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A synoptic survey of microbial respiration, organic matter decomposition, and carbon efflux in U.S. streams and rivers

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Abstract

We analyzed ecoenzyme activities related to organic matter processing in 1879 streams and rivers across the continental U.S. as part of the USEPA's National Rivers and Streams Assessment. Ecoenzymatic stoichiometry was used to construct models for carbon use efficiency (CUE) and decomposition ($-k$). Microbial respiration (R_m) was estimated from sediment organic carbon stocks, CUE and $-k$. The streams and rivers were classified by size (headwaters: 1st-order; streams: 2nd-3rd order; small rivers: 4th-5th order; big rivers 6th-7th order; and great rivers 8th order) and condition class (least, intermediate and most disturbed), and grouped into nine ecoregions. There were ecoregion, stream size, and condition class effects for CUE, $-k$, and R_m , with R_m increasing from eastern ecoregions through the plains to the western ecoregions. CUE, $-k$, and R_m decreased with increasing streams size and increased with increasing disturbance. R_m , CUE, and $-k$ were correlated with water and sediment chemistry; CUE and $-k$ were also correlated with stream bed fine sediments; and CUE was further correlated with catchment land cover. R_m was extrapolated to ecoregional and national scales, and the results suggest that microbial assemblages account for 12% of the total CO₂ outgassing, and nearly 50% of the aquatic metabolism C losses, from U.S. streams and rivers. Cumulative respiratory C losses increased from headwaters to small streams, then decreased with increasing stream size. This U-shaped respiration curve was not evident when streams were viewed by disturbance classes, suggesting that anthropogenic disturbances mask the expected organic matter processing signature of the river continuum.

Keywords

carbon use efficiency; CO₂ efflux; decomposition; ecoenzymes; microbial respiration; river continuum; streams and rivers; U-shaped curve

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Introduction

The distribution and processing of carbon in streams provides the energetic foundation for the longitudinal, ecosystem-scale responses of streams to their environments (Vannote et al. 1980; Benda et al. 2004; Webster 2007). Metabolism of organic matter inputs along stream continua scales positively with stream size (Naiman and Sedell 1980; Sedell et al. 1989; Naiman et al. 1997; Webster 2007; Finlay 2011; Hotchkiss et al. 2015). Organic matter inputs and processing along stream continua reflect a transition in the importance of allochthonous and autochthonous C inputs, and the shifting importance of other environmental drivers, leading to U-shaped curves for organic carbon availability, organic matter decomposition, and accompanying ecosystem respiration along the headwaters to great rivers continuum (Fig. 1; Naiman et al. 1987; Webster 2007; Battin et al. 2009).

The fate of organic carbon in streams, especially leaf litter decay and dissolved organic carbon export, has been extensively documented (Webster and Benfield 1986; Webster et al. 1999; Cole and Caraco 2001). However, the microbial processing of particulate and particle-bound organic carbon has received much less attention, especially across stream sizes and landscapes, and in response to anthropogenic disturbances. Similarly, the role of stream sediment C storage and CO₂/CH₄ evolution has been minimally studied (Cole et al. 2007; Stanley et al. 2016). Given the cumulative extent of stream channels on the landscape, their role in global C budgets should not be ignored (Cole et al. 2007; Butman and Raymond 2011; Butman et al. 2016).

Carbon accrual by stream microbial assemblages is stoichiometrically related to nutrient availability such that anabolic C fixation (primary production) and catabolic C releases (respiration) are often positively correlated with N and P availability (Hill et al. 2002, 2010a, 2012; Sinsabaugh et al. 2009). Microbial assemblages also produce coenzymes to facilitate the degradation of organic matter in order to acquire organically-bound carbon and nutrients (Sinsabaugh and Foreman 2001; Hill et al. 2010a, 2010b, 2012; Sinsabaugh et al. 2009). We are especially interested in glycosidases (used in the acquisition of labile organic carbon), glucosaminidases and peptidases (nitrogen acquisition), and phosphatases (phosphorus acquisition). Ecoenzymatic activity (EEA) has been positively correlated with sediment nutrient concentrations (Sinsabaugh et al. 2009; Hill et al. 2010a, 2012), with organic carbon refractivity (Sinsabaugh and Follstad Shah 2011), and with microbial metabolism (Sinsabaugh et al. 2012).

Microbial carbon use efficiency (CUE), a measure of the relative apportionment of C utilization for microbial growth versus the energetic requirements (as respiration) to support existing microbial biomass, ranges from 0 to 0.60, and decreases with increasing temperature, though the response is weak within the temperature range of most studies (Keiblinger et al. 2010; Manzoni et al. 2012; Sinsabaugh et al. 2013). Carbon and nutrient stoichiometry has proven to be an important regulator of CUE, with increasing C and decreasing N and P availability resulting in lowered CUE (Manzoni et al. 2012; Sinsabaugh et al. 2013; Hill et al. 2014).

Our objectives for this study were to use microbial EEA of sediments collected from 1879 U.S. streams and rivers in enzyme-based decomposition models (EDM) to predict organic matter decomposition rates ($-k$) and microbial respiration (R_m). We explored ecoregional, stream size, and disturbance class differences in CUE, $-k$, and R_m , and used these variables, along with sediment OC standing crop, to test predictions (Fig. 1) of the River Continuum Concept (RCC; Vannote et al. 1980; Minshall et al. 1985) and the U-shaped curve hypothesis (Naiman et al. 1987; Webster 2007) at regional and national scales. Finally, we used R_m to estimate the cumulative microbial contributions to CO_2 efflux from U.S. streams and rivers.

Materials and methods

Study sites

Our study included the 938 Wadeable streams (1st–4th order, Strahler 1957) and 941 non-wadeable rivers (5th–10th order) that were sampled (excluding site re-visits) during the summers of 2008–2009 as part of the EPA Office of Water's National Rivers and Streams Assessment (NRSA; Fig. 2). The sampling design was spatially balanced and employed an unequal site selection based on stream order. The design selected a single point along the stream center line as depicted by the National Hydrography Database (NHD-Plus, <http://horizon-systems.com/nhdplus>). All sites were selected using NHD as the sample frame. Each probability site included in the survey has a sample weight that represents a known stream or river length based on the population of streams included in the survey design, the probability of that site being selected for sampling, and the number of sites actually sampled. These weighted stream and river lengths were summed to estimate the cumulative extent and condition of streams and rivers in the conterminous U.S. (USEPA 2016). The NRSA design allows the assessment of ecological conditions of streams at many hierarchical spatial scales from ecoregional to national. Here we report results nationally and for nine aggregated ecoregions: Coastal Plains (CPL), Northern Appalachian Mountains (NAP), Southern Appalachian Mountains (SAP), Northern Plains (NPL), Southern Plains (SPL), Temperate Plains (TPL), Upper Midwest (UMW), Western Mountains (WMT), and Xeric West (XER; Fig. 2).

