# Source Switching Maintains Dissolved Organic Matter Chemostasis Across Discharge Levels in a Large Temperate River Network

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#### Abstract

Dissolved organic matter (DOM) helps regulate aquatic ecosystem structure and function. In small streams, DOM concentrations are controlled by transport of terrestrial materials to waterways, and are thus highly variable. As rivers become larger, the River Continuum Concept hypothesizes that internal primary production is an increasingly important DOM source, but direct evidence is limited. Recently, the Pulse-Shunt Concept postulated that terrestrial DOM concentrations in larger rivers increase with flow and temperature, which

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seemingly contradicts previously reported DOM chemostasis in large rivers. This study estimates daily gross primary production (GPP) in 13 streams and rivers across the Connecticut River watershed (watershed areas 0.4–25,019 km<sup>2</sup>) from 2015 through 2017. Chemostasis of DOM concentrations is maintained by a switch from autochthonous sources of DOM at low flows to terrestrial sources of DOM at high flows in a large temperate river and to a lesser degree in smaller tributaries. At low flow, autochthonous DOM linked to aquatic GPP is the dominant fraction of the DOM pool in large rivers. This autochthonous DOM maintains chemostasis in the main stem and to a lesser extent upstream. Thus, in larger rivers, low-flow autochthonous production stabilizes DOM concentrations during the summer, a critical time for riverine ecology. Consistent with the Pulse-Shunt Concept, terrigenous DOM is the dominant fraction of DOM during higher flow periods and about 70% of annual DOM fluxes to the coast are terrestrial. This pattern of DOM switching is potentially widespread in temperate watersheds with implications to both inland waters and coastal ecosystems.

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**Key words:** ecosystem metabolism; gross primary production; ecosystem respiration; river network scaling; dissolved organic matter; PARAFAC; environmental flows.

## **H**IGHLIGHTS

- Aquatic photosynthesis alters organic matter composition at low flow in rivers
- Primary production maintains constant organic carbon levels in big temperate rivers
- Main stem DOM flux is 70% terrestrially sourced and highest at high flows

#### INTRODUCTION

Dissolved organic matter (DOM) is a master variable in aquatic environments (Kaplan and Cory 2016). As such, it has interested inland water scientists for almost a century (Birge and Juday 1934). DOM influences aquatic light availability, regulating photosynthesis (Siegel and Michaels 1996) and the breakdown of organic compounds (Opsahl and Benner 1998). Further, DOM influences water chemistry via complexing of inorganic nutrients and metals (Mantoura and others 1978) and pH buffering (McKnight and others 1985).

In freshwaters, organic matter inputs are generally classified into two groups-allochthonous and autochthonous (Thurman 1985)—based on where the carbon was fixed. Allochthonous DOM is defined as carbon that is fixed—converted from CO<sub>2</sub> to organic matter-externally to river systems. Autochthonous carbon, by contrast, is organic carbon that is fixed by primary producers internally within the river network. The sources of allochthonous DOM in inland waters are mainly surface soils and wetlands. Transfer from terrestrial to aquatic environments, particularly in temperate and high latitude systems, is controlled by hydrologic events that flush organic matter sources on the landscape to varying degrees, leading to highly variable dissolved organic carbon (DOC) concentrations in headwaters (McDowell and Likens 1988; Hornberger and others 1994; Boyer and others 1997; Raymond and Saiers 2010; Inamdar and others 2013; Shultz and others 2018), with hydrologic connectivity playing a critical role (Zimmer and McGlynn 2018; Hale and Godsey 2019; Lynch and others 2019). In flowing waters, allochthonous carbon typically has higher apparent

molecular weights, is older, and is more enriched in conjugated compounds—including aromatic rings-compared to autochthonous DOM (Hood and others 2005; Butman and others 2012). Flowing waters are conventionally viewed as containing primarily allochthonous DOM (Kaplan and Cory 2016), but inputs of light and nutrients can lead to high ratios of production to respiration (P:R; Hosen and others 2019) and production of autochthonous DOM, particularly in larger rivers when flow is low or water residence times are high (Bianchi and others 2004; Morling and others 2017). Aquatic primary producers have fewer structural compounds than terrestrial vegetation, meaning that freshly produced algal DOM, as a whole, contains smaller, less-aromatic molecules that are more recently fixed than terrestrial DOM (Hood and others 2005; Fasching and others 2016). Aquatic gross primary production (GPP) is generally expected to increase the concentration of autochthonous DOM (Bertilsson and Jones Jr 2002), though in situ reports of this link in freshwaters are rare (Morling and others 2017).

DOM composition often correlates strongly with terrestrial source (Kaplan and Cory 2016; Lynch and others 2019). As such, although allochthonous and autochthonous are frequently taken to be synonymous with DOM composition, this is often not true. In fact, these two groups represent assemblages of highly heterogeneous compounds. Thus, although allochthonous DOM tends to contain more compounds with high apparent molecular weights, many smaller compounds are present as well (Wagner and others 2015). Further, terrestrially fixed, microbial compounds may resemble autochthonous compounds but come from various other sources, including agricultural lands (Wilson and Xenopoulos 2008), urban areas (Hosen and others 2014), and wastewater effluent (Stedmon and Markager 2005). Thus, terrestrial DOM flushed into headwater streams from watersheds can contain compounds with composition and reactivity that is typically associated with autochthonous DOM (Inamdar and others 2011). Further, terrestrial DOM can be highly modified within inland waters by bio- and photo-degradation such that it begins to resemble autochthonous DOM. Photo-oxidation of terrestrial DOM produces compounds of lower molecular weight and higher microbial bioavailability (Helms and others 2008; Tranvik and Bertilsson 2008).

Additionally, secondary production using terrestrial DOM increases concentrations of proteins and other labile forms of DOM (Fasching and others 2014), making allochthonous DOM appear more autochthonous-like as residence time increases (Lynch and others 2019). In such cases, it could be argued that these compounds are autochthonous as they were produced in situ even though the carbon used to make them was fixed external to aquatic systems. Thus, we recognize that there are generally two groups of compounds that co-vary that are typically referred to as allochthonous and autochthonous, but highlight that the origin of compounds with terrestrial or aquatic character is not necessarily an indicator of organic compound source. Ecosystem respiration (ER) may be an effective indicator of secondary production, but production efficiencies complicate this relationship (Marcarelli and others 2011). Given these uncertainties, we use the terms allochthonous-like and autochthonous-like, in recognition of this shortcoming and the need for new terminology that reflects updated knowledge.

Although many individual processes contributing DOM to river networks are known, how these processes interact to control DOM composition and fluxes in river networks remains an active subject of research (Catalán and others 2016; Raymond and others 2016; Moatar and others 2017; Abbott and others 2018; Hale and Godsey 2019; Hosen and others 2019; Koenig and others 2019; Lynch and others 2019; Wagner and others 2019, among others). As DOM travels through river networks, both biological and photochemical processes contribute to a consistent drawdown of DOM that is broadly correlated to travel time within a fluvial network (Catalán and others 2016). Such observations led to the formulation of the Pulse-Shunt Concept, which contends that during hydrologic events, reactive components of allochthonous DOM are transported long distances due to high water velocities, resulting in shorter travel times in river networks (Raymond and others 2016). The Pulse-Shunt Concept predicts that even large rivers should show increases in terrestrial DOM concentration at high discharges as terrestrial pulses elevate DOC concentrations and high flow velocities-and thus short travel times-curtail removal of DOC during transport through the river network. This pulse of terrestrial DOC was recently observed in the main stem of a large temperate river (Shultz and others 2018).

