

# Untangling the complex issue of dissolved organic carbon uptake: a stable isotope approach

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## SUMMARY

1. We estimated uptake of stream water dissolved organic carbon (DOC) through a whole-stream addition of a <sup>13</sup>C-DOC tracer coupled with laboratory measurements of bioavailability of the tracer and stream water DOC.
2. The tracer, a leachate of <sup>13</sup>C-labelled tree tissues, was added to the head waters of White Clay Creek, Pennsylvania, U.S.A., over a 2-h period and followed 1.27 km downstream to generate mass transfer coefficients for DOC lability classes within the tracer.
3. From the longitudinal <sup>13</sup>C uptake curve, we resolved labile and semi-labile DOC classes within the <sup>13</sup>C-DOC tracer comprising 82% and 18% of the tracer respectively.
4. Plug-flow laboratory bioreactors colonized and maintained with stream water were used to determine the concentration of stream water DOC fractions that had a similar lability to the labile and semi-labile classes within the tracer and we assumed that stream water DOC and tracer DOC with comparable lability fractions in the bioreactors behaved similarly in the stream, i.e. they had the same mass transfer coefficients.
5. A small fraction (8.6%) of the stream water DOC was labile, travelling 238 m downstream before being taken up. The remaining bioavailable stream water DOC was semi-labile and transported 4.5 km downstream before being taken up. These uptake lengths suggest that the labile DOC is an energy source within a stream reach, while the semi-labile DOC is exported out of the reach to larger rivers and the downstream estuary, where it may provide energy for marine microbial communities or simply be exported to the oceans.

*Keywords:* bioavailability, dissolved organic carbon, stable isotopes, streams, tracer addition

## Introduction

In streams and rivers, dissolved organic carbon (DOC) supplies energy and carbon (C) to heterotrophic bacteria, and DOC incorporation into the microbial loop or aggregation into particles affects its transfer to higher trophic levels. These organic molecules constitute the largest pool of organic

matter in rivers, and while they were long considered biologically refractory (Wetzel, 1995), laboratory measurements have shown that humic substances (Volk, Volk & Kaplan, 1997) and lignin (Frazier, Kaplan & Hatcher, 2005) from headwater streams are bioavailable. Additionally, *in situ* gas flux measurements (Cole & Caraco, 2001) and C-isotope analyses (Mayorga *et al.*, 2005) provide evidence that terrestrial C is assimilated and respired in large rivers. Yet, we do not know the length of the river the DOC travels before it is taken up because no study to date has directly measured uptake lengths for total stream water DOC or its constituent lability classes.

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Dissolved organic carbon is a complex mixture of thousands of organic molecules from various sources with differing biological labilities (Kim, Kaplan & Hatcher, 2006). That complexity, combined with simultaneous processes that continually produce, transform and consume DOC molecules in transport, makes *in situ* measurements of DOC uptake challenging. Stable and radioactive isotope tracers have been essential tools for examining *in situ* nutrient dynamics of inorganic nitrogen (Tank *et al.*, 2000) and phosphorus (Newbold *et al.*, 1983). C cycling has been examined *in situ* with  $^{13}\text{C}$ -bicarbonate additions to follow autochthonous C in lakes (Pace *et al.*, 2004), and with  $^{13}\text{C}$ -acetate additions to follow heterotrophic uptake of monomeric organic C substrates in streams (Hall & Meyer, 1998). Neither one of these tracers is ideal for stream DOC because their chemical composition is not reflective of terrestrially produced C, nor is it likely that any tracer is ideal given the heterogeneity of terrestrial C resulting from sorptive fractionation onto mineral surfaces (Aufdenkampe *et al.*, 2001), oxidation by soil and aquatic bacteria, and photo-oxidation during transport (Amon & Benner, 1996). Previously, we synthesized a  $^{13}\text{C}$ -DOC tracer from deciduous trees with monomeric and polymeric constituents (Wiegner *et al.*, 2005a) and injected it into 15-L mesocosms containing streambed sediments and re-circulating stream water to estimate mass transfer coefficients for different DOC lability classes (Wiegner *et al.*, 2005b).

Here, we performed a whole-stream addition of the same  $^{13}\text{C}$ -DOC tracer and paired those data with direct measurements of biological labilities of the tracer and stream water DOC in laboratory bioreactors to estimate the *in situ* uptake of stream water DOC. Although  $^{13}\text{C}$ -DOC leached from labelled trees provides a first-approximation surrogate for the natural DOC found in the stream, its specific mixture of labilities cannot be expected to match those of the natural stream water DOC. Thus, to estimate the *in situ* uptake of natural DOC using the tracer dynamics from the whole stream addition, we compared their uptakes side-by-side in laboratory bioreactors. In the bioreactors, DOC lability fractions in the tracer and stream water were partitioned by residence time. The uptake of the natural DOC was then estimated by applying the whole-stream mass transfer coefficient for a particular DOC lability class from the tracer to the stream water DOC fraction with

the corresponding lability. Our novel approach of using tree-derived  $^{13}\text{C}$ -DOC to determine the distance travelled by terrestrial organic C in a stream provides insight into how DOC metabolically links lotic systems to each other, coastal waters and the ocean, as well as insight into the global C cycle.

