

## Low-level addition of dissolved organic carbon increases basal ecosystem function in a boreal headwater stream

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**Abstract.** Comprehension of basic stream ecosystem function relies on an understanding of aquatic–terrestrial linkages. One major component of such linkages is the incorporation of landscape-derived energy and nutrients into the aquatic food web via microbes. In many boreal streams, wetlands and alder are known to be primary sources of dissolved organic carbon (DOC) and dissolved inorganic nitrogen (DIN), respectively. To simulate the influence of the highly labile portion of wetland-derived DOC subsidies on microbial production and ecosystem processes in a stream with high landscape-derived nutrient inputs, we enriched a boreal headwater stream situated in a high-alder, low-wetland cover catchment (i.e., high DIN, low DOC) with low levels (~0.25 mg/L) of labile DOC (as acetate-C) for 9 weeks. We compared nutrient uptake, bacterial biomass production, and photosynthesis of periphyton and ecosystem metabolism in physicochemically similar upstream (reference) and downstream (treatment) reaches. DIN uptake was greater in the treatment than in reference reach on six out of nine dates during the dosing period. Bacterial biomass production positively responded to C enrichment. Ecosystem respiration increased up to ~50% after dosing began. Gross primary production responded positively to DOC enrichment early in the study when riparian vegetation did not limit light availability, but negatively later on in the growing season. We conclude that even low levels of labile DOC may act as a strong subsidy to headwater stream ecosystems, particularly those with high levels of DIN inputs from alder. Headwater streams influenced by high contributions of both alder and wetlands may represent biogeochemical hotspots, and these landscape features should be viewed as vital and complementary in their roles for ecosystem function.

**Key words:** Alaska; experiment; Kenai Peninsula; organic matter; periphyton; resource subsidies; uptake velocity.

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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, 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).

Dissolved organic carbon 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 DOC quantity and quality, and the availability of necessary nutrients for growth (Bott et al. 1984, 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, 2012, Thouin et al. 2009) 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 base-flow 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 and Tank 2009, Johnson et al. 2009), but such short-term additions are unable to assess the interaction between 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 dissolved inorganic nitrogen (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

Methods here are abbreviated. Complete methods can be found in Appendix S2.