Other studies demonstrate chemostasis, that is, DOC concentrations increase less with discharge in large rivers than small streams, leading to progressively lower variability as watershed size increases (Vannote and others 1980; Godsey and others 2009; Creed and others 2015). Yet, the mechanism behind DOM chemostasis and how this process can be aligned with the Pulse-Shunt Concept is not yet settled. Using the slopes of concentration–discharge (C-Q) relationships, researchers have shown that homogenization of DOM from different upstream sources explains some of this chemostasis (Godsey and others 2009; Moatar and others 2017). Yet, in-channel production of autochthonous DOM may also help buffer DOM concentrations in larger rivers (Creed and others 2015). Where and why chemostasis occurs in rivers remains a topic of debate, with studies of expansive datasets finding evidence for and against DOM chemostasis (Moatar and others 2017).

Here, we use daily in situ measurements of GPP and regular characterization of DOM quantity and composition across 13 watersheds to test the processes that control DOM fluxes in river networks. Specifically, we test whether (1) DOM composition is linked to GPP and ER, (2) scaling of DOM C-Q relationships are correlated to GPP and ER, and (3) DOM chemostasis and the Pulse-Shunt Concept can explain DOM concentration and composition in the main stem of the Connecticut River. Observed links between GPP and ER and/or DOM composition and DOM concentration scaling relationships will indicate a biological role for DOM chemostasis in this river basin. If Pulse-Shunt Concept processes are important, we expect to observe that DOC flux peaks and DOM composition is primarily allochthonous-like in the main stem of the river at high flows.

#### **Methods**

## Study Sites

We studied 13 streams and rivers within the Connecticut River watershed, the largest river basin (29,200 km<sup>2</sup>) in New England, USA (Supplementary Table S1; Supplementary Figure S1). Twelve sites were located within the sixth-order Farmington River watershed in Connecticut (n = 5) or the 6th-order Passumpsic River watershed in Vermont (n = 7). Stream order estimates were obtained from the National Hydrography Dataset Plus High Resolution maps (NHDPlus HR). One site was located on the main stem of the Connecticut River in Thompsonville, CT (USA Geological Survey [USGS] ID #01184000). For further site information, see Hosen and others (2019).

## In Situ Data Collection

Temperature, dissolved oxygen (DO), and specific conductance were measured in situ at 15-min intervals using Eureka Manta 2 water quality son-

des (Eureka Water Probes, Austin, TX) from May 2015 through July 2017. Sensors were calibrated following manufacturer specifications and USGS protocols (Rounds and others 2013). Calibrations were checked at least monthly year-round and twice monthly during summer months. Specific conductance was checked with conductivity standards and DO was checked by measuring air-saturated water. Performance of all sensors was evaluated with side-by-side deployments of a recently calibrated check sonde alongside the deployed field sonde. If readings from the sondes deviated 5% or more during this paired deployment, data were considered unusable and the unit was recalibrated. Once offloaded, sonde data were checked and measurements impacted by bio-fouling or instrument malfunction were removed from the final dataset. On average 13.9% of site data was removed during the quality control step. For additional information regarding sensor data coverage and full metabolism time series, see Hosen and others (2019).

#### Hydrology

At active gage sites, discharge and channel morphology data were obtained from the USGS National Water Information System (NWIS; USGS 2016). At Nepaug River and Phelps Brook, discharge was measured using a combination of salt injections and velocity-area methods, and measurements were used to update existing USGS rating curves. Relationships between discharge and mean channel depth at each site were developed using channel data obtained from NWIS. At Hubbard River, Nepaug River, Pope Brook, and Sleepers River sites, USGS channel transects were not representative of the upstream channel morphology. To address this issue, additional transects were collected at these sites to develop more accurate discharge-channel depth relationships.

#### Ecosystem Metabolism Models

We estimated GPP and ER using Bayesian ecosystem metabolism models (Odum 1956; Appling and others 2018) generated from single-station DO measurements using the streamMetabolizer 0.10.1 package (Appling and others 2017) in R 3.4.3 (Hosen and others 2019). Gas exchange between water and air varies over time and space in flowing waters (Raymond and others 2012) and, thus, is an important parameter in fluvial ecosystem metabolism models (Appling and others 2018). To address this issue, we partially pooled daily estimates of gas exchange rates normalized to a Schmidt number of 600 (K600,  $d^{-1}$ ) in a hierarchical model. We estimated GPP, ER, and K600 using the following equation:

$$\Delta \mathrm{mO}_{i,d} = \left(\frac{\mathrm{GPP}_d}{z_{i,d}} \times \frac{\mathrm{PPFD}_{i,d}}{\overline{\mathrm{PPFD}}_d}\right) \\ + \left[\left(\frac{\mathrm{ER}_d}{z_{i,d}}\right) + f_{i,d}(K600_d) \left(\mathrm{Osat}_{i,d} - \mathrm{mO}_{i,d}\right)\right] \\ \times \Delta t$$
(1)

mO<sub>i.d</sub> represents modeled DO concentration at timestep *i* on day *d*; Osat<sub>*i d*</sub> represents DO saturation at timestep i on day d; GPP<sub>d</sub> and ER<sub>d</sub> are average GPP and ER on day *d* (in units g-O<sub>2</sub> m<sup>-2</sup> day<sup>-1</sup>);  $z_{i,d}$ is the average cross-section depth of each upstream reach at a given timestep;  $K600_d$  is the gas exchanged rate normalized to a Schmidt number of 600 on day *d*;  $PPFD_{i,d}$  is the photosynthetic photon flux density at a given timestep;  $\overline{PPFD}_d$  is the sum of solar insolation on a given day; and  $\Delta t$  is the length of timestep (here, 15 min). To estimate  $z_{id}$ , relationships between discharge and mean channel depth at each site were developed using channel data obtained from NWIS as described above. To estimate  $\overline{PPFD}_d$ , incident photosynthetically active radiation (PAR;  $\mu$ mole photons m<sup>-2</sup> s<sup>-1</sup>) was modeled following Yard and others (2005). Any gaps in the dataset were filled with linear interpolation. If there were more than 3 h of missing data for any variable in a 24-h period that day was excluded from the model input. The resulting streamMetabolizer parameter name "b\_Kb\_oipi\_tr\_plrckm.stan" was used to run these models. Each Bayesian metabolism model was run on four chains with 1000 burn-in steps and 2000 saved steps. The output of the model is a set of daily estimates of GPP, ER, and the associated standard deviation of each value.

Due to limited data from periods impacted by ice, estimates of GPP from winter months are underrepresented at many sites. Thus, to estimate mean GPP at each site, we took the mean GPP for each month. For months where there were less than five site-days of data available, we used linear interpolation to gap-fill (Lepot and others 2017). To estimate mean annual GPP, we applied mean monthly GPP values to a linear model with Newey–West covariance matrices to account for temporal autocorrelation of the data (Newey and West 1987, 1994; Shultz and others 2018; Hosen and others 2019).

