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Measuring heterotrophic respiration rates of suspended particulate organic carbon from stream ecosystems

David C. Richardson[1,2], J. Denis Newbold[2], Anthony K. Aufdenkampe[2], Philip G. Taylor[3], and Louis A. Kaplan[2]*
[1]SUNY New Paltz, 1 Hawk Drive, New Paltz, NY 12561, USA
[2]Stroud Water Research Center, 970 Spencer Road, Avondale, PA 19311, USA
[3]Department of Ecology and Evolutionary Biology, University of Colorado, Boulder, CO 80303, USA

Abstract

Integrated carbon budgets of terrestrial and aquatic ecosystems indicate that particulate organic carbon (POC) plays an important role in the transport, storage, and turnover of carbon during its transit from land to sea. However, little is known about the rates at which POC in suspension is metabolized during downstream transport. We address this deficiency by improving existing respiration methods and models to assess the biological lability of POC suspended in a headwater stream. Our method involves concentration of stream particles by tangential flow filtration, extended incubations (35-40 d) using conditions to ensure particle suspension and prevent particle aggregation, correction for the simultaneous respiration of dissolved organic carbon, and conversion from oxygen measurements into carbon with a respiratory oxidation ratio (*OR*) of 1.30 O_2:C. We include analysis of the choice of *OR*. The POC turnover times estimated with the improved methods in this study are ~10 days. These respiration rates are among the highest reported for either suspended or benthic POC in streams and suggest that suspended POC is mineralized closer to its point of origin than was previously assumed. During incubation, keeping POC in suspension can increase respiration rates as much as 2-fold compared with allowing particles to settle and physical inhibition of POC aggregation can increase rates by 1.2-fold compared with allowing POC to aggregate. Methods that explicitly incorporate suspension and discourage aggregation of POC and longer incubation times during respiration measurements will generate data that improve our understanding of the dynamic role of POC in aquatic ecosystems.

Each year, rivers export approximately 0.2 Pg each of particulate organic carbon (POC) and dissolved organic carbon (DOC) to the oceans (Meybeck 1982; Ludwig et al. 1998; Schlunz and Schneider 2000), and much more POC is deposited annually into lakes and reservoirs (Stallard 1998; Cole et al. 2007; Tranvik et al. 2009). An additional, possibly larger, flux of POC enters or originates within river networks, but is mineralized prior to export (Cole and Caraco 2001; Mayorga et al. 2005), transferring metabolic energy from upstream to downstream ecosystems (Vannote et al. 1980; Battin et al. 2008), and contributing to the > 0.5 Pg y^{-1} of global carbon out-gassing as carbon dioxide (CO_2) from streams and rivers (Richey et al. 2002; Aufdenkampe et al. 2011; Butman and Raymond 2011). Yet, published mineralization rates of stream and river POC, with an average turnover time of 2.6 years, imply that POC would need to travel tens to hundreds of kilometers through river networks before being metabolized by the heterotrophic community (Webster et al. 1999). We suggest that these rates are in error, overestimate the average turnover time, and underestimate the metabolic contribution of POC transported by river systems, largely as a result of the methods currently employed to collect, analyze, and calculate the lability of POC transported in streams.

Most rate measurements of POC mineralization to CO_2 in lotic ecosystems have been made in headwater streams using POC collected from the benthos, where the highest microbial densities are located (Webster et al. 1999). Rates are estimated by placing benthic POC into gas-tight bottles filled with stream water and measuring the change in dissolved oxygen (O_2) over a single time period (Naiman and Sedell 1979; Webster et al. 1999). Incubations are terminated within short time

*Corresponding author: E-mail: richardsond@newpaltz.edu

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periods (<24 h) to avoid anoxia. Rates are typically reported as mass of oxygen consumed per initial mass of carbon per unit time (e.g., Naiman and Sedell 1979; Webster et al. 1999) and converted to carbon turnover time using a molar respiratory oxidation ratio ($OR = O_2{:}CO_2$) of 1.0 mol of O_2 per mol of C (Webster et al. 1999).

There are a number of reasons to suspect that estimates of stream POC mineralization rates and turnover times determined with these methods and calculations do not reflect the in situ rates of POC in transport through stream networks. Due to hydrodynamic particle sorting, the physical, chemical, and biological properties of benthic particles differ from those of suspended particles. Benthic POC has a mean size (>125 µm; e.g., Bonin et al. 2000) that is 6 to 200 times larger than the majority of the particles in the suspended load (0.7 to 20 µm; Naiman and Sedell 1979; Wallace et al. 1991; Kaplan et al. 2006). At baseflow, suspended POC likely represents a subset of benthic particles that are easily suspended and transported downstream including both low-density particles primarily composed of detrital organic matter from diatom cells, bacterial cells, and small leaf fragments and high-density particles primarily composed of mineral clays and silts with some adsorbed and surface-complexed organic matter. Naiman and Sedell (1979) found that respiration rates of drifting particles in a 0.45 to 53 µm size class accounted for 57% to 97% of the total respiration of particles in transport that included 53 to 106 µm and > 106 µm size classes. Studies in large rivers, river deltas, and estuaries have shown that larger particles in those environments are a mixture of high-density, low-surface area, low-carbon mineral particles, and low-density detrital particles with higher C:N and other biochemical signatures of plant fragments (Keil et al. 1997; Hedges et al. 1997; Aufdenkampe et al. 2007). Further, incubations of stream POC occur in the presence of DOC, and to our knowledge, no studies analyzing POC quality explicitly correct for DOC driven respiration, even though microorganisms on particles as well as free-living microorganisms in the incubation water metabolize DOC.

Short incubations can characterize only the rates of the most labile POC with no differentiation of other lability pools. The concept that most organic matter (OM) in the environment is composed of a number of pools spanning a wide range of mineralization rates is well established for marine sedimentary OM (Berner 1995), soil OM (Trumbore 2000), marine DOC (Carlson 2002), and stream DOC (Cummins et al. 1972; Kaplan and Newbold 2003). Because POC migrates rapidly downstream (e.g., at 50 to 150 m d^{-1}, Webster et al. 1999, Newbold et al. 2005), characterizing the size of the POC pool that is converted to biomass or CO_2 on times scales ranging from days to months is critical in assessing contributions of transported POC to downstream ecosystem metabolism. The only way to quantify the size of the carbon pool contributing to measured respiration is by curve fitting data from longer duration incubations (i.e., 7 to 90 d); this has not been done on stream suspended or benthic POC by any published study of which we are aware. Furthermore, published rates are derived from incubations that do not reflect in-stream conditions. Sediment to water ratios are orders of magnitude too high, turbulence is negligible, sediments settle in deposits on the bottle bottom, and temperature is poorly controlled (e.g., Benner et al. 1995; Webster et al. 1999; Bonin et al. 2000).

A primary challenge to collecting and experimenting with suspended POC is that POC is often very dilute and thus O_2 demand is low relative to the precision of selected O_2 measurement methods. For example, an average baseflow suspended POC concentration in the third-order stream from the eastern Piedmont sampled for this study is 0.15 mg POC L^{-1} (Richardson et al. 2009), which would consume < 0.1 mg L^{-1} oxygen during a 24-h incubation, assuming 20% of POC were mineralized. The precision of the O_2 measurement can be increased with the use of potentiometric auto-titration of the classic Winkler reaction to quantify O_2 concentrations, which has a real-world precision of ± 0.25% to ± 0.5% or approximately ± 0.01 to ± 0.08 mg L^{-1} (Graneli and Graneli 1991; Benner 1995; Furuya and Harada 1995; Amon and Benner 1996). However, a 10-fold improvement in O_2 measurement precision alone is not sufficient to develop the high precision data for curve fitting various kinetic models. Tangential flow filtration (TFF) can be used to concentrate POC > 0.1 µm by up to 1000-fold (Benner et al. 1997). By concentrating suspended POC to a target range and measuring small changes in O_2 with precision, the experiments can run for weeks without causing anoxia in the bottles. This allows for the characterization of the semi-labile POC pool with turnover times of weeks to months. Last, field and laboratory evidence suggest that careful attention to mimicking in situ conditions is critical to estimating in situ mineralization rates. Stream particles are normally in a turbulent environment and constantly bounce off the stream bed (McNair and Newbold 2001). Replicating suspension under laboratory conditions facilitates oxygen diffusion to the microorganisms attached to individual particles (Ploug and Grossart 1999).

