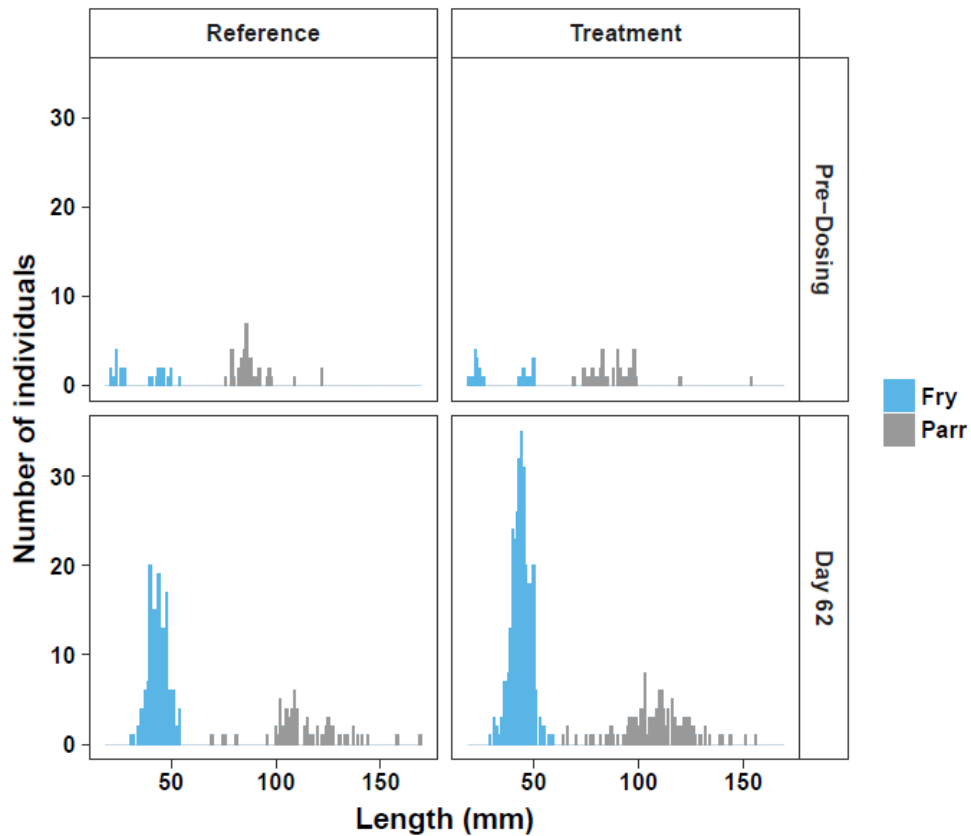


Figure 3.3. Dolly Varden (*Salvelinus malma*) size-frequency histograms for fry (age 0, blue) and parr (age 1+) age classes captured by electroshocking the reference reach and treatment reach on days -17 (pre-dosing) and 62 (post-dosing).

Total dolly varden abundances in the treatment reach also qualitatively followed a gradient response, with total Dolly Varden abundances declining from 174 fish in the top subreach (0 – 25 m) nearest the dosing station to 104 fish in the bottom subreach (50 – 75 m), with abundance in the reference subreaches ranging from 81 – 86 (Table 3.3). Subreach abundance trends were similar for both age classes (Table 3.3).

We recaptured 39 of the 78 Dolly Varden parr captured pre-dosing. One individual migrated (from the reference reach to the treatment reach) and so was excluded from growth analysis. We therefore calculated IGRs for 19 individuals in each reach.

After controlling for initial size, there was a highly significant reach effect on Dolly Varden growth (Fig. 3.4, Reach*log Initial Weight interaction $P=0.006$). Initially larger Dolly Varden parr were more likely to have higher growth rates in the treatment reach than in the reference reach, but initially smaller DV had similar IGRs by reach. IGR for Dolly Varden in the reference reach tended to decrease with initial size, whereas there was no significant decline in growth with initial size in the treatment reach.

Discussion

Our study suggests that environmentally relevant concentrations of labile DOC can dramatically subsidize stream consumers. DOC is inefficiently transferred to metazoan consumers, with much of it being respired (Faithfull et al. 2011; Hitchcock et al. 2016; Hiltunen et al. 2017). This suggests that low quantities of labile DOC may not appreciably subsidize food webs. Yet, we observed substantial increases in macroinvertebrate and fish consumers. Our results therefore suggest that very small