## Methods

### Site description

The study was conducted in a forested reach of White Clay Creek, a third-order stream with intact riparian woodlands. The stream is located in southeastern Pennsylvania, U.S.A. (39°53'N, 75°47'W), in the Piedmont physiographic province. White Clay Creek drains 725 ha of agricultural land (74%) and forest (23%) (Newbold *et al.*, 1997). The dominant trees are beech (*Fagus grandifolia* Ehrh.), red and black oak (*Quercus rubra* L. and *Q. velutina* Lam.) and tulip poplar (*Liriodendron tulipifera* L.). White Clay Creek has a stream gradient of 8 m km<sup>-1</sup> and a mean annual stream flow, water temperature and precipitation of 115 L s<sup>-1</sup>, 10.6 °C and 105 cm, respectively (Newbold *et al.*, 1997). Seasonal average baseflow concentrations of DOC range from 1.3 mg C L<sup>-1</sup> in the winter to 1.7 mg C L<sup>-1</sup> in the summer (Hullar *et al.*, 2006). This experiment, conducted during daylight hours, coincided with a drought in the autumn of 2002, when base flow declined to 15 L s<sup>-1</sup>.

### $^{13}\text{C}$ -DOC tracer generation

Tulip poplar seedlings (*Liriodendron tulipifera* L.) were grown with  $^{13}\text{CO}_2$  (Wiegner *et al.*, 2005a), harvested and dried. Leaves, stems and roots were ground and between 1 and 3 g of tissue were extracted in 1 L of filter-sterilized de-ionized water in the dark at 4 °C for 24 h (Wiegner *et al.*, 2005a). The infusion was centrifuged, filtered, Tyndallized (heated to 70 °C for 0.5 h twice, separated by 24 h at room temperature) to ensure biological stability, and each batch was stored in the dark at 4 °C for *c.* 1.5 months until 120 L were prepared for the stream addition.

### Whole-stream $^{13}\text{C}$ -DOC tracer addition

$^{13}\text{C}$ -DOC (120 L; 20 mg C L<sup>-1</sup>) and bromide (7.27 L; 42.7 mg Br<sup>-</sup> L<sup>-1</sup>) were simultaneously added into a

1.27-km study reach within the 4.2-km long East Branch White Clay Creek over a 2-h period, using peristaltic pumps. The addition was performed in early October (2/10/2002), 3 weeks prior to peak leaf fall. DOC,  $^{13}\text{C}$ -DOC and bromide ( $\text{Br}^-$ ) samples were collected over an 8-h period at eight stations within the study reach, and a reference site, located just below the next upstream riffle, 82.7 m from the addition site. Station distances were selected based on previous  $^{13}\text{C}$ -DOC tracer uptake dynamics measured in 15-L re-circulating mesocosms with water and sediments from White Clay Creek (Wiegner *et al.*, 2005b).  $\text{Br}^-$  was used as a conservative tracer to correct for stream water dilution and to describe stream flow characteristics, including cross-sectional area, streamflow, longitudinal dispersion and transient storage throughout the reach using a one-dimensional advection–dispersion model (Runkel, 1998). Stream width was measured at 19 transects; velocity and depth were calculated from streamflow, channel cross-section and width. The  $^{13}\text{C}$ -DOC addition followed the general approach described for whole-stream solute additions (Webster & Valett, 2006). Background samples for DOC,  $^{13}\text{C}$ -DOC and  $\text{Br}^-$  were taken immediately prior to the solute addition at all eight stations and then hourly during the experiment at the reference site. The solute pulse was sampled downstream once concentrations in the stream stabilized (plateau period). DOC and  $^{13}\text{C}$ -DOC samples were filtered through preashed (6 h, 500 °C) Whatman GF/F filters (Whatman, Inc., Florham Park, NJ, U.S.A.) and  $\text{Br}^-$  samples were filtered through 0.2- $\mu\text{m}$  Gelman HT filters (Pall Corp., East Hills, NY, U.S.A.). DOC was measured by Pt-catalysed persulphate oxidation (OI Analytical Model 1010, College Station, TX, U.S.A.) and  $\text{Br}^-$  was analysed by ion chromatography (Dionex Model 500, Sunnyvale, CA, U.S.A.). C isotope samples were concentrated using rotary evaporation, acidified, lyophilized, combusted and the  $\text{CO}_2$  analysed with an elemental analyser (EA 3000, Eurovector, Milan, Italy) interfaced to an isotope ratio mass spectrometer (GV Instruments, Manchester, U.K.) (Gandhi *et al.*, 2004). Isotope ratios are expressed in per mil,  $\delta^{13}\text{C} = [(R_{\text{SAMPLE}}/R_{\text{PDB}}) - 1] \times 1000$ , where  $R$  is the abundance ratio of  $^{13}\text{C}/^{12}\text{C}$ . The standard was V-PDB and the reproducibility of three replicate samples at natural abundance levels was  $\leq 0.2\text{‰}$  and was 0.03 atom % for enriched samples (1.16–7.53 atom %) (Gandhi *et al.*, 2004; Wiegner *et al.*, 2005a).

### $^{13}\text{C}$ -DOC tracer calculations

The  $^{13}\text{C}$  enrichment (fractional abundance,  $F$ ) in the stream water DOC following the tracer addition was calculated using measured  $\delta^{13}\text{C}$ -DOC values as,  $F = R_{\text{SAMPLE}}/(R_{\text{SAMPLE}} + 1) = {}^{13}\text{C}/\text{C}_T$ , where  $\text{C}_T = {}^{12}\text{C} + {}^{13}\text{C}$ . These data were then used in a mixing model to provide estimates of the fraction of DOC ( $f_T$ ) in the stream originating from the tracer during the addition,  $f_T = [(F_{\text{MIX}} - F_{\text{STREAM}})/(F_{\text{TRACER}} - F_{\text{STREAM}})]$ , where  $F_{\text{MIX}}$  is the measured fractional abundance of  $^{13}\text{C}$ -DOC in White Clay Creek water amended with the  $^{13}\text{C}$ -DOC tracer,  $F_{\text{STREAM}}$  is the measured fractional abundance of  $^{13}\text{C}$ -DOC in White Clay Creek water prior to the  $^{13}\text{C}$ -DOC tracer addition, and  $F_{\text{TRACER}}$  is the measured fractional abundance for the  $^{13}\text{C}$ -DOC tracer. The concentration of  $^{13}\text{C}$ -DOC in White Clay Creek water originating from the tracer ( ${}^{13}\text{C}_{\text{TRACER}}$ ) was calculated as,  ${}^{13}\text{C}_{\text{TRACER}} = f_T \text{C}_T F_{\text{TRACER}}$ .

