

CURRENT EVIDENCE

Biogeochemical tools for characterizing organic carbon in inland aquatic ecosystems

S. L. McCallister ¹* N. F. Ishikawa ² D. N. Kothawala³

¹Department of Biology and Environmental Sciences, Virginia Commonwealth University, Richmond, Virginia;

²Department of Earth Sciences, ETH Zurich, Zurich, Switzerland; ³Department of Limnology, Evolutionary Biology Centre, Uppsala, Sweden

Scientific Significance Statement

Inland waters receive large quantities of organic matter from their watersheds which may be mineralized during transit, buried or passively exported to the marine system. Tracing the source and fate of this terrestrially derived organic carbon in aquatic ecosystems remains a major analytical and methodological challenge. Our study describes promising research approaches that provide unique and significant insights into the diversity, delivery, transformation, and metabolic fate of organic carbon in aquatic systems. We also propose future research questions whose answers will provide a mechanistic understanding of the role of aquatic systems in regional and global carbon budgets.

Abstract

Integration of inland waters into regional and global carbon (C) budgets requires a comprehensive understanding of factors regulating organic carbon (OC) delivery and in situ processing. This study reviews advances in optical, molecular, and isotopic approaches to resolve the sources, ages, and transformations of OC in aquatic systems. OC characterization using excitation emission matrix spectra, Fourier transform ion cyclotron mass spectrometry, and nuclear magnetic resonance provides detailed molecular level insight. Radiocarbon isotopic approaches and compound-specific techniques resolve the input, metabolic fate, and turnover time of OC in ecosystems ranging in size from streams to the open ocean. Accumulating evidence suggests that aquatic OC is composed of diverse biogeochemical components. We conclude with enduring and emerging questions that underscore the role of inland systems in the global C cycle and propose unique combinations of approaches to better discern their role in the delivery and transformation of OC from soils to seas.

Historically, global C models focused on three primary reservoirs, atmospheric, terrestrial, and marine, and exchanges between them. A pivotal study by Cole et al. (2007) prompted

a paradigm shift in our view of inland waters, not as passive conduits, but rather as an active component of the global carbon (C; see Table 1 for the list of abbreviations) cycle.

*Correspondence: slmccalliste@vcu.edu

Author Contribution Statement: The study was conceived by SLM, NFI, and DNK. NFI reviewed radiocarbon approaches, DNK evaluated OM optical and molecular characterization, and SLM reviewed isotopic and additional approaches and collated the manuscript. The final manuscript was reviewed and edited by all.

Data Availability Statement: The data on the trends in publishing of carbon methods are provided in the Supporting Information section.

Additional Supporting Information may be found in the online version of this article.

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

Table 1. Notation.

Acronym	Description
AA	Amino acid
AMS	Accelerator mass spectrometer
C	Carbon
CRDS	Cavity ring down spectroscopy
CRAM	Carboxyl-rich alicyclic molecules
CSRA	Compound-specific radiocarbon analysis
DIC	Dissolved inorganic carbon
DOC	Dissolved organic carbon
DOM	Dissolved organic matter
EA-IRMS	Elemental analyzer-isotope ratio mass spectrometer
EEM	Excitation emission matrix
ESI	Electrospray ionization
fDOM	Fluorescent dissolved organic matter
FLAME	Fast limnology automated measurements
FTICR-MS	Fourier transform ion cyclotron-mass spectrometry
HFM	High-frequency measurement
HMW	High molecular weight
HPLC	High performance liquid chromatography
MS	Mass spectrometry
NMR	Nuclear magnetic resonance
OC	Organic carbon
PARAFAC	Parallel factor analysis
PCF	Permafrost carbon feedback
POC	Particulate organic carbon
RCC	River continuum concept
tCO ₂	Terrestrially derived CO ₂
tDOC	Terrestrial dissolved organic carbon
tOC	Terrestrial organic carbon
tPOC	Terrestrial particulate organic carbon
WRT	Water residence time
yr	Year
yr BP	Years before present (i.e., 1950 AD)

Although inland waters are now included in the Fifth International Panel on Climate Change C budget, the quantification of C storage and efflux at both the regional and global scale remains uncertain (Raymond et al. 2013). Currently, estimates of CO₂ efflux from inland systems to the atmosphere are upward of 2.1 Pg (Cole et al. 2007; Raymond et al. 2013) and are driven in part by in situ respiration of terrestrial organic carbon (tOC; Table 1) (McCallister and del Giorgio 2008).

