Limnol. Oceanogr., 57(1), 2012, 76–89
© 2012, by the Association for the Sciences of Limnology and Oceanography, Inc.
doi:10.4319/lo.2012.57.1.0076

Distinguishing dynamics of dissolved organic matter components in a forested stream using kinetic enrichments

Brian D. Lutz,[a,*] Emily S. Bernhardt,[b] Brian J. Roberts,[c] Rose M. Cory,[d] and Patrick J. Mulholland[e]

[a] University Program in Ecology, Duke University, Durham, North Carolina
[b] Department of Biology, Duke University, Durham, North Carolina
[c] Louisiana Universities Marine Consortium, DeFelice Marine Center, Chauvin, Louisiana
[d] Environmental Sciences and Engineering, University of North Carolina, Chapel Hill, North Carolina
[e] Environmental Sciences Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee

Abstract

Traditional methods for investigating stream solute biogeochemistry measure longitudinal rates of uptake by increasing either the concentration or isotopic composition of solutes. These methods cannot be applied to dissolved organic matter (DOM) because we cannot replicate the heterogeneous native DOM pool. We explored an alternative approach, attempting to displace or enhance benthic uptake of native DOM by supplying an exogenous source of labile carbon or by enriching the stream with inorganic nitrogen. This approach allows us to measure uptake rates of enriched solutes, as well as changes in the concentration and composition of native DOM resulting from the experimental manipulations. We examined DOM composition using fluorescence characterization. We were able to elicit changes in the chemical composition of native DOM by differentially altering the dynamics of autotrophic production and heterotrophic uptake within the second-order reach of Walker Branch, a well-studied stream in eastern Tennessee. Supplying heterotrophs with labile carbon resulted in an increase in fluorescence associated with terrestrially derived DOM. Stimulating algae by adding inorganic nitrogen increased autochthonous production and indirectly displaced heterotrophic demand for terrestrial DOM due to increased in-stream production of bioavailable DOM. While we were able to alter the composition of the native DOM pool, we observed little change in DOM concentrations. The ability to differentiate between DOM subcomponents provides insight into processes controlling DOM production and consumption that cannot be gained by treating DOM as a single bulk pool.

Dissolved organic matter (DOM) represents an important source of energy in stream ecosystems (Fisher and Likens 1973; Cummins 1974), fueling heterotrophic microbial production and establishing the foundation on which stream trophic structure and ecosystem function relies (Minshall et al. 1983; Webster and Meyer 1997). DOM, however, is a single term applied to an extremely heterogeneous pool of organic molecules, with independent subcomponents that can exhibit distinct dynamics. Understanding the role that DOM plays in stream ecosystems requires that we are able to elucidate the processes that govern the dynamics of different DOM subcomponents.

The heterogeneity of native DOM in streams restricts our ability to manipulate DOM in an experimental context because we are unable to synthesize the native DOM pool in its entirety. Nutrient Spiraling Theory (NST; Newbold et al. 1981; Stream Solute Workshop 1990), which has been a powerful tool for investigating solute biogeochemistry in streams, cannot be directly applied because this approach requires that we are able to add the diversity of DOM molecules to a stream in order to measure rates of longitudinal uptake. While several studies have used NST with manipulations of specific subcomponents of the DOM pool, such as litter leachates (McDowell 1985; Wiegner et al. 2005; Bernhardt and McDowell 2008) or simple organic monomers (Bernhardt and Likens 2002; Brookshire et al. 2005), these experiments share a common limitation in that they focus on only a small fraction of the DOM pool that is often highly dissimilar from DOM that is cycling under background conditions.

Recognizing that we cannot add the diversity of native DOM molecules to a stream to directly measure in-stream removal, we have taken an alternative approach in which we have instead attempted to alter the processes controlling in-stream DOM uptake and production through whole-ecosystem enrichment of labile carbon and nitrogen. Assessing how native DOM responds to the resulting alterations in heterotrophic and autotrophic activity should provide similar insights to what could be gained through a traditional spiraling approach were we able to directly manipulate DOM concentrations. In streams, DOM is derived from either the hydrologic flux of terrestrial carbon that is fixed outside of the stream channel (Fisher and Likens 1973) or from the in situ fixation of inorganic C by autotrophs in the water column or attached to the benthos (autochthonous production; Minshall 1978; Fig. 1A). Once in the stream channel, the dominant sink for DOM is heterotrophic uptake, and the portion of the DOM pool that is not consumed is transported downstream (Fig. 1A). While there is little we can do to alter the hydrologic sourcing of terrestrial DOM to the stream channel, we can directly manipulate heterotrophic uptake and autotrophic production rates.

We hypothesized (H_1) that we could displace heterotrophic demand for—and observe the release of—native DOM by supplying heterotrophs with a highly labile source of

* Corresponding author: brian.lutz@duke.edu

non-native reduced C substrate (Fig. 1B). Because DOM cycling under background conditions is likely to be composed of a variety of different compounds that vary in bioavailability, demand for some types of native DOM may be more readily displaced than others. Thus adding an external source of labile DOM in increasing increments may lead to an increase in the concentration of displaced DOM, as well as lead to systematic changes in the composition, or quality, of the DOM that is released.

Similarly, we hypothesized (H_2) that increasing inorganic nutrient and/or light availability would increase autotrophic production of autochthonous DOM, which could then lead to an increased production of algal-derived DOM (Fig. 1C). Autochthonous DOM is typically considered to be much more bioavailable than terrestrially derived DOM (Sun et al. 1997), and following this logic we predicted that small increases in autochthonous production might displace some heterotrophic demand for recalcitrant DOM, while larger increases in autochthonous production could lead to the release of algal-derived, more labile forms of DOM to the water column.

The objective of this study was to evaluate how altering heterotrophic uptake and autotrophic production affects the concentration and chemical composition of native DOM in a well-studied stream, Walker Branch, Tennessee. We conducted these experiments during the spring, prior to tree canopy closure, when both autotrophic production and heterotrophic metabolism tend to be high (Roberts and Mulholland 2007). Autochthonous production in this system has been shown to exhibit some degree of N limitation (Rosemond 1994; Roberts and Mulholland 2007), with increased algal growth often leading to a drawdown of stream-water nitrate concentrations (Mulholland 1992; Roberts and Mulholland 2007). We enriched the stream with inorganic N (nitrate) and then highly labile C (acetate) to differentially alter autotrophic production and heterotrophic uptake through a series of progressive kinetic enrichments, which are back-to-back solute additions at increasing levels of concentration that are sustained for very short periods of time.

We assessed changes in the native DOM pool in response to these sequential nitrate and acetate enrichments by measuring changes in both concentrations of stream-water DOM as well as changes in DOM fluorescence. Rapidly advancing modeling approaches of three-dimensional excitation–emission matrices (EEMs) of organic matter fluorescence have demonstrated considerable utility for characterizing the chemical composition of DOM, providing information on the biogeochemical processes involved in DOM cycling (Cory and McKnight 2005; Stedmon and Markager 2005; Murphy et al. 2008).

Study site

The Walker Branch watershed is located in Eastern Tennessee on the U.S. Department of Energy's Oak Ridge National Environmental Research Park (latitude 35°58′N, longitude 84°17′W). We conducted this experiment in the second-order reach of Walker Branch. The stream drains an aggrading temperate forest dominated by chestnut oak (*Quercus prinus*), tulip poplar (*Liriodendron tulipifera*), red maple (*Acer rubrum*), white oak (*Quercus alba*), and American beech (*Fagus granifolia*) (Johnson 1989). The site is typical of the humid southern Appalachians, with a mean annual temperature of 14.5°C and mean annual precipitation of 1400 mm (Mulholland 2004). The head of the 250-m study reach was located approximately 15 m downstream from the confluence of the first-order streams draining the East and West Forks of the watershed. The stream is moderately oligotrophic, with inorganic N (NO_3-N + NH_4-N), soluble reactive phosphorus (SRP), and dissolved organic carbon (DOC) concentrations rarely exceeding ~ 40 μg N L^{-1}, ~ 4 μg P L^{-1}, and ~ 1.5 mg C L^{-1}, respectively, during this time of year (Mulholland 2004; Pat Mulholland unpubl.). For more information regarding site location and characteristics, *see* Johnson and Van Hook (1989).

Methods

We conducted this experiment over 2 d (08–09 April) in the spring of 2009, immediately before leaf canopy closure. Previous studies at this site have shown that in-stream production during this time of year can be an important source of organic matter to the system (Mulholland 1992) and that the first-order stream (West Fork) exhibits high gross primary production (GPP) and low respiration (with daily GPP occasionally approaching or exceeding daily respiration; Roberts et al. 2007). In-stream production during this time of year is commonly limited by a combination of light, N availability, and grazing pressure (Rosemond et al. 2000).

Solute injections and sampling—On the day prior to the first sequential enrichments (approximately 16 h beforehand), we estimated stream velocity (5.71 m min^{-1}) and discharge (30 L s^{-1}) for a 50-m section of the experimental reach (50–100 m downstream) using a slug injection of NaCl (Gordon et al. 1992). We estimated the travel time between the injection site and the bottom of the sampling reach (250 m downstream) to be ~ 40 min. We estimated the time to reach plateau at the downstream station to be two times the travel time (~ 80 min) and timed sampling for each plateau so that the downstream station was not sampled before this amount of time had elapsed. This travel time assumption was confirmed by examination of the time required to reach stabilization of conductivity at the downstream station during the salt-propane injections described below. From long-term weekly stream-water chemistry monitoring at an upstream site, we estimated that nitrate concentrations would be approximately 15 μg N L^{-1} and DOC concentrations would be approximately 0.5 mg C L^{-1}. We used these estimates of discharge and background concentrations to determine the rates of additions necessary to reach each of our target enrichment levels.