Conversion factors to estimate carbon mineralization from oxygen consumption have been previously used inconsistently. Most textbooks write out the reversible photosynthesis/respiration reaction as $CO_2 + H_2O \leftrightarrow CH_2O + O_2$, in which all carbon is fixed to carbohydrates and the *OR* is exactly 1.0. However, assuming an *OR* of 1.0 ignores the fact that most natural organic matter contains other elements (e.g., nitrogen and sulfur), is more complex than simple carbohydrates, and requires more oxygen to completely oxidize organic matter via heterotrophic respiration. Estimating an *OR* for POC in freshwater ecosystems requires a careful examination of organic matter elemental composition and assumptions of the fate of organic material (Hedges et al. 2002; Berggren et al. 2011). Consideration of these factors contributing to net *OR* is critical for accurate assessments of organic carbon quality and use.

We have developed an improved experimental approach for quantifying in situ kinetics of suspended stream POC mineralization (i.e., pool sizes and rate constants). Our approach integrates solutions from other disciplines to each of the issues described above. We present a modified experimental approach for estimating the in situ mineralization rates of suspended POC in streams by concentrating suspended POC relative to DOC, keeping particles in suspension during the incubation, and correcting for respiration of DOC. We used improved analytical methods to quantify changes in O_2 as a proxy for organic carbon lability, lengthened incubation times to 35-40 days, and tested different incubation conditions. We collected suspended POC from a third-order SE Pennsylvania stream and assessed each of the proposed modifications to the experimental approach: keeping particles in suspension and turbulently mixed compared with treatments where POC was allowed to settle. We advance the mathematical modeling of POC respiration by including the correction for respiration of DOC and assess the choice of *OR* on determination of carbon quality. Finally, we tested whether the process of concentrating POC had an effect on DOC biological lability.

Materials and procedures

Site description

Stream water was collected for three experiments from the upper East Branch White Clay Creek (WCC: N39°51'30", W75°46'60"), a third-order stream draining 7.3 km^2 of eastern Pennsylvania Piedmont, U.S. The watershed has 23% of total area as temperate deciduous forest largely within an intact riparian zone, 52% as pastures or hay fields, and 22% as row crop agriculture (Newbold et al. 1997). At baseflow, WCC discharge averages 85 L s^{-1}, has high nutrient concentrations (3-5 mg NO_3-N L^{-1}; 4-33 µg PO_4-P L^{-1}) from agricultural land use, moderate DOC concentrations (1.5 mg DOC L^{-1}; Newbold et al. 1997), and between 0.1 and 0.4 mg suspended POC L^{-1} at baseflow (Richardson et al. 2009).

Experimental approach

Our overall measurement approach was to collect large volumes of stream water, treat subsets of water by either concentrating or removing particles (described in detail below), then homogeneously dispense water and particles from each treatment into a series of identically filled glass biochemical oxygen demand (BOD) bottles for long-term incubations. Individual BOD bottles were sampled over time to create a time series of O_2 concentrations for each treatment. Specifics for each of these steps are described below.

Sample collection and concentration

Two experiments were conducted to assess methods for measuring heterotrophic respiration of suspended POC and were started on 17 Jan 2006 (*Jan 2006*) and 04 Apr 2007 (*Apr 2007*). A third experiment to test the effects of tangential flow filtration on DOC lability was started on 15 Aug 2007 (*DOC lability*). For each experiment, stream water was collected from WCC at baseflow using a submersible battery-operated pump (Whale Submersible pump GP1352) suspended at 2/3 depth in the water column (~15 cm deep). Water was pumped through a 53 µm sieve to remove any large particles, while still collecting > 90% of baseflow particles (Kaplan et al. 2002; Kaplan et al. 2006). The sieving was performed to avoid the significant challenges of homogeneously subsampling sand-sized suspended particles, and because of the differences in composition of the organic matter in that size range (Keil et al. 1997; Aufdenkampe et al. 2007). We concentrated the particles using tangential flow filtration (TFF; Millipore Pellicon and 0.65 µm pore-size Millipore Pellicon 2 Cassette Filter) (Hoffmann et al. 2000). Approximately 200 L were concentrated to 20 L sample for Jan 2006 and Apr 2007 experiments and 100 L stream water concentrated to 10 L sample for the DOC lability experiment. Although stream waters were concentrated approximately 10-fold, nearly 50% of POC was lost to the TFF membrane, resulting in an effective 5-fold concentration increase.

Experimental treatments

The final volume of concentrated water (20 L) was divided into 5-L aliquots for four treatments tested in each experiment (Jan 2006 and Apr 2007). For the Jan 2006 experiment, the treatments (explained below) included (1) DOC control, (3) settled particles, (4) suspended particles, and (5) dispersed, suspended particles (Table 1). For the Apr 2007 experiment, treatments 1, 3, and 5 were the same as the Jan 2006 experiment and treatment 4 was replaced with a (2) dispersed DOC control treatment (Table 1). We tested the effects of keeping particles suspended by attaching the BOD bottles to a rotating axle (Treatments 4 and 5—'suspended', *see* "Incubations" and Fig. 1) and allowing the particles to settle in static treatments

Table 1. Experimental design for January 2006 and April 2007 experiments. A closed circle indicates the treatment combination was used in that experiment; an X indicates the experimental conditions: POC present in bottle (POC), particles kept in suspension (incubation axle), and particles were dispersed (beads).

Treatment number	Treatment	Jan 2006	Apr 2007	POC	Incubation axle	Beads
1	DOC control	•	•		X	
2	Dispersed DOC control		•		X	X
3	Settled particles	•	•	X		
4	Suspended particles	•		X	X	
5	Dispersed, suspended particles	•	•	X	X	X

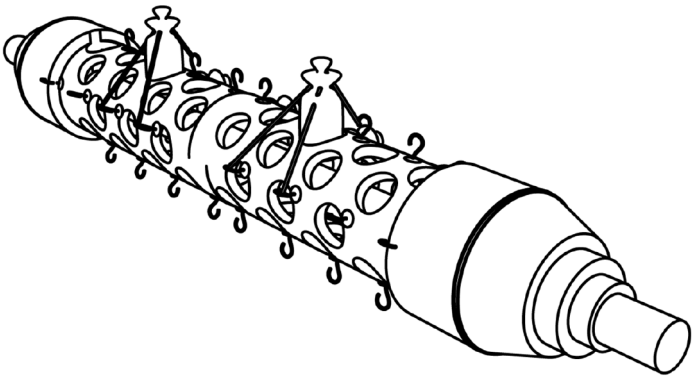

Fig. 1. Schematic of the incubation axle. The axle was placed in a temperature-controlled water bath and rotated by a motor with acetal chain and gear (not shown). BOD bottles were secured to the incubation axle using rubber bands attached to cup hooks.

(Treatment 3—'settled') (Table 1). To test the effect of dispersing particle and bacterial flocs in both POC (Jan 2006) and DOC alone (Apr 2007) treatments (Treatments 2 and 5—'dispersed,' Table 1), we added 20 sterile glass beads (4 mm diameter) to the 60 mL BOD bottles before adding the sample water. Twenty beads provided an additional 9% surface area (50 mm^2 $bead^{-1}$) above the internal surface area of the BOD bottle. In both the Jan 2006 and Apr 2007 experiments, we measured heterotrophic respiration on DOC alone (Treatment 1—'DOC control': Table 1) to correct for respiration of DOC in bottles containing both DOC and POC (Table 1). To remove particles for the DOC control treatments, we vacuum-filtered 5-L subsamples of TFF concentrate water through ashed (4.5 h, 480°C) glass fiber filters (Millipore AP40 with nominal pore-size of 0.7 µm). For the DOC lability experiment, there were only two treatments, both particle-free, to test the effect of the TFF particle concentration step on DOC quantity and quality. Stream water was collected and particles were removed before and after TFF (pre- and post-TFF, respectively).