### *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. 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%)

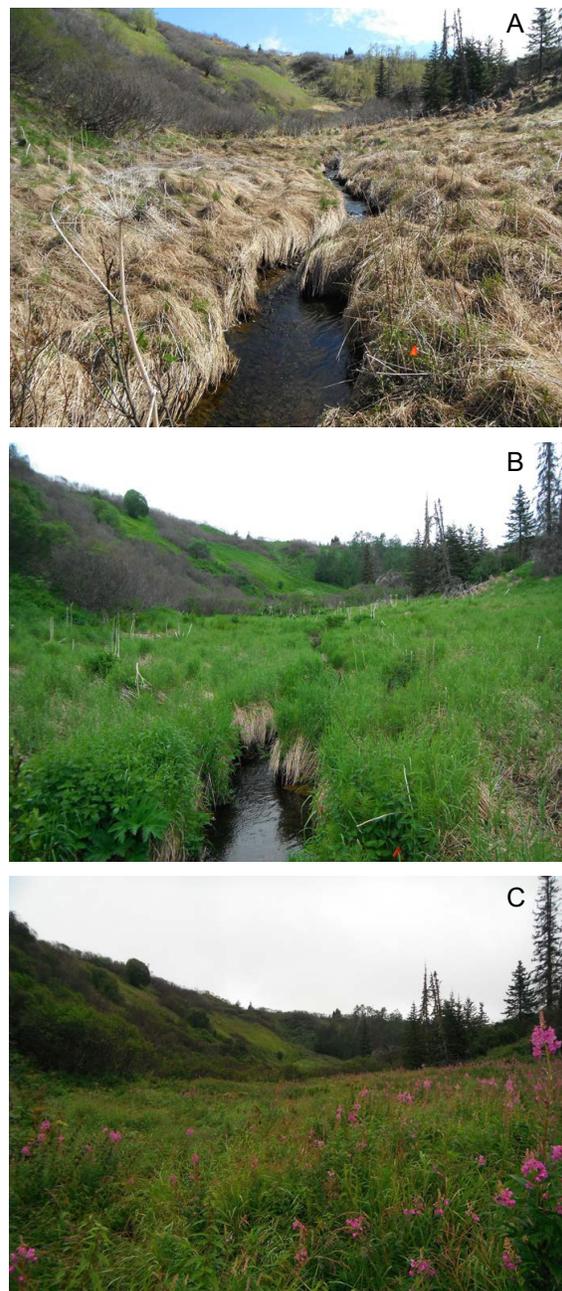


Fig. 1. Photos of the reference reach of ANC-1203, facing upstream, showing encroaching herbaceous vegetation in late June (A), July (B), and August (C) of 2013.

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:  $1.49 \pm 0.26$  m; T:  $1.30 \pm 0.30$  m; mean  $\pm$  SD), mean depth (R:  $0.12 \pm 0.07$  m; T:  $0.12 \pm 0.07$  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 dissolved inorganic carbon (DIC), DIN,  $\text{PO}_4\text{-P}$ , and DOC were very similar between the reaches (Appendix S1: Tables S1 and S2). 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 d, starting on 25 June 2013 (day 1 of dosing) and terminating on 25 August 2013. The dosing solution consisted of sodium acetate ( $\text{C}_2\text{H}_3\text{NaO}_2$ ) as a labile DOC source. Acetic acid (dissociated acetate in  $\text{H}_2\text{O}$ ) 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; R. D. Doyle, 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 model QBG pump (Fluid Metering Inc., Syosset, New York, USA) 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 polyvinyl chloride conduit. The first 5 m of the reach below T0 was 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*).

### 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 4 June (day  $-21$ ) to 19 August (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, Denver, Colorado, USA) through a pre-rinsed cellulose acetate membrane filter (0.45  $\mu\text{m}$ ). 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 QuikChem 8500 series 2 continuous flow injection analyzer (Lachat Instruments, Loveland, Colorado, USA). DOC and TDC were analyzed using a Shimadzu TOC-Vcsh (Shimadzu Corporation, Kyoto, Japan). Dissolved inorganic carbon 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  $V_f = uz/S_w$  where  $u$  is velocity and  $z$  is 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 and photosynthesis***

We used the dual-label radioassay method to measure 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 seven 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, three jars were covered in foil as dark treatments, three jars were left uncovered, and one jar was immediately injected with buffered formalin to a final concentration of 4% as a killed control. Using a 50- $\mu$ L syringe, we injected each jar with 25  $\mu$ 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  $\mu\text{E}$ ). After 1.5 h, we injected 25  $\mu$ L of  $^3\text{H}$ -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 one to three (depending on slurry volume) 50-mL centrifuge tubes. We centrifuged slurries for 45 min 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 (2000) for converting radioactivity to biological activity. Briefly, we filtered slurry onto a polycarbonate filter (0.2  $\mu\text{m}$  pore size) and washed the filter with acid, ethanol, and deionized water. 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, USA). 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 ( $K_{\text{O}_2}$ ) using propane following Bott (2006).  $K_{\text{O}_2}$  was measured by bubbling propane at the top of the reaches and collecting five water samples each at the 10 and 75 m locations after coincident NaCl additions had reached plateau. To estimate  $K_{\text{O}_2}$  for periods of metabolism in which we did not perform propane injections, we regressed measured  $K_{\text{O}_2}$  against  $Q$  for each reach, resulting in linear equations used to predict  $K_{\text{O}_2}$  based on  $Q$  measured on the first and final days of sonde deployment. Dissolved  $\text{O}_2$  was logged at 20-min intervals using two calibrated YSI Exo1 sondes (YSI, Inc., Yellow Springs, Ohio, USA) deployed at T75 and R75 such that full diel curves were generated for dosing days –4 to 0 (pre-dose), 9–13, 23–27, and 37–41. 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 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  $V_{fS}$  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) 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 *lsmmeans* (version 3.1.1; R Core Team, Vienna, Austria). Any effects were considered significant at  $\alpha = 0.05$ . Means are presented  $\pm 1$  standard error.