Tracing the dynamics of terrestrial dissolved organic carbon (tDOC; Table 1) and terrestrial particulate organic carbon (tPOC; Table 1) is inherently difficult given its molecular and structural complexity. Inland waters are extremely diverse in terms of climatology, geomorphology, and topography, thereby complicating comparisons between systems. Implicit

in these geographic differences are large spatiotemporal variations in the hydrological flow path and consequently the concentration, molecular composition, age, and delivery of tOC to individual systems. Once tDOC is introduced to aquatic systems, it is subject to rapid biological and/or photochemical transformation (Findlay et al. 1998; Cory et al. 2014) with subsequent alterations in its chemical composition and bioavailability. Along the longitudinal flow path, there is a dynamic exchange of organic carbon (OC; Table 1) between dissolved and particulate phases as a result of aggregation/dissolution and adsorption/desorption, which further exacerbates our ability to elucidate the fate of tDOC and tPOC. Characterization of OC in terms of its molecular, structural, and isotopic composition is a powerful and necessary tool to gain fundamental insight into the source, transformation, and fate of OC in aquatic systems. In the past, unraveling the biogeochemical transformations of OC has been hampered by: limited analytical capabilities, large sample volume requirements, low sample throughput, time constraints, data handling, and low-resolution instrumentation. Many of these limitations have been resolved, with notable advancements in recent years.

In this article, we start with a brief review of key methods used to discern the source and biogeochemical transformation of tOC in aquatic systems. We then discuss pivotal advances in our fundamental understanding of C transformations in aquatic ecosystems, as derived from these methods. Lastly, we propose some remaining specific research questions, which are critical to forecast the role of inland waters in regional and global C cycles, and highlight methods best able to resolve these lingering gaps in our knowledge.

Promising tools to shed novel insight on the aquatic carbon cycle

As researchers attempt to generate detailed insight regarding the source, molecular composition, and structure of dissolved organic matter (DOM; Table 1), there is a growing need to obtain specialized analytical chemistry skills as well as access to sensitive and expensive instrumentation. Researchers are often faced with counter-balancing two aspects in DOM characterization, the level of molecular and isotopic specificity desired, and degree of complexity to implement (Fig. 1). Approaches resulting in a higher level of molecular and isotopic specificity often yield an impressive and robust set of conclusions that help develop novel insight into DOM processing (Hertkorn et al. 2006; Feng et al. 2017). However, high specificity does not always guarantee novel insight. In some instances, simple optical and isotopic methods (Fig. 1) can be impressive in revealing important patterns, especially when large datasets and wide biogeochemical gradients are involved (Cloern et al. 2002; Wickland et al. 2007).

Fluorescence spectroscopy

A wide suite of optical approaches ranging from simple indexes and peak picking are available for interpretation of

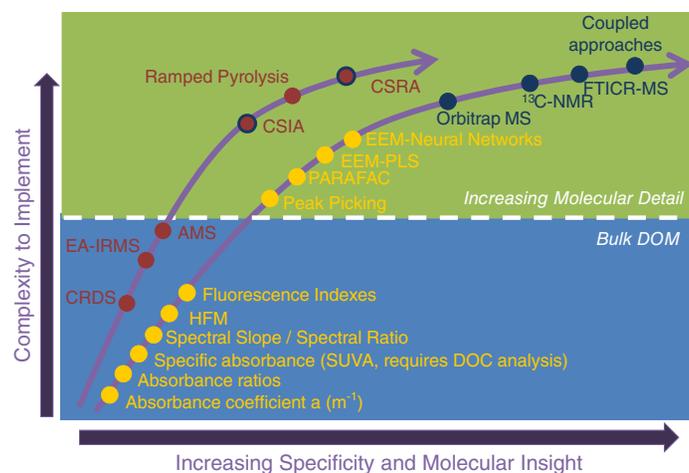


Fig. 1. Conceptual diagram of the relative amount of molecular insight one can expect relative to the level of complexity to implement. Analytical techniques are categorized as isotopic (red), optical (yellow), molecular (blue), or molecular and isotopic (red with blue outline). All approaches below the dashed white line assess bulk DOM, while those above the line attempt to characterize more than one type of DOM. As the level of complexity for most methods will vary with the application, the precise location of methods relative to each other within a box is interchangeable. Note that isotopic approaches are on a different trajectory as they generally require greater complexity to implement but do not provide as detailed molecular insight relative to other approaches such as FTICR-MS. We include abbreviations which are not discussed in text and contained in Table 1: EEM-PLS, Emission excitation matrixpartial least squares; FTIR, Fourier-transform infrared spectroscopy; CSIA, compounds specific stable isotopic analysis.