## DOM Chemistry and Optical Spectroscopy

We collected water samples at least every 2 weeks at each site when sondes were deployed and ice did not limit site access. We filtered samples in the field to 0.2 µm using polyethersulfone (PES) filters (Waterra USA Inc.; Peshastin, Washington, USA). Samples were returned to the laboratory on ice and stored at 4°C until analyzed for DOC concentration and fluorescence and ultraviolet-visible (UV-Vis) spectroscopy. We analyzed water samples for DOC and total dissolved nitrogen (TDN) concentrations (TOC-vCPH with TNM-1; Shimadzu Corporation; Kyoto, Japan). Samples were acidified to 2% of 2 M HCl and sparged for 5 min. UV–VIS absorption spectra and fluorescence excitation-emission matrices (EEMs) were collected using a Horiba Aqualog fluorometer (Horiba Scientific; Edison, NJ). Refrigerated samples were warmed to room temperature in the dark and spectroscopic analyses were performed on an Aqualog spectrometer (Horiba, Inc., Edison, NJ). Samples were measured for UV-Vis absorbance from 200 to 800 nm at 1 nm increments. EEM analysis was conducted at excitation wavelengths ranging from 240 to 800 nm at 3 nm increments. Fluorescence emissions were collected from approximately 247.808 to 828.528 nm at 2.06 nm increments (using integration of 4 CCD pixels). CCD gain was set to medium. Raman scattering area was determined by exciting Type I water obtained from a Milli-Q Advantage A-10 system (EMD Millipore, Billerica, MA) and integrating fluorescence from 247.808 to 828.528 nm for 10 s (Stedmon and others 2003). For all other analysis, integration time was set to 1 s. During analysis, an initial four Milli-Q water blanks were collected with an additional water blank collected every fifteen samples.

## Spectroscopy Statistics and PARAFAC Analysis

We applied UV–VIS absorbance and fluorescence spectroscopy to determine DOM composition. Optical metrics of DOM composition are broadly linked to composition from mass spectrometry (Lavonen and others 2013; Stubbins and others 2014; Wagner and others 2015; Gonsior and others 2016; Martínez-Pérez and others 2017) and to the overall microbial bioavailability (Fellman and others 2008; Balcarczyk and others 2009; Hood and others 2009; Petrone and others 2011; Hosen and others 2014, 2018; Shin and others 2016) of DOM. Thus, we argue that this method offers robust

measures of DOM composition. Analysis of EEMs with parallel factor analysis (PARAFAC) is particularly useful because it decomposes optical DOM fingerprints in a robust and repeatable manner that allows analysis of different fractions of aquatic organic matter (Stedmon and others 2003; Cory and others 2010; Poulin and others 2014; Brezonik and others 2019). We calculated spectral slope ratio  $(S_{\rm R})$ , which is determined from UV–Vis spectra of water samples as the ratio of the log-transformed absorbance slopes at 275-295 nm and 350-400 nm (Helms and others 2008) and is negatively correlated with DOM apparent molecular weight (Wünsch and others 2018). Specific UV absorbance at 254 nm (SUVA<sub>254</sub>) was calculated by taking UV absorbance at 254 nm in absorbance units per centimeter divided by the DOC concentration in milligrams per liter and multiplied by 100. SUVA<sub>254</sub> is reported in units of liter per milligram carbon per meter and correlates with the levels of aromatic or conjugated bonds in DOM (Weishaar and others 2003). Freshness index was determined as fluorescence emission at 380 nm divided by the maximum fluorescence intensity between 420 and 435 nm when exciting a water sample with light at 310 nm (Parlanti and others 2000; Wilson and Xenopoulos 2009).

We conducted PARAFAC analysis following standard methods (Cory and others 2010) using drEEM 0.2.0 (Murphy and others 2013) in MA-TLAB v. R2016b (MathWorks, Inc., Natick MA). The PARAFAC model was run with a total of 1048 EEMs, representing samples collected across the Connecticut River watershed, which produced a validated PARAFAC model that included six components. First, sample and blank EEMs were corrected using manufacturer-defined emission and excitation correction files. Samples were then inner-filter corrected using UV-Vis absorbance data (McKnight and others 2001) and blank subtracted. To normalize for changes in bulb intensity over time, EEMs were converted to Raman units (RU, nm<sup>-1</sup>) by dividing each corrected EEM by the Raman scattering area determined on a given date (Stedmon and others 2003).

PARAFAC models containing 3–8 components were evaluated and a six-component model was selected after the removal of 22 samples with high model leverage. The six-component model was tested for local minima by conducting 10 random initialization model runs and the dataset was split into quarters and three validation tests were performed on different combinations of these splits following the  $S_4C_6T_3$  scheme described in Murphy and others (2013). The three allochthonous-like PARAFAC components were strongly correlated (Supplementary Table S2), but this model was accepted because the spectrum of each fluorophore was discrete (Cory and others 2010). To obtain component scores for all samples, the EEMs removed during model development were projected using the final PARAFAC model.

Each PARAFAC component (C1–C6) represents a different optical signature of DOM found in our water samples (Table 1; Supplementary Figure S2). Components with analogs in the literature were identified via OpenFluor (www.openfluor.org) using a similarity score of 0.98. Protein-like fluorescence was determined by summing fluorescence intensity RU for both tryptophan (C5) and tyrosine (C6) PARAFAC components (Table 1; Supplementary Figure S2). Protein-like fluorescence has been shown to be a strong indicator of biolabile, auto-chthonous DOM (Stedmon and others 2003; Cory and McKnight 2005; Fellman and others 2009; Murphy and others 2011; Osburn and others 2012).

## End Member Mixing Analysis

We applied end member mixing analysis (EMMA; Hooper and others 1990; Inamdar and others 2013) coupled with eigenspace analysis (Dietze and others 2012; Dietze and Dietze 2019) to PARAFAC scores in the Connecticut River (n = 56). We selected this method because it uses an automated approach with objective fit statistics, which are considered critical elements for the development of a robust EMMA model (Barthold and others 2011). Using this approach, we fit EMMA models starting with one end member and ranging up to five end members. Following established methods (Dietze and others 2012), we used plots of the number of variables versus explained cumulative variance and mean total  $R^2$  to select the optimal number of end members (Supplementary Figure S3). To confirm that results are robust, bootstrapped replicates were run using different weight transformations following Klovan and Imbrie (1971). This bootstrapping method was also used to obtain estimates of standard deviation for each end member and end member scores. Two end members-one linked to autochthonous DOM and the other resembling al-