## RESULTS

Mean daily water temperature on sampling dates ranged from 5.83° to 8.65°C. Discharge measured on sampling dates ranged from 13.9 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  $15.6 \pm 1.9$  L/s.

### Water chemistry

Dissolved organic carbon concentrations during our study ranged from 1.85 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:  $2.52 \pm 0.184$  mg/L, T0:  $2.49 \pm 0.182$  mg/L; Appendix S1: Table S1). 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 (Appendix S1: Table S1).

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  $0.254 \pm 0.017$  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:  $23.6 \pm 0.79$   $\mu\text{g/L}$ ; T0:  $21.1 \pm 0.54$   $\mu\text{g/L}$ ; Appendix S1: Table S2).  $\text{NO}_x\text{-N}$  was the dominant component of DIN (R10:  $374 \pm 22.3$   $\mu\text{g/L}$ ; T0:  $369 \pm 24.8$   $\mu\text{g/L}$ ; Appendix S1: Table S2) and ranged from 185 to

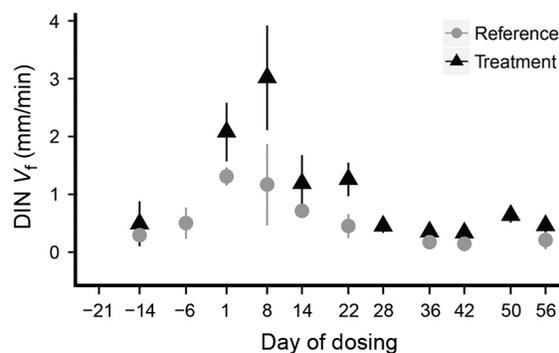


Fig. 2. Dissolved inorganic nitrogen (DIN) uptake velocities ( $V_f$ , mm/s) calculated from longitudinal declines in DIN ( $\text{NH}_4\text{-N} + \text{NO}_x\text{-N}$ ) in the reference reach (gray circles) and treatment reach (black triangles). Error bars are 95% confidence intervals propagated from 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.

441  $\mu\text{g/L}$  (Appendix S1: Table S2).  $\text{PO}_4\text{-P}$  concentrations were relatively high and stable during our study (R10:  $57.6 \pm 2.47$   $\mu\text{g/L}$ ; T0:  $57.3 \pm 2.47$   $\mu\text{g/L}$ ; Appendix S1: Table S2).

### Net nutrient uptake

Dissolved inorganic nitrogen  $V_f$  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). 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 CIs in the reference reach overlapped 0 slightly on days 28 and 50 (both lower CIs  $\sim 0.025$  mm/s). 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). The greatest difference between reaches was on days 1 and 8, when treatment  $V_f$  reached 2.1 and 3.0 mm/s, respectively, compared to 1.31 and 1.17 mm/s on days 1 and 8, respectively, in the reference reach.

$\text{PO}_4\text{-P}$   $V_f$  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.

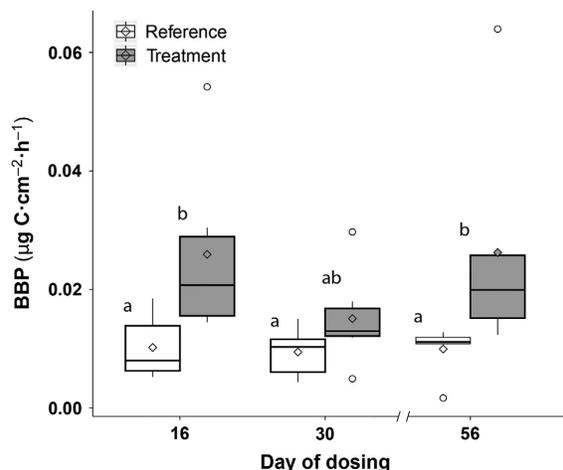


Fig. 3. Bacterial biomass production in reference (white) and treatment (gray) reaches standardized to surface area of the rock ( $\mu\text{g C}\cdot\text{cm}^{-2}\cdot\text{h}^{-1}$ ). Different letters indicate significantly different means ( $P < 0.05$ ). Boxes represent the interquartile range, containing 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.  $n = 6$ .