### $^{13}\text{C}$ -DOC uptake in White Clay Creek

Dissolved organic carbon in stream water is not uniformly biodegradable and the molecules within this pool most probably represent a nearly continuous array of biological labilities. The longitudinal loss rate curve for the  $^{13}\text{C}$ -DOC tracer can be approximated from the sum of a few first-order loss rate curves for different DOC lability classes. In this work, we resolved two longitudinal loss rate coefficients ( $k^L_1, k^L_2$ ) according to the equation,  $[{}^{13}\text{C} - \text{DOC}] = C_1 e^{-k^L_1 x} + C_2 e^{-k^L_2 x}$ , where  $[{}^{13}\text{C} - \text{DOC}]$  is the average background-corrected,  $\text{Br}^-$  normalized  $^{13}\text{C}$ -DOC plateau concentration at distance ( $x$ ) downstream from the addition site and  $C_1$  and  $C_2$  are the added (i.e. at  $x = 0$ )  $^{13}\text{C}$ -DOC concentrations in the respective DOC lability classes. The parameters ( $k^L_1, k^L_2, C_1$  and  $C_2$ ) were estimated by nonlinear least squares analysis (PROC NLIN, SAS 8.1; SAS Institute Inc., Cary, NC, U.S.A.). Because the sample variances among observations at a station were found to decline as a power function of downstream distance, we weighted the observations by the inverse of this function, i.e.  $w(x) = x^{0.76}$ , where  $w(x)$  is the weighting factor applied at distance,  $x$ . We report the approximate asymptotic 95% confidence limits as computed by the least squares analysis (PROC NLIN). We used the extra sums of squares principle (Draper & Smith, 1998) to determine that two lability classes

explained more of the weighted variance ( $r^2 = 0.97$ ) than did a single class ( $r^2 = 0.89$ ,  $P < 0.001$ ), whereas a model involving three lability classes offered no further improvement ( $P > 0.05$ ). From the estimates for  $k_L$ , we calculated the uptake length,  $S_W$  and mass transfer coefficients,  $V_f$ , for each lability class, using the relationships,  $S_W = 1/k_L$  and  $V_f = V_W d k_L$ , in which  $V_W$  is water velocity and  $d$  is water depth (Webster & Valett, 2006). Runkel (2007) pointed out that the estimate of uptake length and its conversion to mass transfer coefficients can be complicated by longitudinal dispersion and longitudinal increase in stream flow. The longitudinal dispersion measured in this study was in a range that affects uptake length by only a few per cent, and the effects of longitudinal dilution were accounted for via the use of a conservative tracer.

#### *<sup>13</sup>C-DOC tracer and stream water DOC lability profiling*

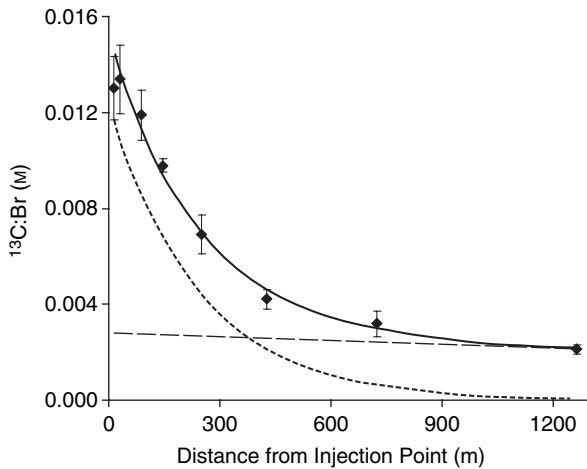
Plug-flow bioreactors were used to measure concentrations of biodegradable DOC (BDOC) (Kaplan & Newbold, 1995) and to separate the BDOC in stream water and the <sup>13</sup>C-DOC tracer into different biological labilities classes (i.e. a lability profile). The bioreactors, chromatography columns filled with sintered glass beads (Siran<sup>®</sup>; Jaeger Biotech Engineering Inc., Costa Mesa, CA, U.S.A.), were kept in the dark and continuously fed in a once through, up-flow mode at 4 mL min<sup>-1</sup> with filtered stream water from White Clay Creek. Filtration was performed with a three-stage glass fibre cartridge system of 75-, 25- and 0.3- $\mu$ m filters in series that removed larger particles, but allowed 95% of the bacteria suspended in the stream water to pass into the filtrate. The filtrate was stored in a reservoir that was changed weekly and served as a continuous source of bacteria and DOC to colonize and maintain the bioreactors. We constructed eight bioreactors with empty bed contact times (EBCT; equal to volume of the bioreactor divided by the flow rate) that increased in a geometric series (0.5, 1.5, 3, 6, 9, 18.5, 37 and 73.8 min) and operated them for 1 year prior to this study. BDOC concentrations were operationally defined as the difference between the DOC concentration in the influent and effluent waters of the bioreactors, as DOC removal within the bioreactors is overwhelmingly a biological process, with abiotic adsorption accounting for *c.* 10% of the BDOC estimate (Kaplan & Newbold, 1995). Two days after

the whole-stream release, freshly collected samples of stream water or stream water amended with <sup>13</sup>C-DOC tracer (7 L White Clay Creek water + 8 mL <sup>13</sup>C-DOC tracer) were pumped into the bioreactors, the influent water was sampled and then the effluent was collected after three bioreactor bed volumes had passed to waste. BDOC concentrations for the <sup>13</sup>C-DOC tracer were calculated from measures of  $\delta^{13}\text{C}$ -DOC, while stream water BDOC concentrations were calculated from the bulk DOC concentrations. To calculate the incremental uptake of DOC from each increase in EBCT, we subtracted the BDOC concentration measured in the smallest bioreactor in the series from the BDOC concentration of the next larger bioreactor, and continued this calculation through the series. BDOC concentrations associated with incremental increases in EBCT were expressed as a percentage of the bulk DOC when divided by the concentration of DOC in the influent water. Concentrations of <sup>13</sup>C-DOC from the influent and effluent waters of the bioreactors were calculated using a similar approach as described in the <sup>13</sup>C-tracer uptake section (the same three equations were used).