Component	Excitation peaks (nm)	Emission peaks (nm)	Description
C1	< 240, (323)	440	Humic, terrestrial, aged, linked to lignin phenols (Allochthonous) Component C1 (Garcia and others 2015); Component C1 (Walker and others 2009); Component C1(Walker and others 2013); Component C (Yamashita and others 2011); Peak C (Coble 1996)
C2	< 240, (335)	444	<ul> <li>Humic, newer/more labile C, photolabile, greater production in warm months (Allochthonous/Autochthonous)</li> <li>Component C1 (Stedmon and Markager 2005); Component C3 (Williams and others 2013); Component C1 (Stedmon and others 2007); Peak A (Coble 1996)</li> </ul>
C3	< 240, (425)	516	<ul><li>Humic, aged terrestrial C; recalcitrant; linked to wetlands, forested water-sheds (Allochthonous)</li><li>Component C2 (Stedmon and Markager 2005); Component C2 (Hosen and others 2014)</li></ul>
C4	(269), 380	465	Humic, terrestrial, linked to lignin phenols (Allochthonous) Component C2 (Walker and others 2013); Component C2 (Yamashita and others 2013); Component C1 (Ohno and Bro 2006); Component C1 (Fellman and others 2008)
C5	< 240, (290)	375	Tryptophan-like fluorescence (Autochthonous) Component C5 (Graeber and others 2012); Component C6 (Murphy and others 2011); Component C7 (Stedmon and Markager 2005); Compo- nent C5 (Walker and others 2013)
C6	(< 240), 272	(< 250), 309	Tyrosine-like fluorescence (Autochthonous) Component C8 (Stedmon and Markager 2005); Component C7 (Murphy and others 2011)

Table 1. Characteristics of the Fluorescent Components Identified by the PARAFAC Model in this Study

Wavelength values in parentheses indicate local maxima. Wavelength loadings and contour plots of the six components are presented in Supplementary Figure S2.

lochthonous DOM—were identified (Supplementary Figure S4). We calculated the percent of each end member for each water sample collected. We combined these values with estimates of DOC concentration to determine the amount of autochthonous DOM and allochthonous DOM in each sample.

## Monte Carlo Simulation

To estimate the flux of DOC past the USGS gage on the Connecticut River at Thompsonville, CT, we conducted a Monte Carlo simulation (Tiwari and Hobbie 1976; Koch and others 2015). We developed linear models between autochthonous and allochthonous DOC concentrations developed using EMMA, river discharge, and water temperature. Water temperature was converted to Boltzmann-normalized temperatures centered around 15°C (288.15 K) and discharge was log-transformed.

We used discharge measurements, simulated temperature data, and the end member linear models to estimate annual loads of allochthonous and autochthonous DOC. We applied discharge data obtained from the USGS gage at Thompsonville, CT (USGS #01184000) over a 25-year span (1993–2017). Temperature data from the sonde deployed in the Connecticut River at Thompsonville, CT was used to develop temperature distributions for each season. We evaluated the fit of normal, gamma, uniform, beta, and skewed normal distributions for each season of temperature data and the distribution that generated the lowest root mean square error (RMSE) when comparing empirical and theoretical cumulative distributions was selected. For autumn, a uniform distribution ranging from 1.85 to 23.7°C best reproduced available temperature data. Winter temperature data were simulated using a gamma distribution with shape 0.86 and rate 0.53. For summer, a skewed normal distribution with mean 24.3°C, standard deviation 3.2, and skewness coefficient  $4.6 \times 10^{-6}$  was applied (Wuertz and others 2016). A uniform distribution was applied for spring ranging from 5.25 to 23°C. The temperature distributions combined with discharge data were applied to the EMMA linear models to obtain a daily estimate of autochthonous and allochthonous DOC concentration. These concentrations were then multiplied by daily discharge to obtain daily flux estimates (kg-DOC day $^{-1}$ ). This process was repeated 1000 times by drawing random temperature values from the distribution described above that are paired with the 25-year discharge

record. To check the validity of our model, we also estimated annual DOC fluxes for the Connecticut River at Thompsonville, CT using LOADEST (Runkel and others 2004) with the LoadRunner application (Booth and others 2007). LOADEST estimates were generated from the same DOC concentration and discharge data used to produce the Monte Carlo estimates.

#### Time Series Statistical Analysis

DO records over as much as 28 days have been shown to predict algal productivity in freshwater systems (Pace and others 2017). In rivers, DOM composition is a product of processing both within a given river reach and processing that has occurred upstream. In this study, we applied the mean of GPP values at a site over the previous 4 days and previous 7 days, in addition to daily GPP values. We used linear models to compare GPP to DOM composition as measured by protein-like fluorescence in RU, Freshness Index, and spectral slope ratio  $(S_R)$  values. At each site, we compared each DOM composition metric to daily GPP, GPP averaged over the previous 4 days, and GPP averaged over the previous 7 days. For each combination of site and DOM composition, we used  $R^2$ estimates to determine the best model-mean daily GPP with no lag or lagged over the previous 4 or 7 days—among those with a significant p value (alpha level of 0.05 was selected a priori). The same analyses were conducted substituting ER for GPP. We selected means of 1, 4, and 7 days for multiple reasons. First, we wanted to limit the number of days in an effort to be conservative and avoid an excessive number of statistical comparisons. Tracer studies of labile and semi-labile DOM indicated uptake times of 1.5 h and 29 h respectively, justifying the single day window (Kaplan and others 2008). Work in UK stream networks indicates that 65% of DOM is removed at 70 h. Network-scale estimates of ambient DOM  $V_{\rm f}$  of 0.26 mm min<sup>-1</sup> (Mineau and others 2016) and 0.03 mm min<sup>-1</sup> (Wollheim and others 2015) have also been reported. Assuming a mean depth of 0.5 meters, this results in turnover times of about 1.5 and about 11.5 days, respectively. Based on these estimates, we selected 7 days since that should represent approximate residence time of most DOM found at a site.

## Linear Modeling

We used linear models to test the relationships between DOM composition and GPP and between DOM composition and discharge and Boltzmann-



GPP g-O<sub>2</sub> m<sup>-2</sup> d<sup>-1</sup>

**◄ Figure 1.** GPP plotted against protein-like fluorescence (Raman Unit; RU; A-C), freshness index (D-F), and spectral slope ratio  $(S_R)$  (**G–I**). Relationships are presented for the Connecticut River at Thompsonville, CT, the Farmington River at Tariffville, CT, and the Passumpsic River at Passumpsic, VT. Measures of GPP were averaged over the previous 7 days with the exception of GPP versus  $S_R$  (**F**) and freshness index (**I**) in the Passumpsic River where daily GPP estimates provided the best fit. We also present  $S_{\rm R}$  to GPP relationships for streams and rivers with smaller watersheds in order of descending size: J Farmington River at Unionville, K Still River, L Moose River, M E. Branch Passumpsic River, N Sleepers River; O Nepaug River, P Bunnell Brook, Q Pope Brook, R Phelps Brook, **S** Pope Brook Tributary W9. Lag indicates length in days of the lagged rolling mean window. Daily values have a lagged mean window of one (lag = 1), the mean of a daily value and the previous 3 days is a lagged mean window of 4 days (lag = 4), and the mean of a daily value and values from the previous 6 days is a lag window of 7 days (lag = 7). Regression statistics for these variables at all sites are reported in Supplementary Table S3.

normalized temperature centered around 15°C, as described above. In cases where there were multiple independent variables, models were initially fit with interaction terms. In all cases, interaction terms were nonsignificant and therefore removed. Protein-like fluorescence, GPP, watershed area, and discharge variables were log-transformed to meet model assumptions of normality and homoscedasticity. An alpha level of 0.05 was selected a priori. All statistical analyses were conducted in R 3.4.3 (R Core Team 2018).