#### Bacterial biomass production and photosynthesis

Mean BBP (Fig. 3) ranged from  $0.009 \pm 0.001 \mu\text{g C}\cdot\text{cm}^{-2}\cdot\text{h}^{-1}$  to  $0.010 \pm 0.002 \mu\text{g C}\cdot\text{cm}^{-2}\cdot\text{h}^{-1}$  in the reference reach, and  $0.015 \pm 0.003 \mu\text{g C}\cdot\text{cm}^{-2}\cdot\text{h}^{-1}$  to  $0.026 \pm 0.008 \mu\text{g C}\cdot\text{cm}^{-2}\cdot\text{h}^{-1}$  in the treatment reach. The magnitude of BBP did not significantly differ across dates within either reach (Fig. 3). BBP was affected by the labile C addition ( $F = 6.49$ ,  $P = 0.002$ ). BBP was consistently higher in the treatment than in the reference reach, and approximately  $2.5\times$  greater on days 16 and 56, reaching  $0.026 \mu\text{g C}\cdot\text{cm}^{-2}\cdot\text{h}^{-1}$  on both dates (Fig. 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  $\sim 30\%$  higher in treatment than in reference incubations on day 30 (Fig. 4). Photosynthesis was very low in both reaches on day 56 (R:  $0.046 \pm 0.065 \mu\text{g C}\cdot\text{cm}^{-2}\cdot\text{h}^{-1}$ , T:  $0.010 \pm 0.008 \mu\text{g C}\cdot\text{cm}^{-2}\cdot\text{h}^{-1}$ ; Fig. 4).

#### Ecosystem metabolism

Measured  $K_{O_2}$  ranged from  $0.0552 \text{ min}^{-1}$  to  $0.0896 \text{ min}^{-1}$ . Because estimated  $K_{O_2}$  was a

function of  $Q$  and the average  $Q$  during each deployment was within the range of  $Q$  observed during propane evasions, estimated  $K_{O_2}$  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 in the reference reach, with reach differences ranging from  $0.59$  to  $0.78 \text{ g O}_2\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ . Acetate addition increased ER in the treatment reach following onset of dosing (Fig. 5). ER was generally  $>25\%$  higher ( $>2 \text{ g O}_2\cdot\text{m}^{-2}\cdot\text{d}^{-1}$  difference) in the treatment than in the reference reach, except day 23 when it was only 11% greater, and reached a maximum of 52% greater on day 27, corresponding to a  $\sim 4.6 \text{ g O}_2\cdot\text{m}^{-2}\cdot\text{d}^{-1}$  difference.

Gross primary production in both reaches decreased throughout the dosing period, peaking on the first day (9) of the first dosing period measurements ( $\sim 8.1\text{--}9.75 \text{ g O}_2\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ , compared to  $<1 \text{ g O}_2\cdot\text{m}^{-2}\cdot\text{d}^{-1}$  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%

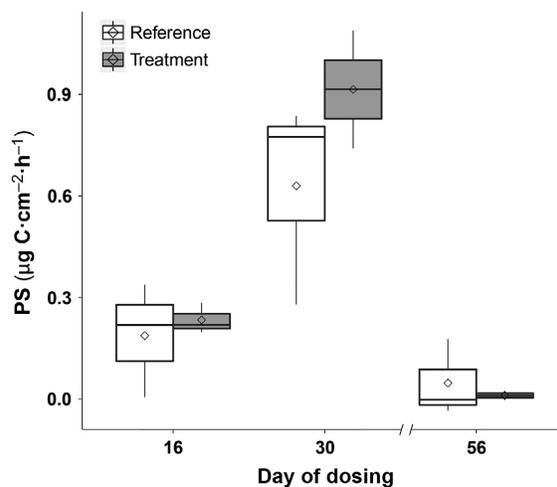


Fig. 4. Photosynthesis in reference (white) and treatment (gray) reaches standardized to surface area of the rock ( $\mu\text{g C}\cdot\text{cm}^{-2}\cdot\text{h}^{-1}$ ). Boxes represent the interquartile range, containing the median (black line) and mean (open diamond). “Whiskers” extend to furthest data point within 1.5 times the interquartile range, while dots are outliers.  $n = 3$ .