#### **Results**

The <sup>13</sup>C-DOC tracer addition increased the stream DOC concentration by 5%, from 1.48 ( $\pm 0.06$ ) (mean  $\pm$  SD) to 1.55 mg C L<sup>-1</sup> ( $\pm 0.05$ ), and enriched the stream <sup>13</sup>C-DOC pool from  $\delta^{13}\text{C}$  -25.8‰ ( $\pm 0.4$ ‰) to 337‰ ( $\pm 35$ ‰; average from stations 1 and 2  $\pm$  SD). From the least squares analysis of the longitudinal <sup>13</sup>C-DOC uptake curves (Fig. 1, Table 1), 82% of the tracer comprised a labile component with an uptake length ( $S_{W_1} = 1/k_{L_1}$ ) of 238 m and a mass transfer coefficient ( $V_{f1}$ ) of 20.3  $\mu\text{m s}^{-1}$ . The second semi-labile, component comprised 18% of the tracer, with an uptake length of 4546 m and a mass transfer coefficient of 1.1  $\mu\text{m s}^{-1}$ .

On the day of the injection, stream flow increased longitudinally from 12.8 L s<sup>-1</sup> at the point of injection ( $x = 0$  m) to 17.6 L s<sup>-1</sup> at the lowermost sampling station ( $x = 1265$  m), as inferred from the conservative tracer injection. Model-fitting of the conservative tracer yielded a cross-sectional area of 0.37 m<sup>2</sup>, a longitudinal dispersion of 0.21 m<sup>2</sup> s<sup>-1</sup>, a transient storage exchange coefficient of  $5.33 \times 10^{-4}$  s<sup>-1</sup>, and a cross-sectional area of transient storage of 0.057 m<sup>2</sup>. From these, the calculated average velocity,  $V_W$ , was

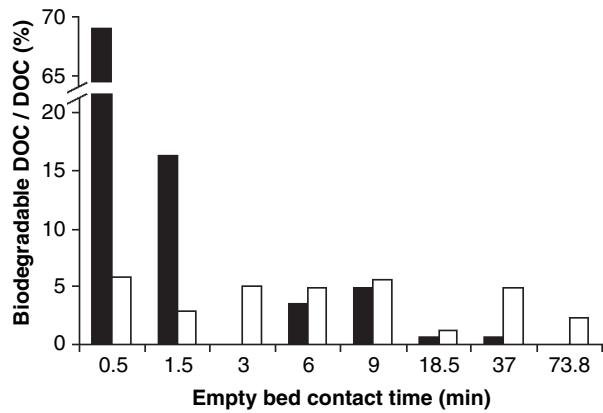


**Fig. 1** Longitudinal uptake of the  $^{13}\text{C}$ -DOC tracer during a whole-stream addition, corrected for stream water dilution using concentrations of bromide, a conservative tracer. The diamonds and error bars show the mean  $\pm$  SD of 5 samples, and the solid line, dotted line and dashed line represent uptake curves for the overall, labile and semi-labile classes of the  $^{13}\text{C}$ -DOC tracer respectively.

$0.044 \text{ m s}^{-1}$  and depth,  $d$  was  $0.11 \text{ m}$  using the measured stream width of  $3.33 \text{ m}$  ( $n = 19$ ,  $\text{SD} = 1.4 \text{ m}$ ). The uptake lengths and average velocity were used to calculate turnover times ( $S_w/V_w$ ) equal to 1.5 and 29 h for the labile and semi-labile components respectively.

To link DOC dynamics in the bioreactors to those in the stream, we matched the lability profile of the  $^{13}\text{C}$ -DOC tracer in the bioreactors with the uptake of the tracer in the stream and then extrapolated stream water DOC dynamics in the bioreactors to the stream. This approach allowed us to scale the uptake of stream water DOC lability classes in the bioreactors to the uptake of stream water DOC lability classes in the stream.

Based on the  $^{13}\text{C}$ -DOC stream addition, 82% of the  $^{13}\text{C}$ -DOC was labile and behaved as a single lability class. Nearly the same fraction (85%) of the  $^{13}\text{C}$ -DOC



**Fig. 2** Lability profiles of dissolved organic carbon (DOC) from White Clay Creek stream water (open bars) and the  $^{13}\text{C}$ -DOC tracer (solid bars) generated in stream water-fed bioreactors as a function of empty bed contact time.

