Estimation of riverine carbon and organic matter source contributions using time-based isotope mixing models

Katie Hossler and James E. Bauer

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[1] Rivers transport globally significant amounts of carbon (C) from terrestrial ecosystems to ocean margins. Understanding and quantifying the sources and respective contributions to riverine C has emerged as an important biogeochemical problem that can be approached through natural-abundance isotope mass balance. Traditionally, the sources of riverine C have been identified either qualitatively or quantitatively through application of static mixing models. However, both source signatures and contributions can vary significantly with time. Here we apply two time-based mixing models to a study of six rivers draining the northeast U.S. In the first model, a time-averaged mixing model (TAMM), we vary only the source isotopic ($\delta^{13}C$ and $\Delta^{14}C$) signatures. In the second model, a time-varying mixing model (TVMM), we allow both isotopic signatures and contributions to vary with time. Based on results from the TVMM, drivers of variation in riverine particulate organic C (POC), dissolved organic C (DOC), and dissolved inorganic C (DIC) include stream discharge, stream discharge and water temperature, and water temperature and vegetation phenology, respectively. Major sources include C$_3$ plant material, algal material and slow-turnover soil OC (“slow SOC”), which together account for 50%–100% (95% CI) of riverine POC; C$_3$ plant material and slow SOC, which together typically account for 60%–100% (95% CI) of DOC; and atmospheric exchange which alone typically accounts for 40%–60% (95% CI) of DIC. Seasonal change in relative contributions from algal material, slow SOC, and photosynthesis (in response to the identified drivers) dominates the observed variation in POC, DOC and DIC, respectively. The TVMM is a novel tool to identify component contributions under more realistic non-static conditions, and with potential application to a broad range of biogeochemical studies.


1. Introduction

[2] Rivers link the terrestrial and oceanic carbon (C) cycles by transporting and transforming globally significant amounts of C and organic matter (OM) from terrestrial net ecosystem production. Although historically viewed as playing only a minor role in the global C cycle, there has been growing awareness of the importance of riverine ecosystems as a significant fate for terrestrial C [Cole et al., 2007; Aufdenkampe et al., 2011] and source of coastal ocean C [Smith and Hollibaugh, 1993; Chen, 2004]. Constraining the various sources of C and OM contributing to riverine systems is thus imperative for more accurate budgeting of the current global C cycle and for forecasting changes in C cycling induced by global climate change and other anthropogenic impacts.

[3] A common method of identifying riverine C and OM sources is to utilize natural-abundance $^{13}C$ and $^{14}C$ isotopes either qualitatively [e.g., Pocklington and Tan, 1987; Raymond et al., 2004; Hélie and Hillaire-Marcel, 2006] or quantitatively through mass balance [e.g., Spiker and Rubin, 1975; Hellings et al., 1999; Cole and Caraco, 2001]. In terms of C budgeting, the latter quantitative analysis is preferred. These analyses, however, have thus far treated the source (i.e., end-member) isotopic signatures and contributions as fixed (static), and do not account for the significant temporal variation in isotopic signatures of the major C pools frequently observed in riverine systems.

[4] Temporal variation in riverine $\delta^{13}C$ and $\Delta^{14}C$ has been observed across numerous studies and is attributed primarily to changes in relative source contributions. In the case of riverine particulate and dissolved organic C (POC and DOC, respectively), for example, primary drivers such as hydrology, temperature and photoperiod may modulate
contributions from general potential sources such as allochthonous (i.e., terrestrial materials from the surrounding watershed) versus autochthonous (i.e., aquatic) inputs and aged versus modern inputs [Pocklington and Tan, 1987; Hellings et al., 1999; Cole and Caraco, 2001; Kendall et al., 2001; Raymond et al., 2004]. Temporal variation in riverine δ13C and Δ14C may also result from changes in the isotopic signatures of the potential contributing C sources. For example, atmospheric CO2, an indirect source for POC and DOC (through plant fixation) and a direct source for dissolved inorganic C (DIC), demonstrates both seasonal variation and annual variation (e.g., general decrease over time because of fossil fuel burning) in δ13C and Δ14C [Allison et al., 2003; Levin et al., 2010].

[5] In the absence of (or assumed absence of) temporal variation, the most basic approach for quantifying the various source contributions uses a linear mixing model based on isotopic mass balance [Schwarcz, 1991; Phillips, 2001]

\[
\begin{align*}
\delta_{\text{mix}}^i &= f_1 \delta_{1}^i + \cdots + f_{N+1} \delta_{N+1}^i \\
\delta_{\text{mix}}^N &= f_1 \delta_{1}^N + \cdots + f_{N+1} \delta_{N+1}^N \\
1 &= f_1 + \cdots + f_{N+1}
\end{align*}
\]

(1)

where \( \delta_{\text{mix}}^i \) is the isotopic ratio of isotope \( i \) (\( i = 1 \ldots N \)) for the mixture; \( \delta_j^i \) is the isotopic ratio of isotope \( i \) for source \( j \) (\( j = 1 \ldots N + 1 \)); and \( f_j \) is the fractional contribution of source \( j \) to the mixture. For any \( N \) isotope, \( N + 1 \) source system in which the source isotopic signatures fully constrain the isotopic signature of the mixture, an exact solution can be determined. This technique has long been employed across various disciplines such as hydrology [Epstein and Mayeda, 1953; Friedman et al., 1964; Dincer, 1968], atmospheric chemistry [Keeling, 1958; Ault et al., 1970], archaeology [Vogel and van der Merwe, 1977], and ecology [Calder and Parker, 1968].

[6] In cases where there are more than \( N + 1 \) sources (i.e., underdetermined), the solution is no longer unique and can only be approached. Approaches to finding approximate solutions include algebraic determination of minimum and maximum possible source contributions [Daubey, 1989; Lubetkin and Simenstad, 2004], and estimation of the range of possible source contributions either by Monte Carlo simulation [Minagawa, 1992] or by iteration over all possible combinations using stepwise increment [Zencich et al., 2002]. The latter method was formalized by Phillips and Gregg [2003] in their Visual Basic program IsoSource. More recent methods utilize Bayesian statistics, which have the additional advantage of accounting for uncertainty in the mixture and source isotopic signatures, as well as allowing incorporation of prior information [Moore and Semmens, 2008; Parnell et al., 2010]. The Moore and Semmens [2008] implementation of IsoSource is available as the Matlab program MixSIR and the Parnell et al. [2010] implementation is available as the R package SIAR [see also Jackson et al., 2009; Semmens et al., 2009]. A more complete overview of isotopic mixing models (although geared toward trophic analysis) can be found in Layman et al. [2012].