Stream classification, catchment land cover, and stream channel fine sediments

We also classified our study streams and rivers into size classes based on stream order (Strahler 1957) as represented on U.S. Geologic Survey 1:100,000 scale maps. Headwaters were 1st order streams; streams: 2nd–3rd order; small rivers: 4th–5th order; big rivers 6th–7th order; and great rivers 8th order). We also classified streams according to their potential stress based on biotic, chemical, and physical characteristics along a gradient of disturbance. Because pristine conditions are uncommon or absent in many streams and rivers, the NRSA defined reference condition as least-disturbed, or those streams and rivers with the best available physical, chemical, and biological condition given the current status of the landscape in which these streams were located (Herlihy et al., 2008). Least, intermediate, and most disturbed stream conditions were established for each of the 9 ecoregions.

Total catchment area for each site was calculated by summing the areas of all NHD-Plus catchments intersected while navigating upstream from each site. Percent of the catchment in forests (% forest) and grasslands (% grassland) were extracted from the National Land Cover Database (NLCD, USGS, 2001). The NLCD, derived from multi-temporal and terrain-corrected satellite imagery, provided consistent land cover estimates for the United States. Targeted assessments found accuracy of land cover estimates ranged from 73 to 77% (Homer et al., 2004).

Stream reach lengths were defined as 40 times their low-flow wetted channel widths, with a minimum reach length 150 m and a maximum reach length of 4000 m. Each sampling reach was sub-divided by 11 equally spaced transects for the purpose of stream sediment and biology sampling. The proportion of each stream channel composed of fine-grained sediments (< 2 mm average particle diameter) was determined by systematic substrate sampling or by visual estimates at each cross-sectional transects (USEPA 2008).

Sediment collection and chemistry

Fine-grained sediments were collected near the 11 cross-sectional transects and combined into a single sediment sample from each site. Sediment samples were dried and ground for nutrient analysis. Total sediment carbon (Sed TC) and nitrogen (Sed N) were determined by the combustion method using an elemental analyzer (Model 1112EA, Carlo Erba Instrumentazione, Milan, Italy). Since total organic carbon (TOC) was not measured on these samples, sediment TOC (Sed OC) was estimated from Sed N (which is predominantly organic N; Schulten and Schnitzer 1998; Allan and Castillo 2009) using a stoichiometric C/N of 14.3 (Cleveland and Liptzin 2007). The resulting Sed OC represented 73% of Sed TC, which is consistent with the proportion of total sediment C that was organic C at more than 1000 sites in the U.S. Geological Survey's National Water Quality Assessment (Horowitz and Stevens 2008). Total sediment phosphorus (Sed P) was determined by first digesting the sample in reagent grade concentrated HNO₃ using a microwave digester (Anton Paar Multiwave, Anton Paar GmbH, Graz, Austria). Samples were neutralized with 10 mL NaOH, diluted to 90 mL with deionized water, and analyzed by the molybdate-ascorbic acid method (APHA, 1998).

Microbial carbon use efficiency and organic matter decomposition

Microbial EEA related to carbon, nitrogen, and phosphorus acquisition were previously reported (Hill et al. 2012) and details of their analyses and interpretation will not be repeated here. We used EEA of the four most commonly measured hydrolase enzymes (BG: β -d-glucosidase, EC 3.2.1.21; NAG: β -N-acetylglucosaminidase, EC 3.2.1.50; LAP: L-leucine aminopeptidase, EC 3.4.11.1; and AP: acid phosphatase, EC 3.1.3.1), and the phenol oxidase enzyme (POX: polyphenol oxidase, EC 1.10.3.1) to estimate the estimate CUE and for inclusion in the enzyme decomposition model.

Because the sediment C pool was expected to become more recalcitrant as organic matter decomposition progressed, which would result in slowed microbial growth, EEA stoichiometric ratios were normalized for POX activity to account for the decrease in the demand for P relative to that for N (Sinsabaugh and Follstad Shah 2012). Regression of

POX-normalized EEA more accurately reflects relative C, N, and P limitation along the decomposition gradient:

$$\ln BG / \ln POX = \ln[NAG + LAP] / \ln POX \quad (1)$$

$$\ln BG / \ln POX = \ln AP / \ln POX \quad (2)$$

with deviations from a slope of 1 indicating relative N (Eq. 1) or P (Eq.2) limitation.

Carbon use efficiency (CUE) was predicted as a saturating function based on N and P supply relative to C availability (Sinsabaugh et al. 2013):

$$CUE = 0.6 * (CN_S * CP_S) / ((K_{CN} + CN_S) * (K_{CP} + CP_S))^{0.5} \quad (3)$$

where CN_S is the C/N of microbial biomass relative to available C/N [8.6/(TOC/TN)], CP_S is the C/P of microbial biomass relative to available C/P [60/(TOC/TP)], and K_{CN} and K_{CP} are the half-saturation constants (0.5).

Organic matter decomposition ($-k$, % d^{-1}) was modeled using an ecoenzyme allocation model (Sinsabaugh and Moorhead 1994). We substituted CUE, as calculated in Eq. 3, for the original static enzyme degradation efficiency ($k_c=0.2$; Sinsabaugh and Moorhead 1994):

$$-k = (CUE * ENZ_{TOT}) / (1 + ([NAG + LAP] / BG) + (AP / BG)) \quad (4)$$

where ENZ_{TOT} is the sum of all of the EEA normalized to their maximum values. Normalized values were used for BG, [NAG+LAP], and AP in the remainder of the equation.

Microbial respiration and carbon processing in U.S. streams

Sediment microbial respiration (R_m , $nmol\ C\ h^{-1}\ g^{-1}\ DW$) associated with the decomposition of sediment organic carbon was estimated as the product of the sediment organic C pool (Sed OC), decomposition rate ($-k$), and carbon use efficiency (CUE):

$$R_m = Sed\ OC * -k * CUE \quad (5)$$

Sed OC ($mol\ C\ m^{-2}$) and R_m ($mol\ C\ m^{-2}\ d^{-1}$) were extrapolated to streambed area by accounting for sediment bulk density (BD, $g\ m^{-3}$; Avinimelech et al. 2001) and scaling this to streambed area (m^2):

$$BD = 1.776 - (0.363 * (\ln \text{Sed OC})) \quad (6)$$

$$\text{Sed OC} = (\text{Sed OC} / BD) \quad (7)$$

$$R_m = R_m * (\text{Sed OC} / BD). \quad (8)$$

We calculated Sed OC (mol C km⁻¹) stocks and R_m (mol C km⁻¹ d⁻¹) per km of stream length:

$$\text{Sed OC} (\text{mol C m}^{-2}) * W * 1000 \quad (9)$$

$$R_m (\text{mol C m}^{-2} \text{d}^{-1}) * W * 1000 \quad (10)$$

where W is stream width (m) and the multiplier of 1000 converts the product to km. Sed OC (mol C km⁻¹) and R_m (mol C km⁻¹ d⁻¹) were further multiplied by their weighting factor to derive the cumulative sediment C stocks (Sed OC_{cum}, Mmol OC) and respiration (R_{cum}, Mmol C d⁻¹):

$$\text{Sed OC}_{\text{cum}} = \sum_{i=1}^n \text{Sed OC} (\text{mol C} / \text{km}) * \text{weight} \quad (11)$$

$$R_{\text{cum}} = \sum_{i=1}^n R_m (\text{mol C} / \text{km} / \text{d}) * \text{weight} \quad (12)$$

where weight is the number of km of streams represented by any given stream site, as prescribed by the spatially balanced, unequal probability survey design (Olsen and Peck, 2008).