## RESULTS

## DOM Composition is Significantly Linked to GPP in Larger Rivers

GPP and DOM composition were more consistently and strongly correlated in the three large rivers that had watershed areas greater than 1000 km<sup>2</sup> than in the ten smaller upstream sites (Figure 1; Supplementary Table S3). GPP is significantly greater in these larger rivers primarily because relative canopy cover decreases with log-transformed watershed size (Hosen and others 2019). Thus, only in larger rivers does GPP become high enough to significantly impact DOM composition. We report on the relationship between GPP and DOM composition at all sites (Supplementary Table S3), but focus here on the three large sites (the Connecticut, Farmington, and Passumpsic Rivers) where auto-

chthonous production and thus the linkage to DOM composition was greatest (Figure 1). Primary production rates were much greater in these downstream sites compared to low productivity upstream sites (Hosen and others 2019) and thus we found the greatest evidence of processing in rivers of order 5 and larger, in keeping with the predictions of the River Continuum (Vannote and others 1980) and Pulse-Shunt Concepts (Raymond and others 2016). Log-transformed protein-like fluorescence was significantly and positively correlated to GPP in the Connecticut River at Thompsonville, СТ (Figure 1A; p < 0.0001; $r^2 = 0.377$ ), the Farmington River at Tariffville, CT (Figure 1B; p = 0.0001;  $r^2 = 0.193$ ), and the Passumpsic River in Passumpsic, VT (Figure 1C; p = 0.0008;  $r^2 = 0.313$ ). Likewise, Freshness Index was positively correlated to GPP in the Connecticut (Figure 1D; p < 0.0001;  $R^2 = 0.562$ ), Farmington (Figure 1E; p < 0.0001;  $R^2 = 0.291$ ), and Passumpsic Rivers (Figure 1F; p = 0.0014;  $R^2 = 0.276$ ).  $S_{\rm R}$  was also positively related to GPP in the Connecticut (Figure 1G; p < 0.0001;  $R^2 = 0.749$ ), Farmington (Figure 1H; p < 0.0001;  $R^2 = 0.308$ ), and Passumpsic Rivers (Figure 1I; p = 0.0019;  $R^2 = 0.406$ ). In smaller water bodies, GPP was much lower and had an increasingly negligible impact on DOM composition with decreasing area (Figure 1J–S; Supplementary watershed Table S3).

The relationship between GPP and DOM composition was best modeled using lagged mean estimates of GPP. We tested DOM composition against daily GPP values (lag window = 1) and rolling mean GPP averaged over a lagged window of 4 days (daily value plus previous 3 days) and 7 days, using  $R^2$  values to select the best significant model among the three. At the three largest sites, the correlation between GPP and DOM composition was strongest when using a rolling mean window for GPP of 7 days for almost all comparisons (Figure 1; Supplementary Table S3). The only exceptions were found in the Passumpsic River for Freshness Index (Figure 1F) and  $S_r$  (Figure 1I). In those cases, daily GPP values provided the best fit. In smaller streams and rivers, DOM composition largely was not correlated to GPP (Figure 1J-S; Supplementary Table S3). When we did find a significant relationship between DOM and GPP in smaller streams, it was using GPP values integrated over shorter windows. For these smaller reaches, we found that daily GPP or values averaged over 4 days best predicted DOM composition (Figure 1; Supplementary Table S3).



Discharge (m<sup>3</sup> s<sup>-1</sup>)

**◄ Figure 2.** Plots relating DOC concentration, protein-like fluorescence, and humic-like DOM fluorescence at all thirteen study sites, arranged from smallest watershed area to largest watershed area. Plots are presented for A site W9, B Phelps Brook, C Pope Brook, D Bunnell Brook, E Nepaug River, F Sleeper's River, G E. Branch Passumpsic River, H Moose River, I Still River, J Farmington River at Unionville, CT, K Passumpsic River, L Farmington River at Tariffville, CT, M Connecticut River at Thompsonville, CT. Protein-like fluorescence is calculated as the sum of PARAFAC components C5 and C6. PARAFAC component C3 was identified as being characteristic of humic-like DOM (Table 1). Temperature was included as a covariate. To visualize temperature dependence, regression lines are plotted for different temperatures: purple (0°C), blue  $(5^{\circ}C)$ , green  $(10^{\circ}C)$ , orange  $(15^{\circ}C)$ , and red  $(20^{\circ}C)$ .

We found relationships between ER and DOM composition as well, but at fewer sites and with generally weaker correlations than for the relationships between GPP and DOM composition (Supplementary Table S4). Significant relationships were found between ER and any of the DOM composition metrics at only 7 of 13 sites. Further, the strongest correlation found, which was between 7-day ER and log-transformed S<sub>R</sub> in the Connecticut River at Thompsonville, CT ( $R^2 = 0.58$ ; Supplementary Table S4) was not as strong as the equivalent relationship between GPP and log-transformed S<sub>R</sub> ( $R^2 = 0.75$ ; Supplementary Table S3).

## DOM Correlated to Discharge

Across the Connecticut River watershed, we found strong correlations between discharge and both DOC concentration and DOM composition. Assuming Arrhenius temperature dependence of DOM variables, we incorporated Boltzmann-standardized temperature as an explanatory covariate. When accounting for temperature, log-transformed DOC concentrations were significantly linked to log-transformed discharge at every site except for the Connecticut River main stem (Figure 2). Logtransformed discharge and Boltzmann-standardized temperature were significantly correlated to log-transformed protein-like fluorescence at all sites (Figure 2). Freshness Index was significantly correlated to discharge and temperature at 11 of 13 sites and  $S_{\rm R}$  at 10 of 13 sites (Supplementary Table S5). Discharge and temperature were significantly and positively correlated to PARAFAC Component C3 values at all sites studied (Supplementary Table S5). PARAFAC component C3 presents a fluorescence signature consistent with allochthonous compounds that are humiclike—terrigenous and enriched in aromatic compounds (Table 1). By contrast, SUVA<sub>254</sub>, another measure of aromatic-rich DOM, was significantly linked to discharge and temperature at only 7 of 13 sites (Supplementary Table S5).

The nature of the relationship between DOM composition and concentration and discharge was dependent on network position (that is, stream size). As log-transformed watershed area increased, the slope of the relationship between DOM variables and discharge changed in a predictable manner. DOC concentrations became less variable in larger rivers, consistent with a trend toward chemostasis (Figure 3; Supplementary Table S5). The slope of the relationship between discharge and DOC concentration significantly decreased with increasing watershed area until the main stem of the Connecticut River, where the slope of DOC concentration to discharge did not differ significantly from zero ( $F_{(1,11)} = 7.42$ ; p = 0.021;  $R^2 = 0.397$ ; Figure 3A). Variability of DOC was also significantly and negatively correlated to logtransformed watershed area, as indicated by a decreasing coefficient of variation (C.V.)  $(F_{(1,11)} = 10.2; p = 0.009; R^2 = 0.481;$  Figure 3D).