[7] Here we present two optimization-based solutions for riverine C isotope mixing models that incorporate temporal variation in both source isotopic signatures and source contributions. By encapsulating temporal variation and pooling data, these two solutions also permit determination of more than \( N + 1 \) sources within a system. In the first solution, the time-averaged mixing model (TAMM), only time-based changes in source isotopic signatures are included and the output is a single set of estimates representing annual average source contributions. This method is similar to recent models developed for trophic system analyses, which use dynamic source signature baselines that vary over time [Pace et al., 2004; Woodland et al., 2012] or space [Rasmussen, 2010] (note that the Woodland et al. [2012] model was solved within a Bayesian framework and the Pace et al. [2004] and Rasmussen [2010] models solved by least squares). In the second solution, the time-varying mixing model (TVMM), we additionally include time-based effects on source contributions (e.g., stream discharge and water temperature) and the output is multiple sets of estimates—one per sample period—representing the temporal source contributions. To our knowledge, no isotope mixing model encompassing dynamic source contributions has yet been developed.

[8] By capturing inherent seasonal and interannual variation in source isotopic signatures and contributions, we aimed to improve source contribution estimation, as well as to further understanding of seasonal patterns in C cycling at the terrestrial-aquatic interface. We measured δ13C and Δ14C of POC, DOC and DIC for six different rivers in the northeastern U.S. on seven different occasions. For POC and DOC we were interested in estimating contributions from six possible sources (\( C_3 \) plant material, \( C_4 \) plant material, algal material, slow-turnover soil organic C (OC), passive-turnover soil OC, and fossil OC), and for DIC, four possible sources (atmospheric exchange, carbonate dissolution, POC remineralization, and DOC remineralization).

2. Materials and Methods

2.1. Study Area

[9] The study originally focused on eight primary river systems discharging to a common ocean margin, the Middle Atlantic Bight (i.e., the region constrained by Cape Cod to the north and Cape Hatteras to the south). These rivers were the Connecticut, Hudson, Delaware, Schuylkill (a tributary of the Delaware), Susquehanna, Potomac, Pamunkey and Roanoke (Figure 1). However, two of the rivers, the Schuylkill and Pamunkey, demonstrated obvious 14C contamination from upstream nuclear power plants and had to be removed from this modeling effort because our selected models were not valid in these systems (but see K. Hossler and J. E. Bauer, 1968). Amounts, isotopic character and ages of organic and inorganic carbon exported from rivers to ocean margins, submitted to Global Biogeochemical Cycles, 2012, for a discussion of these systems). The remaining six rivers covered a range of drainage areas, regional locations, discharges, underlying mineralogies, and anthropogenic impacts. These and other factors helped to optimize the range of potential variability in input signatures of POC, DOC and DIC (Table S1 in Text S1 of the auxiliary material and Table 1; see also Section S1 in Text S1 for a description of drainage area characterization methodologies).
Each river was sampled at approximately 3–4 month intervals in 2005–2007 at the point farthest downstream that was accessible and above the reach of tidal influence. Across the study region, mean annual temperatures ranged from 6.4°C to 13.5°C and annual precipitation ranged from 979 mm to 1160 mm (Section S1).

2.2. Field Sampling

Surface water samples (~0.1 m depth) were collected in 10% HCl-cleaned 20 L polycarbonate carboys by small boat near the midpoint of each river. If samples could not be processed immediately after collection, carboys were kept in the dark on ice to minimize microbial and photochemical alteration of river OM and were processed within 5 h of collection. Samples (~100 mL) for DIC were collected directly from the rivers using 60 mL gas-tight syringes and were injected immediately into 125 mL gas-tight glass serum bottles containing 200 µL of saturated HgCl₂ solution and pre-sparged with ultra high purity N₂ gas. Detailed collection and processing protocols for each sample type (DOC, POC, and DIC) are more fully described in Raymond and Bauer [2001a]. Surface water temperature was measured with either a YSI Model 55 dissolved oxygen and temperature meter or a YSI Model 5739 field oxygen/temperature probe and pH was measured with a Fisher Scientific Accumet AP61 portable pH meter (temperature-corrected).

2.3. Sample Processing and Isotopic Measurements

Details on sample processing and isotopic measurements can be found in Section S2. Briefly, acidified POC and DOC samples from which inorganic carbonates had been removed were oxidized to CO₂ (by dry combustion and high-energy UV irradiation, respectively), then purified on a vacuum extraction line. Acidified DIC samples were sparged to extract the CO₂ gas which was then purified on a vacuum extraction line. Aliquots of CO₂ were collected in sealed Pyrex tubes and analyzed for δ¹³C and Δ¹⁴C at the National Science Foundation-Arizona Accelerator Mass Spectrometry (AMS) Facility. Concentrations of POC and DIC were determined by quantification of the CO₂ yield directly on the vacuum extraction line. DOC concentrations were analyzed independently by high temperature catalytic oxidation.
Table 1. Pedologic, Lithologic and Anthropogenic Characteristics of the Watersheds in the Present Study

<table>
<thead>
<tr>
<th>Soil Orderb</th>
<th>Lithologyc</th>
<th>Anthropogenic Impactsd</th>
</tr>
</thead>
<tbody>
<tr>
<td>River</td>
<td>(%) U</td>
<td>(%) IC OC</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Connecticut</td>
<td>72.6</td>
<td>0.1</td>
</tr>
<tr>
<td>Hudson</td>
<td>84.6</td>
<td>0.0</td>
</tr>
<tr>
<td>Delaware</td>
<td>29.5</td>
<td>24.1</td>
</tr>
<tr>
<td>Susquehanna</td>
<td>21.6</td>
<td>33.9</td>
</tr>
<tr>
<td>Potomac</td>
<td>25.0</td>
<td>47.2</td>
</tr>
<tr>
<td>Roanoke</td>
<td>10.6</td>
<td>73.0</td>
</tr>
</tbody>
</table>

Subregion

Connecticut | 32.4      | 0.1                  | 13.9   | 5.4  | 7    | 6   | 102 | 136 |
Hudson      | 58.5      | 0.2                  | 21.4   | 11.1 | 17   | 10  | 132 | 82  |
Delaware    | 57.3      | 16.9                 | 11.0   | 2.3  | 17   | 6   | 113 | 58  |
Susquehanna | 58.0      | 21.4                 | 14.4   | 7.8  | 27   | 24  | 366 | 141 |
Potomac     | 36.5      | 33.0                 | 34.8   | 6.1  | 34   | 10  | 144 | 126 |
Roanoke     | 18.1      | 53.6                 | 14.3   | 0.3  | 22   | 9   | 38  | 49  |

Summary of pedologic, lithologic and anthropogenic characteristics for the six rivers sampled in this study. Only select properties are described. See also Section S1 in the auxiliary material for further description of the data.