Statistical analyses

We calculated weighted mean values and percentiles for box and whisker plots using the sample selection weights for CUE, $-k$, and R_m. These weighted results make inference to the total population of flowing water in the conterminous U.S. and not just the set of sampled sites. We evaluated the relationships between CUE, $-k$, and R_m, and catchment land cover, stream channel fine sediments, and water and sediment chemistry (see Hill et al. 2012 for physico-chemical data) using Spearman rank correlation to avoid problems associated

with non-normal data distribution. Mean microbial CUE, $-k$, and R_m were compared at the ecoregion, stream size, and condition class levels using a type I general linear model for unbalanced, nested designs. All analyses were done using SAS for Windows, release 9.4 (SAS Institute, Inc., Cary, NC, USA).

Results

Carbon use efficiency, organic matter decomposition, and microbial respiration,

Carbon use efficiency ($CUE \pm SE$) was lowest (0.016) in Northern Plains headwaters and highest (0.299 ± 0.085) in Western Mountain great rivers (Fig. 3a; Supplemental Table 1). Mean CUE varied between ecoregions (Table 1), but with no apparent ecoregional trends (Fig. 3a). CUE also increased with increasing stream size (Fig. 3a; Supplemental Table 1) and increasing disturbance (Fig. 4a) in most ecoregions. CUE was positively correlated with pH and sediment P concentration, and negatively correlated with fine-grained channel sediments (Table 2).

Organic matter decomposition rates ($-k \pm SE$) were slowest ($0.001 \pm 0.001\% \text{ d}^{-1}$) in Western Mountain great rivers and fastest ($0.092\% \text{ d}^{-1}$) in Northern Plains headwaters (Fig. 3b; Supplemental Table 1). Organic matter decomposition rate exhibited differences between ecoregions, with rates increasing through the plains (NPL, SPL, and TPL), Upper Midwest and Western Mountains and Xeric ecoregions compared to the Coastal Plains and Northern and Southern Appalachian ecoregions of the east (Table 1; Fig. 3a). Organic matter decomposition rates exhibited an inverse relationship with stream size in most ecoregions (Table 1; Fig. 3b) and with disturbance classes (Table 1; Fig. 4b). Decomposition rate was positively correlated with % fines, and with sediment OC, N and P concentrations (Table 2).

Microbial respiration ($R_m \pm SE$) ranged from $0.01 \pm 0.01 \text{ mol C m}^{-2} \text{ d}^{-1}$ in great rivers of the Western Mountains ecoregion to $9.76 \pm 5.10 \text{ mol C m}^{-2} \text{ d}^{-1}$ in the headwaters of the Xeric ecoregion (Fig. 3c; Supplemental Table 1). R_m varied between ecoregions and disturbance classes, but not between stream size classes (Table 1; Figs. 3c and 4c). R_m was positively correlated with water total P, and with sediment OC, N, and P concentrations (Table 2).

Mean stream width (W) ranged from 1 to 612 m (Supplemental Table 2), with obvious stream size class differences. Cumulative catchment stream lengths (Cum L) are estimates of the total length of flowing water in each ecoregion or size class. Cum L were shortest (250 km) in Western Mountain great rivers, while Southern Appalachian headwaters had the greatest Cum L (232,309 km; Supplemental Table 2). The product of W and Cum L is cumulative stream bed area (Cum A), which ranged from 1 km^2 for Northern Plains headwaters to 4495 km^2 for small rivers of the Upper Midwest ecoregion (Supplemental Table 2).

Accounting for mean stream width and the cumulative stream length, cumulative sediment OC storage ($Sed \text{ OC}_{cum}$) ranged from 48 Mmol in the great rivers of the Western Mountain ecoregion to 31,332 Mmol in streams of the Coastal Plains ecoregion (Supplemental Table 2). Cumulative microbial respiration ranged from a low of $<1 \text{ Mmol C d}^{-1}$ in great rivers of the Western Mountains ecoregion to a high of $473 \text{ Mmol C d}^{-1}$ in streams of the Coastal

Plains (Table 3). Summing across ecoregions yields respiratory C losses from streams and rivers ranging from 12.6 Mmol C d⁻¹ (Southern Plains) to 527 Mmol C d⁻¹ (Coastal Plains). A similar summing across stream size classes yields estimated respiratory C losses of 540 Mmol C d⁻¹ from headwaters streams; 639 Mmol C d⁻¹ from streams; 324 Mmol C d⁻¹ from small rivers; 232 Mmol C d⁻¹ from big rivers; and 177 Mmol C d⁻¹ from great rivers. The summed cumulative respiratory C losses from the 1,685,193 km (42,356 km² of stream bed) of U.S. streams and rivers was 1912 Mmol C d⁻¹ (8.37 Tg C y⁻¹; Table 3).

Discussion

The River Continuum Concept (RCC; Vannote et al, 1980) proposed a framework for considering the geomorphological continuum from headwater streams to large rivers in the context of their ecosystem responses (organic C stocks, decomposition rates, respiration). They proposed downstream transitions from coarse to fine particulate C, from allochthonous to autochthonous support for ecosystem respiration, resulting in downstream decreases in most measures of ecosystem C availability. Naiman et al. (1987) and Webster (2007) demonstrated that these downstream shifts in resources and ecosystem responses often result in initial decreases (increases) in transitioning from headwaters to larger streams, followed by subsequent increases (decreases) transitioning from larger streams to rivers. Webster (2007) used data from a headwater to mid-sized river continuum to parameterize a model of energy flow and investigated the mechanisms resulting in U-shaped curves for stream energetics. We've captured the RCC and the U-shaped curve in our conceptual model (Fig. 1), which we can now re-evaluate using our sediment OC, CUE, $-k$, and R_m data from streams across the conterminous U.S.