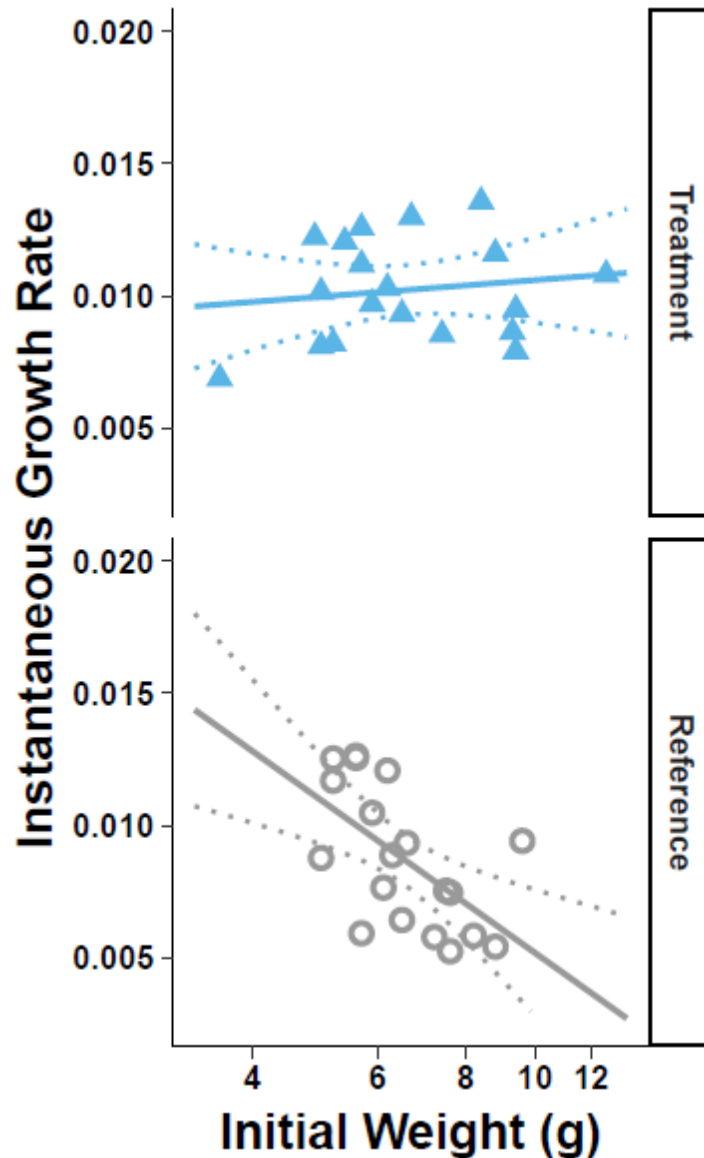


Figure 3.4. Instantaneous growth rates (IGR) of PIT-tagged Dolly Varden (*Salvelinus malma*) parr recaptured in the reference (grey circles) or treatment (blue triangles) reach. IGRs are regressed against Dolly Varden weights at initial capture (log-scaled x axis). Solid lines are predicted ANCOVA slopes and dotted lines are 95% confidence intervals for the predicted slopes.

quantities of labile DOC could supply an appreciable amount of energy for stream consumers, especially when N and P are in relatively high supply.

Benthic invertebrates were ~9x more abundant near the dosing station in comparison to any reference reach sampling locations. Bacterial C, derived from labile

DOC, can be a dominant C source for stream macroinvertebrate consumers (Hall and Meyer 1998; Collins, et al. 2016), and the few labile DOC addition studies looking at macroinvertebrate responses have showed that labile DOC can subsidize macroinvertebrate populations, albeit at very high dosing concentrations (Warren et al. 1964; Fuller et al. 2004; Wilcox et al. 2005). Large increases in *Baetis* spp. and Chironomidae densities in the treatment reach supported our hypothesis that small-bodied, multivoltine taxa would display the strongest responses to the C addition. Populations of multivoltine taxa in the treatment reach probably translated increased survivorship from a bolstered resource base to increased reproductive output, because the common uni- or semi-voltine taxon (*Zapada* spp.) did not significantly increase in response to labile DOC. Even so, abundances in the treatment reach trended greater for all taxa with modelled abundances, as well as most taxa identified, suggesting at least increased survivorship of benthic invertebrates was ubiquitous in the treatment reach.

Substantial increases in macroinvertebrate abundances led to a near doubling of Dolly Varden abundance nearest the dosing station. Greater juvenile Dolly Varden abundance was likely sustained by the substantial increases in *Baetis* and chironomids. Dolly Varden feed on both drifting and benthic individuals of these taxa (Nakano and Furukawa-Tanaka 1994; Nakano et al. 1999). One other study has looked at responses of juvenile salmonids to labile DOC addition in streams and found that production was nearly seven times greater in enriched reaches of an experimental stream (Warren et al. 1964). That study used screens to avoid movement of yearling trout stocked at low densities (compared to the densities found here), whereas our study reaches were unconstrained and fish had freedom to move throughout or between reaches. Fish

congregated closer to the DOC source, where invertebrate biomasses were highest, suggesting that the increased macroinvertebrate abundances sustained greater Dolly Varden biomass. This increased carrying capacity could be due to both increased migration and survivorship. Additionally, parr growth improved in the treatment reach, but only for initially larger individuals. Larger individuals may have better used the increased resource base, possibly outcompeting smaller individuals for space and food (Abbott et al. 1985). Similarly, larger individuals might have had larger mouth gape to cannibalize fry as an additional, highly nutritious food source.

The influence of allochthonous resources on consumer production is largely dependent on the availability of nutritionally-rich and consumer-preferred autochthonous resources (Marcarelli, Baxter, Mineau, and Hall 2011; Karlsson et al. 2012; Tanentzap et al. 2014; Guo et al. 2016). In contrast, bacteria lack numerous essential lipids and are a relatively poor resource on their own (Hiltunen et al. 2017). In our study, increased bacterial biomass from labile DOC enrichment likely supplemented a nutritionally adequate autochthonous resource base fueled by inorganic nutrients. Nitrogen availability was regionally high due to catchment scale N₂-fixing alder and probably not strongly limiting to autotrophic production (Hiatt et al. 2017). We hypothesize that the influence of labile DOC on consumer production could be controlled by nutrient limitation of algal production. However, algal production may be adequate to supply many consumer nutritional demands in all but the most closed canopy streams (Neres-Lima et al. 2017). Intriguingly, results from Robbins et al. (2017) suggest labile DOC could indirectly boost the nutritional value of periphyton by increasing primary production, which may only occur when inorganic nutrients are highly available (Bechtold et al. 2012). In this way,

DOC may not only fuel the brown food web (heterotrophic pathways), but also may spur green (autotrophic) pathways, blurring the lines between two often conceptually distinct trophic pathways. Future work should determine how nutrients, light and labile DOC may interact to influence microbial interactions that could determine the quantity and quality of consumer resources.

Our study suggests a combined role for landscape elements that source labile DOC and nutrients. In our study system, upland stands of alder are the dominant source of N to streams, and peatlands can provide significant quantities of DOC (both labile and recalcitrant, (Walker et al. 2012). N rich streams likely contain numerous C limited compartments, while use of labile C in wetland dominated systems may be limited by low nutrient availability (Burrows et al. 2017). Our study implies that the confluence of catchments with attributes that complementarily alleviate biogeochemical limitations may surge to consumer production (Robbins et al. 2017). Allochthony can be strongly tied to landscape characteristics (Smits et al. 2015; Tanentzap et al. 2017; Jonsson et al. 2018), but we suggest that holistically understanding stream ecosystems, from microbes to top consumers, requires consideration for any specific landscape elements providing significant basal resources (Laudon and Sponseller 2018).

Our study, using an environmentally relevant concentration of labile DOC, demonstrates that labile DOC can play an important role in aquatic food webs, particularly streams. Labile DOC can fuel microbial growth and respiration, in turn cycling N as C is used. Environmentally realistic concentrations of labile DOC can subsidize both autotrophic production and consumers, despite known inefficiencies in heterotrophic pathways. We contend that further understanding the role of DOC in whole

ecosystem functioning will require a greater understanding of microbial (primarily, algal-bacterial) interactions, as these interactions may be the ‘gate-keeper’ for how and whether labile DOC influences nutritionally important autotrophic biofilms (Scott et al. 2008; Kamjunke et al. 2015). We suggest allochthonous DOC ‘modulates the volume’ of aquatic ecosystems more positively than has been appreciated in lakes (Prairie 2008).