fluorescence data. However, more detailed insight into biogeochemical processing is likely with the use of multivariate approaches, such as parallel factor analysis (PARAFAC; Table 1). In the case of PARAFAC, underlying fluorescing components unique to a particular dataset are identified. The intensity of each component can then be related to a specific process or gradient of interest to address pertinent research questions. Thus, as shown in Fig. 1, the level of biogeochemical insight provided by optical approaches is inversely related to the complexity of data interpretation. The growing popularity of optical approaches (Supporting Information Table S1) is due in part to the ease of use, low sample volume requirements, and wide accessibility of absorbance and fluorescence instrumentation. The specifics of data correction and interpretation may be found in Fellman et al. (2010). A key advancement since these reviews is the advent of a spectrofluorometer that simultaneously analyzes absorbance and fluorescence (Aqualog, Horiba, France). Sample analysis times are significantly reduced and data treatment including blank subtraction, inner filter correction, and Raman normalization are performed immediately within the sample analysis software. It is increasingly common to use autosamplers with Aqualog, which further reduces the labor involved. These recent advances have greatly improved the possibilities of collecting

vast amounts of spectral data using fluorescence and makes routine monitoring feasible.

To standardize data treatment and interpretation of excitation emission matrix (EEM; Table 1) data, several handy toolboxes such as DOMFluor (Stedmon and Bro 2008), EEMizer (Bro and Vidal 2011), FDOMcorr (Murphy et al. 2010), and drEEM (Murphy et al. 2013) have made this step easier, especially for novice users. An important evaluation of fluorescence as a tool to characterize DOM involved a large-scale inter-laboratory comparison of natural water samples as well as set of fluorophore standards to compare results generated from different labs (Murphy et al. 2010). Additionally, there is now a library of PARAFAC components that have been reported in the literature that can be easily searched and statistically compared with any newly generated components, called OpenFluor (Murphy et al. 2014).

Mass spectrometry

Mass spectrometry (MS; Table 1) has been used to analyze DOM for several decades with ion trap, quadrupole, and time of flight mass spectrometers. However, arguably the single greatest advancement in the analysis of DOM in recent years has been the development of Fourier transform ion cyclotron MS (FTICR-MS; Table 1) instrumentation. FTICR-MS is typically used with a soft ionization sample introduction system called electron spray ionization (ESI; Table 1), such that molecules are left intact during ionization, rather than being fragmented. The resulting mass spectrum typically includes the mass to charge (m/z) of several thousands of peaks comprising DOM compounds. FTICR-MS is particularly powerful as it can resolve between the nominal masses of several thousands of DOM compounds and allow for the assignment of molecular formula including heteroatoms such as N, S, and P, in addition C, H, and O, which form the backbone of DOM. Sample preparation, peak assignment, and data interpretation are reviewed extensively in Mopper et al. (2007). It should be noted that while ESI-FTICR-MS is rapidly gaining popularity, the total number of applications is still emerging (37 between 2012 and 2017; Supporting Information Table S1), and the field has yet to develop a set of universally accepted validation steps to ensure reproducible results between instruments and data processing. The recent development of an automated compound identification algorithm allows more precise and constant formulae assignments across varied studies (Koch et al. 2007).

Nuclear magnetic resonance spectroscopy

Although nuclear magnetic resonance (NMR; Table 1) spectroscopy remains less prevalent as a technique to characterize DOM than other approaches (Supporting Information Table S1), it should not be overlooked as a particularly promising technique. Some of the earliest discoveries on DOM structure that form our basic conceptual understanding arose from NMR analysis, for example, see Benner et al. (1992). NMR exploits the resonance frequency of atomic nuclei (commonly ^1H , ^{13}C , ^{15}N , or ^{31}P), which is influenced by surrounding electronic structures,

and thus the shift in resonance frequency given by a particular atom gives insight into the presence of surrounding functional groups. More detailed reviews of NMR methodology and its applications to DOM characterization have been previously provided (Mopper et al. 2007; Hertkorn et al. 2013).