Benthic organic carbon in streams and rivers consists of wood, coarse and fine particulate matter embedded in stream sediments, and dissolved organic matter in interstitial waters. In many streams and rivers wood is the dominant benthic organic matter (BOM) pool, averaging as much as 62% of total BOM (Webster et al. 1995; Webster and Meyer 1997). Coarse (CPOM, >1mm diameter) and fine (FPOM, <1mm diameter) are the two remaining particulate pools, each being roughly equivalent in abundance (Hill et al. 1992; Webster et al. 1995; Jones 1997; Webster and Meyer 1997). Reported FPOM in U.S. streams ranges from 1-40 mol C m⁻² (Naiman et al. 1987; Hill et al. 1992; Webster et al. 1995; Jones 1997; Sinsabaugh 1997; Webster and Meyer 1997). Sediment OC values from our synoptic survey of US streams and rivers represent the lower end of this range, but the spatial extent and statistical power of our survey design suggest that previous estimates of BOM may have overstated FPOM storage in US streams. Considering all 1879 sites in our data, U.S. streams and rivers demonstrated a strong linear decrease in Sed OC with increasing stream size, supporting the prediction of the RCC (Fig. 5a). Finlay (2011) suggested that anthropogenic disturbances masked RCC trends in streams. The downstream decrease in Sed OC is still evident when considering only our least disturbed (reference) sites (Fig. 5b), but we also see the U-shaped downstream trend suggested by Webster (2007). Our national scale estimate, based on the weighted means of 1879 sites, of 122,210 Mmol C stored the surface sediments of streams and rivers is in agreement with sediment erosion and storage estimates for US streams (4000 Mmol C y⁻¹; Smith et al. 2001), if one assumes an average FPOM turnover

time (FPOM stocks/[metabolism + export]) of 40 y, a rate that is twice as fast as was reported for FPOM in Oregon streams (Naiman and Sedell 1980).

The biological processing of FPOM, specifically decomposition and the microbial respiration it supports, was the focus of our research. Organic matter (especially leaf litter) decomposition in streams and rivers has been extensively studied (Webster and Benfield 1986; Webster et al. 1995; Young et al. 2008; Tank et al. 2010). Several factors may influence organic matter decomposition in streams, including temperature and the source of the organic matter. Despite widely reported correlations between leaf litter decomposition rates and stream temperatures, Webster et al. (1995) reported no differences between decomposition rates from northern and southern US streams. Organic matter nutrient content, especially nitrogen, and the relative recalcitrance of organic carbon (e.g., lignin content), both related to organic matter origin (leaves, wood, microbial processing), have been shown to be inversely related to decomposition rates (Webster and Benfield 1986; Sinsabaugh et al. 1994; Webster et al. 1995, 1999; Tank et al. 2010), and this is consistent with our reported correlations of CUE, $-k$, and R_m with fine-grained stream bed sediments (reflecting increased sediment surface area for microbial colonization) and sediment C, N and P content (Table 3). Decomposition of FPOM has been less well studied, especially in streams (Sinsabaugh et al. 1994; Webster et al. 1999; Jackson and Vallaire 2007; Tank et al. 2010). These authors reported faster decomposition rates for CPOM than for FPOM, but also cited methodological problems related to enclosing POM in mesh bags, which led to underestimation of ambient decomposition rates, especially for FPOM (Sinsabaugh et al. 1994; Jackson and Vallaire 2007). Sinsabaugh et al. (1994) used direct comparisons of decomposition and microbial enzyme activity on POM enclosed in mesh bags and ambient POM collected from the streambed at the time of litter bag collection to validate their use of an enzyme decomposition model (EDM) as a predictor of POM decomposition rates. The Sinsabaugh and Moorhead (1994) EDM is one of few models linking organic matter, nutrients, and the role of microbial processes in the mediation of energy flow and nutrient cycling (Sinsabaugh and Moorhead 1994; Schimel and Weintraub 2003; Moorhead et al. 2012). These models highlight the tradeoffs of microbial C acquisition to meet metabolic demands and the energetic costs of producing coenzymes for the acquisition of N and P to support that level of metabolism. The applicability of EDM have been demonstrated in a number of aquatic ecosystems (Sinsabaugh et al. 1994; Jackson and Vallaire 2007; Hill et al. 2014).

Carbon use efficiency (CUE) has been shown to be inversely correlated with OC availability (Manzoni et al. 2012; Sinsabaugh et al. 2013; Hill et al. 2014). Since we predicted declining sediment OC with increasing stream size, we subsequently predicted increasing CUE with increasing stream size (Fig. 1), and this was evident in analyses of all stream sites (Fig. 5a) and in our least disturbed sites (Fig. 5b). These results are consistent with predictions based on the RCC, and the least disturbed sites suggest a U-shaped curve with a slight decrease in CUE transitioning from headwaters to larger streams and an uptick in CUE in the great rivers.

We predicted that decomposition rates ($-k$) would decline with increasing stream size, reflecting increasing recalcitrance of the POM, but with significant increase in $-k$ in mid-

order streams related to a pulse of labile OC of autochthonous origins (Fig. 1; Webster 2007). Declining $-k$ with increasing stream size was evident from analyses of all sites (Fig. 5a) and from only the least disturbed sites (Fig. 5b). Contrary to our predictions of a declining $-k$ with stream size, there was a slight increase in $-k$ measured at least disturbed great rivers sites, hinting at the possibility of a U-shaped curve.

Microbial respiration (R_m) supported by streambed POM is widely reported, including two studies which considered broad regional or national scales (Hill and Gardner 1987; Webster et al. 1995, 1999; Sinsabaugh 1997; Hill et al. 2002, 2012; Battin et al. 2009; Young et al. 2008; Wallin et al. 2012; Gomez-Gener et al. 2016). These studies represent a range of methods, including oxygen uptake by sediments enclosed in chambers, open-channel diurnal oxygen fluxes, microbial electron transport system activity, and predictions based on microbial enzyme decomposition models. Webster et al. (1995) reported R_m ranging from 0.11–8.06 g C m⁻² d⁻¹ for Eastern Deciduous Forest streams; Sinsabaugh (1997) and Hotchkiss et al. (2015) reported R_m in U.S. streams and rivers ranging from 0.03–3.28 g C m⁻² d⁻¹. Here we report our modeled respiration data (R_m) which are positively correlated ($r=0.32 - 0.62$; Supplemental Fig. 1) with measured microbial respiration from these same rivers and streams. In all cases R_m is lower than measured respiration, and thus represents a more conservative estimation of microbial respiration. Our R_m , when converted to similar units (range: 0.04 –60.4 g C m⁻² d⁻¹; median: 10.2 g C m⁻² d⁻¹), expands these previously reported ranges, while extending these measurements to a wider geographic distribution and to a greater range of stream sizes. We predicted that R_m would decline with increasing stream order, largely as a function of declining Sed OC (Fig. 1), and this prediction was supported by R_m from all sites (Fig. 5a) and from our least disturbed sites (Fig. 5b). The downstream decrease in R_m , with an increase R_m in small rivers before further declines, supports RCC predictions and the U-shaped curve hypothesis.