The slope of the relationship between DOM composition metrics and discharge decreased with increasing watershed area for variables associated autochthonous production-Protein-Like with Fluorescence, Freshness Index, and S<sub>R</sub>—but not for humic-like PARAFAC Component C3 (Figure 3M-O). In the case of protein-like fluorescence, the slope of the relationship was positive in headwater streams, but became gradually more negative in the largest rivers studied ( $F_{(1,11)} = 5.33$ ; p = 0.41;  $R^2 = 0.326$ ; Figure 3G). For Freshness Index values, a similar pattern was found  $(F_{(1,11)} = 10.7;$ p = 0.007;  $R^2 = 0.494$ ; Figure 3J). Values for slopes of S<sub>R</sub> versus discharge were not significantly related to log-transformed watershed area (Supplementary Table S6).

We found evidence linking GPP to the changing relationships between DOC concentration and composition and discharge that we observed across sites. Mean annual GPP was strongly and positively related to watershed area ( $F_{(1,11)} = 37.3$ ; p < 0.0001;  $R^2 = 0.772$ ). Interestingly, mean annual GPP ( $F_{(1,11)} = 9.84$ ; p = 0.0095;  $R^2 = 0.472$ ; Figure 3B) and mean annual ER ( $F_{(1,11)} = 12.4$ ; p = 0.0048;  $R^2 = 0.531$ ; Figure 3C) at a site were better predictors of the slope of DOC-Q relationships than watershed area (Figure 3A). DOC coefficient of variation (C.V.) also decreased



Watershed Area (km<sup>2</sup>) Mean GPP  $g-O_2 m^{-2} day^{-1}$  Mean ER  $g-O_2 m^{-2} day^{-1}$ 

**Figure 3.** Relationship between watershed area and the slope of the relationship between DOM metrics and discharge (*Q*) at each site. Variables included are **A** DOC concentration versus *Q*, **C** DOC C.V., **E** slope of protein-like fluorescence versus *Q*, **G** slope of Freshness Index versus *Q*, and **I** slope of PARAFAC Component C3 versus *Q*. These same variables are also plotted against mean annual GPP (**B**, **E**, **H**, **K**, **N**) and ER (**C**, **F**, **I**, **L**, **O**) at each site. Watershed area, mean annual GPP, and mean annual ER were log-transformed. Error bars represent standard error of the mean as determined from linear models. Full statistics are presented in Supplementary Table S6.

significantly with increasing mean annual GPP ( $F_{(1,11)} = 7.61$ ; p = 0.019;  $R^2 = 0.409$ ; Figure 3E), but not with mean annual ER. Both ER

 $(F_{(1,11)} = 14.3; p = 0.0031; R^2 = 0.565;$  Figure 3I) and GPP  $(F_{(1,11)} = 6.08; p = 0.0313; R^2 = 0.356;$ Figure 3h) were better correlated to protein-like



**Figure 4.** DOC yield versus catchment area for each site. Each point represents mean DOC yield at a site for a given flow level—high (66th percentile flows and higher), baseflow (33rd through 66th percentile flows), and low (33rd percentile flows and lower). Error bars represent standard error of the mean.

fluorescence *C*–*Q* slopes than watershed area ( $F_{(1,11)} = 5.33$ ; p = 0.0414;  $R^2 = 0.326$ ; Figure 3G). *C*–*Q* slopes for  $S_R$  were only correlated to log-transformed GPP ( $F_{(1,11)} = 6.54$ ; p = 0.0373;  $R^2 = 0.338$ ). In contrast to other composition metrics, Freshness Index *C*–*Q* slopes were best correlated to log-transformed watershed area ( $F_{(1,11)} = 7.61$ ; p = 0.019;  $R^2 = 0.409$ ; Figure 3J). Full summary statistics are available in Supplementary Table S6.

DOC yield increased with watershed area under low-flow conditions. DOC yields are estimates of flux normalized by watershed area and are presented here in units of kg-C  $d^{-1}$  ha<sup>-1</sup>. Using a linear mixed-effects model to test the relationship between DOC yield and catchment area, we found a significant interaction term (p < 0.0001) with discharge level (low: 0-33rd percentile discharge; baseflow: 33rd-66th percentile discharge; high flow: 66th–100th percentile discharge; Figure 4). At high flows, DOC yields were constant from headwaters to the main stem (8th order, 25,019 km<sup>2</sup>) of the Connecticut River, indicating conservative transport of DOC. At moderate (p < 0.0001) and low flows (p = 0.010), DOC yields and concentrations increased significantly with watershed area, indicating an increase in low-flow DOC flux with river size (Figure 4).

## DOM Mixing Model for the Connecticut River

We applied EMMA coupled with eigenspace analvsis (Dietze and others 2012) to PARAFAC scores to test whether DOM composition changes from low flow to high flow in the main stem of the Connecticut River at Thompsonville, CT. We found evidence that the proportion of allochthonous and autochthonous DOM levels change over time in the Connecticut River main stem. A two-endmember mixing model best fit the data (mean total  $R^2 = 0.79$ ; Supplementary Figure S3). End member 1 was high in PARAFAC components C1, C3, and C4, which have previously been identified as aromatic-rich, allochthonous DOM (Table 1; Supplementary Figure S4). End member 2 was high in PARAFAC component C2—a humic-like substance that is the product of primary or secondary production that has been recently released to the environment (Stedmon and Markager 2005) linked in this study to autochthonous production-and the two protein-like components C5 and C6. To test whether groundwater was a strong contributor to either end member, we analyzed nine groundwater



**Figure 5.** Concentration of **A** total DOC, and the fractions of DOC identified as **B** allochthonous, **C** autochthonous versus discharge in the main stem of the Connecticut River. Flux of **D** total DOC, **E** allochthonous DOC, and **F** autochthonous DOC versus discharge in the Connecticut River. Lines represent significant linear regressions and shaded areas represent model standard error. Plots of regression equations comparing **G** the concentration of allochthonous and autochthonous DOC fractions to discharge and **H** flux of allochthonous and autochthonous DOC fractions from panels **B** and **C** were used. For panel **H**, regression equations from panels **E** and **F** were applied. **I** Estimates of DOC fluxes (both Autochthonous and Allochthonous) in the main stem of the Connecticut River from a Monte Carlo simulation were compared to total DOC flux estimates obtained from LOADEST.

samples from USGS wells across the Connecticut River watershed (Supplementary Table S7) with depth that ranged from 5.67 to 35.4 meters (mean: 12.2 meters). Groundwater DOC concentrations (mean: 1.04; se: 0.44 mg-C l<sup>-1</sup>) were significantly lower than all surface water sites (p < 0.0001), including the main stem of the Connecticut River (mean: 3.44; se: 0.21 mg-C l<sup>-1</sup>). Groundwater DOM EEM-PARAFAC scores were applied to the Connecticut River mixing model. Mean composition was 35.2% end member 1 (allochthonous) and 64.8% end member 2 (autochthonous). Thus, while groundwater had slightly more autochthonous-like DOM, concentrations of groundwater DOC indicate this material is a small proportion of overall surface fluxes.