CHAPTER FOUR

Temporal Patterns of Stream Bioavailable Dissolved Organic Carbon Vary with Anthropogenic Sources

Introduction

Dissolved organic matter, usually expressed and measured as dissolved organic carbon (DOC), is the most dominant form of reduced C in stream ecosystems (Fisher and Likens 1973; Wetzel 2001). DOC notably influences a host of environmental variables, such as light availability, metal binding and pH, but its role as a microbial resource has increased with the recognition that freshwater systems are reactors, and not just passively transporting pipes, for terrestrial C, in particular as scientists aim to quantify controls and fluxes of carbon through aquatic systems (Cole et al. 2007; Prairie 2008; Raymond et al. 2013; Bodmer et al. 2016; Winterdahl et al. 2016). DOC is also transformed into microbial biomass, thus forming a nutritive base for food webs (Hall and Meyer 1998; Wilcox et al. 2005), and numerous biogeochemical reactions are fueled by DOC, including methanogenesis, denitrification and assimilative nitrogen uptake (Schlesinger and Bernhardt 2013). Identifying control points for these processes necessitates identifying spatial and temporal patterns of the DOC that drives them, but this additionally requires a recognition that DOC is more than a single compound.

Natural stream DOC is comprised of thousands of different types of molecules, ranging broadly in their size, hydrophobicity, aromaticity and other molecular characteristics (Findlay and Sinsabaugh 2003; Hockaday et al. 2009). Importantly, these compositional variations lead to a continuum of biological lability, where some

compounds are highly bioavailable and some are resistant to degradation (Findlay 2003). It is these labile or bioavailable DOC (BDOC) forms that, at least on short timescales, will generally fuel heterotrophic microbial processes (Wiegner et al. 2005; Kaplan et al. 2008). Thus, identifying patterns in the BDOC pool could be particularly important to identifying when, where and how rapidly many biogeochemical processes, including carbon and nutrient use, are occurring.

Stream BDOC derives from any DOC source, including wetlands, terrestrial root leachate, freshly fallen leaf litter, soil organic matter and algae (Kaplan and Bott 1982; Wiegner et al. 2005; Giesler et al. 2007; Ågren et al. 2008; McLaughlin and Kaplan 2013; Vonk et al. 2015). However, anthropogenic stream alterations and inputs frequently enhance DOC quality in streams, increasing BDOC or the proportion of BDOC in the total DOC pool (Stanley et al. 2012; Williams et al. 2016). Anthropogenic BDOC is typically fresher organic matter that has not had the opportunity to be strongly degraded, such as agricultural (e.g., poultry litter) or residential lawn fertilizers. For instance, row-crop agriculture, pasture cover and urbanized land cover in catchments typically lead to enhanced DOC degradability or proxies of lability in streams (Wilson and Xenopoulos 2009; Williams et al. 2010; Hosen et al. 2014; Parr et al. 2015; Shang et al. 2018). Identifying how anthropogenic sources of DOC influence patterns of stream BDOC may be important to understanding how humans are altering heterotrophic stream ecosystem processes.

Stream DOC quality and quantity are strongly tied to the terrestrial landscape by hydrologic connectivity (Aitkenhead et al. 1999; Ågren et al. 2008; Berggren et al. 2010; Laudon et al. 2011). Increased precipitation shifts flowpaths from deep, DOC poor

mineral soil layers to shallow, organic rich layers (Vidon et al. 2008; Lambert et al. 2013). During periods of high runoff, stream DOM characteristics more reflect a terrestrial signature and can include highly labile compounds (Berggren et al. 2010; Guarch-Ribot and Butturini 2016). This also implies that delivery of anthropogenic BDOC may be influenced by terrestrial-aquatic connectivity (Hu et al. 2016). Increased runoff, and thus shallower flowpaths, reduces terrestrial retention and degradation of potentially higher concentrations of fresh organic matter (Shang et al. 2018). Thus, patterns of stream BDOC in response to land cover may differ depending on the degree of hydrologic connectivity with the landscape.

Studies using optical indices to assess changes in DOM composition are common across land cover gradients. In contrast, very few studies have examined actual BDOC concentrations across land cover gradients and differing hydrologic scenarios. Optical indices are rapid and useful assessments of DOM composition which frequently correlate with BDOC (Cory and Kaplan 2012), but measuring BDOC through assays allows microbes to “do the talking.” In this study, we tested the hypothesis that anthropogenic land use (developed or pasture cover) would increase BDOC concentrations, and that this relationship would be stronger during high flow events (indicating increased contribution of runoff). Our study aims to further the understanding of spatial and temporal stream BDOC patterns by considering major anthropogenic sources of BDOC with changing hydrologic scenarios associated with a winter to spring transition.

Methods

Our study was conducted on 13 mid-order (3rd -to-5th) streams in the Ozark Highlands ecoregion of northwest Arkansas and northeast Oklahoma. Our study streams were not hydrologically connected (i.e., no site was down or upstream from another). Mid-order streams in this region are characterized by gravel/cobble substrate with often significant groundwater inflows resulting in very low turbidity (Leasure et al. 2016). Sites ranged from ~5 to 20 m wide with open canopies and are largely wadeable (Cook et al. 2018).

Anthropogenic land-use in the region is dominated by grazing pasture (Table 1). Developed land cover, such as roads and buildings, is the other major land-use class (Table 1), with significant urbanization occurring in northwest Arkansas. Row-crop agriculture is not significant in this region, so field tile drainage is uncommon. We chose study streams that drained a gradient of anthropogenic land cover, from more forested catchments to greater development or pasture cover. Five sites received upstream municipal WWTP effluent discharge (Table 4.1). Catchment area (km²) was estimated with a 30-m digital elevation model (USGS National Elevation Dataset; available online). We conducted catchment delineation using the watershed function in ArcGIS 10.1 (ESRI, Redlands, California, USA). Catchment areas were estimated using the cumulative areas of the 30-m pixels within each catchment boundary. Catchment land cover estimates were made using the National Land Cover Dataset (NLCD 2011).