The role of streams and rivers in global C budgets has only recently received consideration. Prior to 2001, analyses of global C budgets considered three pools, land, sea and atmosphere (Cole et al. 2007; Aufdenkampe et al. 2011). Since that time several researchers have quantified the magnitude of CO₂ flux (0.19–1.2 Pg C y⁻¹) from inland aquatic environments that is needed to balance the differences between C losses from the terrestrial environment and C inputs to the oceans (Cole and Caraco 2001; Cole et al. 2007; Battin et al. 2009; Aufdenkampe et al. 2011). The most recent studies have employed a bottom-up approach to quantifying the contribution of streams and rivers by extrapolating measured streambed CO₂ efflux to entire stream networks (Battin et al. 2009; Butman and Raymond 2011; Raymond et al. 2013; Hotchkiss et al. 2015; Butman et al. 2016). Battin et al. (2009) used whole ecosystem metabolism data as the basis for their estimation of respiratory CO₂ efflux, whereas Butman and Raymond (2011), Raymond et al. (2013) and Butman et al. (2016) all used models of stream water CO₂ partial pressure ($p\text{CO}_2$) to estimate C efflux from streams and rivers. Hotchkiss et al. (2015) combine the metabolism and $p\text{CO}_2$ methods to determine the proportion of CO₂ evasion from streams that could be attributed to ecosystem respiration. We used the most recent estimate of 69 Tg C y⁻¹, based on a cumulative conterminous U.S. streambed area of 40,600 km² (Butman et al. 2016) to compare with our microbial respiratory C losses over 42,000 km² of cumulative U.S. stream bed. Hotchkiss et al. (2015) concluded that aquatic metabolism accounts for 28% of the CO₂ emissions from

U.S. streams and rivers, ranging from 14% of the C loss in small streams to 54% of the C loss in large rivers. Hotchkiss et al.'s (2015) respiration estimates compare favorably with our microbial respiratory C losses of 12% ($\pm 5\%$) of the $p\text{CO}_2$ efflux, suggesting that microbial respiration may account for nearly half of the total aquatic metabolism C losses from streams and rivers of the conterminous U.S.

Headwaters and smaller streams dominate the cumulative extent of U.S. streams and rivers (Leopold et al. 1964; Nadeau and Rains 2007), and this is reflected in our survey. Headwaters comprise 52% of our weighted, cumulative stream length; streams 31%; small rivers 12%; big rivers 4%; and great rivers <1% (Supplemental Table 2). This spatial distribution, however, does not reflect the results of our weighted, cumulative respiratory C losses, which increase transitioning from headwaters to streams before declining with subsequent increases in stream size, consistent with Webster's (2007) U-shaped curve.

Butman et al. (2016) also reported $p\text{CO}_2$ efflux at the major river basin scale (U.S. Geological Survey two-digit Hydrologic Unit Code [HUC]), with their highest $p\text{CO}_2$ efflux from the drier portions of Pacific Northwest and California river basins, which corresponds to portions of our Western Mountain and Xeric ecoregions, from which we modeled our lowest respiratory CO_2 efflux. This discrepancy suggests that much of the western stream CO_2 evasion is derived from respiration outside of the stream channel which enters the stream as dissolved inorganic C contributing to $p\text{CO}_2$ efflux, rather than the product of in-stream metabolism. Butman et al. (2016) also estimated relatively high $p\text{CO}_2$ efflux from the Missouri River basin, and from Pacific Northwest, South Atlantic, and Gulf of Mexico coastal regions, which corresponded to our high respiratory CO_2 efflux estimates from Temperate and Coastal Plains streams and rivers. Butman et al.'s lowest $p\text{CO}_2$ efflux was reported for the Souris-Red-Rainy river basins in Minnesota, North Dakota, and South Dakota, an area that represents a small portion of our Upper Midwest ecoregion, from which we reported some of our highest rates of respiratory CO_2 efflux.

Conclusions

Our objectives for this study were to investigate the role of microbial coenzymes in facilitating sediment organic matter processing in streams and rivers of the United States, and to use these microbial enzymes in models to predict organic matter decomposition rates and to model microbial respiration. We used these respiration and decomposition estimates to explore ecoregional, stream size, and disturbance class differences in aquatic metabolism, finding an east to west increase in decomposition rates and microbial respiration. We also observed an inverse relationship between stream size and disturbance class with decomposition and respiration. We then used our respiration data to estimate the cumulative annual respiratory C losses from the streambeds of U.S. streams and rivers. The National Rivers and Streams Assessment (NRSA) sampling design and the data collected from each site provided a unique opportunity to investigate the environmental constraints on organic matter decomposition and microbial respiration at the site, ecoregion, and national scale. The NRSA probability design also allowed us to extrapolate site-specific data to stream networks and to all streams in the conterminous United States. Our study represents the first statistically-based assessment of organic matter decomposition in headwater streams to great

rivers, and in all streams across the conterminous United States. Finally, our study partitions the role of stream biota in C cycling and CO₂ evasion from streams and rivers, highlighting the contribution of stream microbial respiration related to organic matter decomposition.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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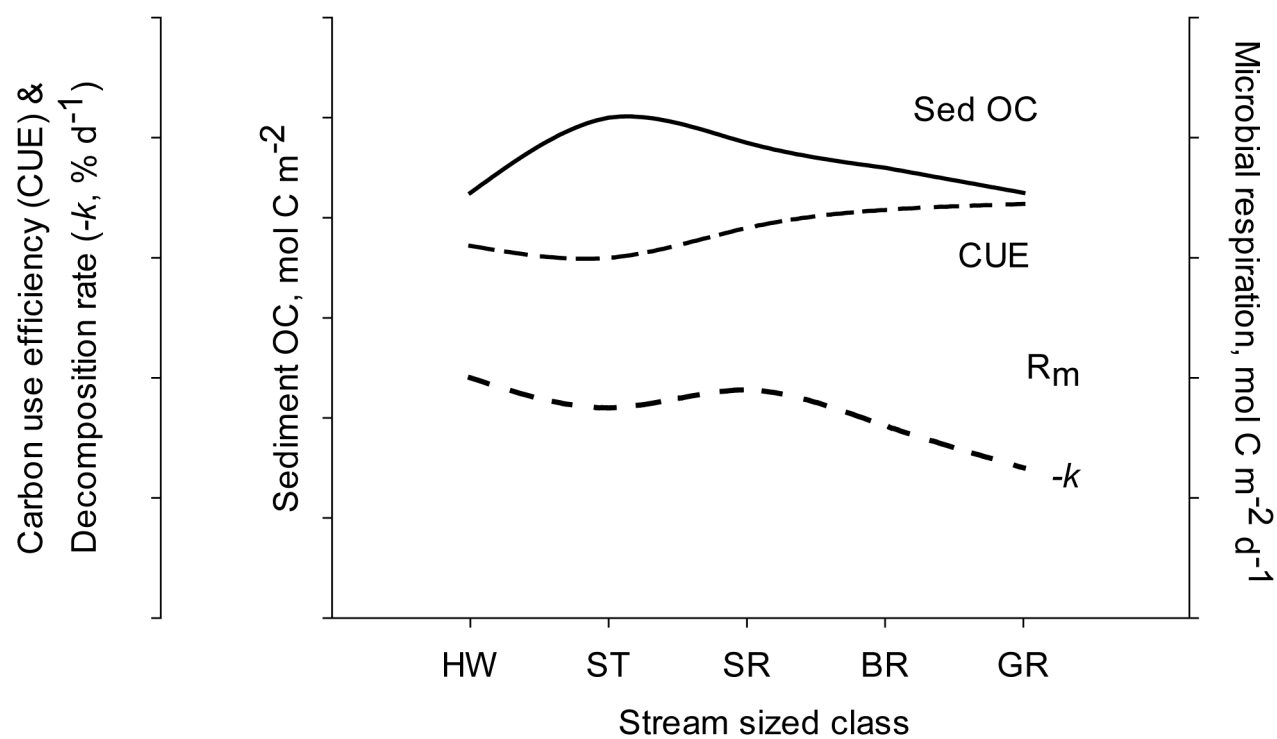
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**Fig 1.**