The 5-L aliquots were left in a water bath (<8 h) to equilibrate with the incubation temperature (18°C) and then the 5-L bottles were shaken vigorously by hand for 5 min to saturate the water with oxygen before dispensing into the BOD bottles. Sample water from each 5-L bottle was divided into 21 precombusted (4.5 h, 480°C) borosilicate glass 60-mL BOD bottles (Wheaton) using a 5-L churn splitter (USGS 2002) to ensure homogeneous distribution of POC into each BOD bottle. These 21 bottles were used as replicates to be sampled over the duration of the experiment with three bottles randomly chosen every time period. Each bottle was filled with a tube extending from the churn splitter outflow to the bottle bottom at a slow but steady rate to overflow the bottle while avoiding surface turbulence and air bubbles. Each bottle was sealed with a glass stopper with no headspace or air bubbles. Water was added to the BOD bottle lip to keep the ground glass seal wet and eliminate any potential for atmospheric exchange with sample water, and this was contained by a plastic cap placed over the stopper.

Carbon measurements

Approximately 60 mL aliquots of sample water were taken from the churn splitter at the beginning, middle, and end of each sample distribution, analyzed for POC and DOC, and averaged to obtain initial carbon concentrations. The aliquots for all treatments were filtered through stacked pairs of identical pre-weighed and pre-ashed (480°C for 4.5 h) glass fiber filters (Millipore AP40 with nominal pore-size of 0.7 µm). The filters were dried for 12-18 h at 60°C and subsamples (~50% to 70%) were taken from each filter using cork borers with known areas and were analyzed for carbon mass using an Elemental Analyzer (Costech Model 4010). We calculated the POC concentration for each sample by multiplying the carbon mass by the inverse of the subsample proportion and dividing by the volume filtered. DOC concentration was measured from the filtrate water (< 0.7 µm) using UV catalyzed persulfate oxidation detected by conductivity (Sievers 800 or 900 TOC analyzers equipped with an inorganic carbon removal module).

Incubations

BOD bottles were incubated in the dark to prevent autotrophic carbon fixation and submerged in a water bath with a chiller and heat exchange coils to maintain the water temperature at 18°C. For the settled particle treatment, BOD bottles were placed on the water bath floor and particles were allowed to settle to the bottom of the bottle. The remaining bottles (suspended treatments) were strapped to a rotating incubation axle on a stand submerged within the water bath to keep particles in suspension (Fig. 1). The incubation axle is a nested 2 m PVC axle; an inner PVC pipe (outer diameter: 2.54 cm) supports an outer PVC pipe (outer diameter: 10.8 cm) with maximum capacity of 108 60-mL BOD bottles. Each hole for BOD bottles is 4.4 cm in diameter; the hole centers are spaced ~6 cm apart. The axle, connected to a motor by a plastic gear and chain, rotates bottles end over end at 5 revolutions per minute to keep particles in constant suspension.

O_2 measurements

We quantified bacterial respiration by measuring changes in oxygen concentration over time using the Winkler technique with potentiometric auto-titration (Bryan et al. 1976; Graneli and Graneli 1991; Biddanda et al. 1994). Each bottle was treated with 0.4 mL manganous sulfate (252 g L^{-1}) and 0.4 mL of alkaline iodide (136 g L^{-1} NaI and 500 g L^{-1} NaOH) solutions followed by 0.8 mL of hydrosulfuric acid (18 N; Bryan et al. 1976). A 25 mL aliquot was drawn from the acidified solution with a volumetric pipette and was titrated using sodium thiosulfate (0.0125 N). Titration equivalence points were determined potentiometrically using an auto-titrator (Mettler DL-50) equipped with a platinum combination electrode (Mettler DM 140-SC; Graneli and Graneli 1991; Furuya and Harada 1995; R. Benner pers. comm.).

Three randomly selected replicate bottles were removed at 7 time periods for Jan 2006 and Apr 2007 experiments

(approximate sampling times after initialization of both experiments: 0 h, 5-7 h, 24-36 h, and 7, 14, 28, and 35 d). Only the first 5 time periods were sampled for the DOC lability experiment.

Model fitting and treatment comparisons

We modeled first-order carbon decay dynamics in each treatment assuming two or three pools of carbon quality pools. Measured oxygen data were used to derive parameters for the carbon dynamics models instead of direct measurements of carbon because we lacked enough carbon mass and analytical sensitivity to measure carbon changes in the bottles. Parameters estimated for respiration of DOC alone were used as background during estimation of POC rate constants.

Changes in total organic carbon concentrations over time due to heterotrophic respiration, *C(t)*, are typically modeled by fitting a multiple component first-order decay model, given in generalized form as:

$$dC/dt = -k_1 \times C_1 - k_2 \times C_2 \ldots - k_n \times C_n \quad (1)$$

having the solution:

$$C(t) = c_0 + c_1\, e^{-k1\times t} + c_2\, e^{-k2\times t} + \ldots + c_n\, e^{-kn\times t} \quad (2)$$

with first-order decay rate coefficients (k_1 to k_n) for each pool of carbon given the total initial carbon concentration is $c_0 + c_1 + c_2 + \ldots + c_n$. Based on our sampling frequency and total incubation time, we simplified the generalized model to models with 2 and 3 carbon pools of different qualities. The simplest model,

$$C(t) = c_0 + c_1\, e^{-k1\times t} \quad (3)$$

assumes two pools of carbon quality. Pool 0 is inert and has a constant concentration of c_0, and pool 1 is bioavailable with an initial concentration of c_1 and a first first-order decay rate coefficient of k_1. The total initial carbon concentration is equal to $c_0 + c_1$. The three pool model,

$$C(t) = c_0 + c_1\, e^{-k1\times t} + c_2\, e^{-k2\times t} \quad (4)$$

assumes three pools of carbon quality pools (pool 0: inert, pool 1: labile, and pool 2: semi-labile) with first-order decay rate coefficients, k_1 and k_2, respectively (Eq. 4).

To adapt these equations to our measured O_2 data requires use of the *OR* for conversion from O_2 respired to carbon consumed. We discuss the calculations behind choosing an *OR* and the assumptions and implications below. Starting with the simple 2-pool model and using data from our filtered treatments, we calculate that respiration of DOC consumes oxygen according to $dC = (1/OR)dO_2$, we can write

$$O_2(t) = O_2(0) + \int_0^t \frac{dO_2}{dT}\,dT = O_2(0) + OR\int_0^t \frac{dC}{dT}\,dT \quad (5)$$

where $O_2(0)$ is oxygen concentration at time *0* and *OR* is the respiratory oxidation ratio of O_2 consumed relative to carbon. After integrating Eq. 5, the two compartment model for oxygen dynamics due to DOC respiration becomes

$$O_2(t) = O_2(0) - B\left(1 - e^{-k_{doc}t}\right) \quad (6)$$

which contains three parameters: the initial concentration of oxygen [$O_2(0)$], the first-order decay rate of DOC (k_{doc}), and the concentration of oxygen used for consumption of DOC in the bioavailable pool ($B = c_{1doc}\times OR_{doc}$). We estimated the three parameters [$O_2(0)$, k_{doc}, and B] with data from the particle-free incubations using nonlinear least squares analysis (proc NLIN, SAS v9.1 Level 1M3, 2007, SAS Institute).