2.4. Model Descriptions

2.4.1. Modeled C Sources for POC, DOC and DIC

For POC and DOC, we assumed six primary sources of C: modern C3 plant material (C3 OC), modern C4 plant material (C4 OC), modern algal material (algal OC), slow-turnover soil OC (slow SOC), passive-turnover soil OC (passive SOC), and fossil OC. We distinguish the “modern” sources as that C having recently been fixed by photosynthesis (e.g., within one year) and acknowledge that these “modern” sources will still have a “non-modern” component to the signatures that is inherited from the inorganic C (IC) reservoirs utilized (e.g., atmospheric or dissolved CO2). Note also that the “modern C3” and “modern C4” sources refer primarily to terrestrial plants, but for practical purposes the Δ13C and Δ14C signatures are indistinguishable from some aquatic plants such as emergent aquatic species which will also fix atmospheric CO2 by C3 or C4 metabolism [e.g., Osmond et al., 1981; Marcenko et al., 1989]. These two modern sources of riverine OC are distinguished from “modern algal fixation” which represents sources of riverine OC from primarily nonvascular, aquatic species fixing aqueous forms of IC (e.g., dissolved CO2 or carbonate) by C3 metabolism. However, vascular, aquatic C3 plants (e.g., submergent vegetation) would also be included in this latter category if utilizing aqueous IC. For DIC, the modeled sources included atmospheric exchange, carbonate dissolution, POC remineralization, and DOC remineralization. Additional details on the POC, DOC and DIC source isotopic signatures are provided in Section S3.

2.4.2. Time-Averaged Mixing Model

We first applied a two-isotope time-averaged mixing model (TAMM) to estimate source contributions to POC, DOC and DIC in each watershed as

$$δ_{i,p}^j(t) = \sum_{j} f_{s,p,j} δ_{i}^j(t) + ε_{j,p}^i(t)$$

where $δ_{i,p}^j(t)$ is the measured isotopic signature $i$ (i.e., $δ^{13}$C or $Δ^{14}$C) for site $s$ (i.e., Connecticut, Hudson, Delaware, Susquehanna, Potomac or Roanoke) and carbon pool $p$ (i.e., POC, DOC or DIC), and varies with time $t$ (i.e., the sample collection date, with seven sample collections per site); $f_{s,p,j}$ is the fractional contribution from source $j$ (e.g., C3 OC, atmospheric exchange) for site $s$ and carbon pool $p$, and has the added constraints that as a proportion it must be between 0 and 1, and that for site $s$ and carbon pool $p$, the sum is 1; $δ_i^j(t)$ is the isotopic signature $i$ for source $j$ and time $t$; and $ε_{j,p}^i(t)$ is the residual error (i.e., unexplained variance) for isotopic signature $i$ from site $s$, carbon pool $p$ and time $t$. Note that as indicated, the source isotopic signatures were allowed to vary with time in this model (i.e., modeled $δ_i^j(t)$ rather than $δ_i^j$; see also Section S3). This signature variation was key to providing an adequate number of linearly independent equations to estimate the four to six fractional contributions from a two-isotope system.

Source contributions (i.e., $f_{s,p,j}$) were estimated by iteratively Reweighted Least Squares [Kutner et al., 2004], which minimized a weighted-residual sum-of-squares ($SST_w$) combining the $ε_{j,p}^i(t)$ from equation (2). See Section S4 for details on this method and choice of the weighting factor $w$.

Model fitting was performed in R 2.9.1 [R Development Core Team, 2009] using the “optim” function with “L-BFGS-B” method. Model fit was evaluated with a coefficient-of-determination ($R^2_w$) based on the weighted total and residual sum-of-squares ($SST_w$ and $SSE_w$, respectively)

$$R^2_w = 1 - \frac{SSE_w}{SST_w}, \quad \text{with}$$

$$SST_w = SST_1 + w \cdot SST_2,$$

and

$$SST_i = \sum_{j=1}^{N} \left\{ δ_{i,p,j}(t) - \text{mean}\left[ δ_{i,p,j}(t) \right] \right\}^2,$$

where $SSE_w$ and $w$ are as described for equation (S4) and $SST_i$ is the total sum-of-squares of isotope $i$ (i.e., $1 = Δ^{13}$C and $2 = Δ^{14}$C) for site $s$ and carbon pool $p$ based on the $N$ samples collected over time $t$. (Note that $R_w^2$ can range from less than 0 to 1, unlike the more familiar linear regression $R^2$ which is bounded by 0 and 1. When the model fits the data exactly, $R_w^2$ will be 1 (same as for the linear regression $R^2$). When the model fit is worse than the null model (e.g., $ε_{j,p}^i(t)$ is not a function of $t$; equation (2)), $R_w^2$ will be less than 0.)

Confidence intervals (95% CI) for the estimated $f_{s,p,j}$ were obtained by bootstrap. The $N$ samples were resampled with replacement 10,000 times and $f_{s,p,j}$ re-estimated (source isotopic signatures were also randomly resampled with...
each iteration to account for uncertainty in the signature estimates). The 25th and 975th quantiles provided the 95% CI (see Section S7 for additional details).

### 2.4.3. Time-Varying Mixing Models

To improve model fit and source contribution estimates, we next accounted for temporal variability in source contributions by including effects of stream discharge, water temperature, atmospheric \( CO_2 \) concentration, and vegetation phenology in the form of a time-varying mixing model (TVMM). A fractionation effect induced by the photosynthetic utilization of DIC was also introduced into the DIC TVMM (see Section S5). As with the TAMM, we iteratively solved for source contributions which best fit the observed data. The source contributions, however, could now vary temporally depending on the effects included in the mixing model (i.e., now modeled \( f_{s,p,t} \) rather than \( f_{s,p,j} \) as was modeled in the TAMM (equation (2)); note also that source isotopic signatures could still vary temporally in the TVMM as in the TAMM). To incorporate temporal effects on source contributions, we first rewrote the TAMM (equation (2)) in terms of actual source contributions \( c \), as opposed to fractional source contributions \( f \), obtaining

\[
\delta_{t}^{*}(t) = \sum_{j} c_{s,p,j}(t) \delta_{t}^{*}(t) + \epsilon_{t}^{*}(t),
\]

where \( c_{s,p,j}(t) \) is the actual contribution from source \( j \) for site \( s \), carbon pool \( p \) and time \( t \); and where the fractional contribution is defined as \( f_{s,p,j}(t) = c_{s,p,j}(t) / \sum_{j} c_{s,p,j}(t) \). Then, to include a specific temporal effect such as stream discharge for example, we modified the discharge-affected source contribution \( c_{*}^{s,p,k}(t) \) within the mixing model

\[
c_{*}^{s,p,k}(t) = c_{s,p,k}(t) \times f_{s}(t),
\]

with

\[
\delta_{t}^{*}(t) = \frac{c_{*}^{s,p,k}(t) \delta_{t}^{*}(t)}{c_{s,p,k}(t) + \sum_{j} c_{s,p,j}(t) \delta_{t}^{*}(t) + \epsilon_{t}^{*}(t)},
\]

where \( c_{*}^{s,p,k}(t) \) is the discharge-affected contribution from source \( k \) for site \( s \), carbon pool \( p \) and time \( t \); and \( f_{s}(t) \) is the stream discharge for site \( s \) at time \( t \). Daily stream discharge data were obtained for each site from the nearest U.S. Geological Survey water monitoring station (see Section S1). Other temporal effects (i.e., water temperature, atmospheric \( CO_2 \) concentration, and vegetation phenology) were similarly incorporated into the TVMM and were also specific for each site (see Section S6 for details).