On each day we performed a sequential enrichment experiment in which we elevated either nitrate (Day 1) or bioavailable DOC concentrations (Day 2) by approximately 2, 4, 8, and 16 times their background concentra-

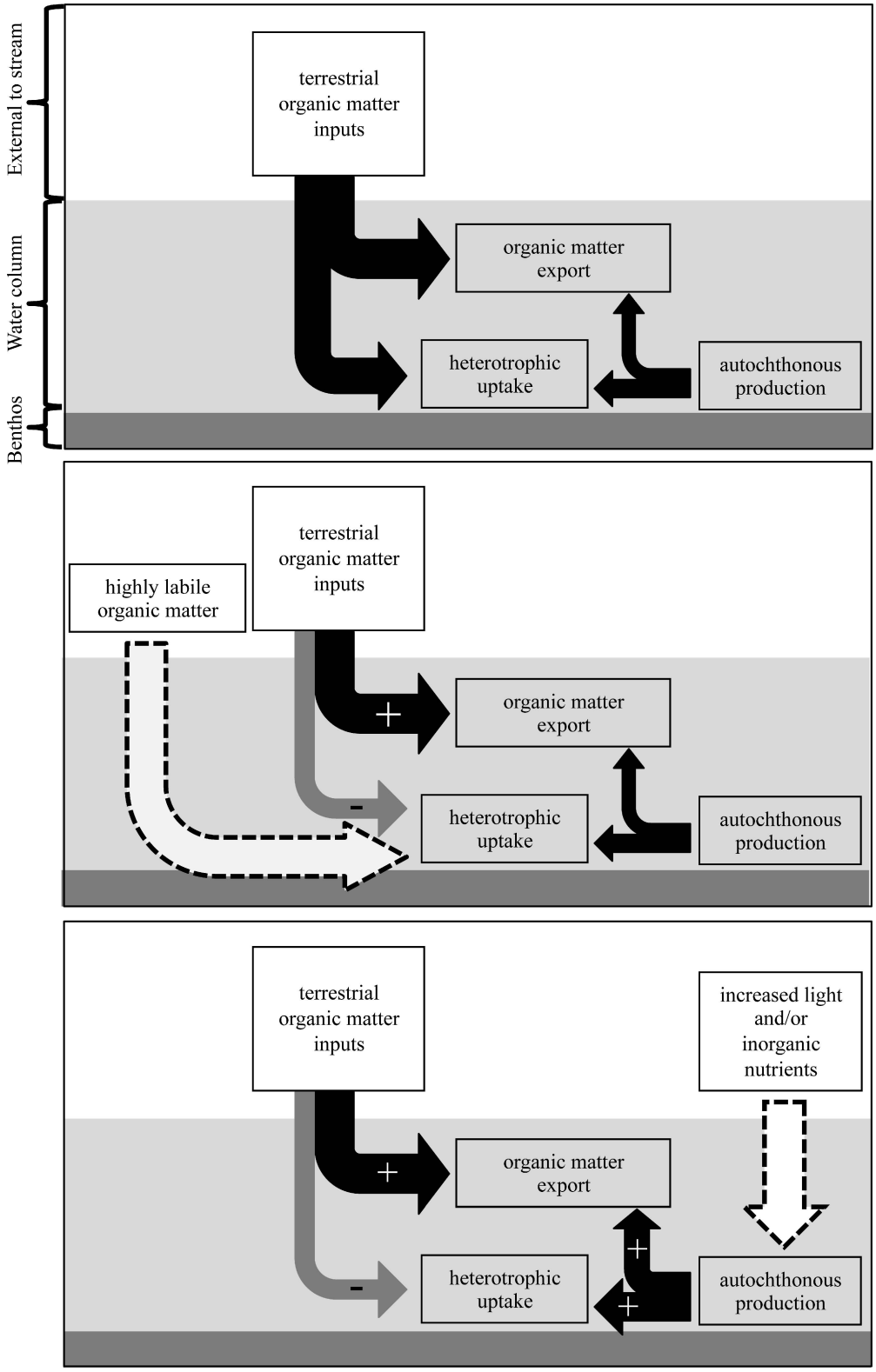

Fig. 1. Conceptual model for DOM sourcing, uptake, and loss within a stream channel. (A) DOM can be derived from two principal sources (terrestrial material or autochthonous production) and has two principal fates (heterotrophic uptake or loss to the water column). (B) Adding a source of highly bioavailable reduced carbon to the stream can displace heterotrophic demand for both terrestrial DOM and autochthonous DOM (H_1). (C) Increases in light availability or inorganic nutrients limiting in-stream production can result in an increase in

tions. We reversed this order and presented DOC results before nitrate results throughout to improve clarity and facilitate data interpretation. The confluence of the East and West Forks of Walker Branch serves as the reference point (i.e., 0 m) for all stream sampling locations. The injection site was located 15 m downstream of the confluence, with the first sampling station located 5 m upstream of the injection site (Sta. 1 = 10 m). At each enrichment-level plateau we collected stream-water samples from the thalweg at six downstream stations distributed longitudinally along the study reach (50, 75, 110, 155, 200, and 250 m). All additions were performed using a single batch of carbon or nutrient solution with a conservative tracer (Br^-) added to assess dilution rates. We increased the pump rate for each enrichment level in order to achieve target stream-water concentrations rather than making multiple solutions. The DOC solution was a 10-L volume of 270 g C L^{-1} as CH_3CO_2K with 281 g Br L^{-1} as KBr. The stock nitrate solution was a 10-L volume of 82 g NO_3-N L^{-1} as KNO_3 and contained 281 g Br L^{-1} as KBr.

Additions were begun at approximately 09:00 h on both mornings. A peristaltic metering pump with a pump range of 5–60 mL min^{-1} (520N pump; Watson-Marlow) was used for the additions. The pump addition rates were verified several times during each addition in the field using graduated cylinders to ensure accuracy and no drift through time. Complete mixing of the solute injections at the first downstream station was verified by taking several measures of specific conductance across the stream channel. Approximately 1 h following the initiation of each addition, we commenced sampling at the top of the reach (Sta. 1 was located ~ 5 m upstream of the injection site) and worked in a downstream direction. This was not a Lagrangian approach of following a nutrient pulse in a specific water parcel; the addition was sustained to plateau in the traditional Eulerian-style solute addition typical of stream spiraling studies (Stream Solute Workshop 1990; Doyle and Ensign 2009). However, not having to wait for the furthest downstream station to reach plateau before commencing sampling at the upstream station allowed us to minimize the addition durations, and care was taken to avoid entering the stream or disturbing sediments as sampling occurred. The short addition durations (1.5 h) were a priority to avoid potential changes in the stream benthic community associated with changes in the resource supply resulting from the enrichments.

Samples were field filtered through a Whatman GF/F 0.7-μm inline filter (Whatman) connected with Tygon R-1000 tubing (Saint-Gobain Ceramics and Plastics) through a peristaltic pump driven by a cordless electric hand drill to minimize sampling time (Woessner 2007). Samples were collected into two new 250-mL high-density polyethylene bottles (soaked for 3 d, rinsed with deionized water, and rinsed with a small amount of filtered sample water in the field prior to use) and one 40-mL I-Chem vial (Thermo Fisher Scientific) at each station. Samples were acidified in the field within 30 min of collection with 1% 2 mol L^{-1} HCl by volume and kept on ice until returned to the laboratory (< 12 h), where they were stored at 4°C until analysis.

Stream physical and hydrodynamic parameters and reaeration—We determined hydrodynamic parameters and reaeration coefficients (k_{O_2}) for upstream (50–150 m) and downstream (150–250 m) subreaches of the stream on the afternoon of 08 April 2009 using co-injections of propane gas (volatile tracer) and a concentrated NaCl solution (conservative tracer) as in Roberts et al. (2007). Background conductivity was recorded at the site of each sonde deployment (50, 150, and 250 m) before the additions using a Yellow Springs International (YSI) Model 30 conductivity meter. During the injection, a concentrated solution of NaCl was introduced at the injection site used in the kinetic enrichment experiments using a battery-powered fluid metering pump (Fluid Metering) while propane gas was simultaneously injected at a constant rate through a 30-cm × 6-cm aeration stone. We monitored specific conductance at three stations downstream of the injection site at 15 s (50 m) or 30 s (150 m and 250 m). Each injection continued until specific conductance at the downstream station had clearly reached plateau (specific conductance did not increase more than 0.1 μs cm^{-1} over 5 min). Travel time for each subreach was calculated as the distance between sampling sites divided by the amount of time it took between observations of the maximum rate of change in conductivity at the upstream and downstream site for that subreach (e.g., 50 m and 150 m for the upstream subreach). Stream velocity was calculated as the subreach length divided by the water travel time for each subreach. Stream discharge rate at each station (50, 150, and 250 m) was determined from the increase in stream-water specific conductance during the injection as described in Roberts et al. (2007). There was a small amount of drainage from a culvert passing under the access road entering the stream channel 113 m from the reach head, resulting in a higher dilution rate in the upper subreach (3.6 L s^{-1}) than in the lower subreach (2.0 L s^{-1}). Stream width was determined as the average of wetted-width measurements taken every 5 m along the two subreaches (3.53 ± 0.55 and 3.63 ± 0.60 m for the upstream and downstream subreaches, respectively). Mean depth (Z_{mean}, m) was calculated from discharge (Q), velocity (u), and average stream width (w) using the equation $Z_{mean} = Q/(1000 \times u \times w)$ and was 0.084 m and 0.101m, respectively, for the upstream and downstream subreaches.