To derive heterotrophic respiration on POC alone, we modeled oxygen consumption (Eq. 6) as a sum of oxygen consumption due to DOC and additional consumption due to POC (Eq. 7) where $O_2(0)$ is initial oxygen concentration and *OR* is the respiratory oxidation ratio for conversion from O_2 respired to carbon consumed for DOC or POC.

$$O_2(t) = O_2(0) + OR_{doc}\int_o^t \frac{dC_{doc}}{dT}\,dT + OR_{poc}\int_0^t \frac{dC_{poc}}{dT}\,dT \quad (7)$$

After integrating Eq. 7, the oxygen consumption for the two-compartment model for POC (Eq. 8) includes parameters estimated from the DOC curve (*B* and k_{doc}). Here, we estimated POC curve parameters [$O_2(0)$, $E = c_1/OR_{poc}$, and k_1] using nonlinear least squares analysis as above, where *E* is the concentration of oxygen used for consumption in the labile component.

$$O_2(t) = O_2(0) - B\left(1 - e^{-k_{doc}t}\right) - E\left(1 - e^{-k_1 t}\right) \quad (8)$$

The three-compartment model also uses parameters estimated from the DOC control, but also includes two additional parameters for estimation of an additional POC pool (Eq. 9). Although Eq. 8 has a form similar to a 3-pool model, it operates as a 2-pool model as only three model parameters are fit (and the other 2 parameters are taken from DOC fit). Here, we estimate five total parameters: $O_2(0)$, the concentration of oxygen used for consumption in the labile ($E = c_1\times OR_{poc}$) and semi-labile pools ($F = c_2\times OR_{poc}$) and the first-order decay constant for both pools (k_1 and k_2, respectively).

$$O_2(t) = O_2(0) - B\left(1 - e^{-k_{doc}t}\right) - E\left(1 - e^{-k_1 t}\right) - F\left(1 - e^{-k_2 t}\right) \quad (9)$$

The reciprocal of each first-order decay constant, *k*, is the observed biological turnover time for the pool of DOC or POC of that quality. The turnover times (1/k) represent the turnover time of that pool of carbon (i.e., the average time until that pool is converted to CO_2 via cellular respiration).

Model fits (r^2, *P* values from *F*-statistics) were calculated for each curve using the extra sums of squares principal (Draper and Smith 1998). We report 95% confidence limits for all

model parameters as calculated by the least squares analysis. Comparisons between treatments (paired *t* tests) were made using estimates of the rate coefficients and standard errors from nonlinear least squares analysis.

Calculating OR

The basic respiration/photosynthesis reaction, $CO_2 + H_2O \leftrightarrow CH_2O + O_2$, illustrates the production of carbohydrates from carbon dioxide and provides an *OR* of 1.0. Redfield et al. (1963) expanded this reaction stoichiometry to include nitrogen and phosphorus for marine systems where these nutrients are supplied as or mineralized to nitrate (NO_3^-) and phosphate (PO_4^{-3}), respectively. In the last decade, a number of studies have more accurately assessed carbon:oxygen stoichiometries in organic matter and elucidated how *OR* depends on the carbon oxidation state, the oxidation state of involved inorganic nitrogen species, and to a lesser extent, sulfur species (Hedges et al. 2002; Masiello et al. 2008; Hockaday et al. 2009).

Thus, with knowledge of the elemental ratio of the organic matter pool and the final nitrogen species, it is possible to calculate *OR* exactly using the following equations, which are the first published error-free stoichiometries that include sulfur and phosphorus and assume nitrate as the nitrogen end product (Hockaday et al. 2009; Masiello and Hockaday pers. comm.):

$$\begin{aligned} \mathrm{C_xH_yO_zN_wS_mP_n} + \left(\frac{4\mathrm{x} + \mathrm{y} - 2\mathrm{z} + 5\mathrm{w} + 6m + 5n}{4}\right)\mathrm{O_2} \\ \leftrightarrow x\mathrm{CO_2} + \left(\frac{y - w - 2m - 3n}{2}\right)\mathrm{H_2O} + w\mathrm{HNO_3} \\ + mH_2SO_4 + nH_3PO_4 \end{aligned} \quad (10)$$

Variables represent the molar quantities of each element and the coefficients in the O_2 term represent the oxidation state of each respective element's oxidized form divided by the oxidation state of carbon in CO_2. The coefficients in the H_2O term represent total hydrogen less the number of hydrogen atoms in the oxidized form of each respective element, all divided by the number of hydrogen atoms in H_2O. The average oxidation state (C_{ox}) of carbon in the organic matter is calculated from balancing oxidation states of the elements in organic matter:

$$\mathrm{C}_{ox} = \frac{2\mathrm{z} - \mathrm{y} + 3\mathrm{w} + 2\mathrm{m} - 5\mathrm{n}}{\mathrm{x}} \quad (11)$$

This presumes that organic N predominately has a –3 oxidation state (i.e., amine) and that organic P has a +5 oxidation state (i.e., phosphate ester). We also assume that organic S predominately has a –2 oxidation state (i.e., thiol, sulfide) and that natural sulfate esters with a +6 oxidation state contribute negligibly to algae, fresh vascular plant detritus, and other components of suspended POM. However, sulfate ester contributions are not well constrained, could be as high as 30% in some agricultural soils, and forces the coefficient of *m* in Eq. 11 to vary between +2 and –6 (Hockaday et al. 2009). A simplified formulation of *OR* can be attained by rearranging the ratio of the stoichiometric coefficients of O_2 and CO_2 to the following equation:

$$OR = 1 - \frac{C_{ox}}{4} + \frac{2w}{x} + \frac{2m}{x} \quad (12)$$

Organic sulfur and phosphate contribute minimally to natural organic matter, and ignoring these terms, only introduces small errors (Hockaday et al. 2009). For example, using data for marine plankton from Hedges et al. (2002) with Eq. 12 including sulfur and phosphorus raises the *OR* by only 0.39%. However, knowledge of organic sulfur and phosphate content would improve estimates of *OR*, especially for any organic matter with high sulfur or phosphorus content.

Many aquatic studies (e.g., Amon and Benner 1996; Webster et al. 1999) use an *OR* of 1.0 (Berggren et al. 2011). Yet other aquatic studies (i.e., Williams and del Giorgio 2005) take the physiological perspective that organisms excrete reduced nitrogen (i.e., urea or NH_3), and therefore, ignore the rapid oxidation of inorganic nitrogen to nitrate in oxic aquatic systems by chemolithotrophs. In terrestrial systems, nitrogen is often supplied as N_2 or NH_4^+ depending on the importance of nitrogen fixation or fertilization. These net community transformations of nitrogen need to be explicitly considered in calculating *OR*. For example, the *OR* of a typical protein, ignoring organic sulfur ($m = 0$), can be 1.04 (Eq. 6 from Masiello et al. 2008) or 1.56 (Eq. 12 above) depending on whether the organic nitrogen is transformed to ammonium or nitrate, respectively. The *OR* for scaling from respiration of oxygen to carbon removal can be most accurately estimated using Eq. 12 or other equations with knowledge of the fate of organic nitrogen and the elemental composition of organic matter. Recent studies have refined the elemental ratios of fixed/respired organic matter to better reflect actual biochemical compositions of marine plankton (Hedges et al. 2002; Baldock et al. 2004) and soil organic matter and plant tissue residue (Baldock et al. 2004; Hockaday et al. 2009). Assuming the fate of organic nitrogen were nitrate, we recalculate the *OR* from these reported elemental ratios using Eq. 12 for marine plankton ($1.32 \leq OR \leq 1.45$, midpoint = 1.39) and plant residue in soils ($1.04 \leq OR \leq 1.2$, midpoint = 1.12). Suspended POC is likely a mixture of detrital organic matter particles with characteristic elemental and biochemical compositions that closely reflect their algal or vascular plant leaf sources and mineral-complexed organic matter that has a characteristic low C:N and protein-rich composition (Aufdenkampe et al. 2007; Aufdenkampe et al. 2011; Richardson et al. 2009).

Modeling results indicate that 88% to 94% of POC from this system is low-density detrital organic matter particles (leaf and algal carbon) while the remaining 6% to 12% of carbon is associated with high-density particles (Richardson et al. 2009), which in turn have distinctly different elemental and biochemical compositions (Aufdenkampe et al. 2007; Richardson

et al. 2009). From elemental and mixing model analyses, we calculated that about 30% of the detrital organic matter particles were composed of terrestrial vegetation while the remaining 70% were of algal origin (D. C. Richardson unpubl. data). Therefore, we can calculate an *OR* = 1.3 for POC for this study given that 30% of the material has an *OR* like soil + plant residues (~1.12 from above) and 70% has an *OR* similar to marine plankton (~1.39). The elemental and biochemical composition of DOC, on the other hand, is typically much more oxidized (Gu et al. 1994, 1995; Kim et al. 2006), supporting *OR* = 1.0 for DOC.