We visited the study streams on four different sampling events in 2017, January 9-11, February 18-19, March 20-21 and April 23-24, to consider changes in seasonal weather conditions on bioavailable DOC. Early January through late April is a full winter to

spring transition in the Ozarks. The January sampling event was very cold, with some ice lingering in stagnant waters, and streams were largely at their lowest flows. Stream flow and temperature generally increased through February and March. April temperatures were relatively warm and most streams were measured at high discharge, post flood or near-flood. Thus, we sampled across a gradient of hydrologic connectivity (low discharge with large groundwater contributions to very high discharge dominated by overland flow).

At each site, we used YSI EXO1 (Yellow Springs, OH, USA) data sondes to measure pH, specific conductivity, dissolved oxygen and water temperature. Discharge at gaged sites was taken from either USGS or Army Corps of Engineers online data. At ungaged sites, we measured discharge by measuring flow velocity and depth at regular increments across the stream width using a Marsh-McBirney flow mate (Hach, Inc., Loveland, CO, USA) and top setting wade rod. At several sites (BALL, LSAL, SALI, SPRG3) during the April event, high flow precluded wading or caused gage malfunction, so we estimated discharge on these dates by extrapolating regressions of flows at similar nearby sites across a range of safely wadeable flows at the same sites during a previous study (Cook et al. 2018). The r^2 values for these regressions were all >0.7 , although we were necessarily extrapolating beyond the data range. These discharge values were not used as statistical predictors, but rather only used to approximate relative flows (discharge at a site divided by its lowest observed flow during our sampling). We interpret flows as indicating general hydrologic connectivity across sites during each event, similar to antecedent precipitation. Instantaneous discharge at a site would be not be a comparable measure of hydrologic connectivity to other sites due to varying

catchment characteristics influencing the timing and magnitude of water transport through the catchment. We would expect discharge to correlate poorly with DOC exports during low flow events due to internal processing, and during strong runoff events would be subject to hysteretic patterns beyond the scope of our study. Instead, we used relative discharge across sites to approximate hydrologic connectivity by event, so the seasonal transition was a temporal gradient of hydrologic scenarios.

Water samples were collected at each site to measure dissolved nutrient concentrations. Samples were filtered through a pre-rinsed 0.45 μm polypropylene luer-lock filter and syringe and stored on ice in the dark, and frozen within two days of collection. Nutrients ($\text{PO}_4\text{-P}$, $\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$) were analyzed following (APHA 1998).

Bioavailable carbon (BDOC) assays were conducted as a measurement of stream DOC lability following methods similar to (Servais et al. 1995) and (Vonk et al. 2015). We filtered 30 mL of stream water on-site through a syringe and luer-lock filter (0.7 μm GFF, Whatman) into six, pre-combusted 40 mL amber glass vials. Filtering immediately upon collection ensured capturing the “super labile” DOC fraction that could be metabolized within hours if samples were transported to the lab prior to starting assays (Pollard 2013). Three vials were acidified immediately with 60 μL 6N HCl ($\text{pH} < 2$) to measure initial DOC concentration. Within 2 days of collection, vials for initial DOC concentrations were placed in a fridge at 4° C, and the other vials for measuring DOC declines were loosely capped and placed in a dark incubator at 20° C. Incubating vials were vigorously shaken every 3-4 days to prevent anoxic conditions. After 28 days from collection, 20 mL from each incubated vial was filtered through a syringe and luer-lock filter (0.7 μm GFF, Whatman) into glass vials and acidified with 40 μL 6N HCl.

Table 4.1. Summaries of variables for 13 Ozark Highland streams used in this study. Catchment variables (Pasture cover, developed land cover, forest cover, area), mean and standard deviation (SD) of water chemistry (DIN (dissolved inorganic nitrogen = $\text{NO}_3\text{-N} + \text{NH}_4\text{-N}$)), $\text{PO}_4\text{-P}$, DOC (dissolved organic carbon)), and whether the site received wastewater treatment plant (WWTP) effluent upstream.

Site Code	Stream Name	Pasture Cover (%)	Developed Cover (%)	Forest Cover (%)	Area (km ²)	DIN (µg/L)	DIN (SD)	$\text{PO}_4\text{-P}$ (µg /L)	$\text{PO}_4\text{-P}$ (SD)	DOC (mg/L)	DOC (SD)	WWTP
SALI	Saline Creek	26.3	4.0	60.0	270	745	435	13.0	10.1	0.89	0.49	No
ILLI1	Illinois River	36.9	4.5	55.6	69	291	254	6.4	4.2	1.58	0.51	No
EVAN	Evansville Creek	39.9	4.3	52.4	164	509	490	12.3	6.1	1.03	0.62	No
LSAL	Little Saline Creek	34.9	3.3	50.9	62	785	471	10.1	5.0	0.81	0.74	No
SPRG3	Spring Creek (OK)	40.4	4.7	50.4	297	618	411	10.6	5.4	0.84	0.78	No
BARR1	Baron Fork	48.3	4.2	45.4	106	1552	719	16.9	18.9	1.79	0.83	Yes
BEAT	Beaty Creek	61.7	5.0	29.8	153	2660	767	49.6	37.7	0.91	0.74	No
FLIN2	Flint Creek	58.1	9.1	27.6	146	2533	832	32.8	43.3	1.34	1.14	No
GOOS	Goose Creek	49.2	23.5	26.1	35	3826	1558	44.4	14.4	3.56	0.83	Yes
BALL	Ballard Creek	67.7	7.9	23.2	90	1796	753	71.1	68.3	2.20	1.81	No
SPAR	Spring Creek (AR)	42.7	44.0	11.7	92	3120	409	100.8	13.6	2.60	0.43	Yes
SAGE	Sager Creek	53.6	35.5	9.0	46	3649	1849	96.8	81.2	3.03	0.76	Yes
OSAG1	Osage Creek	34.6	56.5	7.3	101	3397	956	37.2	14.2	2.29	0.21	Yes

Filters and syringes were pre-rinsed with 30 mL DI and at least 5 mL of incubated water from each vial. We corrected incubated DOC measurements for evaporative solute concentration based on gravimetric measurements of water loss throughout incubation.