After the conductivity at all stations reached plateau, six replicate samples were collected for propane analysis at the 50-, 150-, and 250-m sampling stations. Six milliliters of stream water was injected into 7-mL pre-evacuated vacutainers, and air-equilibrated headspace gas from each

←
autochthonous DOM lost to the water column or, because autochthonous DOM is often highly bioavailable, the indirect displacement of terrestrially derived DOM (H_2).

vacutainer was analyzed on a Hewlett Packard Model 5890 Series II gas chromatograph equipped with an Agilent High Performance–Porous Layer Open Tubular (HP-PLOT) Al_2O_3 column and a flame ionization detector. Propane reaeration coefficients ($k_{propane}$) for each subreach were calculated as the difference between the natural log values of the upstream and downstream propane concentrations divided by reach length and multiplied by average water velocity. O_2 reaeration coefficients (k_{O_2}) were calculated from $k_{propane}$ using the standard conversion $k_{O_2} = k_{propane} \times 1.39$ (Rathbun et al. 1978).

Ecosystem metabolism—Ecosystem metabolism rates were determined for two subreaches of the sampling reach (50–150 m and 150–250 m) using the two-station approach described in Roberts et al. (2007) for the first-order West Fork of Walker Branch. Dissolved O_2 (DO), temperature, and conductivity were measured and recorded using either YSI model 600 OMS (50 m), 6920 (150 m), or 6600V2-4 (250 m) sondes equipped with Model 6150 ROX optical oxygen sensors (YSI) deployed in well-mixed locations along the reach. DO sensors were calibrated in water-saturated air at the beginning and end of the deployments, DO data were corrected for barometric pressure recorded during calibration, and consecutive calibrations were used to detect instrument drift over deployment. The sonde located at 150 m was deployed from 18 March–14 April 2009 and recorded readings every 15 min. The upstream (50 m) and downstream (250 m) sondes were deployed from 07–10 April 2009 and recorded measurements every 1 min. These finer temporal resolution measurements allowed for more precise calculations of metabolism using the two-station approach since we were able to account for the travel time between the upstream and middle (17 min) and between the middle and downstream (19 min) sondes, which was determined from the salt-propane injections described above.

Ecosystem metabolic rates were calculated every 15 min for each subreach from the rate of change in DO concentration, using the equation: $\Delta DO = GPP - ER + E$, where ΔDO is the change in DO concentration (g O_2 m^{-3}), GPP is volumetric gross primary production (g O_2 m^{-3}), ER is volumetric ecosystem respiration (g O_2 m^{-3}), and E is net exchange of O_2 with the atmosphere (g O_2 m^{-3}) between the upstream and downstream sondes. The net exchange of O_2 with the atmosphere is the product of the O_2 reaeration coefficient (k_{O_2}, determined from salt-propane injections described above) and the average DO deficit (DO concentration at 100% saturation minus the DO concentration in stream water) over the measurement interval. Daytime ER was estimated by averaging ER during the hour before dawn and the first hour after dusk (Roberts et al. 2007). GPP for each daytime interval was the difference between the net metabolism flux and interpolated ER. Daily volumetric GPP and ER rates (g O_2 m^{-3} d^{-1}) were calculated as the sum of the 15-min rates over each 24-h period. Volumetric rates were converted to areal units (g O_2 m^{-2} d^{-1}) by dividing by the mean water depth (determined from NaCl injections described above) of stream subreaches.

Chemical analysis—Dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) concentrations were measured on a Shimadzu TOC-V_{CPH} total carbon analyzer with a Total Nitrogen Module-1 nitrogen module (Shimadzu Scientific Instruments). The method detection limits (MDLs) for DOC and TDN were 0.01 mg C L^{-1} and 3.0 μg N L^{-1}, respectively. These low detection limits were achieved by analyzing large (2-ml) sample volumes. Solution concentrations of NO_3^-, Br^-, and acetate (CH_3CO_2-C) were determined by ion chromatography (MDL 1.0 μg NO_3-N L^{-1}; Dionex ICS-2000 with eluent generation on an AS18 column, Dionex Corporation). Ammonium concentrations were determined using fluorescence detection following reaction with orthophthaldialdehyde (Holmes et al. 1999). The MDL for ammonium detection is 1.0 μg N L^{-1}. Concentrations of dissolved organic nitrogen (DON) were determined by difference [DON = TDN − (NO_3-N + NH_4-N)]. Concentrations of non-acetate DOC (DOC_N) during the acetate additions were also determined by difference [DOC_N = DOC − CH_3CO_2-C). Hereafter we always use "DOC" to indicate the non-acetate, or native, DOC concentration, and we report no DOC concentrations from the acetate treatments in which the added acetate has not been subtracted from the total DOC measurement.

Organic matter fluorescence and modeling—Fluorescence EEMs were collected for each sample within 4 weeks of returning the samples to the lab. All EEMs were measured on a Fluoromax-3 fluorometer (Jovin Yvon [now Horiba Scientific]) at excitation wavelengths ranging between 240 nm and 450 nm (at 10-nm increments) and emission wavelengths ranging between 300 nm and 600 nm (at 2-nm increments) using methods outlined in Cory and McKnight (2005) and Cory et al. (2010). No samples had an absorbance greater than 0.04 at 300 nm in a 1-cm cuvette, and, because of this, no efforts to correct for potential inner filter effects were made (Stedmon and Bro 2008). All EEMs were corrected for instrument bias and normalized to the area under the Raman peak at 350 nm. The complete dataset consisted of 70 EEMs. Parallel Factor (PARAFAC) Analysis was conducted in MATLAB (Mathworks) using the DOMFluor Toolbox (Stedmon and Bro 2008). The PARAFAC model was validated via a four-way split analysis according to Stedmon and Bro (2008; Fig. 2).

In order to assure that the elevated acetate or nitrate concentrations did not alter the modeled fluorescence component values, both deionized water and stream-water samples were spiked with two levels of KNO_3 (50 μg N L^{-1} and 300 μg N L^{-1}) and two levels of CH_3CO_2K (0.5 mg C L^{-1} and 3.5 mg C L^{-1}). EEMs were collected to ensure that adding these solutions did not produce fluorescence signals within the deionized water or alter the fluorescence patterns in stream water relative to the unamended samples.

Samples were acidified in the field to a pH of ~ 4 to limit microbial decomposition of DOM prior to sample analysis. However, altering pH has been demonstrated to alter the spectrophotometric properties of DOM (Spencer et al. 2007). Because all samples are from the same location with

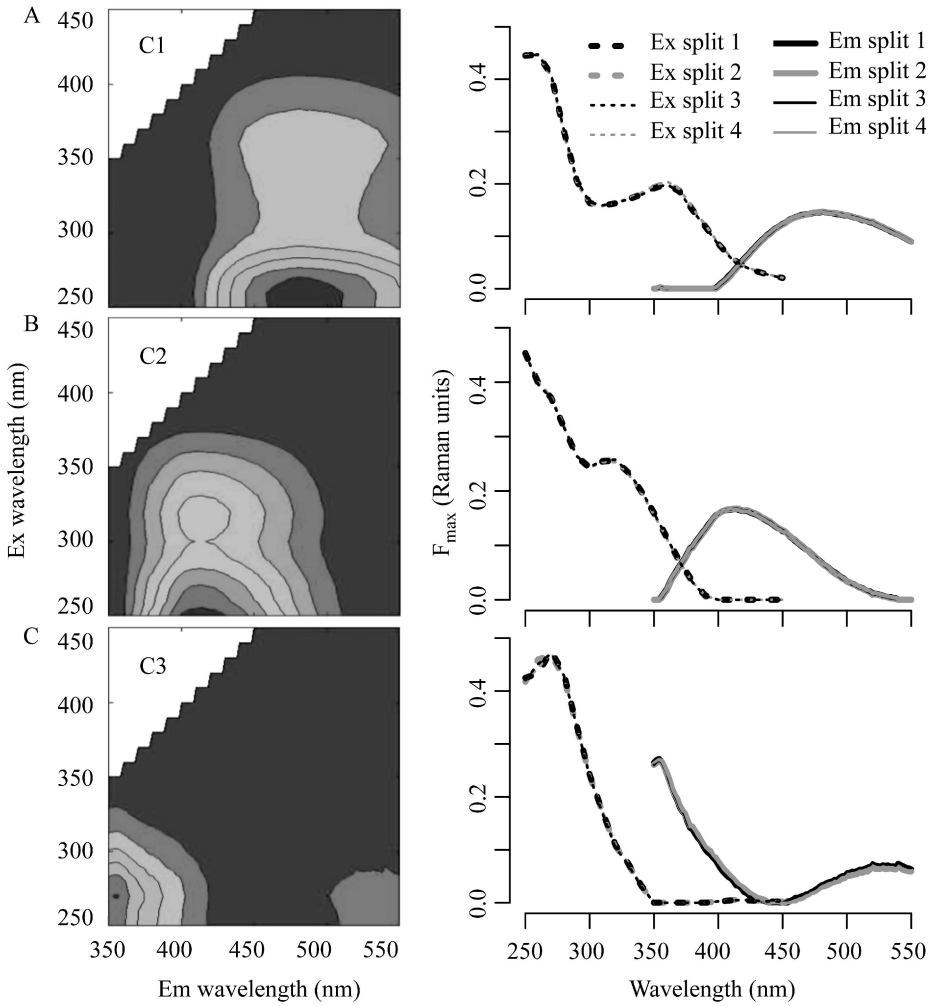

Fig. 2. Excitation–emission plots (left) of the three fluorescence components identified in this study. Line plots (right) represent the split-half validations of the PARAFAC model.

similar DOC sources and were acidified equally, we assume that this impacted all of our samples similarly and had minimal influence on our results.