Currently, there is heightened potential for continued advancements including detailed molecular analysis of DOM after chromatographic separation, for example, using high-performance liquid chromatography (HPLC)-NMR (Simpson et al. 2004). Although the high resolution of FTICR-MS provides empirical formulae with impressively high precision, we are still unable to confidently extrapolate these data to exact structural composition. Knowledge of DOM structural stereochemistry is an essential next step, as subtle alterations in molecular structure can shift the functional group composition and modify enzymatic activity, and thus result in profound changes to its biological and physicochemical reactivity. Ultimately, detailed knowledge of the molecular architecture of DOM, will allow geochemists to infer the potential reactivity toward a diverse suite of microbial enzymes and mineral surfaces. Continued use of NMR in conjunction with FTICR-MS will likely pioneer our future understanding of the diverse molecular structures comprising DOM and their biogeochemical function (Hertkorn et al. 2013).

High-frequency measurements

Over the past 15 yr, there have been rapid advances in electrochemical and optical sensor technology, which was spurred in large part, from the transition of analogue to digital technologies (Meinson et al. 2016). Now, due to reduced costs, increased sensitivity, and decreased maintenance requirements, the aquatic research community is poised to exploit the new sensor technology for the high-resolution biological and chemical monitoring of diverse inland aquatic systems. In situ high-frequency measurements (HFM; Table 1) of aquatic $p\text{CO}_2$ gas are routinely measured for extended periods of time (months) and when coupled with optical measurements of chromophoric dissolved OC (DOC) as a proxy for DOC (Table 1), provide a powerful tool to assess vertical and lateral C fluxes.

Additional insights into the hydrological and biogeochemical controls on C dynamics in aquatic systems are frequently gleaned through isotopic analysis (see below). The conventional method for measuring $\delta^{13}\text{C}$ - $p\text{CO}_2$ in aquatic systems requires the collection of discrete samples, which are then analyzed by large laboratory-based isotope ratio mass spectrometer (IRMS; Table 1). Although this method provides high-precision data, it requires a time-consuming headspace equilibration, is costly, and results are frequently not available for weeks to months after sample submission. Cavity ring down spectroscopy (CRDS; Table 1) is a portable laser-based spectroscopy technique that employs the optical absorbance characteristics of individual gases to measure their concentration and isotopic signature. Although CRDS is not as precise as IRMS, it nevertheless enables high throughput isotopic

analysis capable of resolving rates and pathways of C cycling (Maher et al. 2013).

Exploitation of new developments in optical, colorimetric, and isotopic sensor technology will help to identify “hot spots” and “hot moments” in OC biogeochemical transformations which better connect aquatic and terrestrial C fluxes at the landscape level. HFM remain invaluable as a quantitative means to unravel the role of continental drainage basins in the global carbon cycle and provide background information for which targeted experimental manipulations should be superimposed. Heightened collection, curation, and public availability of long-term HFM datasets, open source tools for data reduction, and intercomparison and continued model development is essential to illuminate the current, and forecast the future, role of inland waters in the global carbon cycle.

Natural abundance isotopic approaches

Stable carbon isotope ratios ($\delta^{13}\text{C}$) have been routinely used to quantify contributions of tOC to aquatic realms. However, the $\delta^{13}\text{C}$ values for aquatic primary producers (e.g., benthic algae, phytoplankton) show considerable variations in space and time and frequently overlap with terrestrial materials (e.g., Ishikawa et al. 2012). This is primarily because $\delta^{13}\text{C}$ of aquatic OC is strongly affected by isotopic fractionation associated with algal photosynthesis (Finlay et al. 1999). To better differentiate OC sources, we review two alternative and complementary isotopic tools: radiocarbon signatures ($\Delta^{14}\text{C}$) and compound-specific ^{13}C and ^{14}C measurements.