Biodegradable DOC (BDOC)

From initial trials with TFF, we found that DOC was concentrated above initial levels despite nominal TFF cutoff size of 0.65 µm. We used the method proposed above to assess the lability of the DOC before and after TFF. We wanted to compare these results to existing methods to determine bioavailable DOC (BDOC) using plug-flow bioreactors (Kaplan and Newbold 1995). Briefly, the bioreactors are 600 mL glass chromatography columns filled with sintered glass beads; they are continuously fed WCC stream water at ~4 mL min^{-1} filtered to 0.3 µm to remove any particles while allowing ~95% of the bacteria into the bioreactors (Kaplan and Newbold 1995). On 15 Aug 2007, 2-L aliquots of preTFF and postTFF water from the DOC lability experiment were pumped into two different bioreactors that had been continuously running on WCC stream water for > 4 months. After 3 bed volumes of sample passed through the bioreactor to waste, we took subsamples of influent and effluent water for DOC analysis. The BDOC for each sample is defined as the DOC removed by the bioreactor: influent concentration minus effluent concentration.

Assessment

Collection and concentration of POC

TFF concentrated small (>0.65 µm) particles 5-fold above stream concentrations. Using measurements of POC and DOC from before and after TFF, we calculated that POC, as % of total organic carbon (POC + DOC), increased from 11% to 44% for Jan 2006 and 9% to 33% for Apr 2007. TFF was necessary because methods to collect POC with nets or sieves can only concentrate particles sizes that are ≥ 15-20 µm and recovery of vacuum-filtered particles from glass fiber or membrane filters has proven to be challenging and ineffective (D. C. Richardson unpubl. data). TFF might not be necessary to measure POC quality when POC concentrations are high, such as in samples from turbid rivers like the Amazon or Mississippi, anthropogenically impacted areas, pond and reservoir outflows, or stream waters collected during storms (e.g., Whiles and Dodds 2002). However, for small headwater streams that have high DOC:POC ratios and low POC concentrations at baseflow (Kaplan et al. 2006), respiration of the larger DOC pool could mask oxygen consumption from POC respiration. Increasing suspended POC concentration above naturally occurring concentrations is necessary to adequately measure POC respiration rates.

Although TFF was advantageous in elevating POC concentrations, recovery of POC from TFF was only 50% to 60%. This recovery was less than optimal, but similar to other recovery rates reported in the literature for POC (e.g., <56% in Roland et al. 2009). Poor recovery may be due to adsorption of particles to the membrane (Minor et al. 1999), and TFF filter cleaning with detergents after sample processes always liberated substantial amounts of particles. All filtration processes may change particle structure and size; TFF has the potential to do so through sheer forces generated by the particles passing through tubes and across the filter. However, several studies examined the effects of TFF on environmental particle size and structure including prokaryotic cells (Giovannoni et al. 1990), viruses (Alonso et al. 1999), soil particles (Tang et al. 2009), and freshwater particles (Morrison and Benoit 2004). In general, these studies found that TFF minimally affected particle size and composition compared with other concentration techniques. Alternative concentration techniques (e.g., continuous flow centrifugation) may have higher recovery, but then particles compressed within the pellet would need to be dispersed. Further, TFF resulted in an unintended concentration of DOC by as much as 62% (Table 2), discussed below (*see* "DOC lability experiment").

Table 2. Initial particulate and dissolved organic carbon concentrations (mean ± SD) for all experiments. N.A. indicates that POC concentration was not measured.

Experiment	Treatment	POC (mg L^{-1})	DOC (mg L^{-1})
Jan 2006	DOC control	<0.03	1.5 ± 0.05
	Settled particles	0.98 ± 0.04	1.5 ± 0.02
	Suspended particles	0.92 ± 0.10	1.5 ± 0.04
	Dispersed, suspended particles	0.99 ± 0.06	1.5 ± 0.02
Apr 2007	DOC control	<0.03	2.1 ± 0.08
	Dispersed DOC control	<0.03	2.2 ± 0.09
	Settled particles	1.12 ± 0.08	2.1 ± 0.08
	Dispersed, suspended particles	0.97 ± 0.07	2.1 ± 0.05
DOC lability	preTFF	N.A.	1.3 ± 0.02
	postTFF	N.A.	2.1 ± 0.08

Oxygen measurements

Oxygen consumption due to heterotrophic respiration of suspended POC can be difficult to measure due to low concentrations of POC relative to DOC. Therefore, we used the Winkler method with potentiometric auto-titration when measuring the oxygen concentrations in each bottle. This is an efficient, accurate, and precise method to measure oxygen concentration, especially when attempting to detect small amounts of oxygen consumption (Furuya and Harada 1995). Auto-titration was precise within 0.05 ± 0.009 mg O_2 L^{-1} (mean ± SE, n = 22) for oxygen concentrations ranging from 3.5 to 8.5 mg O_2 L^{-1}, or 0.5% ± 0.2% (mean coefficient of variation ± SE, n = 22) comparable to precision seen in other studies for Winkler measurements (e.g., Ploug et al. 2002). This precision is especially important during the high-frequency sampling required for the initial 24 hours of oxygen measurements when concentrations changed by < 0.5 mg L^{-1} (Fig. 2a, 3a). Auto-titration is advantageous over previously used oxygen concentration measurements such as the Gilson respirometer (Webster et al. 1999) or polargraphic electrode probes, which lack the precision and accuracy necessary to measure the small changes in oxygen concentration (CWT 2004). Alternatively, with recent advances in technology, luminescent dissolved oxygen (LDO) probes are now being used in marine and freshwater ecosystem research (e.g., Heffernan and Cohen 2010), and these sensors may be preferable to auto-titration because the measurements are easy, rapid, and have similar levels of precision (0.05 to 0.2 mg O_2 L^{-1}, Tengberg et al. 2006).

Oxygen and carbon consumption curves and model fits

For both experiments, heterotrophic oxygen consumption rates (k) were highest in the POC + DOC treatments (for Jan 2006 experiment: dispersed, suspended particles > suspended particles > settled particles; for Apr 2007 experiment: dispersed, suspended particle = settled particle) and lowest in the DOC

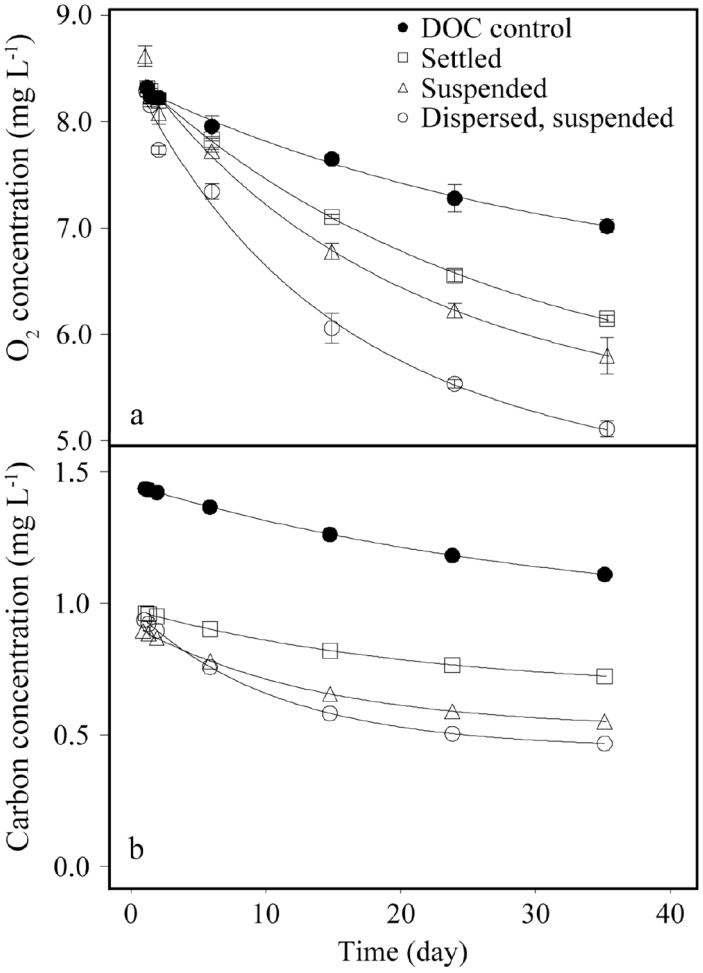

Fig. 2. Jan 2006 experiment (a) measured O_2 consumption, where lines indicate model fits, and (b) modeled carbon dynamics. POC concentrations dynamics are corrected for respiration of DOC. Treatments are DOC control, settled particles, suspended particles, and dispersed, suspended particles. Error bars are standard errors.