C and N uptake calculations—Uptake lengths (S_w) for acetate and nitrate were calculated for enrichment levels as the inverse of the slope value of the ln-transformed equation describing the longitudinal decline in solute concentration per unit length (m) downstream of the addition point:

$$\ln N_x = \ln N_0 - k_x \quad (1)$$

where N_0 is the solute concentration above the addition point and N_x is the concentration x meters downstream, with k representing the rate of decline per meter (Newbold et al. 1981). Concentrations were corrected for groundwater dilution by dividing by the concentration of the conservative tracer, Br^- (Stream Solute Workshop 1990). The vertical mass transfer coefficients were calculated as:

$$V_f = (Q/w)/S_w \quad (2)$$

where Q is discharge (L s^{-1}) and w is mean stream width (m). Areal uptake rates (U) were calculated as:

$$U = V_f \times C \quad (3)$$

where C is the background solute concentration at the head of the reach.

Statistical analyses—Data for the station upstream of the addition point were analyzed separately from downstream stations in order to isolate treatment effects associated with the enrichments. For the upstream station data, simple linear regression (SLR) was used to assess potential changes in either stream-water concentrations or DOM fluorescence throughout the day based on sampling time, since each level of addition occurred sequentially within a day. Where upstream values exhibited a significant change, all downstream values were centered (via subtraction) to the value of the upstream station for each enrichment level to isolate diel effects not associated with the solute manipulations. SLR was used to assess longitudinal trends in solute concentrations and DOM fluorescence. If no significant longitudinal

trends were observed at any level of enrichment, mean downstream concentrations or fluorescence component signals across enrichment levels were assessed using a one-way analysis of variance (ANOVA) with enrichment level as the factor. Tukey's honestly significant difference (HSD) post hoc test was used to determine differences among enrichment levels when significant differences existed.

Results

PARAFAC model: fluorescence components—PARAFAC modeling revealed three distinct fluorescent components (Table 1). Component 1 (C1), which accounted for the greatest amount of variation in EEMs across samples, had a dominant peak with maximum excitation to emission values ($Ex_{max}:Em_{max}$) of 260 nm : 480 nm and a second smaller peak at $Ex_{max}:Em_{max}$ = 350 nm : 480 nm (Fig. 2A). Component 2 (C2) also has two distinct peaks, though both peaks (peak 1: $Ex_{max}:Em_{max}$ = 250 nm : 420 nm; peak 2: $Ex_{max}:Em_{max}$ = 320 nm : 420 nm) were blue shifted (i.e., located at lower wavelengths) relative to C1 (Fig. 2B). The final component (C3), which captured the least amount of variation across EEMs, had a peak at $Ex_{max}:Em_{max}$ = 270 nm : 355 nm (Fig. 2C). These three components have been previously described as a recalcitrant terrestrial humic (C1), a more bioavailable humic of either autochthonous or terrestrial origin (C2), and an amino acid (C3; *see* Discussion). It is important to note that component scores indicate the *relative concentration* of fluorescent DOM represented by each component. Because the components cannot be attributed to specific organic compounds with known quantum efficiencies, component scores cannot be converted to actual concentrations (Stedmon et al. 2003).

We did not detect background fluorescence of either acetate or nitrate when added at low (50 μg N L^{-1} and 0.5 mg C L^{-1}) or high (300 μg N L^{-1} and 3.5 mg C L^{-1}) concentrations to deionized water, within the excitation–emission space quantified by our three components. Slight fluorescence was measured in the Ex : Em 240 nm : 350 nm region (data not shown) for the highest enrichment levels (300 μg N L^{-1} and 3.5 mg C L^{-1}), likely due to fluorescence from impurities in these stock solutions. We did not observe any detectable shift in component concentration or distribution to stream-water EEMs upon addition of acetate or nitrate.

Ecosystem metabolism—There were no significant differences in meteorological variables between days that would limit our ability to compare metabolism measures across days. Walker Branch is a spring-fed stream with groundwater supplies limiting the range of stream-water temperature variation. Mean stream-water temperatures were 11.4°C (range: 9.6–14.1°C) and 12.3°C (range: 10.3–14.8°C) for 08 and 09 April, respectively. Throughout the course of the experiment there was no measurable precipitation, and photosynthetically active radiation on the first day of the experiment (45.4 mol photons m^{-2} d^{-1}) was very similar to that on the second day of the experiment (45.3 mol photons m^{-2} d^{-1}; http://mercury.ornl.gov/EDORA/).

The metabolism data exhibit a clear diel pattern with in-stream O_2 production peaking at approximately 14:00 h. For the upper subreach, GPP rates were 2.29 g O_2 m^{-2} d^{-1} and 2.21 g O_2 m^{-2} d^{-1} and ER rates were −5.98 g O_2 m^{-2} d^{-1} and −6.47 g O_2 m^{-2} d^{-1} during the nitrate and acetate additions, respectively. Values were similar for the lower subreach, where GPP rates were 2.35 g O_2 m^{-2} d^{-1} and 2.16 g O_2 m^{-2} d^{-1} and ER rates were −5.50 g O_2 m^{-2} d^{-1} and −4.78 g O_2 m^{-2} d^{-1}. While significant amounts of C were being fixed within the stream channel throughout the experiment, upper and lower subreaches were net heterotrophic (ER > GPP) on both days. We were unable to resolve any significant treatment effects in the metabolism data on the short timescales (~ 1.5 h) of any enrichment level for either acetate or nitrate additions.

Pre-enrichment and upstream station data—Background concentrations of DOC (~ 0.375 mg C L^{-1}) and nitrate (~ 10.5 μg N L^{-1}; Table 2) were similar to the values we expected based on the historical long-term data (Mulholland 2004; Pat Mulholland unpubl.). During both days of additions, DOC concentrations at the upstream station increased significantly by ~ 0.10 mg C L^{-1} (Fig. 3A,B), representing an increase of ~ 25% throughout the day. DON concentrations did not exhibit a significant diel pattern at the upstream station on the day of acetate additions (Fig. 3C). However, DON concentrations at the upstream station declined significantly throughout the day during nitrate additions, though this decline was modest (~ 10 μg N L^{-1}; Fig. 3D).

Similar to DOC concentrations, at the upstream sampling station (5 m above addition site) all fluorescence components exhibited an increase in concentration over the course of each day (Fig. 4). This increase was significant for both C1 and C2, but only marginally significant ($0.05 < p < 0.15$) for C3. There were no significant longitudinal patterns in any of the fluorescence components at the downstream stations under background conditions (i.e., before either acetate or nitrate was added; Fig. 5).

Acetate enrichment effects—During the enrichment, acetate concentrations, as measured at the first downstream station from the addition point, were very close to targeted enrichment concentrations (Table 2). The longitudinal decay rate (k_L) declined with increasing rates of acetate addition, and demand appeared to be saturated ($k_L \sim 0$) at the highest level of acetate addition (Table 2). The vertical mass transfer rate (V_f) declined with increasing enrichment concentrations, and areal rates of acetate uptake ranged between 0.450 mg C m^{-2} min^{-1} and 0.491 mg C m^{-2} min^{-1} (Table 2).