Radiocarbon is a useful tool for studying the exchange and metabolic fate of tOC in aquatic systems on two timescales, and its ecological use is reviewed elsewhere (Guillemette et al. 2017). Briefly, radiocarbon (^{14}C) is formed from the interaction of cosmic rays with ^{14}N in the stratosphere and has a half-life of 5730 yr. Measurement of the radiocarbon signature will provide an estimate of the time elapsed since autotrophic fixation of the OC by radioactive decay and is useful on centennial to millennial time scales. Additionally, explosion of thermonuclear weapons in the atmosphere, beginning in the 1950s and its subsequent cessation years later, allows us to infer the timescales for C fixation on annual to decadal timescales. Ingalls and Pearson (2005) provide a detailed description of radiocarbon background and nomenclature.

In contrast to high throughput $\delta^{13}\text{C}$ measurements using an elemental analyzer IRMS, $\Delta^{14}\text{C}$ data acquisition is often limited by time-consuming sample preparations, which consist of combustion (particulate) or UV-oxidation (dissolved) of OC, cryogenic purification of evolved CO_2 on a vacuum line, and graphitization. $\Delta^{14}\text{C}$ is typically determined by an accelerator mass spectrometer (AMS; Table 1) with 1 σ of 2‰ and ^{14}C age of up to 50,000 yr before present (yr BP; Table 1).

The measurement of $\Delta^{14}\text{C}$ in select organic compounds, rather than bulk measurements, is a more sensitive means to determine the source and turnover of C at the molecular level (Ingalls and Pearson 2005) (Fig. 2). OC in fresh and marine

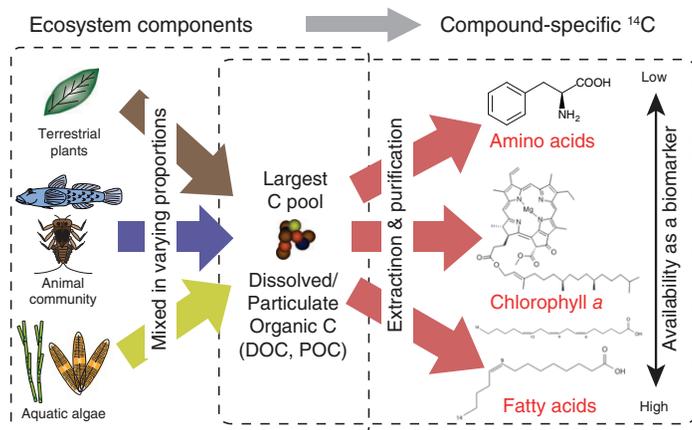


Fig. 2. Schematic for a potential application of the CSRA to future biogeochemical C cycling studies in freshwater systems.

environments is highly heterogeneous and is usually a mixture of multiple compounds that have a continuum of sources and turnover times. Bulk isotope analysis of OC does not allow one to isolate target molecules of interest. Compound-specific radiocarbon analysis (CSRA; Table 1) of particular biomarkers provides source specificity and has the potential to overcome the limitations of bulk ^{14}C analysis.

Radiocarbon measurements have increasingly been employed in aquatic systems to trace the source and turnover time of OC sources; however, we anticipate an acceleration in this trend with the dramatically reduced size and operational costs of new instrumentation coupled with higher sample throughput resulting from interfaces allowing direct CO_2 analysis (continuous flow and moving wire) and the elimination of the cumbersome graphitization step (Sessions et al. 2005). Further, an exciting development for freshwater DO^{14}C analysis is the replacement of time consuming UV-oxidation by a wet oxidation technique derived from Zhou et al (2015) and Lang et al. (2016). Although high blanks have previously been associated with this technique, method development at Lalonde AMS Lab has lowered the blank to $<1\text{pMC}$ with a corresponding daily throughput of 5–8 samples per day (S. Murseli pers. comm.). Lastly, breaking open the age spectrum of marine and freshwater POC using ramped pyrolysis will aid our understanding of its source and turnover time (Rosenheim and Galy 2012; Follett et al. 2014).

Significant findings arising from the above approaches

In the previous sections, we reviewed critical features of five promising analytical tools for studying sources and biogeochemical transformations of aquatic OC. Here, we provide an abbreviated review of significant findings, which were generated through use of these analytical methods and approaches. Thus, we now place the available analytical tools

within the context of several broad research questions common to aquatic C biogeochemistry.