POC and DOC components \( C_3 \) OC, \( C_4 \) OC and algal OC were allowed to vary with stream discharge, water temperature and vegetation phenology; slow SOC, passive SOC and fossil OC were allowed only to vary with stream discharge (see e.g., Tables 2–3). Of the DIC components, atmospheric exchange was allowed to vary with atmospheric \( CO_2 \) concentration; carbonate dissolution could vary with stream discharge; POC and DOC remineralization could vary with stream discharge and water temperature; and photosynthesis was allowed to vary with water temperature and vegetation phenology (see e.g., Table 4). The best model for

### Table 2. Comparison of Time-Averaged and Select Time-Varying Mixing Models for POC in the Six Study Rivers

<table>
<thead>
<tr>
<th>Model</th>
<th>Con</th>
<th>Hud</th>
<th>Del</th>
<th>Sus</th>
<th>Pot</th>
<th>Roa</th>
<th>Temporal Variation in Component Contribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 0</td>
<td>0.12</td>
<td>0.02</td>
<td>0.16</td>
<td>0.11</td>
<td>0.01</td>
<td>0.08</td>
<td>( C_3, C_4, slo \sim F )</td>
</tr>
<tr>
<td>Model 1</td>
<td>0.16</td>
<td>−0.05</td>
<td>0.29</td>
<td>−0.07</td>
<td>−0.08</td>
<td>0.26</td>
<td>( C_3, C_4, slo \sim F )</td>
</tr>
<tr>
<td>Model 2</td>
<td>0.47</td>
<td>0.11</td>
<td>0.26</td>
<td>0.11</td>
<td>0.07</td>
<td>0.07</td>
<td>( C_3, C_4, slo \sim T )</td>
</tr>
<tr>
<td>Model 3</td>
<td>0.15</td>
<td>−0.10</td>
<td>0.08</td>
<td>−0.24</td>
<td>−0.04</td>
<td>0.15</td>
<td>( C_3, C_4, slo \sim T )</td>
</tr>
<tr>
<td>Model 4</td>
<td>−0.04</td>
<td>−0.10</td>
<td>0.11</td>
<td>−0.25</td>
<td>−0.11</td>
<td>0.24</td>
<td>( C_3, C_4, slo \sim F )</td>
</tr>
<tr>
<td>Model 5</td>
<td>0.08</td>
<td>−0.01</td>
<td>0.40</td>
<td>−0.06</td>
<td>0.61</td>
<td>0.16</td>
<td>( C_3, C_4, slo \sim T )</td>
</tr>
<tr>
<td>Model 6</td>
<td>0.32</td>
<td>−0.02</td>
<td>0.26</td>
<td>0.12</td>
<td>−0.33</td>
<td>0.13</td>
<td>( C_3, C_4, slo \sim F )</td>
</tr>
</tbody>
</table>

*Reported are the \( R^2 \) (equation (3)) values for time-averaged (Model 0) and select time-varying (Models 1–6) mixing models per river (abbreviated by first three letters of river name); the highest \( R^2 \) value per river is in bold; Models 0 (i.e., time-averaged) includes only temporal variation in isotopic signatures of the six components: \( C_3 \) OC \( (C_3) \), \( C_4 \) OC \( (C_4) \), algal OC \( (alg) \), slow SOC \( (slo) \), passive SOC \( (pas) \), and fossil OC \( (fos) \). Models 1–6 (i.e., time-varying) additionally incorporate temporal variation in component contributions through effects of stream discharge \( (F) \), water temperature \( (T) \), and vegetation phenology \( (V) \). (Note: 13 time-varying DOC mixing models were actually applied to the data, but only results from the six best-fitting TVMM (based on \( R^2 \)) across all six rivers are presented.)

### Table 3. Comparison of Time-Averaged and Select Time-Varying Mixing Models for DOC in the Six Study Rivers

<table>
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<th>Model</th>
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</tbody>
</table>

*Reported are the \( R^2 \) (equation (3)) values for time-averaged (Model 0) and select time-varying (Models 1–6) mixing models per river (abbreviated by first three letters of river name); the highest \( R^2 \) value per river is in bold; Models 0 (i.e., time-averaged) includes only temporal variation in isotopic signatures of the six components: \( C_3 \) OC \( (C_3) \), \( C_4 \) OC \( (C_4) \), algal OC \( (alg) \), slow SOC \( (slo) \), passive SOC \( (pas) \), and fossil OC \( (fos) \). Models 1–6 (i.e., time-varying) additionally incorporate temporal variation in component contributions through effects of stream discharge \( (F) \), water temperature \( (T) \), and vegetation phenology \( (V) \). (Note: 13 time-varying DOC mixing models were actually applied to the data, but only results from the six best-fitting TVMM (based on \( R^2 \)) across all six rivers are presented.)
each site and carbon pool was evaluated by $R_w^2$, the proportion of variance explained by the model (see equation (3)).

### 3. Results and Discussion

[19] Riverine C pools (i.e., POC, DOC and DIC) from the present study were almost fully constrained (119 out of 126 total samples) by the respective 6-component (POC and DOC) and 4-component (DIC) mixing model sources (Figure 2). For POC and DOC, algal OC and either C$_4$ OC or passive SOC typically provided lower and upper bounds for $\delta^{13}$C signatures, respectively. Fossil OC or passive SOC provided lower bounds for $\Delta^{14}$C signatures; and slow SOC, or in some cases algal OC, provided upper bounds for $\Delta^{14}$C signatures. For DIC, either POC or DOC remineralization and atmospheric exchange provided lower and upper bounds for $\delta^{13}$C signatures, respectively, while carbonate dissolution and either atmospheric exchange or DOC remineralization

![Figure 2. POC (circles), DOC (triangles) and DIC (crosses) isotope data (black coloration denotes sample is fully constrained by model components and orange coloration denotes the sample is unconstrained) with modeled C source components. Source isotopic means for POC and DOC are indicated with empty boxes: C$_3$ OC (blue), C$_4$ OC (green), algal OC (yellow), slow SOC pool (purple), passive SOC pool (cyan), and fossil OC (red). Source isotopic means for DIC are indicated with solid boxes: atmospheric exchange (blue), carbonate dissolution (green), POC remineralization, and DOC remineralization (red). (See Section S3 for details on determination of the C source isotopic signatures.) Solid lines indicate ±2 standard deviations for the respective C source. Note that the C source isotopic signatures have been included to indicate the general ranges (i.e., mean and ±2 standard deviation) to assist the reader, but most actually had time-varying isotopic signatures within the models (i.e., not static mean signatures). Only slow SOC, passive SOC, and fossil OC sources were modeled as non-varying means. For all C sources some portion of the indicated uncertainty in isotopic signatures was also incorporated into the estimated contribution confidence intervals (see Section S7).]
typically provided lower and upper bounds for $\Delta^{14}C$ signatures, respectively.