Conceptual model of stream sediment carbon, microbial respiration (R_m) and carbon use efficiency (CUE), and organic matter decomposition rate ($-k$) as hypothesized by the River Continuum Concept (Vannote et al, 1980; Minshall et al.1985; Sedell et al. 1989) and the U-shaped curve (Naiman et al. 1987; Webster 2007).

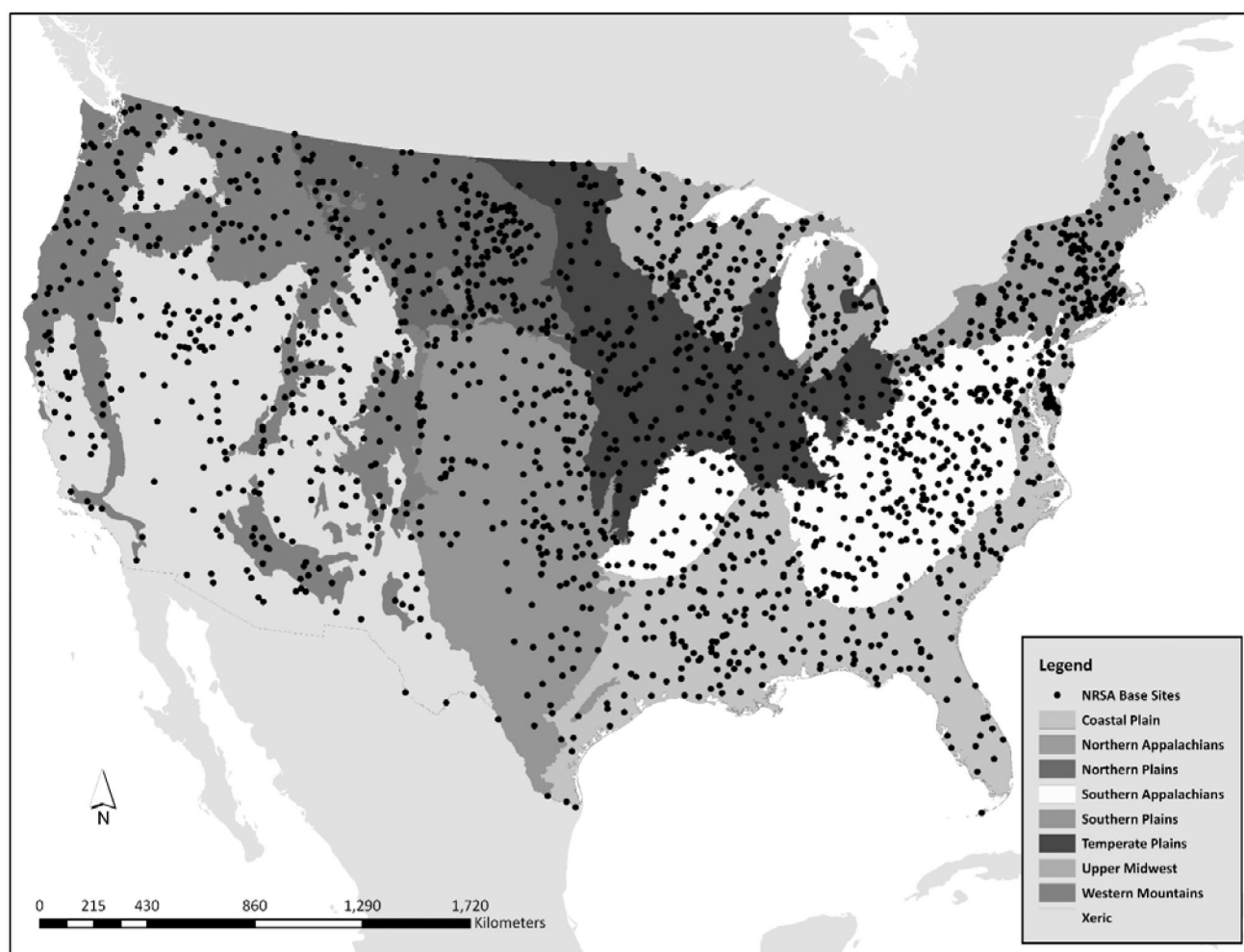


Fig 2.

Map of the conterminous United States with the nine ecoregions delineated and with the locations of the 1916 National Rivers and Streams Assessment sites which were sampled in 2008-2009. Ecoregion abbreviations are: Coastal Plains (CPL); Northern (NAP) and Southern (SAP) Appalachian Mountains; Northern (NPL), Southern (SPL), and Temperate (TPL) Plains; Upper Midwest (UMW); Western Mountains (WMT) and the Xeric west (XER).