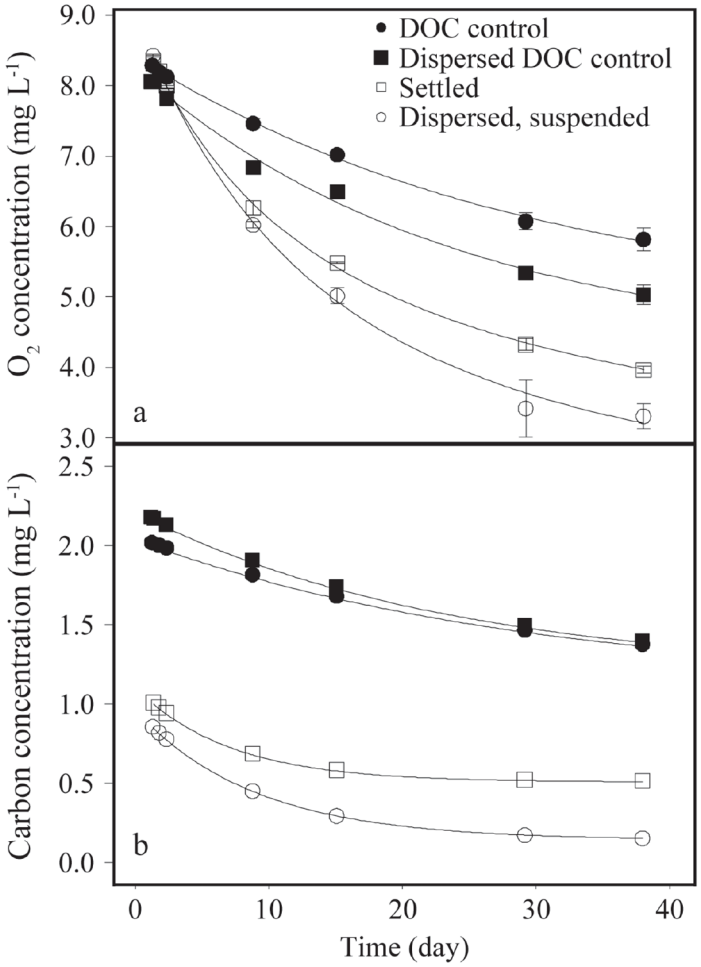

Fig. 3. Apr 2007 experiment (a) measured O_2 consumption, where lines indicate model fits, and (b) modeled carbon dynamics. POC concentrations dynamics are corrected for respiration of DOC. Treatments are DOC control, dispersed DOC control, settled particles, and dispersed, suspended particles. Error bars are standard errors.

control treatments (Table 3). The two compartment first-order POC decay model that fits the data to a bioavailable POC pool and an inert POC pool (Eq. 8, with DOC parameters taken from fitting Eq. 5 to DOC only incubations) was an excellent fit for all curves in both experiments (Table 3, $r^2 > 0.97$). A three-pool POC model (Eq. 9) of the Jan 2006 and Apr 2007 experimental results provided little, if any, improvement to the fit of the curve. For most of the treatments, the second decay rate constant in the three-pool model was forced to 0 by the fitting procedure, and we were unable to divide the bioavailable component into labile and semi-labile organic matter. Extending the length of the experiment would allow for better estimation of the size of the various lability classes (i.e., pools) using these modeling procedures. Measurements over much shorter time periods than this study (<24 h) are adequate for measuring initial rates but do not allow for separation of labilities into two or more pools (Berner 1995; Trumbore 2000; Kaplan and Newbold 2003). Carbon concentration (DOC or POC alone) was modeled using the two-pool model (Eq. 3); although DOC had higher starting concentrations, the initial decrease of total concentration, due to the bioavailable component, was more rapid in most POC treatments (Fig. 2b and 3b).

Carbon turnover

Using the first-order decay rate coefficient (k) and standard errors for the Jan 2006 experiment (Table 3), we calculated the biological turnover time ($1/k$) for POC in each of the treatments. The shortest turnover time in the Jan 2006 experiment was POC in the dispersed, suspended particle treatment, followed by the suspended particle treatment and settled particle treatment (Table 4). For the Apr 2007 experiment, turnover times were shortest in the dispersed, suspended particle and settled particle treatments, and those turnover times were not significantly different from each other (Table 4; $t = -1.43$, df = 33, $P = 0.16$).

We were unable to find any other studies of POC lability that corrected for respiration of DOC during incubations. Without correction for DOC respiration, the POC treatments would simply be estimated using Eq. 6 rather than Eq. 9, resulting in an overestimation of carbon turnover time. For example, the turnover time for the dispersed, suspended particles in the Jan 2006 experiment was 10.6 days; without correction for DOC, the carbon turnover time would be calculated at 20.8 days, an increase of almost 100%.

Table 3. Oxygen consumption curve parameter fits for each treatment and experiment. DOC control fits use Eq. 7; POC fits use Eq. 9 and are corrected for the DOC control for each experiment. k is first-order decay rate coefficients for POC or DOC, B and E are the concentration of oxygen used for consumption of DOC and POC, respectively, in the bioavailable pools, and $O_2(0)$ is the estimated initial oxygen concentration. Estimates (95% confidence intervals) for parameters are included with model fits (r^2). All model fits were significant ($P < 0.001$).

Exp.	Treatment	k_{poc} or k_{doc} (h^{-1})	B or E (mg O_2 L^{-1})	$O_2(0)$ (mg O_2 L^{-1})	r^2
Jan 2006	DOC control	0.0013 (0.0003 to 0.0023)	2.0 (1.1 to 2.8)	8.28 (8.20 to 8.35)	0.96
	Settled particles	0.0019 (0.0011 to 0.0028)	1.2 (1.0 to 1.4)	8.31 (8.27 to 8.35)	0.99
	Suspended particles	0.0033 (0.0011 to 0.0055)	1.6 (1.2 to 1.9)	8.42 (8.28 to 8.57)	0.97
	Dispersed, suspended particles	0.0039 (0.0023 to 0.0055)	2.1 (1.8 to 2.3)	8.19 (8.06 to 8.31)	0.98
Apr 2007	DOC control	0.0014 (0.0008 to 0.0021)	3.6 (2.8 to 4.5)	8.27 (8.17 to 8.37)	0.98
	Dispersed DOC control	0.0018 (0.0012 to 0.0023)	4.0 (3.4 to 4.6)	8.04 (7.93 to 8.15)	0.98
	Settled particles	0.0059 (0.0048 to 0.0070)	2.3 (2.2 to 2.5)	8.35 (8.27 to 8.43)	0.99
	Dispersed, suspended particles	0.0047 (0.0033 to 0.0061)	3.2 (2.9 to 3.5)	8.4 (8.24 to 8.57)	0.99
DOC lability	preTFF	0.0090 (0.0011 to 0.0168)	0.6 (0.5 to 0.8)	8.24 (8.19 to 8.28)	0.96
	postTFF	0.0033 (–0.0026 to 0.0091)	1.5 (–0.1 to 3.1)	8.21(8.16 to 8.25)	0.92

Table 4. Biological turnover times ($1/k$) for only POC; POC turnover is corrected for DOC respiration. Turnover times of treatments with the same letter were not significantly different when analyzed using all pairwise t tests within each experiment where $P < 0.05$. t tests were completed using estimates of k and standard errors (Table 3) generated from nonlinear least squares analysis.

Exp.	Treatment	Turnover time (1/k, d)	Within experiment grouping	Bioavailable pool size (mg L^{-1}); % of total carbon	Inert pool size (mg L^{-1}); % of total carbon
Jan 2006	Settled particles	21.5	a	0.35; 35.9%	0.63; 64.1%
	Suspended particles	12.7	b	0.45; 48.8%	0.47; 51.2%
	Dispersed, suspended particles	10.6	b	0.60; 61.0%	0.38; 39.0%
Apr 2007	Settled particles	7.1	a	0.67; 60.1%	0.45; 39.9%
	Dispersed, suspended particles	8.9	a	0.92; 95.2%	0.05; 4.80%

Rotation and mixing of BOD bottles increased oxygen consumption and decreased bioavailable oxygen turnover time over static bottles for both Jan 2006 and Apr 2007 experiments (Fig. 2, 3). Diffusion of oxygen to particle-attached microorganisms and POC is faster for suspended rather than settled particles since layering in settled treatments limits solute exchange to lower layers of particles (Ploug and Grossart 1999), thereby slowing decomposition by encouraging the formation of anoxic microsites. For example, Ploug and Grossart (1999, 2000) found that bacterial production on marine diatom aggregates increased 5- to 10-fold when particles were kept in suspension during incubations compared with those under static conditions.