[20] The few unconstrained samples included POC samples 0.2‰ to 4‰ more depleted in $\delta^{13}C$ than modeled components and DIC samples 4‰ to 48‰ more enriched in $\Delta^{14}C$ than modeled components (Figure 2). These exceptions are attributed to limitations of the model rather than to missing components (i.e., unmodeled C source). The depleted $\delta^{13}C$-POC samples, for example, could be constrained by algal OC if the modeled photosynthetic fractionation effect were $-24\%$ rather than $-20\%$. A $-24\%$ fractionation effect would be consistent with the study by Finlay (2004), which observed algae to be depleted in $\Delta^{14}C$ by 4‰ to 18‰ relative to $\text{aq-CO}_2$ and by 5‰ to 28‰ relative to $\text{HCO}_3^-$ depending on algal species and availability of the C-source. C pool signatures and modeled components were also based on either mean values or single measurements, such that some unconstrained samples were still within the range of uncertainty (e.g., standard deviations for replicate POC and DIC $\Delta^{14}C$ signatures were around 24‰ and 7‰, respectively).

[21] Some lack of constraint, however, was not generally problematic for the mixing models which found the best solution (i.e., the solution which minimized the weighted sum-of-squares) among the 7 samples per river for each major C pool (i.e., POC, DOC and DIC). For the TAMM, overall model fit was only modest with $R^2_w$ values ranging from $-0.3$ to $0.5$, but typically around 0.1 (Tables S2–S4). The poor fit for the TAMM presumably reflected the additional, unaccounted for seasonal and annual variability in source contribution, e.g., from changes in stream discharge or water temperature. Incorporating temporal drivers of variation (e.g., see Figure S1 in the auxiliary material) into the TVMM improved model fit, accounting for up to 70% more variance in the POC models, 60% more variance in the DOC models, and 60% more variance in the DIC models (Tables 2–4). For the TVMM, $R^2_w$ values ranged from $-0.1$ to 0.9 and averaged around 0.4.

3.1. Mean Annual Source Contributions

[22] The default output of the TAMM was a single set of estimates representing mean annual source contributions (Figure 3, Tables S2–S4). For the TVMM, the default output was multiple sets of estimates (i.e., one set per sample period), which were first combined into a single set of flux-weighted source contribution estimates to facilitate both comparisons with TAMM estimates and inferences regarding riverine C source importance on an annual basis (Figure 3, Tables S5–S7). Confidence intervals (95%) for both TAMM and flux-weighted TVMM source contribution estimates were often broad reflecting not only the seasonal variation, but also the uncertainty in source isotopic signatures (see e.g., Section S7), the sometimes poor discernment between certain C sources (e.g., $C_3$ OC and slow SOC; Figure 2), and unaccounted for variance such as unmodeled (e.g., TAMM) or inadequately modeled (e.g., TVMM) drivers of temporal variation. With few exceptions, mean annual source contribution estimates were similar between the TAMM and flux-weighted TVMM. Hence, while the following discussion of riverine C source importance on an annual basis focuses on the TVMM flux-weighted estimates, the interpretations are also applicable to the TAMM estimates.

3.1.1. POC and DOC

[23] On an annual basis, the majority of riverine POC was derived from $C_3$ OC and algal OC (Figure 3a, Table S5). For DOC, $C_3$ OC and slow SOC were the primary sources of OC, except for in the Delaware and Susquehanna rivers where algal OC became dominant (Figure 3b, Table S6). (Note that isotopic signatures were similar for these three potential sources (Figure 2) and not entirely distinguishable from one another—hence the broad confidence intervals.)

[24] Several studies of riverine C and OM have suggested soil OC to be a major contributor to POC on the basis of N and C isotope and chemical markers: e.g., the Yangtze River in China [Wu et al., 2007], multiple U.S. rivers draining to the Gulf of Mexico [Onstad et al., 2000], and the Amazon River in South America [Hedges et al., 1986a, 1986b]. Using $\delta^{13}C$ measurements exclusively, terrestrial sources of OC in general have been inferred to dominate riverine POC in systems such as the Orinoco River in Venezuela [Tan and Edmond, 1993] and the Sava River in southeast Europe [Kanduč et al., 2007], and DOC in systems such as the St. Lawrence River [Hélie and Hillaire-Marcel, 2006]. Other studies have observed a predominance of algal OC inputs to POC in the St. Lawrence River [Barth et al., 1998; Hélie and Hillaire-Marcel, 2006; but see also Pocklington and Tan, 1987] and for several large rivers across the U.S. [Kendall et al., 2001].

[25] $C_4$ OC was estimated to be a relatively minor component of riverine OC, although estimated annual contributions ranged up to 47% (95%) for POC in the Potomac River and up to 51% (95%) for DOC in the Delaware River (Figures 3a and 3b, Tables S5 and S6). Corn (a $C_4$ crop) production is a minor industry in all six watersheds and likely the primary source of $C_4$ OC for these rivers [Still et al., 2003; National Agricultural Statistics Service, 2009; Still et al., 2009].

[26] Passive SOC and fossil OC contributions ranged from 0% up to 30% (95% CI) for POC and from 0% up to 22% (95% CI) for DOC (Figures 3a and 3b, Tables S5 and S6). Unexpectedly, passive SOC contributions to POC and DOC were lowest (or near lowest) in the Roanoke River, which had the highest percentage of Ultisols—a very old (700,000 yr to 1,600,000 yr), highly weathered soil order [Howard et al., 1993] (Table 1). This apparent discrepancy, however, likely reflects differences in hydrologic flow paths [Hinton et al., 1998; Schaff et al., 1998] and factors affecting soil erodibility such as land relief or agricultural practices [McCool et al., 1997; Longworth et al., 2007; Smith et al., 2007; Boix-Fayos et al., 2009; Siekman et al., 2010]. In the Ronaoko watershed subregion, for example, agricultural activities are moderate relative to the other five watersheds (Table 1) and the terrain is mostly of low relief and less sloped (Figure 1, Table S1).