The mixing model demonstrates that the end member rich in autochthonous DOM dominated during low flows and the end member rich in allochthonous DOM dominated during high flows in the main stem of the Connecticut River (Figure 5A–F). At the Connecticut River site, total DOC concentrations did not vary with discharge (Figure 5a), but the allochthonous fraction of DOC was positively correlated to discharge (Figure 5B;  $F_{(1,30)} = 25.6; p < 0.0001; R^2 = 0.46$ ) and the autochthonous fraction was negatively correlated to discharge (Figure 5C;  $F_{(1,30)} = 30.9$ ; p < 0.0001;  $R^2 = 0.51$ ). Total DOC fluxes were strongly correlated to discharge (Figure 5D;  $F_{(1,30)} = 262$ ; p < 0.0001;  $R^2 = 0.90$ ), as were allochthonous DOC fluxes (Figure 5E;  $F_{(1,30)} = 208$ ; p < 0.0001;  $R^2 = 0.87$ ). As flow increased, greater water flux overcame decreasing autochthonous DOC concentrations. Thus, autochthonous DOC fluxes increased slightly with discharge (Figure 5F;  $F_{(1,30)} = 7.42; p = 0.011; R^2 = 0.198)$ , but to a much lesser extent than allochthonous DOC.

According to the mixing model results, during low flow in the main stem of the Connecticut River-when discharge reached its lowest levels—DOC concentration was about 3 mg-C l<sup>-1</sup> and was comprised of material that is about 70% autochthonous (~ 2 mg-C  $l^{-1}$ ) and 30% allochthonous (~ 1 mg-C  $l^{-1}$ ; Figure 5G, H). At the highest discharge levels for the Connecticut River, DOC concentrations remained largely unchanged (mean: 3.44; SE: 0.21), but the percent of DOM that was allochthonous increases to about 90% (Figure 5G, H). Using the relationships between discharge and concentration of the two fractions of DOM (Figure 5B, C), we constructed a Monte Carlo model to estimate annual fluxes of DOC and the fraction of this material that is allochthonous versus autochthonous (Figure 5I). The higher proportion of terrestrial DOM during high flows suggests that terrestrial DOM dominates riverine export (Figure 5). We found that total fluxes in the Connecticut River from 2015 to 2017 averaged around 134,000  $\pm$  15,000 kg-C day<sup>-1</sup> with about 30% of the material being autochthonous in nature and about 70% allochthonous.

#### DISCUSSION

We found multiple lines of evidence that autochthonous primary production significantly alters the composition of the freshwater DOM pool, within large rivers when discharge is low (Figs. 1, 4). In situ measurements of GPP provided the most direct evidence that autochthonous production influenced DOM composition in the three large rivers. Three indicators of recently produced, autochthonous organic matter were significantly linked to GPP when accounting for hydrology. Protein-like fluorescence (sum of PARAFAC components C5 and C6), S<sub>R</sub>, and the Freshness Index were significantly and positively correlated to GPP at the three largest river sites (Figure 1). Proteinlike DOM has been connected to primary production in lakes (Yao and others 2011). S<sub>R</sub> increases with decreasing apparent molecular weight of DOM (Helms and others 2008; Wünsch and others 2018), which is often a result of autochthonous DOM production (Zhang and others 2013). The Freshness Index is linked to DOM that is the product of primary or secondary production and has been recently released to the environment (Parlanti and others 2000; Wilson and Xenopoulos 2009) and does not increase with photo-degradation (Hansen and others 2016). Hence, these measures of DOM composition all support the assertion that, if GPP is high enough, autochthonous photosynthesis can shift the overall DOM pool to newer, smaller, more protein-rich, and likely more microbially bioavailable compounds. Although it is likely that some of this shift is due to photodegradation of DOM, secondary production (Lynch and others 2019) or contributions from aged groundwater, mechanisms related to secondary production, photo-degradation, hydrologic mixing, and groundwater do not explain the increasing DOC yields as watershed area increases that we find at lower flows (Figure 4). These mechanisms either involve mineralization of DOC and should decrease yields (photo-degradation, secondary production [Hansen and others 2016]) or involve hydrologic mechanisms which should not change DOC yields [groundwater, hydrologic mixing (Moatar and others 2017; Abbott and others 2018; Hale and Godsey 2019)]. Thus, our findings indicate that DOM composition shifts, as measured by S<sub>R</sub>, Freshness Index, and protein-like fluorescence, are controlled, at least in part, by autochthonous production in flowing waters.

In these temperate rivers, peak GPP is limited to low-flow summer periods, which are associated with long water travel time, lower turbidity, higher temperatures, and no canopy cover in larger rivers with favorable light conditions (Hosen and others 2019). These drivers of GPP also impact DOM concentration and quality. As low-flow GPP increased with increasing watershed area, so did the concentration of autochthonous DOM. This caused the relationship between discharge and protein-like fluorescence, S<sub>R</sub>, and Freshness Index to become increasingly negative as GPP and ER increased (Figure 3). In small streams and rivers, protein-like fluorescence was positively correlated to discharge. In these low-order streams with high canopy cover and low levels of photosynthesis, recently fixed allochthonous DOM is likely flushed into streams during storm events (Inamdar and others 2011; Hosen and others 2018; Lynch and others 2019), leading to the positive relationship between protein-like fluorescence and discharge. As travel time increases downstream from headwaters to larger rivers, the most bioavailable DOM is preferentially metabolized (Lynch and others 2019), but during low flows, increased fresh autochthonous DOM appears to be produced by algal primary production (Figure 2). This causes the relationship between protein-like fluorescence and discharge to become negative in large rivers where GPP is highest (Figure 3). This is significant because Freshness Index and  $S_{R}$  are compositional indices, but increases to the intensity in RU of protein-like fluorescence during low flows indicate increasing protein-like quantity (Baghoth and others 2011). This shift in DOM composition means that low-flow groundwater contributions and photo-degradation of DOM, both of which should reduce concentrations, cannot explain our observations. DOC yields and concentrations were positively correlated to watershed area during median and low-flow conditions (Figure 4) and levels of protein-like (Figure 2M) and autochthonous DOM (Figure 5) increased during low flows in the main stem of the Connecticut River. A sampling of nine USGS wells from across the Connecticut River basin (mean depth: 12.2 m; Supplementary Table S7), had a mean groundwater DOC concentration of  $1.04 \pm 0.44$  mg-C l<sup>-1</sup>. Thus, groundwater inputs appear unlikely to support low-flow DOC concentrations ( $\sim$  3 mg-C l<sup>-1</sup>) in the Connecticut River.

Interestingly, DOC concentration and proteinlike fluorescence C-Q relationships were best explained by log-transformed mean annual ER at each site (Figure 3C, I). These relationships may suggest that ER is an indicator of DOM transformation, though support for this link is weak (Marcarelli and others 2011). We argue that it is more likely that increasing ER is an indicator of heterotrophic response to recently produced algal DOM. Heterotrophic ER has been shown to be strongly correlated to autochthonous production (Huryn and others 2014; Wagner and others 2017). Further, as discussed above, it is impossible for processes like ER that result in mineralization of DOC to support increasing concentrations or yields of DOC. Instead, addition of aquatic photosynthetic products appears to drive changes to large-river DOM composition at low flows (Figure 1).