Addition of beads to disperse flocs did not significantly ($P > 0.05$) affect the loss rate of POC (Table 4), although the estimated rate for dispersed particles (0.0039 h^{-1}) was 18% higher than for the bead-free suspension (0.0033 h^{-1}). In treatments without beads, particles in the BOD bottles aggregated into flocs, which were visible to the naked eye in the bottles rotating on the incubation axle. This may be similar to marine environments where flocs are generated by physical collisions of cells and detritus settling at different rates and without turbulence to break the flocs up (Jackson 1990). No flocs could be seen with the naked eye in the WCC water column, in water samples collected from WCC, or through microscopic observations of WCC suspended particles. While the beads acted to increase internal mixing and break up flocs, the 9% increase in available surface area for bacterial attachment did not appear to influence the use of DOC because the resulting DOC mineralization profiles with and without the beads were not significantly different from each other (Table 4, Fig. 3b). Conditions of mass transfer and chemical microenvironments for particles are likely more similar to natural stream conditions due to suspension and mixing of particles through rotation and disaggregation with glass beads.

Comparing the Jan 2006 and Apr 2007 experiments, the turnover time for DOC control was not statistically different ($t = 0.14$, df = 36, $P = 0.88$). However, when comparing dispersed, suspended particle treatments from both experiments, the turnover time for Apr 2007 was 16% shorter than for the Jan 2006 experiment (Table 4) and the corresponding k values were statistically different ($t = 3.2$, df = 33; $P = 0.003$). Furthermore, for the Jan 2006 experiment, the bioavailable pool was calculated to comprise 61% of total initial carbon, and for the Apr 2007 experiment, the bioavailable pool comprised 95% of total carbon. We calculated that respiration of that carbon greatly reduced the total carbon pool by the end of the 40-d experiment (Fig. 3b). The observed difference in heterotrophic respiration, carbon turnover, and bioavailable carbon pool size may reflect seasonal differences in the composition of POC resulting from increases in algal-derived carbon. In early April, the stream canopy is open, light levels are higher, and periphyton growth is at a maximum on the streambed leading to high chlorophyll *a* and carbon content of seston (Pozo et al. 1994; Richardson et al. 2009). In this study, this difference in bioavailability could be linked to an increase in the proportion of particles that were algal cells (D. C. Richardson unpubl. data; Pozo et al. 1994). Similarly, in a eutrophic lake, suspended POC quality dramatically increased during warm season algal blooms (Parparov et al. 1998) and increasing nutrient content of benthic POC was correlated with increasing heterotrophic respiration rates (Fuss and Smock 1996).

OR choice and carbon pools

The choice of *OR* for DOC or POC does not change the estimation of carbon turnover time or the model fits. The modeling fits and parameters are calculated independently of *OR*. However, the choice of *OR* will change the estimates of the carbon content in each pool (bioavailable and inert) and the carbon curves (Fig. 2b, 3b). We wanted to test this assumption by examining the carbon pool sizes over a range of theoretical *OR* values to see how the choice of *OR* affects the division of carbon into different pools. We selected this range to encompass molecular compositions found in wetland DOC (*OR* = 0.95; Gu et al. 1995), carbohydrates (*OR* = 1), plant tissue and soil organic matter (*OR* ~ 1.2; Hockaday et al. 2009), marine plankton (*OR* ~ 1.4), and proteins (*OR* = 1.55 and above depending on nitrogen and sulfur content). For the Jan 2006 and Apr 2007 experiments and the dispersed, suspended particle treatment, we estimated carbon pool size over an *OR* range of 0.7 to 1.7 (Fig. 4). Below *OR* = 0.8, the estimation of carbon pools exceeds calculation bounds for the two pools with bioavailable carbon composing more than 100% of the total pool. At *OR* = 1.30, bioavailable carbon accounted for 55% of total initial carbon whereas inert carbon was the remaining 45%. At our highest *OR* (*OR* = 1.7), the bioavailable carbon was 46% of the total initial carbon. The appropriate choice of *OR* depends on the composition and stoichiometry of organic material and fate of organic nitrogen, which can differ among biogeochemical regions like marine or terrestrial ecosystems. We suggest that *OR* = 1.3 is an appropriate intermediate choice for mixed lotic organic matter with organic nitrogen transforming to nitrate in this system.

DOC lability experiment

TFF creates electrostatic gradients that repel and concentrate DOC molecules that are much smaller than the membrane pore size (Benner et al. 1997). In all experiments, TFF concentrated DOC, and we used this postTFF DOC as the DOC controls in all above experiments. In preTFF and postTFF treatments, the first-order decay model (Eq. 1) was a significant fit for both treatments ($r^2 > 0.92$; Table 3). Carbon turnover for preTFF DOC (4.6 d) and postTFF (12.8 d) were not significantly different (t = 1.27, df = 24, $P = 0.21$) despite heterotrophic consumption of oxygen being higher in postTFF than preTFF treatments (Fig. 5). Furthermore, using plug-flow bioreactors, we determined that the concentration of biodegradable DOC (BDOC) in the postTFF water was 0.88 mg C L^{-1} (41% of DOC) compared with 0.42 mg C L^{-1} (33% of

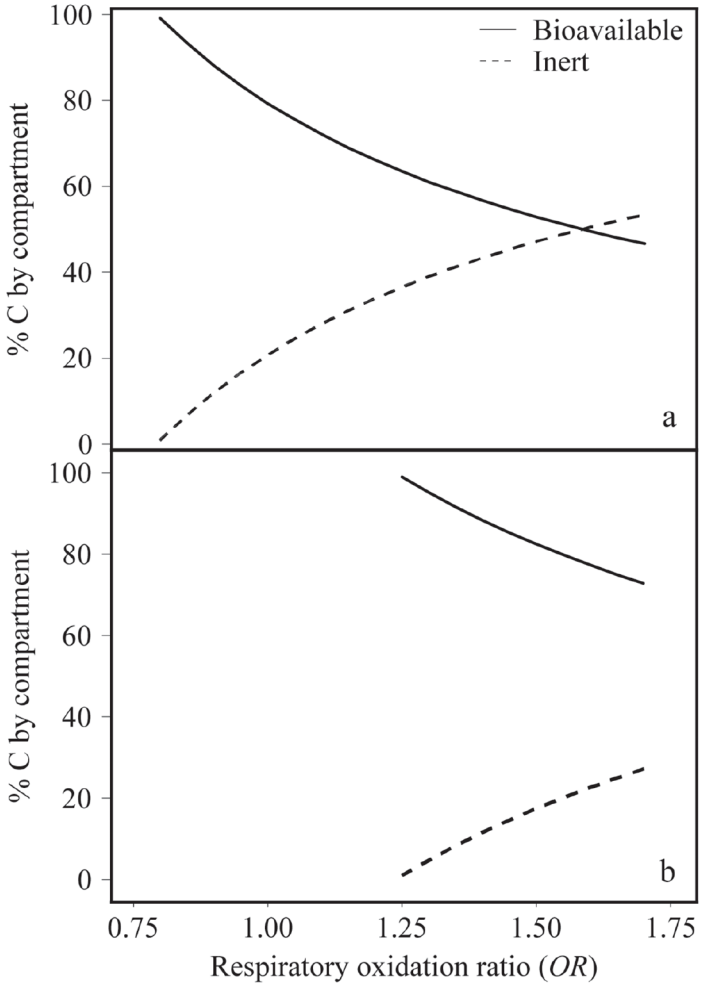

Fig. 4. Sensitivity analysis of respiratory oxidation ratio (*OR*) and effect on the calculated compartmental breakdowns of bioavailable (solid line) and inert (dashed line) organic matter for dispersed, suspended particles from both (a) Jan 2006 and (b) Apr 2007 experiments. The percentage of total organic carbon by compartment was calculated using derived model parameters for the bioavailable component.