was consumed in the two bioreactors with EBCT times of  $\leq 1.5 \text{ min}$  (Fig. 2). These two bioreactors, when fed stream water alone, consumed 8.6% of the stream water DOC or  $0.155 \text{ mg C L}^{-1}$ . Thus, we inferred that  $0.155 \text{ mg C L}^{-1}$  of the stream water DOC was equivalent in lability to the lability class that accounted for 82% of the  $^{13}\text{C}$ -DOC tracer with a mass transfer coefficient ( $V_{f1}$ ) of  $20.3 \mu\text{m s}^{-1}$ , and so represented an uptake flux,  $U$ , of  $272 \text{ mg C m}^{-2} \text{ day}^{-1}$  ( $0.155 \text{ mg C L}^{-1} \times V_{f1}$ ) from the water column to the streambed. Additionally, 11% of the  $^{13}\text{C}$ -DOC tracer was consumed in bioreactors with EBCT  $\geq 1.5 \text{ min}$  and  $\leq 73.8 \text{ min}$ , a percentage comparable to the 18% of the  $^{13}\text{C}$ -DOC tracer that was semi-labile in the stream injection. The same bioreactors, when fed stream water alone, consumed 24% of the stream water DOC or  $0.42 \text{ mg C L}^{-1}$ . Thus, we inferred that  $0.42 \text{ mg C L}^{-1}$  of the stream water DOC was equivalent in lability to the semi-labile class of the  $^{13}\text{C}$ -DOC tracer that had a mass transfer coefficient ( $V_{f2}$ ) of  $1.1 \mu\text{m s}^{-1}$ , and therefore represented an uptake flux,

**Table 1**  $^{13}\text{C}$ -DOC tracer uptake parameters during whole-stream addition

Lability class	C ( $\mu\text{mol } ^{13}\text{C L}^{-1}$ )	Variable			
		$k_L$ ( $\text{km}^{-1}$ )	$S_w$ (m)	$V_f$ ( $\mu\text{m s}^{-1}$ )	$U$ ( $\text{mg C m}^{-2} \text{ day}^{-1}$ )
Labile	0.553 (0.44–0.66)	4.20 (2.65–5.75)	238	20.3	272
Semi-labile	0.123 (–0.002–0.249)	0.22 (–0.60–1.0)	4546	1.1	40

Estimates (95% confidence intervals) for initial concentrations ( $C_{1,2}$ ) of the labile and semi-labile  $^{13}\text{C}$ -DOC classes shown with the uptake rate coefficients ( $k_{L1}$ ,  $k_{L2}$ ). Calculated uptake lengths ( $S_{w1,2}$ ), mass transfer coefficients ( $V_{f1,2}$ ) and total DOC uptake ( $U$ ) are also shown.

$U$ , of  $40 \text{ mg C m}^{-2} \text{ day}^{-1}$  ( $0.42 \text{ mg C L}^{-1} \times V_{L_2}$ ) to the streambed.

## Discussion

With a turnover time of 1.5 h and an uptake length that is *c.* 5.7% of the third-order reach length (4.2 km), labile DOC in stream water is an energy source that is consumed during downstream transport at the reach scale. This result supports the paradigm that a small pool of labile organic molecules cycles rapidly and satisfies a large portion of the energy demands of aquatic heterotrophic bacteria (Kaplan & Newbold, 2003). In contrast, the uptake length for the semi-labile DOC class was fourfold longer than the distance of the experimental reach.

Although the semi-labile component contributed a significant 8% of the explained weighted variance in the two lability class model, the 95% confidence limits for the parameters  $C_2$ , and  $k_{L_2}$  associated with this component were large and included zero (Table 1). The concentration of the labile component exceeded that of the semi-labile component at all but the three most downstream stations (Fig. 1), thus limiting our ability to detect a longitudinal loss in the semi-labile component within our designated experimental reach. Previous measures of the uptake of the same  $^{13}\text{C}$ -DOC tracer by White Clay Creek sediments in mesocosms (Wiegner *et al.*, 2005b) support the whole-stream results concerning a semi-labile DOC fraction. In the mesocosms, the semi-labile DOC class comprised 12% of the tracer, comparable to the 18% estimated from the field injection. The uptake rate of the semi-labile component in the mesocosms averaged 0.051 that of the labile component, nearly identical to the ratio of  $k_{L_2}/k_{L_1}$  (0.052) from this study (Table 1).

While only a small amount of heterotrophic respiration could be supported at the reach scale by the semi-labile DOC class, this lability class may have an important role at the catchment scale. Specifically, the behaviour of the semi-labile DOC in the bioreactors and stream supports the conceptual model that a large pool of DOC degrades slowly during transport through river networks (Kaplan & Newbold, 2003), providing a degree of metabolic stability within stream ecosystems (Wetzel, 2003). We know little about the sources of labile and semi-labile DOC forms in streams, and even less about potential interactions and photolytic transformations of these DOC lability

classes while in transport. However, the uptake lengths of the labile and semi-labile DOC classes suggest that they provide an important energy link between upstream and downstream ecosystems at the reach and catchment scales respectively. The idea that downstream DOC exports influence the structure and function of stream ecosystems was originally proposed in the River Continuum Concept (Vannote *et al.*, 1980), and our findings support the assertion that headwater streams are critical to downstream river ecosystems (Meyer *et al.*, 2003).

It is important to note that our estimates were based on a single whole-stream release, and that the release was performed under drought-exacerbated low flow conditions. Higher stream flow velocities and water depths in non-drought conditions would extend the uptake lengths of each DOC lability class, simply based on hydrodynamic scaling. Nevertheless, the uptake velocity for the labile DOC class of the tracer falls between those for readily labile monomeric carbohydrates, including glucose ( $55 \mu\text{m s}^{-1}$ ) and arabinose ( $21 \mu\text{m s}^{-1}$ ) added to 10 streams in upstate New York (Newbold *et al.*, 2006), glucose ( $26\text{--}46 \mu\text{m s}^{-1}$ ) and arabinose ( $9\text{--}12 \mu\text{m s}^{-1}$ ) in White Clay Creek (L.A. Kaplan & J.D. Newbold, unpubl. data), the disaccharide sucrose ( $146 \mu\text{m s}^{-1}$ ) added to Hugh White Creek (Munn & Meyer, 1990), the volatile fatty acid acetate ( $16\text{--}47 \mu\text{m s}^{-1}$ ) added to two first-order streams at Coweeta Hydrologic Laboratory (Hall & Meyer, 1998) and the amino acid, glutamic acid ( $23 \mu\text{m s}^{-1}$ ) added to Hugh White Creek (Brookshire *et al.*, 2005). Additionally, we recognize that our description of the uptake of bulk DOC as the sum of uptake rates associated with two distinct carbon pools is an over simplification. The complex dynamics associated with the thousands of DOC molecules present in stream water (Kim *et al.*, 2006) would probably more closely resemble a suite of uptake curves representing a series of increasingly resistant C compounds (Cummins *et al.*, 1972) if we had the ability to make such measurements.