[27] Fossil OC contributions to POC were highest in the Susquehanna and Hudson rivers, which also had the highest percentages of subregional bedrock lithology containing fossil OC (Table 1). Other potential sources of fossil OC included wastewater treatment plants [Griffith et al., 2009] and fossil-fuel burning power plants [Hsueh et al., 2007], both of which were most abundant in the Susquehanna watershed subregion (Table 1). The presence of a small amount of aged OC, particularly for POC, is consistent with previous findings in this region [Raymond and Bauer, 2001b;
Figure 3. Barplots indicating estimated source contributions and 95% CI from the time-averaged (lightly shaded) and time-varying (darkly shaded) mixing model analyses for (a) POC, (b) DOC, and (c) DIC. Potential C sources for POC and DOC are C3 OC, C4 OC, algal OC, slow SOC, passive SOC, and fossil OC. For DIC, potential C sources are atmospheric exchange, carbonate dissolution, POC remineralization, and DOC remineralization, with additional potential removal through photosynthesis (TVMM only). Plots are separated by C source and estimates plotted per river; estimated source contributions per river sum to 100%. (Note, however, in Figure 3c only the first four plots are DIC sources and sum to 100%; the final plot is photosynthesis which is a fate, not source, of DIC and plotted values indicate percentages of DIC consumed). For each river C source, the vertical bar boundaries indicate the 95% CI range (obtained by bootstrap with 10,000 resamplings), while the horizontal black bar within each vertical bar indicates the mixing model estimate. Dashed horizontal lines indicate mean source contributions across the six rivers from the TAMM (lightly shaded) and TVMM (darkly shaded) estimates (note that the dashed horizontal lines may be overlapping). (Note also for the TVMM results, the contribution estimate and 95% CI are for the flux-weighted averages of the per-sample- contribution estimates.)
Raymond and Hopkinson, 2003; Raymond et al., 2004; Longworth et al., 2007] and has been correlated with agricultural intensity (e.g., greater erosion of deep, older soil) and presence of shale outcrops [Raymond et al., 2004; Longworth et al., 2007].

3.1.2. DIC

[25] Typically, approximately half of the DIC in the six rivers derived from atmospheric exchange annually (Figure 3c, Table S7). Estimates ranged from 0%–57% (95% CI) in the Connecticut to 50%–60% (95% CI) in the Hudson. Regardless of whether the riverine system is a net source or sink of CO₂, diffusive exchange of CO₂ between the water body and the atmosphere leads to air-water equilibration of the respective isotopic signatures over time. The importance of atmospheric exchange as a source of DIC has been established in several previous studies, particularly under conditions of long residence times or inflow from lakes or reservoirs [Pawellek and Veizer, 1994; Hélie et al., 2002; Finlay, 2003; Brunet et al., 2005; Wachniew, 2006; Zhang et al., 2009].

[29] Mean residence time (i.e., stream length/velocity; Table S1) and number of impoundments (Table 1) varied considerably among the six rivers. Based on these two factors, we would expect atmospheric exchange and isotopic equilibration to be highest in the Susquehanna River (e.g., 590 d residence time and 141 large dams) and lowest in the Delaware River (e.g., 120 d residence time and 58 large dams). Indeed, at least among the better constrained systems, estimated atmospheric contributions were relatively higher in the Susquehanna River and lower in the Delaware River (Figure 3c, Table S7).

[30] The remaining DIC source contributions varied by river (Figure 3c, Table S7). Carbonate dissolution was minor in the Roanoke River, but contributed as much as 32% (95% CI) to the DIC pool in the Delaware River. Indeed, the Delaware River has a relatively carbonate-rich lithology at the subbasinal level (Table 1). The Roanoke River, however, also has a moderately to extremely carbonate-rich lithology at the subregional and subbasinal levels which appears inconsistent with the estimated carbonate contribution. As discussed earlier for passive SOC, this apparent inconsistency is likely a consequence of watershed flowpaths and factors affecting soil or bedrock erodibility.

[31] Remineralization of POC and DOC contributed up to 44% and 96% (95% CI), respectively, to the riverine DIC pools (Figure 3c, Table S7). Previous isotopic-based studies have also suggested that remineralization of OM can be a significant source of riverine DIC [Pawellek and Veizer, 1994; Atekwana and Krishnamurthy, 1998; Hellings et al., 1999; Telmer and Veizer, 1999; Finlay, 2003; Raymond and Hopkinson, 2003; Raymond et al., 2004; Brunet et al., 2005; Kanduć et al., 2007; Zhang et al., 2009; Zeng et al., 2011]. The OM remineralization may occur in situ or terrestrial (with subsequent leaching into the river) and biotically or abiotically (e.g., photochemical degradation) [Pawellek and Veizer, 1994; Amon and Benner, 1996; Wiegner and Setzinger, 2001; Kanduć et al., 2007].

3.2. Photosynthesis

[32] A photosynthetic effect was included in the TVMM only. Photosynthesis consumed an estimated 0%–53% (95% CI) of the DIC pool on an annual basis (Figure 3c, Table S7). In-stream photosynthesis appeared to be a particularly important process within the Connecticut and Roanoke rivers. The DIC photosynthesis estimates often appeared in contrast with estimates of algal OC contribution in the POC and DOC mixing models. In the Susquehanna River, for example, the estimated photosynthetic consumption was 0%–9% (95% CI) of the DIC pool, while the estimated algal OC contribution was up to 69% and 92% (95% CI) in the POC and DOC pools, respectively (Figure 3, Tables S5–S7). Estimated contributions, however, are relative, so while in-stream photosynthesis may be a significant sink for DIC, as a source for POC or DOC, the process may be minor in comparison to other contributing sources (e.g., C₃ OC and slow SOC).

3.3. Drivers of Temporal Variation

3.3.1. POC and DOC

[33] To account for temporal variation in source contributions we incorporated various effects of stream discharge, water temperature and vegetation phenology into the POC and DOC TVMM (Tables 2 and 3). We expected that discharge, in particular, would have a dominant effect on contributions from C₃ and C₄ OC and slow SOC. These surficial and shallow forms of terrestrial OC would likely increase during high flow conditions, while deeper OC materials (e.g., passive SOC and fossil OC) would reflect constant base flow conditions [e.g., Sanderman et al., 2009]. Several studies have observed direct correlations between stream discharge and POC and DOC concentrations, primarily due to increased runoff and erosion during periods of high flow [Lewis and Grant, 1979; Pocklington and Tan, 1987; Frank et al., 2000; Kendall et al., 2001; Wu et al., 2007; Sanderman et al., 2009; Raymond and Saiers, 2010]. We modeled discharge effects as either direct for C₃ and C₄ OC and slow SOC, or inverse (i.e., a dilution effect) on algal OC, passive SOC and fossil OC. Some form of discharge effect appeared in the best-fitting POC and/or DOC TVMM for all six river systems, suggesting that stream discharge is indeed an important source of variation in OC contributions (Tables 2 and 3).

[34] Temperature and vegetation phenology were modeled as direct effects on C₃, C₄ and algal OC. The temperature effect was incorporated first given its well-established role in primary production dynamics [e.g., Went, 1953; Duarte, 1995; White et al., 1997] and as a simple attempt to capture seasonal variation in plant and algal productivity. We also modeled interactive effects between temperature and discharge on source contributions. Temperature was included as a factor for DOC (best-fitting TVMM) in four of the six river systems (and in one best-fitting POC TVMM; Tables 2 and 3). We next fit the models using patterns of vegetation phenology [Zhang et al., 2001] because other environmental factors such as photoperiod and light intensity can also be important drivers of primary productivity [e.g., White et al., 1997; Jones, 1997; Kennedy and Whalen, 2008]. Incorporating the phenology patterns improved model fit for POC and DOC in one of the systems (the Susquehanna), but was generally less effective than temperature in capturing the variation in primary production contributions (Tables 2 and 3).