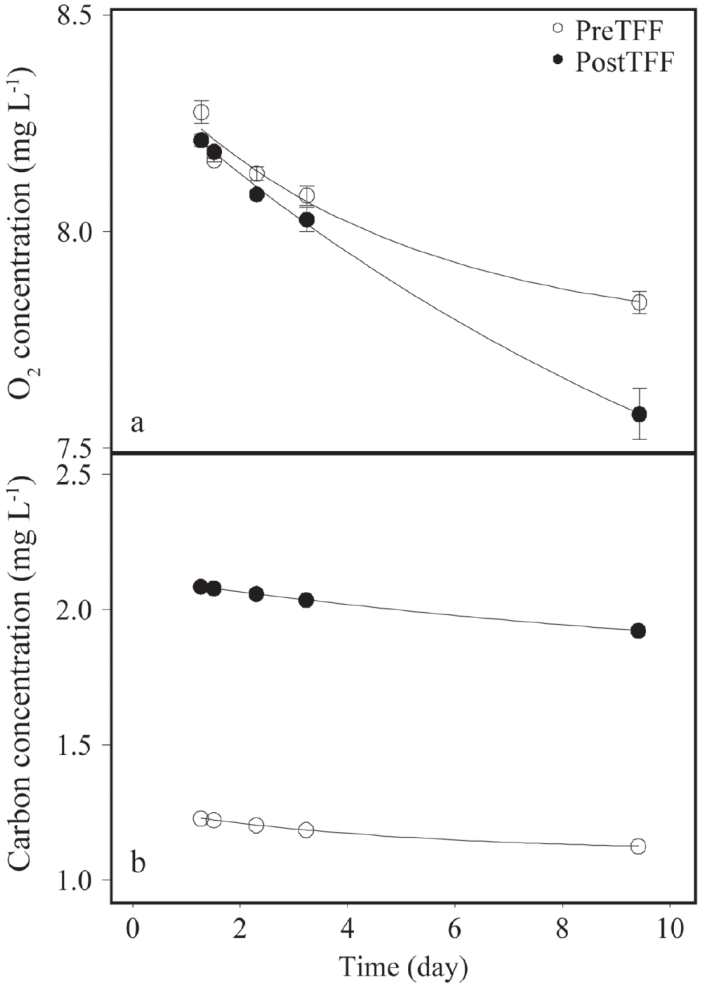

Fig. 5. DOC lability experiment (a) measured O_2 consumption, where lines indicate model fits, and (b) modeled carbon dynamics. Treatments are pretangential flow filtration (preTFF) and post-tangential flow filtration (postTFF). Error bars are standard errors.

DOC) in unconcentrated preTFF water. Although our results were not significantly different, the TFF may have slightly modified DOC composition through biased concentration of DOC molecules or desorption of molecules adhered to particles or a combination of both processes.

However, we could not detect significant differences in *k* between pre- and postTFF DOC in the DOC lability experiment (Table 3), and these rates differ from those estimated for labile (1.5 h) and semi-labile (29 h) DOC measured in whole stream experiments (Kaplan et al. 2008). Further, by removing suspended particles from the bottles, we were removing a significant portion of heterotrophic microbes that would consume DOC. By measuring respiration on short time-scales (<7 d), bacterial populations were not given the enough time to grow and metabolize DOC. Therefore, whereas our use of postTFF DOC as the DOC correction for all POC treatments is appropriate, we avoided interpreting any analysis of DOC lability from the incubations and recommend avoiding comparison of results of DOC from this study to other DOC lability estimates.

Discussion

Respiration rate comparisons

We measured biological turnover times of approximately 10 days for a bioavailable suspended organic carbon pool corresponding to more than half of suspended stream POC (Table 4). Such rapid turnover times have not been previously measured for stream POC or organic carbon collected from other aquatic environments (Table 5). The turnover times for suspended stream POC measured using the method reported here were 2-5 times shorter than for eutrophic lake seston (Parparov et al. 1998) or coastal marine DOC (Biddanda et al. 1994), 20-

Table 5. Ranges of respiration rates for different carbon sources in aquatic ecosystems. Carbon turnover time was either taken directly from the study or was calculated using respiration rates (mg O_2 mg C^{-1} d^{-1}) assuming *OR* of 1.3.

Carbon source	Carbon turnover time (d)	Citation
Stream suspended POC	8.9, 10.6	This study
Marine diatom aggregates	5 to 35	Ploug and Grossart 2000
Lake suspended POC	14-56	Parparov et al. 1998*
Stream benthic POC	180-2700	Webster et al. 1999*
Stream benthic POC	190-760	Bonin et al. 2000
Stream benthic POC, leaves, wood	520-1340	Fuss and Smock 1996
Leaves and wood	180-1610	Stelzer et al. 2003
Tree tissue leachate DOC	0.06, 1.2	Kaplan et al. 2008
Wetland DOC	201-4530	Opsahl 2005
Marine DOC	27-38	Biddanda et al. 1994

*See citation for literature summary of respiration rates.

400 times shorter than for wetland DOC (Opsahl 2005) and about 2 orders of magnitude shorter than the 2.6 years reported by Webster et al. (1999) for benthic stream POC.

One reason for our estimates of high POC mineralization rates is that ours is the first study to measure suspended stream POC turnover times in a realistic context (i.e., in a continually dispersed suspension at near natural suspended concentrations by precision O_2 measurements). Other reasons include collection of naturally suspended stream POC versus benthic stream POC, correction for DOC mineralization, explicit calculation of the size of the labile POC pool by fitting 35-40 days of incubation data versus assuming mineralization of the entire POC pool, and using a more realistic *OR* to convert between oxygen and carbon.

The differences between measurements of suspended POC from this study and benthic POC from other studies (Table 5) are consistent with a conceptual model of selective resuspension of POC from the benthos resulting in a high quality food source that travels downstream. Therefore, sampling the suspended POC pool and keeping the particles suspended during incubation is critical when making calculations of POC to determine contributions of headwater streams to downstream metabolism.

A comparison between spring and fall respiration rates suggests that our method can distinguish between labile POC pools associated with seasonal shifts in POC supply. Rapid turnover times (~1 week) measured in the Apr 2007 experiment from this study could have been driven by vernal algal blooms with high quality organic matter whereas the remaining POC could have been more refractory POC including mineral-organic complexes (i.e., clay particles with adsorbed organic carbon) and leaf fragments.

Directions for future research

By explicitly addressing the lability of suspended POC, we suggest that at a turnover time of 10 days (this study), in a stream where small particles move downstream at 150 m day^{-1} at baseflow (Newbold et al. 2005), more than half of POC (i.e., all labile POC) should be metabolized or converted to biomass after traveling only 1.5 km downstream. This travel distance is far shorter than the 42 km estimated by Webster et al. (1999) for benthic POC in streams smaller than the one from this study and falls between the uptake lengths of 238 m for labile DOC and 4.5 km for semi-labile DOC measured in White Clay Creek (Kaplan et al. 2008). This could explain a portion of the CO_2 out-gassing in large streams and rivers through heterotrophic respiration of carbon from upstream and terrestrial sources (e.g., Cole and Caraco 2001; Battin et al. 2008; Aufdenkampe et al. 2011).

Further, we suggest that our method be extended to a wide range of stream ecosystems, ranging from streams with high primary production (e.g., open-canopy streams) to those dominated by inputs of terrestrially derived organic matter (e.g., small forested headwaters). Incubation times could be increased to estimate multiple pool model parameters (e.g., Eq. 2 and 4), but nutrient and oxygen limitation must be considered. Nutrients and oxygen could be added to a separate aliquot throughout the incubation to alleviate these concerns. Finally, this method could be used to explore the contribution of different particle size classes to overall POC degradation and lability. In this way, application of this method will advance the understanding of POC as a bioenergetic subsidy to river networks and the role of river networks in controlling the transformations of allochthonous and autochthonous POC during transit from the land to the ocean (Cole et al. 2007).

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