We used our measured DOC uptake lengths in White Clay Creek to assess the importance of DOC in transport to stream metabolism by comparing the mass flux of DOC in transport to heterotrophic respiration. Historic stream community respiration data for White Clay Creek measured during the autumn (early October) prior to peak leaf fall were used to estimate heterotrophic respiration. It was

important to match the season, as community respiration is lower just prior to leaf fall, when the standing stock of benthic organic matter is at or near an annual low point. To estimate heterotrophic respiration ( $263.5 \text{ mg C m}^{-2} \text{ day}^{-1}$ ), we corrected measured community respiration ( $0.85 \text{ g O}_2 \text{ m}^{-2} \text{ day}^{-1}$ ; Bott *et al.*, 1985) for algal respiration ( $0.178 \text{ g O}_2 \text{ m}^{-2} \text{ day}^{-1}$ ), assuming that algal respiration is *c.* 25% of gross primary production (Geider & Osborne, 1989), and converted this estimate from units of  $\text{O}_2$  to C, assuming a respiratory quotient of 0.85. Additionally, these estimates were made in mesocosms that exclude the hyporheic zone, so we corrected the estimates of community respiration for hyporheic zone respiration, which we estimated to be 41% of the community respiration (Battin *et al.*, 2003), resulting in a corrected estimate of  $447 \text{ mg C m}^{-2} \text{ day}^{-1}$  for heterotrophic respiration ( $263.5 \text{ mg C m}^{-2} \text{ day}^{-1} / 0.59 = 447 \text{ mg C m}^{-2} \text{ day}^{-1}$ ). This means that the uptake flux of labile bulk DOC fraction ( $272 \text{ mg C m}^{-2} \text{ day}^{-1}$ ) could account for 61% of the heterotrophic community respiration, with the semi-labile bulk DOC fraction ( $40 \text{ mg C m}^{-2} \text{ day}^{-1}$ ) accounting for an additional 9% of the heterotrophic respiration in White Clay Creek. A comparison of lability profiles (Fig. 2) shows that our tracer did not capture the full complexity of stream water DOC lability classes, and the balance of stream ecosystem respiration could be supported by lability classes not included in our tracer as well as particulate organic carbon (Hedin, 1990).

Our finding that DOC supports a significant portion of stream ecosystem metabolism is consistent with the results of a previous field manipulation and a mesocosm experiment that involved stream water and sediments from White Clay Creek (Bott, Kaplan & Kuserk, 1984; Wiegner *et al.*, 2005b). In the field manipulation, benthic bacterial biomass in a spring seep decreased by 55% when sediments from a higher DOC environment were transferred and incubated in a lower DOC environment, suggesting that DOC was critical in supporting benthic bacterial biomass production (Bott *et al.*, 1984). In the mesocosm experiment, the same  $^{13}\text{C}$ -DOC tracer as used in this study was injected into 15-L mesocosms with streambed sediments and re-circulating stream water and was taken up at rates sufficient to support 51% of the bacterial respiration (Wiegner *et al.*, 2005b). The mass transfer coefficients for the two lability classes were 2.6- to 3-fold higher in the mesocosm

experiment than the values reported here that were measured directly in the stream. These differences are most probably an artifact of the sediment handling in the mesocosm study. Specifically, we held streambed sediments trays for 18 days in flumes prior to the experiments, which permitted benthic algal growth on sediments and protected the biofilms from storm disturbances. The respiration rates measured for the sediment trays in the mesocosms were 2.5-fold higher than respiration rates previously measured in White Clay Creek, a difference that was similar in magnitude to the increased microbial demand for DOC (expressed as  $V_f$ ) in the mesocosms compared to the stream. Thus, we attribute the values measured in the mesocosms to be overestimates of the metabolic demands in the stream as a result of biota changes in the sediments during incubation in the flumes.

The bioreactors used in our study are laboratory tools that facilitate measurements of DOC uptake without the confounding issues of algal growth and excretion, photolysis, inputs from ground water or tributaries, leaching of benthic organic matter and excretions from animals. In this manner, the bioreactors permit measurements that cannot be made *in situ*. Qualitative and quantitative gradients in DOC and bacterial densities, community composition, and metabolism form from the inflow to the outflow within the bioreactors, as the most biologically labile DOC molecules are rapidly metabolized (over short bioreactor volumes) and support high bacterial densities, while the more semi-labile DOC molecules are metabolized after longer exposures (Kaplan & Newbold, 1995; Riemann & Søndergaard, 2004). By establishing a series of bioreactors with increasing EBCTs, we were able to collect DOC samples from the chemical gradient that would form in a single large volume bioreactor without the disturbance associated with removing samples from multiple ports along the length of a bioreactor. The geometric series we established allowed us to deconstruct a cumulative DOC uptake curve into individual BDOC lability classes based on bioreactor residence time. That is, EBCT, or the length of time DOC is available for uptake, became a surrogate for DOC biological lability, and by comparing the behaviour of the  $^{13}\text{C}$ -DOC tracer in the bioreactors and the stream, we derived an appropriate scaling that facilitated the transfer of information obtained in the laboratory to the environment.