[35] While the best-fitting TVMM varied from river to river, the model providing the best overall improvement for
POC included a positive effect of discharge on C_3 and C_4 OC and slow SOC inputs (Table 2, POC Model 1). For DOC, the overall best model included a positive effect of temperature on C_3, C_4 and algal OC inputs, and a positive effect of discharge on C_3 and C_4 OC and slow SOC inputs (Table 3, DOC Model 5). Use of the TVMM captured much of the observed temporal variation in POC and DOC $\delta^{13}$C and $\Delta^{14}$C signatures in several of the rivers (e.g., Connecticut, Delaware and Potomac; Figures 4a, 4b, 5a, and 5b).

3.3.2. DIC

[46] For the DIC TVMM, one of the main improvements over the TAMM was the addition of photosynthesis. Photosynthesis with some form of temporal variation appeared in four of the six best-fitting TVMM (Table 4). The net effect of photosynthesis on DIC was enrichment of the $\delta^{13}$C signature (see Section S5).

[47] Drivers of temporal variation in DIC source contributions included stream discharge, water temperature, vegetation phenology and atmospheric CO$_2$ concentration (Table 4). Similar to POC and DOC, we expected that stream discharge would have a direct effect on surficial or shallow components susceptible to runoff and erosion (e.g., plant litter) and an inverse, or dilution, effect on deeper components (e.g., groundwater dissolution of carbonate bedrock). Wachniew [2006], for example, in a study of the Vistula River in Poland observed depletions in DIC-$\delta^{13}$C with flushing events because of increased biogenic $\delta^{13}$C-depleted IC inputs. Consequently, we modeled direct discharge effects on POC and DOC remineralization and inverse discharge effects on carbonate dissolution. Discharge, however, appeared in only two of the best-fitting models (as a factor for POC remineralization in interaction with temperature; Table 4).

[48] Several studies have observed seasonal patterns in DIC-$\delta^{13}$C reflecting relative rates of photosynthesis and respiration [Pawellek and Veizer, 1994; Atekwana and Krishnamurthy, 1998; Hellings et al., 1999; Hélie et al., 2002; Wachniew, 2006; Kanduc et al., 2007; Zhang et al., 2009]. We applied direct effects of temperature to POC and DOC remineralization and photosynthesis and direct effects of vegetation phenology to photosynthesis. Temperature in particular substantially improved model fit and appeared in all six of the best-fitting models (Table 4).

[49] As a temporal factor for DIC atmospheric exchange contributions, we incorporated a seasonally varying pattern based on atmospheric CO$_2$ concentration (see Section S6). None of the best-fitting TVMM, however, included an effect of atmospheric CO$_2$ concentration (Table 4).

[50] The TVMM providing the best overall improvement included only an effect of vegetation phenology on photosynthesis (Table 4, DIC Model 3). Similar to POC and DOC, use of the TVMM characterized much of the temporal variability in DIC $\delta^{13}$C and $\Delta^{14}$C signatures evident in several of the rivers (e.g., Delaware, Hudson and Susquehanna; Figures 4c and 5c).

3.4. Seasonal Source Contributions

3.4.1. POC

[51] The value in the TVMM over the TAMM (in addition to elucidating potential drivers of variation) was the derivation of time-varying source contributions. The dominant pattern observed for riverine POC was a seasonal change in the relative contribution from algal OC (Figure S2). Algal OC comprised a larger proportion of POC in the summer than in the winter, most typically in response to an inverse effect of stream discharge, either alone or in combination (e.g., Connecticut, Hudson and Delaware). Other drivers included stream temperature (e.g., Hudson) and vegetation phenology (e.g., Susquehanna). In the Potomac River, the variation in algal OC contributions was an indirect result of seasonal change in other components.

[52] Specifically, fractional contributions to POC from algal OC tended to be 0.3 to 0.7 higher in the summer under conditions of low flow, high temperature and peak phenology (Figure S2). This finding is in general agreement with other riverine studies using isotope-based analyses which have observed increases in autochthonous relative to allochthonous POC in summer [Pocklington and Tan, 1987; Hellings et al., 1999; Kendall et al., 2001].

[53] Other time-varying component contributions to riverine POC included C_3 and C_4 OC and slow SOC, which tended to be higher during elevated winter discharge. The winter increase in fractional contribution for these three components ranged from 0 to 0.5 for C_3 OC, 0 to 0.3 for C_4 OC, and 0 to 0.6 for slow SOC (Figure S2). In contrast, passive SOC and fossil OC contributions tended to be higher during low summer discharge by 0 to 0.2 and 0 to 0.3, respectively.

[54] The seasonal pattern in algal OC contribution was the primary driver for the observed increase of 3 \% to 9 \% in $\delta^{13}$C-POC signatures from summer to winter (Figures 4a and S2). The summer depletion in $\delta^{13}$C-POC, for example, was the result of an increase of 0.3 to 0.7 in the fractional contribution of algal OC, which had an extremely depleted $\delta^{13}$C signature (relative to other POC components). Underlying this pattern also was a decrease of 3 \% to 9 \% in the algal $\delta^{13}$C signature from winter to summer which tracked the winter-enriched and summer-depleted $\delta^{13}$C-DIC signatures (i.e., the C reservoir for algal fixation; see section 3.4.3).

[55] Algal $\Delta^{14}$C signatures were moderately depleted, at least relative to C_3 and C_4 OC and slow SOC sources, and likewise explained some of the variation in observed $\Delta^{14}$C-POC signatures (Figures 5a and S2). This pattern was somewhat obscured, however, because the $\Delta^{14}$C-DIC signature underlying the algal $\Delta^{14}$C signature was more variable (see section 3.4.3).

[56] Also influencing the variation in $\Delta^{14}$C-POC signatures were seasonal changes in fossil OC contributions. Fossil OC contributions tended to be higher in summer as either a direct inverse response to stream discharge (e.g., Connecticut, Hudson and Delaware) or as an indirect response to other component contributions (e.g., Susquehanna, Potomac and Roanoke). Although the fractional contributions from fossil OC varied only slightly (e.g., typically by less than 0.1), because the fossil OC $\Delta^{14}$C signature was extremely depleted (~1,000 \%), small fluctuations in contribution induced large fluctuations in $\Delta^{14}$C-POC (Figures 5a and S2).

3.4.2. DOC

[57] In contrast to POC, the dominant observed pattern in DOC source contributions was the seasonal change in slow SOC inputs (Figure S3). Fractional contributions from slow SOC were higher in winter by up to 0.7 as either a direct result of stream discharge (e.g., all except the Hudson) or in indirect response to changes in other component contributions (e.g., Hudson). A similar pattern was observed by
Sanderman et al. [2009] in their study of a small coastal watershed in California, USA.

Other regionally consistent patterns included summer peaks in algal OC, passive SOC and fossil OC contributions (Figure S3). Ranges in relative contributions from these three sources were on the order of 0.2, 0.03 and 0.1, respectively. Seasonal changes in relative C3 and C4 OC contributions ranged from 0 to 0.5 and from 0 to 0.4, respectively, and in contrast to other components, exhibited little seasonal coherence across the six rivers.