We measured DOC on a Shimadzu TOC-Vcsh (Tokyo, Japan). Inorganic carbon was removed from each acidified sample by vigorously bubbling N₂ gas into the sample for 3 minutes, and then foil-capped immediately prior to analysis to prevent new CO₂ dissolving into the sample. We followed the approach of Casas-Ruiz et al. (2017) to define significant DOC concentration declines during incubations: DOC measurement precision (0.03 mg/L) was calculated as the standard deviation of re-analyzed samples (i.e., exact replicates). Propagating the standard error of differences between two terms (i.e., initial DOC – incubated DOC) lead us to consider any difference in initial and incubated DOC <0.042 mg/L as non-detectable, and was set to 0.02 mg/L. Because the minimum detection limit of our TOC method was 0.5 mg/L, we also considered incubations where initial DOC values <0.5 mg/L as non-detectable, and set the BDOC value to 0.02 mg/L.

Because our dataset was limited by many measurements that were less than detection limits (but spatial and temporal extent of sampling was similar to other studies, e.g., Hosen et al. 2014; Shang et al. 2018), we kept our modelling approach simple, focusing on our hypothesis regarding large-scale patterns of BDOC in response to anthropogenic land cover and changing hydrologic connectivity through a winter-to-spring transition. BDOC and %BDOC were highly correlated ($\rho = 0.86$), so we focused on patterns of the total BDOC concentration rather than the proportion of bulk DOC that was BDOC. We used linear mixed models in R (R Core Team 2017) with the package

lme4 to account for repeated measurements at each of the 13 sites (site treated as a random intercept). To test our hypothesis, we predicted log-transformed BDOC concentration with an interaction of sampling event and either pasture cover (%) or log-transformed developed cover (%). We did not attempt to parse variation in BDOC due to pasture cover vs developed cover because that would require a much larger dataset. We log-transformed developed cover to better conform to a normal distribution based on visualization of the predictors, and log-transformed BDOC to improve residuals. We used the package *phia* to assess significance of slopes by sampling event. We set $\alpha=0.05$.

Results

Stream flow and weather conditions varied throughout the sampling events under a winter-spring transition. January stream temperatures were $\sim 9^{\circ}\text{C}$, February $\sim 12^{\circ}\text{C}$ and March and April both $\sim 16^{\circ}\text{C}$. Normalized flow, the ratio of measured flow to the lowest measured flow for a site, increased from a mean of 1.4 in January, to 3.3 in February, 5.2 in March and 83 in April. This indicates that streams in January were mostly at their lowest flows, but flows increased considerably through spring. Hydrographs and visual observations (heavy scouring, turbidity, sediment and debris deposited above banks) of sites during the April event suggested events occurred post-flood or post-large spate. Unlike other events, most streams were too dangerous to wade during April.

BDOC in mid-order Ozark Highland streams was significantly, positively related to developed cover in all sampling events except April ($P>0.05$, Fig. 4.1). In contrast, BDOC was not significantly related to pasture cover in any sampling event, nor was there a main effect of pasture cover on BDOC concentrations (all $P<0.05$, Fig. 4.1).

After analyzing our data, it became apparent that the presence of upstream municipal wastewater discharge from wastewater treatment plants (WWTPs) was driving patterns in BDOC response to developed cover and possibly confounding pasture cover analysis for two reasons: 1) BDOC concentrations were generally elevated and invariable

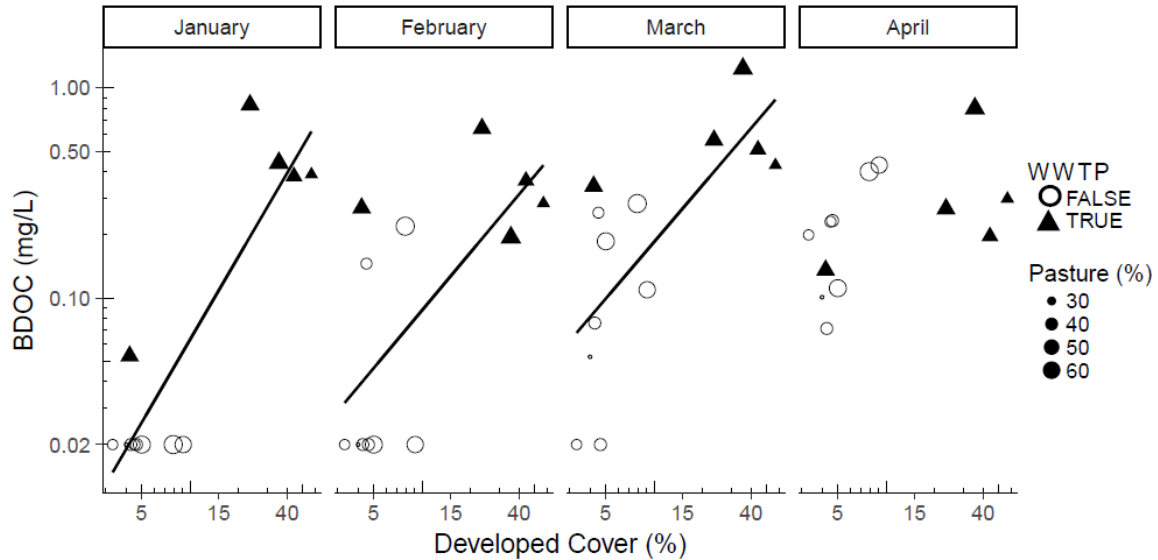


Figure 4.1. Bioavailable dissolved organic carbon (BDOC, mg/L) in mid-order Ozark Highland streams across a gradient of catchment developed cover (%) from January through April 2017. Solid triangles are sites that receive WWTP effluent upstream. Plotted lines are significant ($P < 0.05$) predicted fits by event. X and Y axes are log scaled. Point sizes are scaled by pasture cover (%), which did not predict BDOC in any sampling event (all $P > 0.05$).

across sampling events compared to non-WWTP sites and 2) four out of five WWTP sites fell at the far upper end of developed cover percentages and WWTP grouped in the middle of the pasture cover gradient (Fig. 4.1). Thus, BDOC in WWTP sites appeared unrelated to strict land cover patterns. Because we were also interested in a stricter influence of land cover on BDOC concentrations, we tested our hypothesis on a dataset excluding WWTP sites using the same modelling approach. We did not log-transform

BDOC for the WWTP-excluded analysis because untransformed BDOC had better model residuals.