Acetate enrichment did not result in any measurable changes in the concentrations of non-acetate DOC or DON concentrations within the treatment reach. Further, when averaged across all downstream stations, mean DOC concentrations did not change significantly across enrichment levels, but had increasing variation (increase in SE) with increasing enrichment level (Fig. 3A). DON concentrations averaged across downstream stations did not show

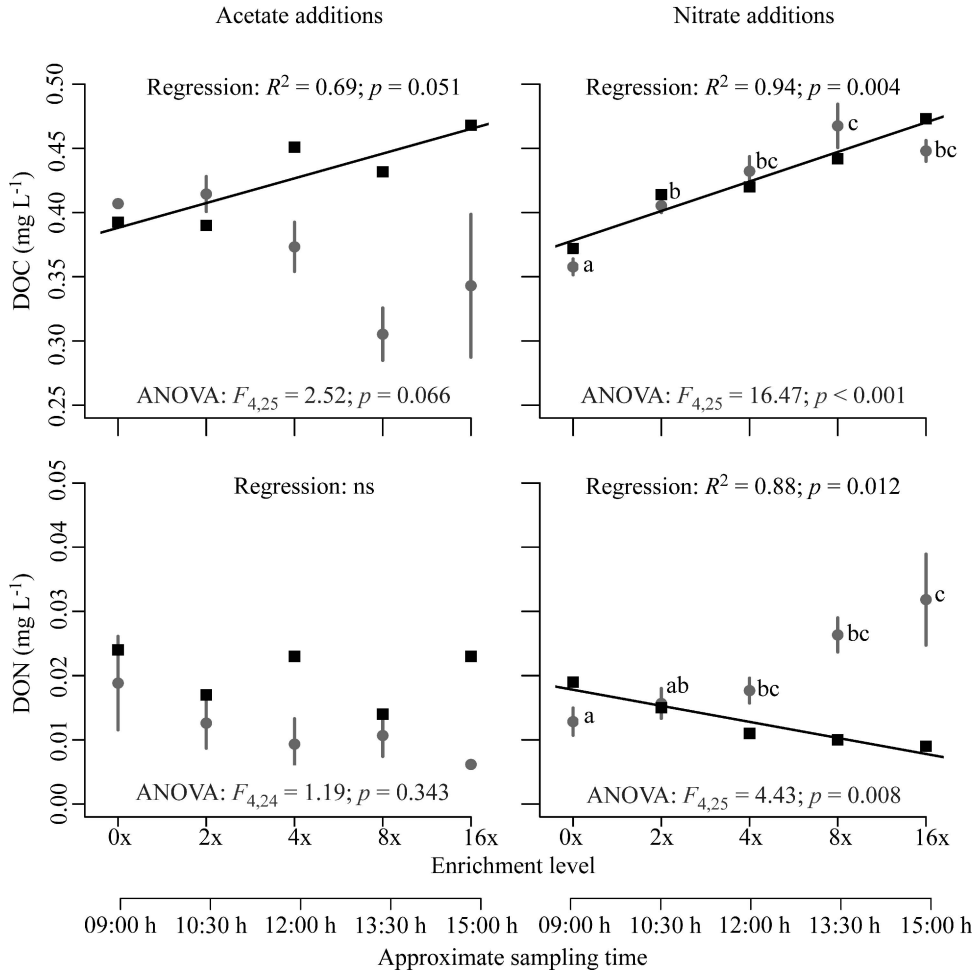

Fig. 3. Concentration data as measured at the sampling station above the point of addition (black squares) and averaged across downstream sampling stations (gray circles). DOC values were corrected for added acetate. Where present, lines indicate significant trend in concentration data at the upstream station between addition levels based on simple linear regression ("ns" indicates no significant trend). For downstream station data, error bars indicate ± 1 SE and letters indicate significant differences between addition levels based on Tukey's HSD (alpha = 0.05).

any significant patterns across acetate enrichment levels (Fig. 3C).

Because significant diel increases in all fluorescence components were observed (Fig. 4), in order to assess the effects of solute enrichments on the fluorescence patterns across downstream stations we subtracted the upstream station data for each enrichment level conducted throughout the day from all downstream station data collected at the corresponding times (Fig. 5). In contrast to the lack of trend in DOC concentrations, acetate additions led to longitudinal increases in the amount of C1 fluorescence (Fig. 5A–E). Conversely, C2 did not exhibit a longitudinal pattern during any of the acetate additions, with downstream values at all enrichment levels being similar to the upstream station (Fig. 5F–J). C3 showed no clear longitudinal pattern in response to acetate additions (Fig. 5K–O).

Nitrate enrichment effects—During the nitrate enrichment experiment, nitrate concentrations at the first downstream station were close to each of our targeted values. At the highest level of enrichment, nitrate uptake appeared to be saturated ($k_L \sim 0$; Table 2). The V_f declined sharply with each level of addition, and areal uptake of nitrate ranged between 33 μg N m^{-2} min^{-1} and 66 μg N m^{-2} min^{-1} (Table 2).

There were no significant longitudinal trends in either DOC or DON across downstream stations during any level of nitrate enrichment. However, when averaged across all downstream stations, mean DOC concentrations increased significantly throughout the day with each level of enrichment, with this increase being very similar to the increase observed in DOC concentrations at the upstream station (Fig. 3B). DON concentrations, when averaged across all downstream stations, also exhibited a significant increase with each level of enrichment, which was not observed in the data from the upstream sampling station (Fig. 3D).

Both C1 and C2 fluorescence components exhibited an abrupt increase at the first downstream station during the

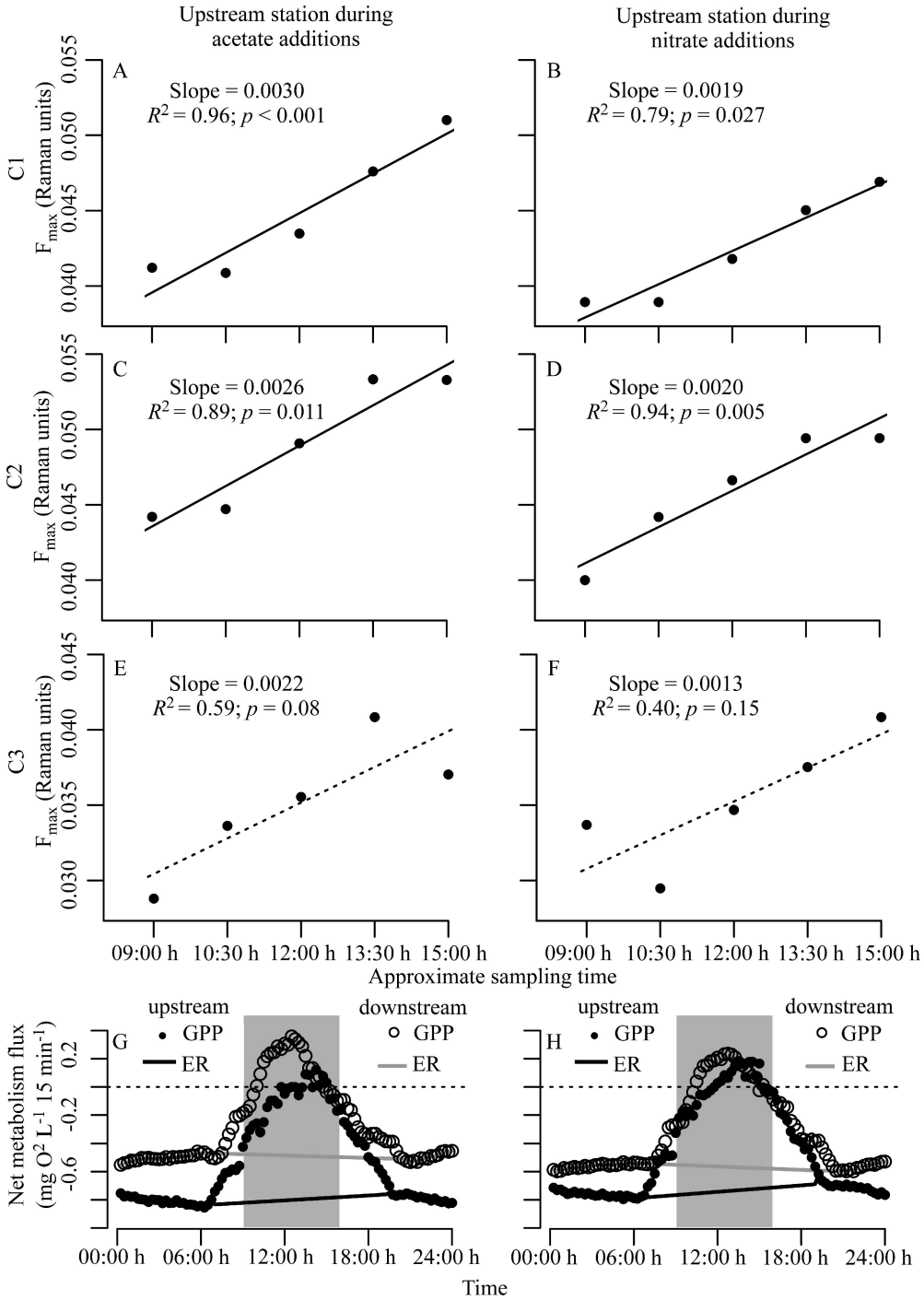

Fig. 4. (A–F) At the upstream station, there were significant increases during both the acetate and nitrate additions in the fluorescence intensities of each of the terrestrial- and autochthonous-like DOM components throughout the day. Increases in the amino acid–like component were marginally significant ($0.05 < p$-value < 0.15; indicated by dashed lines). (G–H) Net metabolic fluxes for each day of solute additions. Gray shaded areas indicate approximate time periods during which additions were performed.

nitrate additions, which was not observed during the acetate additions (Fig. 5A–J). Further, this effect was similar at all levels of nitrate enrichments. Unlike C1, which showed no clear longitudinal pattern following the initial increase, C2 showed a small longitudinal decline downstream during each addition (Fig. 5F–J). Similar to the acetate additions, during the nitrate additions C3 was highly variable across the downstream stations though observed values were typically greater relative to the upstream station (Fig. 5K–O).