Advances in our knowledge of DOM composition, structure, and biogeochemical cycling

EEM-PARAFAC is a particularly attractive tool to examine DOM cycling as it allows researchers to assess the fate and transport of numerous DOM pools which would not be detectable based solely on bulk DOM measurements, and yet, remains a relatively simple, high throughput approach. Hydrology plays a critical role in the delivery of the source and character of DOM to aquatic systems. Inamdar et al. (2011) used a PARAFAC approach to assess the effects of storm events on headwaters streams in a forested catchment. Groundwater sources dominated during baseflow and were characterized by a high % of protein-like fluorescence while storm events showed an increase in humic constituents likely derived from litter leachate and soil water. Seasonal changes in the DOM composition of alpine lakes were shown using PARAFAC and demonstrated the mobilization of terrestrial inputs during snowmelt and algal-derived autochthonous DOM predominate during spring and late summer (Miller and McKnight 2010). Coupled bioassays and PARAFAC analyses in agro-urban streams found bioavailable DOC to range from 2% to 57% and was negatively correlated with percent humic-like fluorescence but positively related to protein-like fluorescence (Petroni et al. 2011). PARAFAC has also proven useful for assessing the trophic status of high mountain lakes (Zhang et al. 2010). For example, oligotrophic lakes were dominated by protein-like spectral characteristics while humic-like DOM-dominated systems that were more highly eutrophied. In addition, a microbial-like component that was correlated with chlorophyll *a* concentrations showed the greatest abundance in eutrophic lakes. To better assess the relationship between DOM reactivity and its chemical composition, fluorescence spectroscopy was used to explore DOM quality from 560 lakes across Sweden and encompassed a wide climatic gradient. PARAFAC components with longer emission wavelengths, or red-shifted components, were most reactive while protein-like components were most persistent within lakes (Kothawala et al. 2014). Collectively these studies suggest that fluorescence spectroscopy is a powerful tool to examine the quality and reactivity of individual DOM fractions how these may respond to changes in hydrology and land cover.

FTICR-MS provides highly detailed insight into DOM compounds and exploits regions of van Krevelen diagrams that are associated with compound groups including lipids, proteins, unsaturated hydrocarbons, polyphenolic aromatics, and lignins (Hertkorn et al. 2013). FTICR-MS is a particularly sensitive technique for discerning alterations in DOM due to in situ biotic and abiotic processing. For instance, Stubbins et al. (2010) determined that photo-exposed Congo River DOM displayed an increased aliphatic character more closely aligned to marine DOM, which reflected a significant loss of aromatics and a

reduction in molecular diversity. In some cases, previously unrecognized compound groups are increasingly studied. For example, global biomass burning results in the production of millions of tons of charcoal, and FTICR-MS analysis found that following its loss from soils via dissolution represents 10% of the global riverine flux of DOC (Jaffé et al. 2013).

While NMR provides among the most detailed insight regarding functional group composition, it has yet to expand and become as widely used an approach. In perhaps the first compositional study of marine DOC, Benner et al. (1992) employed ^{13}C -NMR to identify polysaccharides as the most abundant and reactive components of high molecular weight (HMW; Table 1) DOC. A similar approach dispelled the notion that the molecular composition of marine HMW DOC was distinct from that of freshwater systems due to the influence of soil-derived humics when they found the molecular-level chemical properties to be indistinguishable between the two systems (Repeta et al. 2002). Applying ^1H -NMR to severely OC deplete rainwater Cottrell et al. (2013) determined it was comprised primarily of carboxylic acids, carbohydrates and N-containing compounds. In a ^{13}C -NMR study of DOC from the everglades, O-alkyl concentrations differentiated the primary source of DOC as phytoplankton and seagrass derived, and lignin abundance provided insight into the diagenetic state (Maie et al. 2005). Marine DOC analyzed using solution-state ^1H -NMR revealed the presence of carboxyl groups, aromatic groups, carbohydrates, and most importantly carboxyl-rich alicyclic molecules (CRAM; Table 1), material derived from linear terpenoids, which is the most abundant component of deep sea DOC ever identified. NMR is an invaluable tool for DOM characterization as it is the only approach which provides structural context.