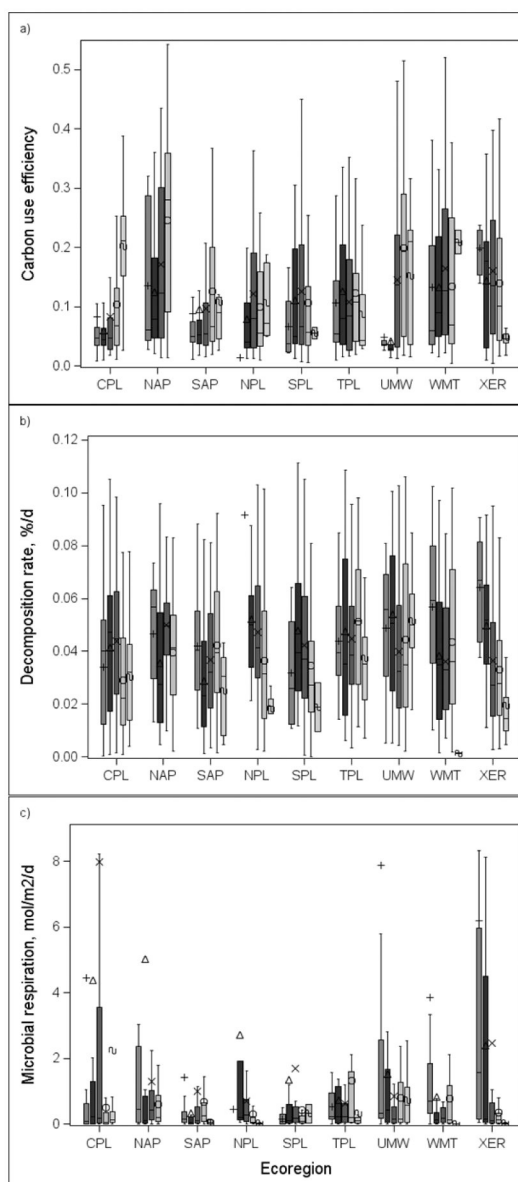
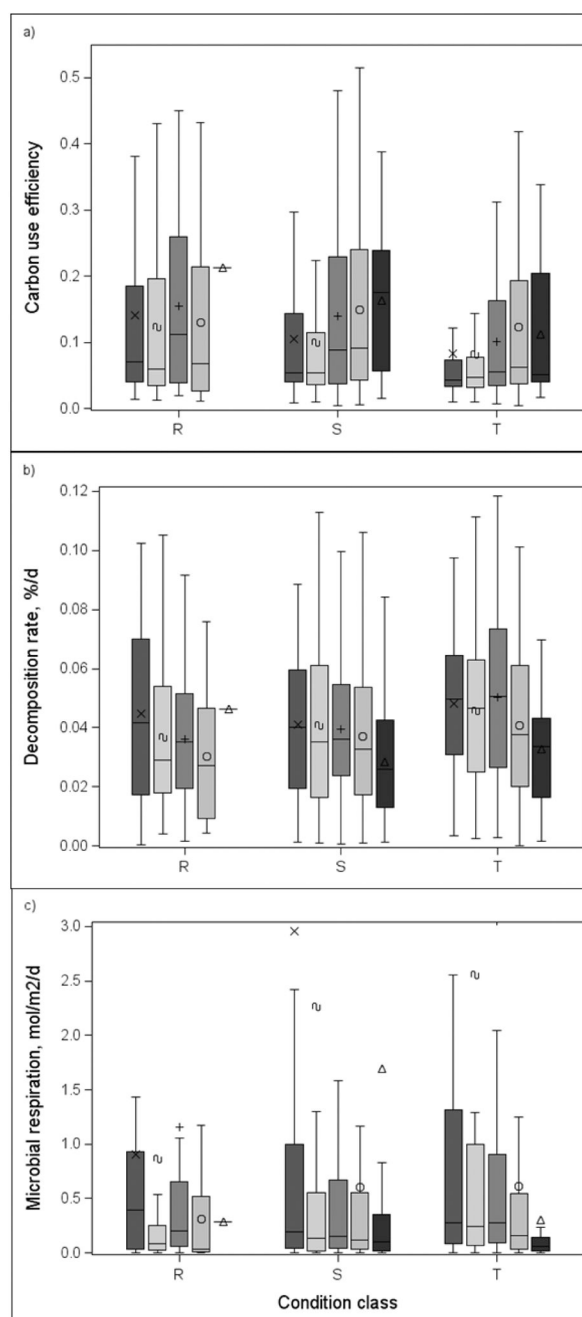


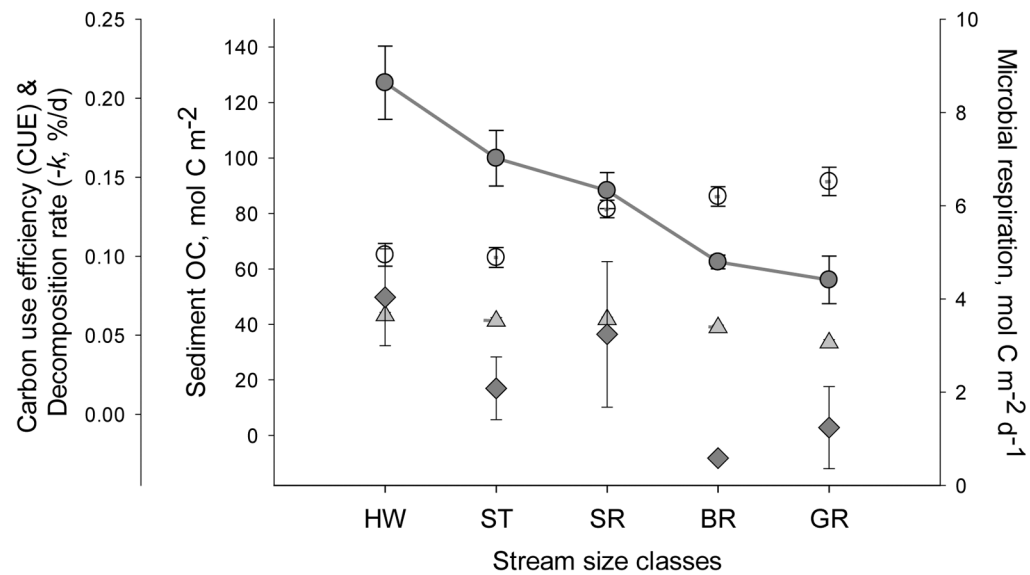
Fig 3.

Box and whisker plots for a) carbon use efficiency, b) decomposition rate, and c) microbial respiration by ecoregion. Boxes represent the 25th and 75th percentiles; the bar across each box is its median value; the symbol associated with each bar is the mean; and the whiskers represent the minimum and maximum values. The first bar in each cluster is for headwaters (light gray bar), followed by streams (medium gray), small rivers (dark gray), big rivers (black), and great rivers (pale gray).