The  $^{13}\text{C}$ -DOC tracer, derived from tulip poplar trees, is a natural product that is present in White Clay Creek. However, our goal was not to assess the importance of tulip poplar leachate to stream respiration, and an appropriate tracer could be any organic molecule or suite of organic molecules that can be metabolized by heterotrophic bacteria in the bioreactors and the stream. Since our primary interest was to determine the behaviour of the complex mixture of natural DOC in the stream water, a tracer with multiple DOC lability classes facilitated this effort. The validity of our DOC lability profiling scheme rests on a primary assumption regarding the fidelity of the bioreactors as models of stream processes, i.e. that organic molecules of similar lability at the bioreactor scale will have similar lability in the stream; the assumption being that the ordering of DOC lability is preserved across systems and scales. Given the complexity of mechanism-based up-scaling, which involves issues of both hydrodynamics and microbial processes, and the current lack of understanding of these issues, we believe that our empirical approach offers a far more robust method of describing whole stream dynamics than would, for example, the construction of an ecosystem-level model parameterized from bioreactor dynamics and fine-scale hydrodynamic measurements.

While our research was limited to a headwater stream ecosystem, implications of our inquiries extend well beyond the energy dynamics of the stream itself. Each year *c.* 0.25 Pg of terrigenous DOC enters the global ocean through riverine transport, the largest transfer of reduced C from the continents to the ocean (Cauwet, 2002). Once organic matter (dissolved and particulate) enters the river system, 50–90% gets metabolized in transit to the ocean (Cole & Caraco, 2001; Richey *et al.*, 2002) and 80% of the riverine DOC that reaches the coast may be mineralized over the continental shelves with a half-life of approximately a decade (Hansell, Kadko & Bates, 2004). Thus, riverine transport and processing of DOC may play a much more important role in terrestrial C cycling and the global C cycle than commonly appreciated (Grace & Malhi, 2002). Lastly, our empirical approach to assessing the biological lability of DOC can be applied to other ecosystems where metabolism of DOC contributes to ecosystem respiration.

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## References

- Amon R.M.W. & Benner R. (1996) Photochemical and microbial consumption of dissolved organic carbon and dissolved oxygen in the Amazon River system. *Geochimica Cosmochimica Acta*, **60**, 1783–1792.
- Aufdenkampe A.K., Hedges J.L., Richey J.E., Krusche A.V. & Llerena C.A. (2001) Sorptive fractionation of dissolved organic nitrogen and amino acids onto fine sediments with the Amazon Basin. *Limnology and Oceanography*, **46**, 1921–1935.
- Battin T.J., Kaplan L.A., Newbold J.D. & Hendricks S.P. (2003) A mixing model analysis of stream solute dynamics and the contribution of a hyporheic zone to ecosystem function. *Freshwater Biology*, **48**, 995–1014.
- Bott T.L., Kaplan L.A. & Kuserk F.T. (1984) Benthic bacterial biomass supported by stream water dissolved organic matter. *Microbial Ecology*, **10**, 335–344.
- Bott T.L., Brock J.T., Dunn C.S., Naiman R.J., Ovinck R.W. & Petersen R.C. (1985) Benthic community metabolism in four temperate stream systems, an inter-biome comparison and evaluation of the river continuum concept. *Hydrobiologia*, **123**, 3–45.
- Brookshire E.N., Valett H.M., Thomas S.A. & Webster J.R. (2005) Coupled cycling of dissolved organic nitrogen and carbon in a forest stream. *Ecology*, **86**, 2486–2496.
- Cauwet G. (2002) Dissolved organic matter in the open ocean. In: *Biogeochemistry of Marine Dissolved Organic Matter* (Eds D.A. Hansel & C.A. Carlson), pp. 579–609. Academic Press, San Diego, CA.
- Cole J.J. & Caraco N.F. (2001) Carbon in catchments, connecting terrestrial carbon losses with aquatic metabolism. *Marine and Freshwater Research*, **52**, 101–110.
- Cummins K.W., Klug J.J., Wetzel R.G., Petersen R.C., Suberkripp K.F., Manny B.A., Wuycheck J.C. & Howard F.O. (1972) Organic enrichment with leaf leachate in experimental lotic ecosystems. *BioScience*, **22**, 719–722.