High-frequency measurements increase spatiotemporal resolution of C cycling in aquatic systems

There is still a great deal of uncertainty regarding the hydroecological controls of carbon dynamics in diverse aquatic systems. Sobczak and Raymond (2015) deemed the temporal mismatch in DOC concentration and composition relative to stream discharge as the Achilles' heel of DOC export science. However, HFM now provide novel insights into the coupled temporal dynamics of discharge and the quantity and quality of DOC export. For example, using in situ HFM deployable UV absorbance sensors, Jeong et al. (2012) found that in mountainous streams, particulate organic carbon (POC; Table 1) was preferentially mobilized during intense storm events whereas DOC dominated the export during baseflow and small storm events. Additionally, in situ fluorescent dissolved organic matter (fDOM; Table 1) measurements paired with laboratory optical properties revealed the rapid export of HMW, soil derived, humic-like DOM from an agricultural watershed during pulsed episodic events (Saraceno et al. 2009). In the San Joaquin River, Spencer et al. (2007) employed in situ optical sensors to distinguish a diurnal pattern in the composition and concentration

of DOC, likely a result of biological and photochemical processes, which was not discernible from discrete measurements of bulk DOC. Collectively, these temporal HFM provide a more comprehensive understanding of the short-term variability in OC composition and export and enhances our ability to integrate DOM biogeochemistry with hydrological drivers on time scales from minutes to millennia (Sobczak and Raymond 2015).

Crawford et al. (2015) developed fast limnology automated measurements (FLAME; Table 1) as a platform to provide high-frequency, spatially explicit, real-time measurements of surface waters to gain a better understanding of C cycling at the ecosystem scale. FLAME is a novel flow through system which when integrated with multiple electrochemical, colorimetric, and optical sensors supplies spatially explicit data to identify biogeochemical "hot spots" such as the aquatic terrestrial interface. Using a suite of biogeochemical sensors, Crawford et al. (2015) were able to identify spatial variations in pCO_2 and DOC which challenges previous notions of horizontal homogeneity in small lakes.

CRDS when paired with an air-water equilibrator provides high-quality, spatially integrated measurements of dissolved CO_2 and its isotopic signature in aquatic ecosystems at high temporal resolution (Webb et al. 2016). CRDS field data generated by Maher et al. (2013) provided insight into the rates and pathways of aquatic C cycling on scales from microbial to ecosystem scales. Specifically, an estuarine survey identified a trend of decreasing pCO_2 concentrations and increasing $\delta^{13}\text{C}\text{-CO}_2$ in the downstream direction, which likely reflects surface isotopic CO_2 equilibration with the atmosphere. Similar spatial surveys over two tidal cycles by Maher et al. (2015) in the same estuary found that overlapping tidal and diurnal shifts in surface pCO_2 may result in a significant overestimation of the water-air CO_2 flux. Spatial sampling approaches like the ones reviewed above help to better resolve the hydrological and biogeochemical controls on the aquatic C cycle.

Radiocarbon approaches to evaluate the source and age of OC supporting aquatic food webs

Chemical weathering of carbonates provides ^{14}C -dead-dissolved inorganic carbon (DIC; Table 1) to stream water columns, which when fixed by photoautotrophs, imparts an aged ^{14}C signature to contemporary primary production. In contrast, tOC such as leaf detritus reflects modern atmospheric $^{14}\text{CO}_2$ values. Therefore, $\Delta^{14}\text{C}$ can be used for isotopically separating the two major carbon sources for stream ecosystems (e.g., benthic food webs), in situ production, and tOC subsidies (Fig. 2; Ishikawa et al. 2014, 2016). Ishikawa et al. (2012) found the $\Delta^{14}\text{C}$ values of benthic algae and plant leaves to be identical to those of DIC and atmospheric CO_2 , respectively, and the $\Delta^{14}\text{C}$ values of invertebrates and fish were bracketed by the end members (Ishikawa et al. 2014, 2016). These results indicate that stream food webs are fueled by both the terrestrial and aquatic primary production in varying proportions (Fig. 2). In larger rivers and lakes,

previous studies have employed $\Delta^{14}\text{C}$ to trace C through trophic pathways. Highly aged OC was assimilated by zooplankton in the Hudson River (Caraco et al. 2010), Lake Schewerlin (Fernandes et al. 2013), and a humic lake (Keaveney et al. 2015). Ancient glacier-derived OM was incorporated into invertebrates and fueled biological production in stream food webs (Fellman et al. 2015) dispelling the notion of a direct linkage of age and bioavailability. This uncoupling of age and bioavailability has also been shown in the North Pacific, northern lakes, temperate rivers, and permafrost regions (Cherrier et al. 1999; McCallister et al. 2004; McCallister and del Giorgio 2012; Mann et al. 2015). Conversely, ^{14}C age of zooplankton in Lake Superior was modern (Zigah et al. 2011), suggesting watershed specific controls on the delivery and bioavailability of tOC.