Removing WWTP sites significantly altered the relationship between BDOC and developed cover. There was a significant interaction between developed cover and sampling event ($P=0.016$). BDOC increased significantly with developed cover only in April ($P<0.001$, Fig. 4.2). Removing WWTP sites also caused pasture cover to positively predict BDOC concentrations overall ($P=0.034$), but this relationship was not strongly influenced by sampling event despite trending toward more significant responses in March and April ($P>0.05$, Fig. 4.3).

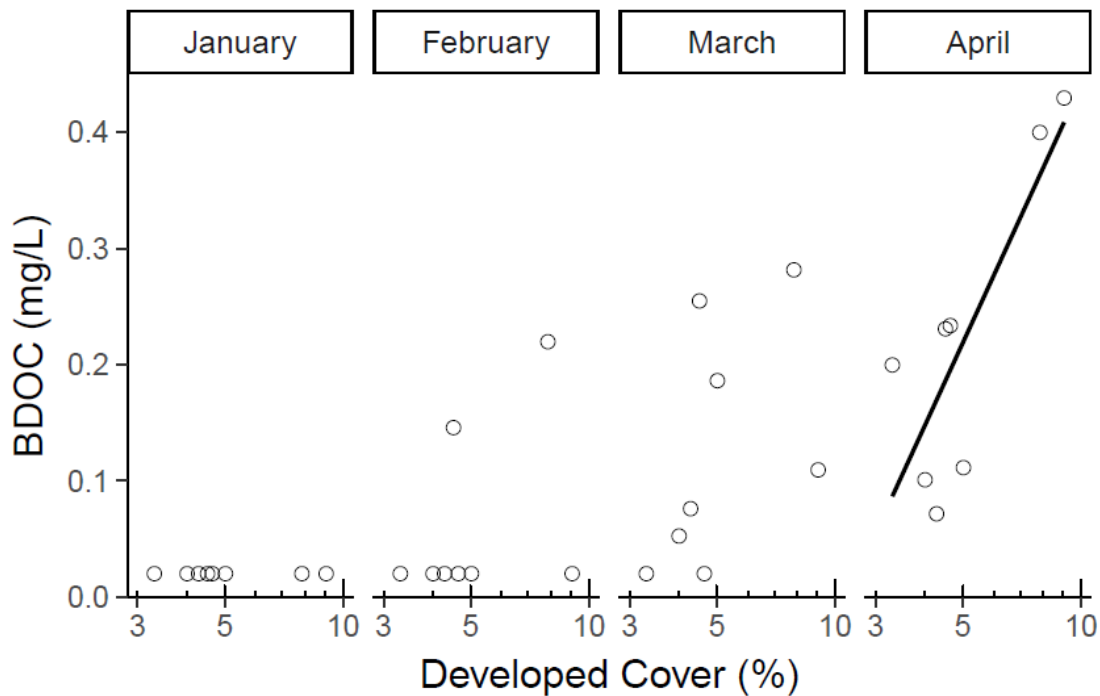


Figure 4.2. Bioavailable dissolved organic carbon (BDOC, mg/L) in mid-order Ozark Highland streams that did not receive upstream WWTP effluent, across a gradient of catchment developed cover (%) from January through April 2017. Plotted line is the only significant ($P<0.05$) predicted fit by event. X axes are log scaled.

Discussion

Our hypothesis that anthropogenic land cover would increase BDOC concentrations was largely supported, but our hypothesis that high flows would result in a stronger relationship between anthropogenic land cover gradients and BDOC was not supported until we reanalyzed the data without WWTP sites (those receiving municipal effluent discharge). Excluding WWTP sites revealed highly contrasting responses of BDOC

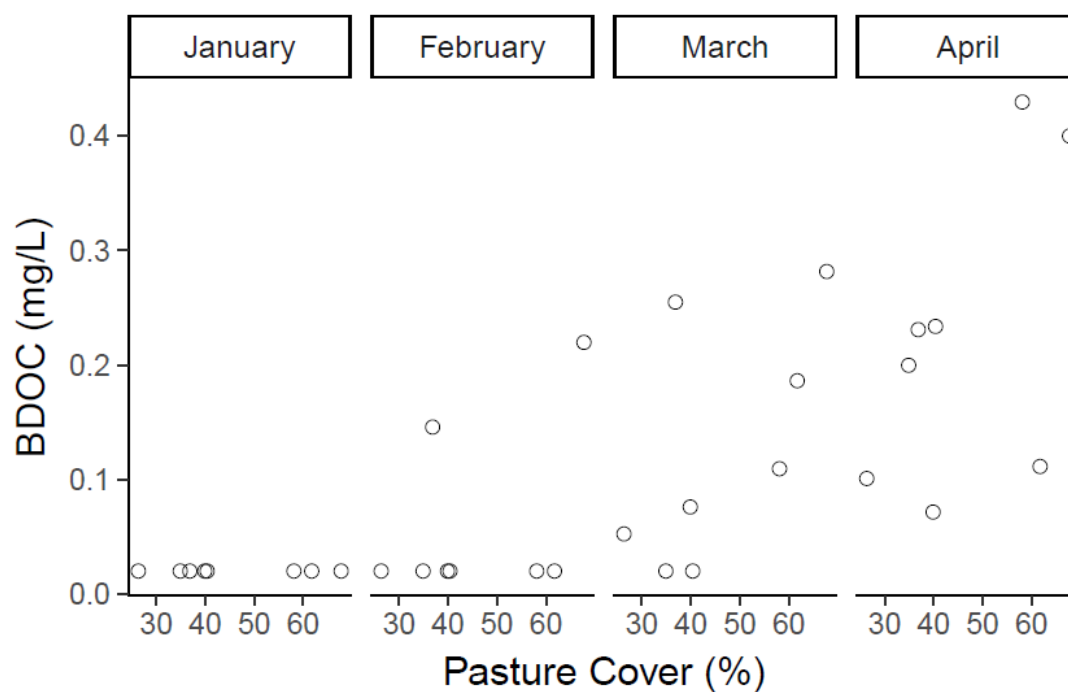


Figure 4.3. Bioavailable dissolved organic carbon (BDOC, mg/L) in mid-order Ozark Highland streams that did not receive upstream WWTP effluent, across a gradient of catchment pasture cover (%) from January through April 2017. Pasture cover never predicted BDOC in any one sampling event, but there was a main effect of pasture cover on BDOC ($P=0.034$).

patterns to point and presumably non-point sources of BDOC under contrasting scenarios of hydrologic connectivity.