The observed summer peak in $\delta^{13}C$-DOC appeared to be driven primarily by the winter to summer decrease in the proportional contribution from slow SOC (Figures 4b and S3). The decreased contribution from slow SOC, which had a relatively depleted $\delta^{13}C$ signature ($\sim-28 \%$), had the net effect of enriching the bulk summer $\delta^{13}C$-DOC signature.

Summer depletions in DOC $\Delta^{14}C$ could likewise be attributed to the decreased contribution from slow SOC, which had a relatively enriched $\Delta^{14}C$ signature ($\sim 160 \%$). Minor summer increases in passive SOC and fossil OC contributions, both of which had extremely depleted $\Delta^{14}C$ signatures, also contributed to the seasonal depletion in bulk DOC $\Delta^{14}C$ (Figures 5b and S3; see also section 3.4.1).

3.4.3. DIC

The dominant pattern in source contribution (or removal) observed for DIC was the seasonal variation in photosynthesis, which corresponded directly with stream temperature and vegetation phenology (Figure S4). Photosynthetic consumption of DIC was 0 to 0.2 higher in summer. Other modeled DIC components changed by less than 0.1 seasonally on average and were more variable between systems (i.e., no broad regional patterns).

Figure 4. Time series plots by river of $\delta^{13}C$ signatures for (a) POC, (b) DOC, and (c) DIC. (Note: scales vary by parameter and river.) Measured values are indicated by solid black lines, time-averaged mixing model estimates by dotted black lines, and time-varying mixing model estimates by dashed black lines (note that some of the lines may be overlapping). Shading indicates winter months (i.e., December (D) through March (M)); spring, summer and fall months are unshaded (e.g., June (J) and September (S)).
While seasonal change in photosynthesis was the predominant pattern in DIC source contribution or removal, its effect on bulk $\delta^{13}C$-DIC signatures was counter to the observed variation (Figure S4). Rather, the generally observed pattern of summer-depleted and winter-enriched $\delta^{13}C$-DIC signatures was primarily a function of seasonal variation in the $\delta^{13}C$ signatures associated with atmospheric exchange, POC remineralization and DOC remineralization (Figures 4c and S4). For atmospheric exchange, the effect was a result of isotopic fractionation during dissolution of atmospheric CO$_2$, in which the $\delta^{13}C$ signature of the dissolved CO$_2$ becomes enriched. This fractionation effect is greater at lower temperatures, resulting in a relatively summer-depleted and winter-enriched $\delta^{13}C$ signature [Mook et al., 1974].

For POC and DOC remineralization, $\delta^{13}C$ signatures mirrored those of the bulk POC and DOC pools and so were indirectly attributable to changes in stream discharge, water temperature, and vegetation phenology (see sections 3.4.1 and 3.4.2). Variations in bulk POC and DOC $\Delta^{14}C$ signatures also dictated much of the observed change in $\Delta^{14}C$-DIC (Figures 5c and S4). The remaining change in $\Delta^{14}C$-DIC was attributable to small fluctuations in relative carbonate contribution, which had a constant, but extremely depleted $\Delta^{14}C$ signature.

3.5. Model Caveats

As with all mixing models, the primary caveat for the TVMM (and TAMM) is the assumption that all contributing sources are known and accurately modeled. The possibility remains, however, that important major sources of C and OM may be missing from the model or inadequately modeled. Missing sources may in some cases be obvious and the...
selected model will clearly no longer be applicable (e.g., radiocarbon contamination from nearby nuclear power plants leading to elevated $\Delta^{14}$C signatures forced us to exclude the Schuylkill and Pamunkey rivers in this modeling effort). In other cases, missing sources are not obvious and we must rely on the correctness of existing theory. In a similar manner, we represent the isotopic signatures of proposed sources as accurately as possible in the model based on the present literature. Source isotopic means are used to determine model estimates and source isotopic variances are used to obtain confidence intervals on the estimates.

Another caveat for the TVMM (as well as the TAMM and other mixing models in general) is that the contributing sources are proportionately reflected in the bulk C fractions. In other words, we assume that there is no preferential loss of a particular source between input to the stream and sample collection. Violation of this assumption, however, while hindering estimates of terrestrial C and OM losses, would not impact estimates of C and OM exports to the coastal ocean.

### 4. Summary

The time-varying mixing model (TVMM) developed in the present study confers several advantages over time-averaged, as well as the traditional static, mixing models. First, the TVMM accounts for temporal variation in both the isotopic signatures and relative contributions of each potential source, thus improving model fit to observed data. Second, through the procedure of fitting the TVMM to the data, one can test proposed drivers of temporal variation, thus gaining insight into which processes are ecologically significant within a system. Finally, the TVMM output is a set of time-varying source contributions which, because of the temporal variation, more accurately represent the behavior of the system and modeled components.

From our application of TVMM to six rivers draining the northeastern U.S., we observed the primary drivers of terrestrial and OM inputs (and subsequent exports) to be stream discharge in the case of POC, stream discharge and temperature for DOC, and either stream temperature or vegetation physiology for DIC. The major sources to each pool included (1) C$_3$ OC, algal OC and slow SOC for POC; (2) C$_3$ OC and slow SOC for DOC; and (3) atmospheric exchange for DIC. These findings were largely consistent with those from previous studies in the existing literature. An examination of the temporal patterns in source contributions and removal, however, suggests that variations in the isotopic signatures of the POC, DOC and DIC pools were dominated by changes in algal OC (peaked in summer), slow SOC (peaked in winter), and photosynthesis (peaked in summer), respectively. In the case of POC and DOC, these contributional patterns translated to their bulk $\delta^{13}$C, and sometimes $\Delta^{13}$C, signatures. For DIC, the observed variation in $\delta^{13}$C and $\Delta^{14}$C signatures was dictated by the underlying component isotopic signatures for atmospheric exchange, POC remineralization and DOC remineralization.

While TVMM fit ($R^2_m$) was in some cases as good as 0.9, the average was 0.4 and sometimes as low as $-0.1$. Percent reduction in sum-of-square error ranged from 3% to 170% for $\delta^{13}$C and from 24% to 1600% for $\Delta^{14}$C. These metrics suggest that much of the underlying variation was not captured by the models, such that perhaps modeling of the temporal drivers (e.g., stream discharge) could be improved. For the DIC TVMM, for example, one approach might be to weight POC and DOC remineralization by their relative concentrations [see e.g., Phillips and Koch, 2002]. Another possibility is that additional drivers and components (e.g., anthropogenic radiocarbon source) need to be included. Despite its general limitations and current shortcomings, the TVMM is an instrumental tool to identify components and contributions under the more realistic non-static conditions encountered in streams, rivers and other ecosystems. Consequently, the TVMM should be of use to the biogeochemical and ecological communities and improved upon in the future.

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