- Draper N.R. & Smith H. (1998) *Applied Regression Analysis*. J. W. Wiley, Hoboken, 736 pp.
- Frazier S.W., Kaplan L.A. & Hatcher P.G. (2005) Molecular characterization of biodegradable dissolved organic matter using bioreactors and [ $^{13}\text{C}/^{12}\text{C}$ ] tetramethylammonium hydroxide thermochemolysis GC-MS. *Environmental Science and Technology*, **39**, 1479–1491.
- Gandhi H., Wiegner T.N., Ostrom P.H., Kaplan L.A. & Ostrom N.E. (2004) Isotopic ( $^{13}\text{C}$ ) analysis of dissolved organic carbon in stream water using an elemental analyzer coupled to a stable isotope ratio mass spectrometer. *Rapid Communications in Mass Spectrometry*, **18**, 903–906.
- Geider R.J. & Osborne B.A. (1989) Respiration and microalgal growth: a review of the quantitative relationship between dark respiration and growth. *New Phytologist*, **112**, 327–341.
- Grace J. & Malhi Y. (2002) Carbon dioxide goes with the flow. *Nature*, **416**, 594–595.
- Hall R.O. Jr & Meyer J.L. (1998) The trophic significance of bacteria in a detritus-based stream food web. *Ecology*, **79**, 1995–2012.
- Hansell D.A., Kadko D. & Bates N.R. (2004) Degradation of terrigenous dissolved organic carbon in the western Arctic Ocean. *Science*, **304**, 858–861.
- Hedin L.O. (1990) Factors controlling sediment community respiration in woodland stream ecosystems. *Oikos*, **57**, 94–105.
- Hullar M.A.J., Kaplan L.A. & Stahl D.A. (2006) Recurring seasonal dynamics of microbial communities in stream habitats. *Applied and Environmental Microbiology*, **72**, 713–722.
- Kaplan L.A. & Newbold J.D. (1995) Measurement of stream water biodegradable dissolved organic carbon with a plug-flow bioreactor. *Water Research*, **29**, 2696–2706.
- Kaplan L.A. & Newbold J.D. (2003) The role of monomers in stream ecosystem metabolism. In: *Aquatic Ecosystems, Interactivity of Dissolved Organic Matter* (Eds S.E.G. Findlay & R.L. Sinsabaugh), pp. 97–119. Academic Press, New York.
- Kim S., Kaplan L.A. & Hatcher P.G. (2006) Biodegradable dissolved organic matter in a temperate and a tropical stream determined from ultra-high resolution mass spectrometry. *Limnology and Oceanography*, **51**, 1054–1063.
- Mayorga E., Aufdenkampe A.K., Masiello C.A., Krusche A.V., Hedges J.L., Quay P.D., Richey J.E. & Brown T.A. (2005) Young organic matter as a source of carbon dioxide outgassing from Amazonian rivers. *Nature*, **436**, 538–541.
- Meyer J.L., Kaplan L.A., Newbold J.D. et al. (2003) *Where Rivers Are Born, the Scientific Imperative for Defending Small Streams and Wetlands*. American Rivers and Sierra Club, Washington, D.C., and San Francisco, CA.
- Munn N.L. & Meyer J.L. (1990) Habitat-specific solute retention in two small streams: an intersite comparison. *Ecology*, **71**, 2069–2082.
- Newbold J.D., Elwood J.W., O'Neill R.V. & Sheldon A.L. (1983) Phosphorus dynamics in a woodland stream ecosystem, a study of nutrient spiralling. *Ecology*, **64**, 1249–1265.
- Newbold J.D., Bott T.L., Kaplan L.A., Sweeney B.W. & Vannote R.L. (1997) Organic matter dynamics in White Clay Creek, Pennsylvania, U.S.A.. *Journal of the North American Benthological Society*, **16**, 46–50.
- Newbold J.D., Bott T.L., Kaplan L.A., Dow C.L., Martin L.A., Van Horn D.J. & de Long A.A. (2006) Uptake of nutrients and organic C in streams in New York City drinking-water-supply watersheds. *Journal of the North American Benthological Society*, **25**, 998–1017.
- Pace M.L., Cole J.J., Carpenter S.R., Kitchel J.F., Hodgson J.R., Van de Bogert M.C., Bade D.L., Kritzberg E.S. & Bastviken D. (2004) Whole-lake carbon-13 additions reveal terrestrial support of aquatic food webs. *Nature*, **427**, 240–243.
- Richey J.E., Melack J.M., Aufdenkampe A.K., Ballester V.M. & Hess L.L. (2002) Outgassing from Amazonian rivers and wetlands as a large tropical source of atmospheric  $\text{CO}_2$ . *Nature*, **416**, 617–620.
- Riemann L. & Søndergaard M. (2004) Profiles of bacterial community composition and metabolic potential through a plug-flow bioreactor fed with lake water. *Journal of Plankton Research*, **26**, 973–978.
- Runkel R.L. (1998) One dimensional transport with inflow and storage (OTIS), a solute transport model for streams and rivers. *U.S. Geological Survey Water-Resources Investigations Report*, **98-4018**, 1–73.
- Runkel R.L. (2007) Toward a transport-based analysis of nutrient spiraling and uptake in streams. *Limnology and Oceanography Methods*, **5**, 50–62.
- Tank J.L., Meyer J.L., Sanzone D.M., Mulholland P.J., Webster J.R., Peterson B.J., Wollheim W.M. & Leonard N.E. (2000) Analysis of nitrogen cycling in a forest stream during autumn using a  $^{15}\text{N}$ -tracer addition. *Limnology and Oceanography*, **45**, 1013–1029.
- Vannote R.L., Minshall G.W., Cummins K.W., Sedell J.R. & Cushing C.E. (1980) The river continuum concept. *Canadian Journal of Fisheries and Aquatic Sciences*, **37**, 130–137.
- Volk C.J., Volk C.B. & Kaplan L.A. (1997) The chemical composition of biodegradable dissolved organic matter in stream water. *Limnology and Oceanography*, **42**, 39–44.

- Webster J.R. & Valett H.M. (2006) Solute dynamics. In: *Methods in Stream Ecology* (Eds F.R. Hauer & G.A. Lamberti), pp. 169–185. Academic Press, San Diego, CA.
- Wetzel R.G. (1995) Death, detritus, and energy flow in aquatic ecosystems. *Freshwater Biology*, **33**, 83–89.
- Wetzel R.G. (2003) Dissolved organic carbon, detrital energetics, metabolic regulators, and drivers of ecosystem stability of aquatic ecosystems. In: *Aquatic Ecosystems Interactivity of Dissolved Organic Matter* (Eds S.E.G. Findlay & R.L. Sinsabaugh), pp. 455–477. Academic Press, New York.
- Wiegner T.N., Kaplan L.A., Newbold J.D. & Ostrom P.H. (2005a) Synthesis of a  $^{13}\text{C}$ -labeled tracer for stream DOC, labeling tulip poplar carbon with  $^{13}\text{CO}_2$ . *Ecosystems*, **8**, 1–11.
- Wiegner T.N., Kaplan L.A., Newbold J.D. & Ostrom P.H. (2005b) Contribution of dissolved organic C to stream metabolism, a mesocosm study using  $^{13}\text{C}$ -enriched tree tissue leachate. *Journal of the North American Benthological Society*, **24**, 48–67.

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