Ozark Highland streams without WWTP effluent inputs were highly variable temporally. In the January sampling event, BDOC was undetectable in non-WWTP streams. In contrast, BDOC generally increased in non-WWTP streams from February through April. In January, air temperatures the night before most sites were sampled were below freezing, with visible ice in stagnant side pools, and stream discharge was very low. Freezing temperatures could halt overland flow processes, leaving the streams dominated by deeper, low DOC groundwater (Vidon et al. 2008; Lambert et al. 2013). In addition, low temperatures can slow both terrestrial and aquatic production of labile DOC compounds (e.g., root or algal exudates; Kaplan and Bott 1982, Giesler et al. 2007). Streams received more overland flow (higher discharge) and catchments were exposed to higher temperatures as the spring progressed, shifting overland flow paths to organically rich (and diagenetically fresh) shallow soil layers (Lambert et al. 2013; Lu et al. 2013). This likely increased the contribution of runoff –derived BDOC into streams, especially in April (McLaughlin and Kaplan 2013). Alternatively, nutrients from natural or anthropogenic sources could have spurred algal production of BDOC, which might have explained some of the variation in BDOC in February and March (Kaplan and Bott 1982); however, BDOC was unmeasurable in all non-WWTP sites in January, and April DOC was very likely dominated by terrestrial sources due to sampling during the descending limb of spates. Autochthonous BDOC may be particularly important to BDOC patterns in low DOC systems, such as the streams in this study, under warm temperatures and base flows. We thus interpret at least the extremes in BDOC as being indicative of runoff dependent loading of terrestrial BDOC.

Upstream WWTP effluent inputs were a significant source of BDOC in Ozark Highland streams, and BDOC in WWTP streams was particularly elevated compared to non-WWTP streams during periods of low flow. Wastewater effluent has long been known to source labile organic compounds to streams (Servais et al. 1999; Shon et al. 2006). The continuous piping of BDOC from WWTPs provided a constant source of BDOC to streams in this study, essentially releasing them from dependence on runoff-generated BDOC that was observed in non-WWTP streams. The relationship between developed cover and BDOC was generally driven by WWTP presence, which were (largely) associated with the most developed catchments. WWTP sites were indicative of high degrees of development (i.e., cities in northwest Arkansas), but the elevated BDOC in the sites were not strictly a result of increasing developed cover. Thus, it was necessary to account for WWTP sites to understand the role of strict land cover on patterns of anthropogenic BDOC in streams.

Developed land cover in non-WWTP sites was positively related to BDOC concentrations only in April, when overland flow was high. Developed land is likely to have high quantities of fresh organic matter (e.g., grass clippings, fertilizer), and high levels of compacted or impermeable substrates probably shunt BDOC to streams rather than percolating to deep, C-consuming flowpaths (Kalbitz et al. 2005; Zimmermann et al. 2006). A lack of overland flow probably limited the transport of anthropogenic DOC to our streams during times of low flow. Although models indicated that pasture cover interacted with sampling event significantly, it was clear that pasture cover had no discernible influence in January, when BDOC was below detection, but did have an overall positive influence on BDOC concentrations. Pasture in the Ozark Highlands is

notably fertilized with poultry litter in the spring, and could also have compacted soils that shunt fresh organic matter to streams, but pasture influences on BDOC may be dependent on grazing pressures, spatial location in a catchment (i.e., distance from sampling location to bulk of pasture cover) or some other factor that we did not measure that could have caused pasture to more variably influence BDOC concentrations (King et al. 2005). Regardless, pasture cover and developed cover in non-WWTP streams were highly, positively correlated ($\rho = 0.77$), so it would be impossible to totally separate the effect of these individual sources without a vastly more extensive spatial and temporal sampling regime.

The role of anthropogenic land cover in driving stream BDOC may be more complicated than is often conceptualized (Stanley et al. 2012). For example, (Hosen et al. 2014) similarly found no relationship between the proportion of BDOC in the total DOC pool and catchment urbanization during colder months, but did not discuss the result in light of hydrologic connectivity. Lu et al. (2013) found no relationship between human land use and stream BDOC, but instead stream BDOC was explained better by in-stream temperature that they interpreted as a control on diagenetic status. Our study now demonstrates that delivery mechanisms can be important to understanding BDOC in human-modified catchments. Accounting for specific anthropogenic sources, especially if they mechanistically differ in hydrologic connection to the stream (i.e., land cover vs effluent discharge), is necessary to identify the role of anthropogenic activity on stream BDOC concentrations.

Our results overall point to stream BDOC dependence on hydrologic connectivity interacting with anthropogenic sources, whether precipitation-driven non-point inputs or

“short circuiting” by directly pumping human-modified water into streams. These transport mechanisms may be even more important in groundwater-dominated streams, where terrestrial BDOC signals are probably very weak under baseflow conditions or can still be heavily diluted even during minor runoff events. Determining how, when and where anthropogenic activity determines bioavailable C in stream ecosystems will lead to a more predictive framework for understanding the intersection of human activity and DOC-driven stream processes, such as riverine CO₂ emissions or biomass production in heterotrophic food webs.

CHAPTER FIVE

Summary and Conclusions

Synthesis

Results from Chapter Two and Three of this dissertation strongly suggest that naturally-occurring quantities of labile DOC play a significant role in stream ecosystems. Chapter Two suggests that labile DOC is an important driver of multiple basal ecosystem facets, including nitrogen uptake, ecosystem respiration and even microbial production that can be transferred to higher trophic levels. Chapter Three went on to confirm that this increased microbial biomass can dramatically translate to production of metazoan consumers. Thus, environmentally relevant levels of labile DOC can fuel significant biogeochemical function, but also cause substantial, positive bottom-up effects on biomass (i.e., a “trophic upsurge”, Tanentzap et al. 2014). Whole ecosystem tests had previously been unable to confirm this significant role of labile DOC due to unrealistic dosing concentrations.