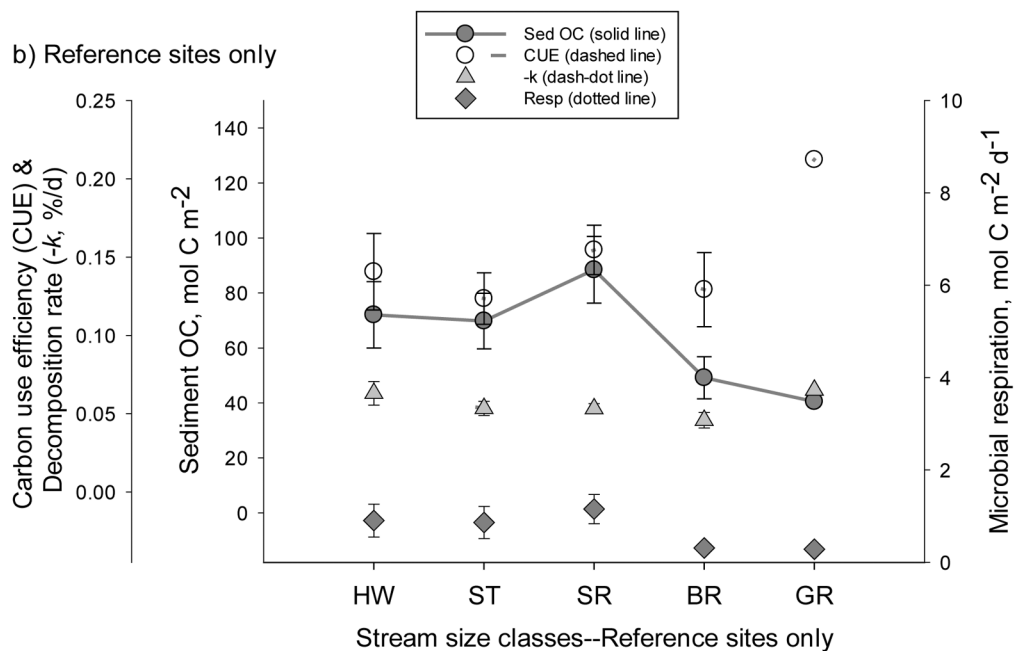
**Fig 4.**

Box and whisker plots for a) carbon use efficiency, b) decomposition rate, and c) microbial respiration by stream condition class (R=least disturbed; S= intermediate disturbance; T=most disturbed). Boxes represent the 25th and 75th percentiles; the bar across each box is its median value; the symbol associated with each bar is the mean; and the whiskers represent the minimum and maximum values. The first bar in each cluster is for headwaters (dark gray bar), followed by streams (pale gray), small rivers (medium gray), big rivers (light gray), and great rivers (black).

a) all sites



b) Reference sites only

**Fig 5.**

A comparison of the fit of the combined ecoregional stream sediment carbon standing crop, carbon use efficiency, organic matter decomposition rates, and microbial respiration data for all sites (a) and for reference sites only (b) to the conceptual model (Fig. 1) as predicted by the River Continuum Concept (Vannote et al, 1980; Minshall et al.1985) and the U-shaped curve (Webster 2007).

Table 1.

Summary statistics of the Type I General Linear Model analysis of an unbalanced, nested (Ecoregion, stream size class, condition class) sampling design for the weighted means for carbon use efficiency (CUE), organic matter decomposition ($-k$, % d⁻¹), and microbial respiration (R_m , mol C m⁻² d⁻¹) in streams and rivers of the continental United States. All variables are natural log transformed before analysis.

Variable	Effects	df	F	P
CUE	Ecoregion	8	7.32	<0.0001
	Stream size class	4	13.2	<0.0001
	Condition class	2	11.5	<0.0001
	Size (Condition)	8	1.42	0.1828
$-k$	Ecoregion	8	9.00	<0.0001
	Stream size class	4	9.70	<0.0001
	Condition class	2	23.1	<0.0001
	Size (Condition)	8	0.50	0.8600
R_m	Ecoregion	8	5.44	<0.0001
	Stream size class	4	1.07	0.3724
	Condition class	2	6.03	0.0025
	Size (Condition)	8	1.16	0.3198

Table 2.

Spearman rank correlation coefficients of weighted means a) between microbial carbon use efficiency (CUE, organic matter decomposition ($-k$, % d⁻¹), and microbial respiration (R_m , mol C m⁻² d⁻¹) with catchment area, catchment land cover and channel fines, water and sediment chemistry, and the lignocellulose index (LCI) in US streams and rivers. Catchment land cover, channel sediments, and water and sediment chemistry were previously reported (Hill et al. 2012). Correlation coefficients that are significant ($p < 0.05$) and have an $r > 0.15$ are in ***bold italic*** type.

	CUE	$-k$	R_m
<i>Stream order, catchment land cover and channel sediments</i>			
Stream order	0.065	-0.101	-0.038
Forest, %	-0.008	-0.053	-0.018
Grassland, %	0.066	-0.072	-0.051
Fines, %	<i>-0.207</i>	<i>0.268</i>	0.094
<i>Water and sediment chemistry, lignocellulose index</i>			
ANC, $\mu\text{eq L}^{-1}$	0.060	<i>0.159</i>	0.120
pH	<i>0.240</i>	0.040	0.007
Total N, $\mu\text{mol L}^{-1}$	-0.038	0.067	0.085
Total P, $\mu\text{mol L}^{-1}$	-0.048	0.087	<i>0.216</i>
SO ₄ , $\mu\text{mol L}^{-1}$	-0.029	0.037	0.021
Sed OC, mmol kg ⁻¹	-0.095	<i>0.383</i>	<i>0.672</i>
Sed N, mmol kg ⁻¹	-0.095	<i>0.382</i>	<i>0.672</i>
Sed P, mmol kg ⁻¹	<i>0.623</i>	<i>0.211</i>	<i>0.204</i>
LCI	-0.096	<i>-0.201</i>	-0.112

Table 3.

Weighted mean (\pm SE) cumulative microbial respiration (R_{cum} , Mmol C d^{-1}) for US streams and rivers. Ecoregion^a and stream size class sums for are also presented.

Stream size class	CPL	NAP	SAP	NPL	SPL	TPL	UMW	WMT	XER	<i>Size class sums</i>
R_{cum} Headwaters	15.1 (7.47)	287 (84.6)	3.84 (0.74)	0.63 (---)	0.18 (0.19)	6.07 (1.12)	90.8 (72.6)	104 (44.0)	32.1 (17.4)	540 (228)
Streams	473 (131)	93.5 (70.6)	9.60 (4.03)	6.57 (2.67)	1.55 (1.06)	11.3 (4.91)	19.1 (9.78)	5.45 (2.84)	18.5 (7.88)	639 (233)
Small rivers	10.8 (6.39)	25.4 (9.14)	10.1 (2.50)	3.82 (1.28)	4.24 (1.80)	13.9 (6.40)	55.3 (14.4)	130 (102)	70.3 (40.8)	324 (183)
Big rivers	7.95 (3.33)	13.5 (5.53)	36.1 (11.0)	22.7 (6.80)	2.28 (0.65)	110 (55.6)	6.74 (2.66)	18.3 (5.09)	14.3 (5.98)	232 (96.6)
Great rivers	19.9 (21.8)	--- (---)	6.39 (2.86)	1.52 (0.65)	4.32 (3.65)	123 (71.4)	21.6 (8.93)	0.01 (---)	0.31 (0.06)	177 (109)
<i>Ecoregion sums</i>	527 (170)	419 (170)	66.0 (21.1)	35.2 (11.4)	12.6 (7.35)	264 (135)	194 (108)	258 (154)	136 (72.1)	1912 (849)

^a Coastal Plains (CPL); Northern (NAP) and Southern (SAP) Appalachian Mountains; Northern (NPL), Southern (SPL), and Temperate (TPL) Plains; Upper Midwest (UMW); Western Mountains (WMT) and the Xeric west (XER).