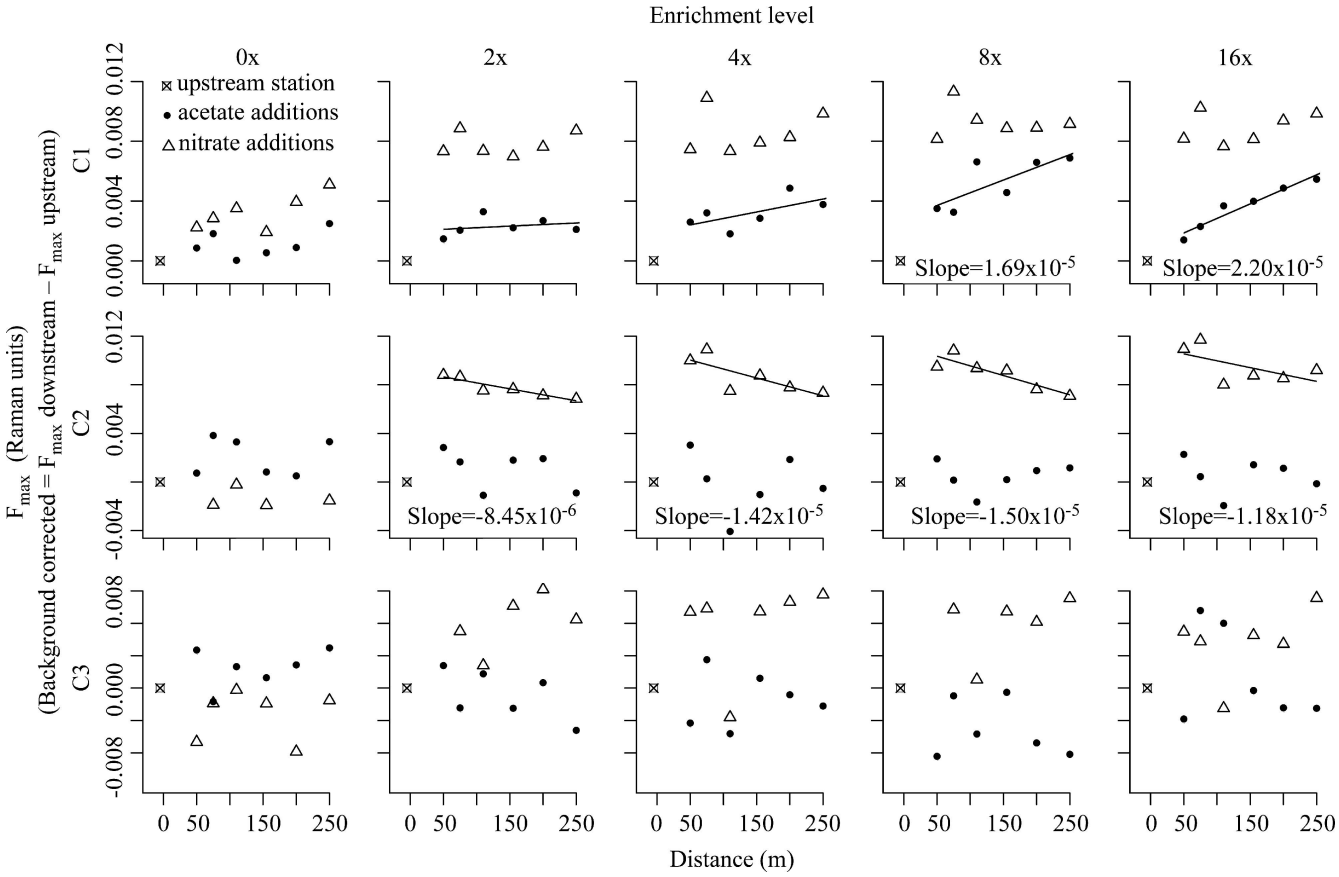

Fig. 5. Longitudinal trends in fluorescence components in response to the acetate and nitrate additions. All data were corrected for the diel effect (Fig. 4) by subtracting the values from the upstream station, which were collected throughout the course of each day at each level of enrichment; upstream values (open diamonds) are always equal to zero, reflecting this correction. Lines do not always represent significant trend but are included to aid in visualization of longitudinal patterns. Where regressions are significant, slope values are indicated on each plot where *p*-values were < 0.05.

Discussion

PARAFAC modeling: fluorescence components—The fluorescence components identified in the PARAFAC model have been ascribed to different DOM sources or functional forms by previous studies that have investigated the optical properties of DOM throughout a variety of freshwater and marine ecosystems. Component 1 (Table 1; Fig. 1A), having lower excitation energies (i.e., longer excitation wavelengths), is characteristic of molecules having a high proportion of aromatic carbon that is largely exclusive to lignin and other terrestrial plant–derived precursor material (Coble 1996; Cory and McKnight 2005). Component 2 (Table 1; Fig. 1B) has a clear shift in fluorescence peaks towards lower excitation and emission wavelengths. This shift is attributed to lower aromatic carbon content and is indicative of the less-conjugated structure of more recently produced DOM (Coble 1996). This component has historically been referred to as marine-like because it was first identified in open ocean waters (Coble 1996). However, more recently C2 has been identified in both terrestrial soil solutions and vegetation extracts (Ohno and Bro 2006), as well as in a variety of marine and freshwater ecosystems where autochthonous production is an important source of DOM (Murphy et al. 2008). Thus, based on literature citations, it is unclear if C2 is of terrestrial or autochthonous origin (or both), but in general C2 is considered to be more bioavailable than C1.

Table 1. Characteristics of the fluorescence components identified within the PARAFAC model, as well as a brief description of each component as others have described them.

PARAFAC component	Primary peak		Secondary peak		Description	References
	Ex_{max} (nm)	Em_{max} (nm)	Ex_{max} (nm)	Em_{max} (nm)		
C1	260	480	350	480	Terrestrial	Cory and McKnight (2005)
C2	250	420	320	420	Terrestrial or autochthonous	Murphy et al. (2008)
C3	270	355	—	—	Amino acid–like	Cory and McKnight (2005)

Table 2. Nitrate, acetate, and non-acetate DOC (DOC_N) concentrations, as measured at the first downstream station from the addition point, for each level of addition for both acetate and nitrate additions. Spiraling metrics are listed for each acetate and nitrate, at each level of enrichment.

	Enrichment level	NO_3-N (μg L^{-1})*	CH_3CO_2-C (mg L^{-1})*	DOC_N (mg L^{-1})*,†	k_L (m^{-1})	S_w (m)	V_f (mm min^{-1})	U (mg m^{-2} min^{-1})
Acetate additions	0×	10.4	0	0.402	—	—	—	—
	2×	13.8	0.431	0.365	−0.0031	322.6	1.5	0.469
	4×	10.7	0.982	0.307	−0.0013	769.2	0.6	0.450
	8×	9.8	2.118	0.229	−0.0006	1666.7	0.3	0.491
	16×	11.1	4.030	0.365	−0.0001	10,000.0	<0.1	0.162‡
Nitrate additions	0×	10.4	—	0.359	—	—	—	—
	2×	39.4	—	0.423	−0.0027	370.4	1.3	0.033
	4×	77.9	—	0.393	−0.0021	476.2	1.0	0.052
	8×	143.3	—	0.440	−0.0012	833.3	0.6	0.066
	16×	318.1	—	0.421	−0.0004	2500.0	0.2	0.047

* Concentrations as measured at first downstream station (50 m below point of addition).
† DOC concentrations reported for acetate additions are for non-acetate DOC (*see* Methods: Chemical analysis).
‡ This value was excluded from any results reported in the text due to dissimilarity (probable inaccuracy due to enrichment level).

Component 3 (Table 1; Fig. 1C) is easily distinguishable from the first two components and has been closely associated with the fluorescence properties of tyrosine and tryptophan (Cory and McKnight 2005).

Diel patterns—Previous work at this site has demonstrated that autochthonous production in the West Fork of Walker Branch is highest during the spring prior to tree canopy closure. Continuous metabolism measures have indicated that the first-order stream is least heterotrophic during this time of year, with the rate of carbon fixation (GPP) occasionally approaching or even exceeding ER (i.e., production : respiration > 1; Roberts et al. 2007). Our finding that DOC concentrations increase during daylight hours by approximately 0.1 mg C L^{-1} is consistent with previously reported values (Mulholland 1992) and represents a $\sim$ 25% increase within a 6-h period. This diel increase in DOC concentrations, caused by increases in primary production within the stream channel, suggests that the chemical composition of DOM, and the ratio of autochthonous to terrestrial DOM sources, is also likely to increase during the daytime. Other studies in temperate forest ecosystems in this region have similarly found that spring algal production can lead to diel increases in DOC prior to tree canopy closure (Kaplan and Bott 1982). DON concentrations at the upstream station either showed no trend (acetate additions) or a very small decline (nitrate additions), with the latter likely being spurious given that the decline is very close to detection limits. That we did not observe a commensurate diel increase in DON concentrations with the increase in DOC concentrations is not unexpected. Given the magnitude of the DOC increase, even if the DOM released by the increased algal production had a low molar C : N ratio (i.e., was N-rich), say C : N = 8 (Sun et al. 1997), the expected increase in DON concentrations would be less than 10 μg N L^{-1}, which is near our analytical detection limits.