The impact of residence time on internal production of DOM is evident because of the time lag in the GPP-DOM quality correlations. DOM composition in large rivers appears to reflect processes occurring in a water mass over about 1 week of travel time to the downstream river study site (Figure 1). In fact, the longer travel time for these large systems may be a prerequisite for autochthonous primary producers to reach a critical mass where DOM can accumulate to significant levels (Bianchi and others 2004; Morling and others 2017; Paerl and others 2018). This increased residence time also allows allochthonous DOM to be compositionally altered to autochthonous-like material via secondary production (Lynch and others 2019) and photo-oxidation (Hansen and others 2016). These processes likely enhance the fraction of DOM that is autochthonous-like when discharge is lowest and residence times are longest.

In accordance with a large and growing number of studies, we demonstrate an increase in the terrestrial signal of the DOM pool at high flows (Aitkenhead-Peterson and others 2003; Raymond and Saiers 2010; Yoon and Raymond 2012; Kaplan and Cory 2016; Hosen and others 2018; Shultz and others 2018; Wagner and others 2019). Unlike autochthonous-like DOM, aromatic-rich, humiclike DOM (PARAFAC Component C3) was always positively correlated to discharge (Figure 2). The slope between humic-like/terrestrial DOM fluorescence and discharge was also not correlated to watershed area, GPP, or ER (Figure 3M-O), and nor was SUVA<sub>254</sub> (Supplementary Table S6). This indicates more conservative behavior for aromatic DOM that is aged and has a high molecular weight, because the consistent C-Q relationships with scale indicate the mechanisms driving DOM transport do not change (Abbott and others 2018; Hale and Godsey 2019).

The opposing correlations in the main stem of the Connecticut River, with autochthonous-like DOM decreasing with discharge and allochthonous-like DOM increasing, maintains DOM concentrations and leads to chemostasis. That is, in the main stem of the Connecticut River, GPP rates became so high during low flows that autochthonous production was responsible for maintaining DOC concentrations (Figure 2M, Figure 5G). This was not evident in smaller rivers and streams. Smaller streams of order 5 or less had mean annual GPP less than  $1.0 \text{ g-O}_2 \text{ m}^{-2} \text{ d}^{-1}$ (Supplementary Table S5), indicating a potential threshold channel size for chemostasis in this large temperate watershed. The EMMA shows that the balance between autochthonous-like production and allochthonous transport results in chemostatic DOC concentrations (Figure 5). At low flows, delivery of allochthonous DOM from upstream decreases, just as GPP rates are maximized. Hence, during low flow, the concentration of autochthonous-like DOM dominates, making up more than two-thirds of bulk DOM (Figure 5G). As flows increase, the proportion of autochthonous-like DOM decreases, while the concentration of allochthonous-like DOM increases so that at high flows, DOM concentrations were approximately 90% allochthonous (Figure 5G). This balance between two DOM types ultimately maintained DOC concentrations in the Connecticut River main stem at 3.44  $\pm$  0.21 mg l<sup>-1</sup> from drought to stormflow (Figure 5).

Temperature dependence of DOM composition enhanced the effect of flow on DOM. Temperatures are highest in the Connecticut River when discharge is lowest, which is true of many temperate river networks (Hosen and others 2019). Discharge-normalized DOC concentrations, proteinlike DOM fluorescence,  $S_R$ , and Freshness Index values all increased with temperature (Figure 2; Supplementary Table S5), likely due to the temperature dependence of GPP (Gillooly and others 2001; Yvon-Durocher and others 2012). Thus, the negative covariance between temperature and discharge may be critical to supporting enhanced autochthonous production at low flows and supporting DOM chemostasis.

Our finding of a transition from autochthonouslike to allochthonous-like DOM from low to high flow indicates that primary production at low flows results in substantial inputs of DOM. These inputs of autochthonous DOM at low flow stabilize DOM concentrations by providing increased DOM when allochthonous inputs are lowest, which provides mechanistic support for biological influence on DOM chemostasis (Godsey and others 2009; Creed and others 2015). Autochthonous photosynthesis appears to be largely constrained to low-flow conditions in large temperate rivers due to light limitations in smaller streams coupled with longer residence times and temperature and light condi-

tions in larger rivers that are more favorable to GPP (Bernhardt and others 2018; Hotchkiss and others 2018; Hosen and others 2019). We find that increased autochthonous primary production during these periods of longer residence time acts to stabilize DOM concentrations, as allochthonous DOM concentrations decrease due to disconnection of surface soils from the water table (Hornberger and others 1994) and promotion of upstream processing via longer residence times (Raymond and others 2016; Lynch and others 2019). This is in contrast to headwater streams where we did not observe chemostasis because GPP is low and terrestrial contributions to DOC are large and strongly correlated to discharge (Figure 2; Supplementary Table S5). Thus, this pattern is both spatial and temporal. As water flows downstream from headwaters, chemostasis gradually increases as GPP increases and instream processes regulate DOC concentrations.

Although there is chemostasis of DOC concentrations in the Connecticut River, there is no chemostasis of autochthonous and allochthonous DOC concentrations or fluxes. Instead, the flux (Shultz and others 2018) and composition (this study) of DOC are driven by discharge, as terrestrial material is flushed from watersheds into river networks (Aitkenhead-Peterson and others 2003: Raymond and Saiers 2010; Zarnetske and others 2018) and autochthonous DOM production is limited during high flows. Thus, the "Pulse" of terrestrial DOM at coastal sites recently hypothesized by the Pulse-Shunt Concept (Raymond and others 2016) also occurs, despite a chemostasis of concentrations. Furthermore, because the percentage of terrestrial DOM is highest at high flow and high-flow periods dominate fluxes, more allochthonous DOM is delivered to the ocean each year than autochthonous and autochthonous-like, riverine DOM.

These findings reconcile an apparent contradiction between the Pulse-Shunt Concept and chemostasis that has been mentioned by others (Moatar and others 2017). It also has important implications to the ecology of river ecosystems. We estimate that autochthonous DOM dominates during lower flows in the Connecticut River main stem around 40% of the year, which expands to about 80% of the summer when discharge is lowest and temperatures are highest. Production of autochthonous DOM in river networks is associated with warm temperatures, which is when higher order organisms are most ecologically active (Nixon and others 2009). Thus, this stabilization of DOC concentrations by inputs of autochthonous DOM may be critical to the ecology of such rivers (Roberts and others 2007). Whether this is true of rivers across ecoregions remains to be seen. In the Connecticut River watershed, the influence of autochthonous production was only substantial in larger rivers. In more arid climates with lower canopy cover, discharge, and inputs of allochthonous DOM (Bogan and others 2013; Hall and others 2015), the impact of GPP on DOM composition is likely to be greater in smaller rivers and streams compared to temperate regions. Further, these findings have particularly strong implications for a changing climate, with more frequent low- and high-flow events and increased temperatures predicted in rivers of the northeastern USA and elsewhere (Sweet and others 2017).

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