Current conceptual frameworks of bottom-up ecosystem control are frequently nutrient-centric. Inorganic nutrients drive autotroph growth, which in turn is responsible for nutrient retention and secondary production. DOC is thus relegated to the role of a modulator of the nutrient-driven effects, mostly through indirect effects on biota (light attenuation, metal complexing, etc., Prairie 2008), and rarely considered to fuel significant portions of food webs except in heavily shaded streams. In contrast, chapters Two and Three suggest a need to revise and upgrade the role of DOC in controlling stream ecosystems. DOC still plays important, indirect “modulating” roles, but there

should be increased recognition of the direct bottom-up control by labile DOC as a resource. Labile DOC-driven heterotrophic pathways can be a critical avenue of control on entire stream ecosystem patterns and processes. (Stanley et al. 2012) called for a shift toward managing DOC in rivers, but mostly because of its many modulating roles, with little recognition of possible trophic upsurges. Given that both biogeochemical and food web processes were significantly altered by relevant levels of labile DOC, DOC should be considered as potentially having similar, yet more varied and complex, roles as nutrient availability.

The extension of Chapters Two and Three is that landscape elements that source labile DOC to streams could be strong drivers of stream ecosystem function. Chapters Two and Three were designed to simulate the level of labile DOC found in peatland (wetland)-draining streams. Chapter Two's results confirm that wetland labile DOC could serve as a significant energy source, spurring considerable respiratory losses of CO₂ and downstream nitrogen retention. Further, Chapter Three suggests that wetland labile DOC can support substantial consumer production, including that of economically critical juvenile fish species. Landscape elements, including wetlands and riparia, should be considered as strong subsidizers of stream ecosystem communities through inputs of labile DOC.

While Chapters Two and Three set out to establish roles of labile DOC in stream ecosystems, and confirmed its importance to ecosystem function, Chapter Four identified patterns of labile DOC in streams due to anthropogenic sources within catchments. Results from Chapter Four confirms what has often been found in other labile DOC studies – anthropogenic activity increases the lability of stream DOC; however, Chapter

Four uniquely modulates this consistency by establishing hydrologic connectivity as a key mechanism driving these patterns in some systems. Labile DOC quantity in mid-order streams might be directly driven by non-point sources (e.g., developed land cover) only when precipitation shifts flow paths to shallower, fresher soil layers. In contrast, constant hydrologic connectivity from wastewater effluent discharge can disconnect stream BDOC patterns from the landscape and precipitation.

Combining the results from the experimental and observational studies here suggests that labile DOC from human modifications could have highly significant consequences for stream ecosystem function. Increasing stream carbon quality is a consistent influence of human catchment modifications (Stanley et al. 2012; Williams et al. 2016). Human inputs of labile DOC could therefore increase stream respiration and nutrient retention. As streams experiencing anthropogenic labile DOC inputs receive concomitant nutrient inputs, this may provide an unintentional stoichiometric mitigation of downstream nutrient transport (Stutter et al. 2018). The most novel finding from these studies is that relevant concentrations of labile DOC can spur significant metazoan survivorship and growth. This suggests that anthropogenic alterations to the landscape could have consequences for secondary production. For example, reduction of catchment wetlands may reduce the quantity of labile DOC exported to streams, thereby limiting an important consumer energy source. Increasing anthropogenic sources of labile DOC, such as through urbanization, may alternatively subsidize higher trophic levels. On the other hand, these relationships are dependent on multiple factors, such as nutrient availability, light availability and the temporal nature of changes to labile DOC quantities.

Future Directions

The three studies comprising this dissertation clarify the patterns and roles of labile DOC in stream ecosystems, but much work remains to understand how, when and where labile DOC is important in stream ecosystems.

One considerable question remaining is the combined role of labile DOC and nutrients on the microbial interactions that influence basal resource quantity and quality. The influence of any environmental variable on an entire ecosystem will always be dependent on other environmental variables. The experimental studies presented here added relevant levels of labile DOC to a stream with ample inorganic nitrogen and phosphorus. When labile DOC is available in stoichiometric excess to nitrogen and phosphorus, bacteria and fungi have lower growth efficiency, respiring the additional labile DOC rather than converting it to biomass (Del Giorgio and Cole 1998). Additionally, bacterial-algal interactions could be influenced by labile DOC availability. When labile DOC is high relative to inorganic nutrient availability, bacteria may outcompete algae for nutrients (Bechtold et al. 2012). Thus, had labile DOC been added to a low nutrient stream, essential lipid-rich autotrophic production may have been significantly stunted, nutritionally limiting consumers. No study currently exists comprehensively examining algal-bacterial interactions across gradients of both DOC and nutrient availability, or how these interactions may change over time (i.e., with biofilm development), but could be critical to understanding how DOC may subsidize consumer growth.

Chapter Four also suggested that substantial hydrologic connectivity may be required for anthropogenic sources of terrestrial labile DOC to be substantially present in

stream ecosystems. This presents an interesting and difficult temporal aspect to the idea of “managing” for DOC (Stanley et al. 2012). In particular, most labile DOC (on an annual basis) will be quickly shunted through streams in infrequent but significant flood pulses when biological reactivity will be severely limited due to low retention time and disturbance. In the Ozark Highland streams studied in Chapter Four, labile DOC was undetectable at very low flows, and could very well have derived from autochthonous sources during normal base flows. This suggests that managing for terrestrial labile DOC inputs could require more sensitive monitoring techniques (e.g., fluorometry) to measure or approximate labile DOC at low flows (e.g., Coble et al. 2014). Continuously discharging point sources, such as municipal wastewater effluent, nevertheless provide a constant source of anthropogenic labile DOC. Future work should also consider how both spatial and temporal variability in DOC quality and quantity is influenced by different land uses (and their spatial arrangements) within the same catchment.

These two future directions point to the complexity of labile DOC in stream ecosystems. No one factor in ecology works in isolation. Assessing the role of labile DOC in a framework including “multiple stressors” will be critical to understanding the way that landscapes, and how humans alter them, affect stream ecosystem function. This dissertation suggests labile DOC could be an underappreciated driver of stream ecosystem function, and that the quantity of that labile DOC in streams can be driven by an interaction between humans and hydrology, but more work is needed to understand how these relationships are altered by other critical environmental variables, such as availability of other basal resources like light and nutrients, climate change impacts on

hydrology, and stream water retention time due to hyporheic extent, all of which can be altered by anthropogenic stream modification.

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