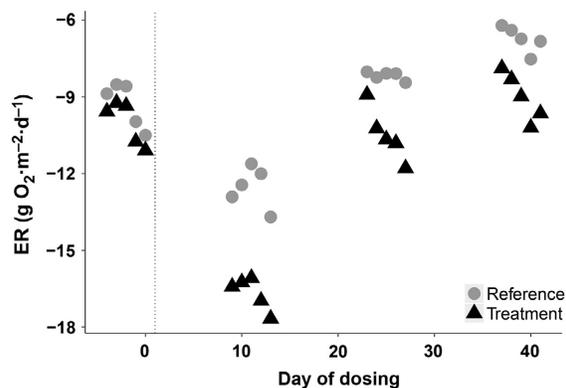


Fig. 5. Ecosystem respiration ( $\text{g O}_2\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ ) in the dosed treatment reach (black triangles) and the reference reach (gray circles) on each date of ecosystem metabolism monitoring. Fine dashed vertical line is the date on which dosing commenced.

higher rates (differences of  $1.1\text{--}1.6 \text{ g O}_2\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ ) 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. 6) when it was raining (R. S. King, *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 (days 37–41; Fig. 6).

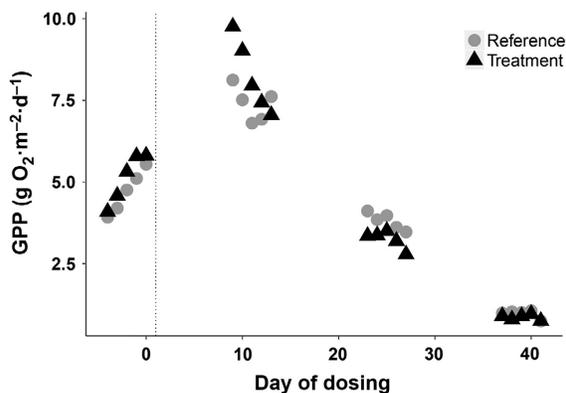


Fig. 6. Gross primary production ( $\text{g O}_2\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ ) in the dosed treatment reach (black triangles) and the upstream reference reach (gray circles) on each date of ecosystem metabolism monitoring. Fine dashed vertical line is the date on which dosing commenced.

## 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-labeled 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).

Dissolved organic carbon 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  $7\times$  increase in  $\text{CO}_2$  production when adding  $5\text{--}7 \text{ mg/L}$  of acetate-C to a stream. Johnson et al. (2012) elicited a  $2\times$  increase in ER with a  $6 \text{ mg/L}$  acetate addition. Oviedo-Vargas et al. (2013) dosed a stream with  $1 \text{ mg/L}$  of acetate and observed  $4\text{--}23\%$  increases in ER. At a maximum dosing concentration of  $\sim 0.30 \text{ mg/L}$  acetate-C, our dosing concentration was between  $\sim 5\%$  (Bernhardt and Likens 2002, Johnson et al. 2012) and  $\sim 30\%$  (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 manuscript*). 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 CO<sub>2</sub>, possibly benefiting algal photosynthesis (Hasler et al. 2016), especially if heterotrophs utilizing acetate were tightly associated with 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 with 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<sub>2</sub> changes, as well as uncertainty in K<sub>O<sub>2</sub></sub> measurement. The upstream O<sub>2</sub> footprint of the treatment reach (length = ~0.5 v/K, ~40% measured O<sub>2</sub> 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 manuscript*), 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  $\sim 2 \text{ g O}_2 \cdot \text{m}^{-2} \cdot \text{d}^{-1}$ , 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. 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, 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  $\text{NH}_4$  to slow enough for heterotrophic release of  $\text{NH}_4$  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., *C. canadensis*, Shaftel 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.

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## SUPPORTING INFORMATION

Additional Supporting Information may be found online at: <http://onlinelibrary.wiley.com/doi/10.1002/ecs2.1739/full>