The experiments reported here provide new insights into these diel patterns in DOM through measuring changes in DOM fluorescence signatures. The metabolism data and the diel increase in DOC concentrations (Fig. 3A–B) indicate an increase in autochthonous production during the course of each day. Other studies have observed increases in amino acid–like fluorescence with increases in autochthonous production (Stedmon and Markager 2005), and the marginally significant increase in C3 during the daytime (Fig. 4E–F) is also likely associated with diel changes in the rate of algal production. While there have been conflicting reports as to whether C2 is of terrestrial or autochthonous origin, the clear diel increase we observed in C2 (Fig. 4C–D) would lend support to the latter interpretation. However, there is also a diel increase in C1 (Fig. 4A–B), which is unambiguously of terrestrial origin. This response is not immediately intuitive because increasing light availability does not alter the rate of hydrologic transport of terrestrial DOM to the stream channel. DOM with these fluorescence characteristics has been shown to readily photodegrade (Opsahl and Benner 1998; Stedmon and Markager 2005); and, based on light patterns alone, we would predict a decrease in this terrestrial-like component

during the daytime. Thus, because we cannot hypothesize a potential abiotic mechanism that would result in this diel increase in C1, it is possible that this increase is biotically driven. Since terrestrial material is necessarily derived from outside of the stream channel, an increase in this component suggests the potential for decreased in-stream demand (i.e., net release) for terrestrial DOM during the day as autochthonous production increases. We find further support for such close coupling of autochthonous and terrestrial DOM dynamics in the results from the acetate and nitrate manipulations (*see* below).

Effects of acetate and nitrate enrichments—Despite nearly saturating the reach with acetate and nitrate (i.e., k_L values at highest additions close to zero, Table 2), DOC and DON concentrations remained remarkably little affected by the enrichments. During the acetate additions, DOC concentrations showed no clear response to enrichment other than greater variation at higher levels of addition (Fig. 3A). This lack of trend could, in part, have resulted from needing to subtract the acetate concentrations from these downstream stations to estimate the concentration of non-acetate DOC (*see* Methods: Chemical analysis). With each increased level of enrichment, as acetate begins to dominate the total organic carbon pool, the *percent error* for each of the total organic carbon and acetate measurements becomes larger relative to the *absolute concentration* of DOC. These limitations of estimating the concentration of a solute by difference from multiple independent measures have been detailed elsewhere (Lee and Westerhoff 2005) and likely contribute to the increasing variation we observed at higher enrichment levels.

During the nitrate additions, DOC concentrations increased at the downstream stations (Fig. 3B). This increase, however, is highly similar to the increase observed at the upstream station (Fig. 3B) and, because of this, is more likely the result of the diel effect related to in-stream production rates than a nitrate enrichment effect. DON concentrations also increased across downstream stations with each increase in nitrate enrichment level and, because this effect was not observed upstream or during the acetate additions (Fig. 3D), likely represents an increase in DON production resulting from greater nitrate availability. This indicates that the stream is, to some degree, limited by N availability and is consistent with previous findings that spring algal production removes inorganic N from the water column (Mulholland 1992; Mulholland 2004; Roberts and Mulholland 2007) and further suggests that a portion of this inorganic nitrogen uptake is rapidly converted to DON exports.

The limited response of DOC and DON concentrations to the acetate and nitrate additions may be due to the relatively low background concentrations of DOM in Walker Branch (*see* Study site). The same methods applied in systems where there is a greater mass of native DOM being consumed may result in measurable concentrations being released. Conversely, the fluorescence data are much more sensitive to changes in the DOM pool than are bulk concentration measurements. While longitudinal trends downstream of the addition point could not be resolved in DOC or DON concentrations, both the C1 and C2 components exhibited clear responses (Fig. 5A–J). Thus, systematic changes in the chemical composition of the DOM pool occurred despite lack of measurable changes in the concentration of the DOM pool.

The longitudinal increase in C1 during the acetate additions indicates that we were able to displace some ecosystem demand for terrestrial DOM through the addition of a more labile C source (H_1, Figs. 1B, 5A–E). Alternatively, C2 fluorescence did not change as a result of the acetate additions (H_1, Fig. 1B). We did not expect that acetate additions would alter either autotrophic production rates or the rate at which terrestrial organic matter enters the stream channel; thus, our manipulations of labile DOC availability provide little direct evidence as to whether C2 is of terrestrial or autochthonous origin. Regardless of source, however, the lack of change also suggests that we were unable to alter rates of heterotrophic uptake of C2. That there was no significant effect on the uptake of C2 in response to increasing the availability of acetate, which is highly bioavailable, suggests that C2 is also highly bioavailable. Because of this, we indirectly infer that C2 is more likely of autochthonous origin since terrestrial DOM is subject to extensive heterotrophic processing within catchment soils and typically depleted of most bioavailable DOM before entering the stream.

In contrast to the acetate additions, when we added nitrate we observed an abrupt increase in the fluorescence intensities of both C1 and C2 immediately downstream of the addition point (Fig. 5A–J). Since algae are commonly limited by N availability during this time of year (Mulholland and Rosemond 1992; Rosemond 1994), the observed increase in C2 in response to the nitrate additions but not in response to the acetate additions lends further support that C2 is likely of autochthonous origin. C2 also shows a consistent downstream decline following the initial increase and C1 does not (Fig. 5A–J), demonstrating that C2 is more bioavailable and preferentially consumed from the bulk DOM pool relative to C1. It is also interesting to note that we observe no additional effect of increased enrichment beyond the first doubling of nitrate concentrations (Fig. 5F–J). Walker Branch is oligotrophic (i.e., low nutrient solute concentrations; *see* Study site) with algae very near to co-limitation by both N and P (Rosemond 1994). The first doubling of N availability may have been sufficient to alleviate N-limitation of algal growth.

The similarity in the responses of C1 and C2 to the nitrate additions is striking (Fig. 5A–J), particularly given that these components are of terrestrial versus autochthonous origin, respectively. However, the increase in C1 in response to stimulating algal production with added nitrate is consistent with our observation of the diel increase in C1 at the upstream site with the diel increase in GPP due to changes in light availability. Thus, even though C1 is terrestrially derived, in both cases it shows a response to variation in autochthonous production. These results suggest a very tight coupling of these two distinct DOM sources such that terrestrial DOM consumption is measur-

ably reduced during times of high autochthonous DOM production (H_2, Fig. 1C).

We observed little response of C3 to either the acetate or nitrate additions at any level of enrichment (Fig. 5K–O). This amino acid–like component is typically associated with proteinaceous carbon, and strong correlations between C3 and native DOM bioavailability have been found in previous studies (Guillemette and Del Giorgio 2011). Background DOC concentrations in Walker Branch are low, and we have demonstrated that there is high heterotrophic demand for labile DOC. Yet even at our highest level of acetate addition, which nearly saturated acetate uptake capacity (Table 2), we did not observe an increase in the highly bioavailable C3 component (Fig. 5K–O). Further, amino acids and their associated proteins can serve as important N sources since they have a high density of N atoms (i.e., low C : N ratios). While there did appear to be a small increase in C3 across downstream stations when demand for N was alleviated during the nitrate additions, this increase was modest and not significantly different from the upstream station values (Fig. 5K–O). Thus, while we were able to elicit systematic changes in the composition of more aromatic and highly conjugated DOM subcomponents, we acknowledge that this displacement approach is limited in that we may not be able to alter the dynamics of the most bioavailable pools of native DOM.

Differentiating DOM subcomponents: implications—Our understanding of the ecological role of DOM within a given stream ecosystem depends on determining why one portion of the DOM pool is consumed, while another resists uptake and is transported downstream. Unlike other solutes, which are typically single element ions or specific molecules, the dynamics of the DOM pool cannot be expressed solely as a function of relative availability, or concentration, but must also take into consideration quality, or DOM chemical composition. Our data demonstrate that we can elicit changes in DOM composition independently of changes in concentration by altering the relative availabilities of labile C and inorganic N.

As opposed to other studies of DOM biogeochemistry in stream ecosystems, which have typically relied on adding some form of DOM and measuring its rate of uptake or removal from the water column, our focus was on assessing the *response of native DOM* to labile C or inorganic N availability rather than the *fate of either the acetate or nitrate* being supplied. While fluorescence measures have limited quantitative information since DOM fluorescence intensities cannot be directly translated into concentration values (Stedmon and Bro 2008), they provide insight into the structuring of the interactions between different native DOM subcomponents that cannot be gained by observing uptake rates of specific DOM molecules. While we are currently limited in our abilities to quantify qualitative changes in the native DOM pool, both fluorescence data (Stedmon et al. 2003), as well as a broad variety of alternative analytical approaches (Seitzinger et al. 2005; Abdulla et al. 2010), are rapidly closing this gap.

Acknowledgments

We thank Lou Kaplan for comments that were helpful for interpreting this dataset. Two anonymous reviews, as well as reviews from members of the Bernhardt Lab at Duke University, improved this manuscript. This work was partially funded by a President's Award from the North American Benthological Society's General Endowment Fund, as well as a Grant-in-Aid of Research from Sigma Xi (both to B.D.L.). A National Science Foundation Graduate Research Fellowship funded B.D.L. This research effort was supported by the U.S. Department of Energy's Program for Ecosystem Research. Oak Ridge National Laboratory is managed by the University of Tennessee–Battelle Limited Liability Company for the U.S. Department of Energy under contract DE-AC05-00OR22725.

References

Abdulla, H., E. Minor, and P. Hatcher. 2010. Using two-dimensional correlations of ^{13}C NMR and FTIR to investigate changes in the chemical composition of dissolved organic matter along an estuarine transect. Environ. Sci. Technol. **44:** 8044–8049, doi:10.1021/es100898x

Bernhardt, E., and W. McDowell. 2008. Twenty years apart: Comparisons of DOM uptake during leaf leachate releases to Hubbard Brook Valley streams in 1979 versus 2000. J. Geophys. Res. Biogeosci. **113:** 1–8, doi:10.1029/2007JG000618

Bernhardt, E. S., and G. E. Likens. 2002. Dissolved organic carbon enrichment alters nitrogen dynamics in a forest stream. Ecology **83:** 1689–1700, doi:10.1890/0012-9658(2002)083[1689:DOCEAN]2.0.CO;2

Brookshire, E. N. J., H. M. Valett, S. A. Thomas, and J. R. Webster. 2005. Coupled cycling of dissolved organic nitrogen and carbon in a forest stream. Ecology **86:** 2487–2496, doi:10.1890/04-1184

Coble, P. 1996. Characterization of marine and terrestrial DOM in seawater using excitation emission matrix spectroscopy. Mar. Chem. **51:** 325–346, doi:10.1016/0304-4203(95)00062-3

Cory, R. M., and D. M. McKnight. 2005. Fluorescence spectroscopy reveals ubiquitous presence of oxidized and reduced quinones in dissolved organic matter. Environ. Sci. Technol. **39:** 8142–8149, doi:10.1021/es0506962

———, M. P. Miller, D. M. McKnight, J. J. Guerard, and P. L. Miller. 2010. Effect of instrument-specific response on the analysis of fulvic acid fluorescence spectra. Limnol. Oceanogr.: Methods **8:** 67–78, doi:10.4319/lom.2010.8.0067

Cummins, K. 1974. Structure and function of stream ecosystems. BioScience **24:** 631–641, doi:10.2307/1296676

Doyle, M. W., and S. H. Ensign. 2009. Alternative reference frames in river system science. BioScience **59:** 499–510, doi:10.1525/bio.2009.59.6.8

Fisher, S. G., and G. E. Likens. 1973. Energy flow in Bear Brook, New Hampshire: An integrative approach to stream ecosystem metabolism. Ecol. Monogr. **43:** 421–439, doi:10.2307/1942301

Gordon, N. D., T. A. McMahon, and B. L. Finlayson. 1992. Stream hydrology: An introduction for ecologists. John Wiley and Sons.

Guillemette, F., and P. A. Del Giorgio. 2011. Reconstructing the various facets of dissolved organic carbon bioavailability in freshwater ecosystems. Limnol. Oceanogr. **56:** 734–748, doi:10.4319/lo.2011.56.2.0734

Holmes, R. M., A. Aminot, R. Kerouel, B. A. Hooker, and B. J. Peterson. 1999. A simple and precise method for measuring ammonium in marine and freshwater ecosystems. Can. J. Fish. Aquat. Sci. **56:** 1801–1808.

Johnson, D. W. 1989. Site description, p. 6–20. *In* D. W. Johnson and R. I. Van Hook [eds.], Analysis of biogeochemical cycling processes in Walker Branch watershed. Springer-Verlag.

———, and R. I. Van Hook. 1989. Analysis of biogeochemical cycling processes in Walker Branch Watershed. Springer-Verlag.

Kaplan, L. A., and T. L. Bott. 1982. Diel fluctuations of DOC generated by algae in a piedmont stream. Limnol. Oceanogr. **27:** 1091–1100, doi:10.4319/lo.1982.27.6.1091

Lee, W. T., and P. Westerhoff. 2005. Dissolved organic nitrogen measurement using dialysis pretreatment. Environ. Sci. Technol. **39:** 879–884, doi:10.1021/es048818y

McDowell, W. 1985. Kinetics and mechanisms of dissolved organic carbon retention in a headwater stream. Biogeochemistry **1:** 329–352, doi:10.1007/BF02187376

Minshall, G. W. 1978. Autotrophy in stream ecosystems. BioScience **28:** 767–770, doi:10.2307/1307250

———, R. C. Peterson, K. W. Cummins, T. L. Bott, J. R. Sedell, C. E. Cushing, and R. L. Vannote. 1983. Interbiome comparison of stream ecosystem dynamics. Ecol. Monogr. **53:** 1–25, doi:10.2307/1942585

Mulholland, P. J. 1992. Regulation of nutrient concentrations in a temperate forest stream: Roles of upland, riparian, and instream processes. Limnol. Oceanogr. **37:** 1512–1526, doi:10.4319/lo.1992.37.7.1512

———. 2004. The importance of in-stream uptake for regulating stream concentrations and outputs of N and P from a forested watershed: Evidence from long-term chemistry records for Walker Branch Watershed. Biogeochemistry **70:** 403–426, doi:10.1007/s10533-004-0364-y

———, and A. D. Rosemond. 1992. Periphyton response to longitudinal nutrient depletion in a woodland stream: Evidence of upstream-downstream linkage. J. N. Am. Benth. Soc. **11:** 405–419, doi:10.2307/1467561

Murphy, K., C. Stedmon, T. Waite, and G. Ruiz. 2008. Distinguishing between terrestrial and autochthonous organic matter sources in marine environments using fluorescence spectroscopy. Mar. Chem. **108:** 40–58, doi:10.1016/j.marchem.2007.10.003

Newbold, J. D., J. W. Elwood, R. V. O'Neill, and W. Van Winkle. 1981. Measuring nutrient spiralling in streams. Can. J. Fish. Aquat. Sci. **38:** 860–863, doi:10.1139/f81-114

Ohno, T., and R. Bro. 2006. Dissolved organic matter characterization using multiway spectral decomposition of fluorescence landscapes. Soil Sci. Soc. Am. J. **70:** 2028–2037, doi:10.2136/sssaj2006.0005

Opsahl, S., and R. Benner. 1998. Photochemical reactivity of dissolved lignin in river and ocean waters. Limnol. Oceanogr. **43:** 1297–1304, doi:10.4319/lo.1998.43.6.1297

Rathbun, R. E., D. W. Stephens, D. J. Shultz, and D. Y. Tai. 1978. Laboratory studies of gas tracers for reaeration. J. Environ. Eng. **104:** 215–229.

Roberts, B. J., and P. J. Mulholland. 2007. In-stream biotic control on nutrient biogeochemistry in a forested stream, West Fork of Walker Branch. J. Geophys. Res. Biogeosci. **112:** 1–11.

———, ———, and W. R. Hill. 2007. Multiple scales of temporal variability in ecosystem metabolism rates: Results from 2 years of continuous monitoring in a forested headwater stream. Ecosystems **10:** 588–606, doi:10.1007/s10021-007-9059-2

Rosemond, A. D. 1994. Multiple factors limit seasonal variation in periphyton in a forest stream. J. N. Am. Benth. Soc. **13:** 333–344, doi:10.2307/1467363

———, P. J. Mulholland, and S. H. Brawley. 2000. Seasonally shifting limitation of stream periphyton: Response of algal populations and assemblage biomass and productivity to variation in light, nutrients, and herbivores. Can. J. Fish. Aquat. Sci. **57:** 66–75, doi:10.1139/f99-181

Seitzinger, S. P., H. Hartnett, R. Lauck, M. Mazurek, T. Minegishi, G. Spyres, and R. Styles. 2005. Molecular-level chemical characterization and bioavailability of dissolved organic matter in stream water using electrospray-ionization mass spectrometry. Limnol. Oceanogr. **50:** 1–12, doi:10.4319/lo.2005.50.1.0001

Spencer, R. G. M., L. Bolton, and A. Baker. 2007. Freeze/thaw and pH effects on freshwater dissolved organic matter fluorescence and absorbance properties from a number of UK locations. Water Res. **41:** 2941–2950, doi:10.1016/j.watres.2007.04.012

Stedmon, C., and R. Bro. 2008. Characterizing dissolved organic matter fluorescence with parallel factor analysis: A tutorial. Limnol. Oceanogr.: Methods **6:** 572–579, doi:10.4319/lom.2008.6.572

———, and S. Markager. 2005. Tracing the production and degradation of autochthonous fractions of dissolved organic matter by fluorescence analysis. Limnol. Oceanogr. **50:** 1415–1426, doi:10.4319/lo.2005.50.5.1415

———, ———, and R. Bro. 2003. Tracing dissolved organic matter in aquatic environments using a new approach to fluorescence spectroscopy. Mar. Chem. **82:** 239–254, doi:10.1016/S0304-4203(03)00072-0

Stream Solute Workshop. 1990. Concepts and methods for assessing solute dynamics in stream ecosystems. J. N. Am. Benth. Soc. **9:** 95–119, doi:10.2307/1467445

Sun, L., E. M. Perdue, J. L. Meyer, and J. Weis. 1997. Use of elemental composition to predict bioavailability of dissolved organic matter in a Georgia river. Limnol. Oceanogr. **42:** 714–721, doi:10.4319/lo.1997.42.4.0714

Webster, J. R., and J. L. Meyer. 1997. Organic matter budgets for streams: A synthesis. J. N. Am. Benth. Soc. **16:** 141–161, doi:10.2307/1468247

Wiegner, T. N., L. A. Kaplan, J. D. Newbold, and P. H. Ostrom. 2005. Contribution of dissolved organic C to stream metabolism: A mesocosm study using ^{13}C-enriched tree-tissue leachate. J. N. Am. Benth. Soc. **24:** 48–67, doi:10.1899/0887-3593(2005)024<0048:CODOCT>2.0.CO;2

Woessner, W. 2007. Building a compact, low-cost, and portable peristaltic sampling pump. Ground Water **45:** 795–797, doi:10.1111/j.1745-6584.2007.00346.x

Associate editor: Peter Hernes

Received: 11 March 2011
Accepted: 26 September 2011
Amended: 26 September 2011