Ubiquitous antenna pigment found in almost all the photoautotrophs can be used as a source specific tracer, and as an index of in situ primary production as it is rapidly degraded when inactive. Isotopic composition of chlorophyll *a* can be regarded as synonymous of *in situ* primary producer (Ohkouchi et al. 2005). Tracing the relative importance of algal vs. terrestrial OC to higher trophic levels and their ultimate fate in aquatic systems is challenging due to the chemical heterogeneity of OC from multiple allochthonous and autochthonous sources. Chlorophyll *a*-specific $\Delta^{14}\text{C}$ in periphyton (benthic biofilm) in comparison with bulk $\Delta^{14}\text{C}$ can differentiate the proportion of algae material relative to other OC contributions in the chemically complex matrix (Fig. 2; Ishikawa et al. 2015). In addition, $\delta^{13}\text{C}$ of essential fatty acids indicate that these compounds are not metabolized by heterotrophs so they are useful in tracing C flow in food webs (Fujibayashi et al. 2016).

Amino acid (AA; Table 1) ^{14}C analysis has been used to radiocarbon date a single AA, hydroxyproline, in a few archaeological studies (Marom et al. 2013), though it is also a promising biogeochemical tracer. Heterotrophs cannot synthesize some of the essential AA. Isotopic composition of each of 20 biogenic AA may reflect different functional roles and thus indicate varied turnover times in aquatic systems. Because AA metabolism in organisms is closely related to their nitrogen utilization, $\Delta^{14}\text{C}$ of AA may also provide information on nitrogen turnover in aquatic ecosystems although the methodology using HPLC (Table 1) is still in its infancy (Bour et al. 2016). Although $\Delta^{14}\text{C}$ of AA from aquatic realms has not yet been reported, Larsen et al. (2013) found that the $\delta^{13}\text{C}$ analysis of AA followed by a principal component analysis is useful to characterize different taxonomic groups in aquatic environments (Fig. 2; e.g., algae, fungi, bacteria, and vascular plants).

^{14}C controls on the source, age, and transformation of OC delivered to aquatic systems

Without knowledge of the radiocarbon signature of tOC exported from tropical, temperate, and boreal rivers we

cannot predict how the size of marine and terrestrial C sinks will respond to climate change and the key role of inland water systems in regulating the redistribution of tOC.

Bulk ^{14}C OC values are composed of a continuum of OC with varying sources and ages, for example, petrogenic (^{14}C -free), algal OC from fixation of modern or ancient DIC, and modern and preaged tOC across a spectrum of ages (Raymond and Bauer 2001). $\Delta^{14}\text{C}$ measurements of DIC, DOC, and POC are useful to differentiate sources and estimate turnover time of C in aquatic water columns (Mayorga et al. 2005; Raymond et al. 2007). The ^{14}C age of both DOC and POC has been shown to vary significantly with discharge. Typically, during baseflow, groundwater inputs are more significant and are reflected in older tDOC whereas high flow events are associated with shallow flow paths and more modern tDOC (Schiff et al. 1997; Raymond et al. 2007). Based on DI^{14}C measurements, Mayorga et al. (2005) determined that CO_2 degassed from the Amazon River was primarily young (< 5 yr old) recently fixed C, suggesting rapid turnover of fresh tOC in aquatic systems.

In order to more accurately trace the source and age of C within and exported from aquatic systems, researchers have employed CSRA of individual components such as lignin, fatty acids, and alkanes which are diagnostic for distinct autochthonous and allochthonous OC sources.

In the Delaware River, the ^{14}C signature of long chain fatty acids (vascular plant and soil derived OC) biomarkers indicates a radiocarbon age which was older than the water and sediment residence time implying a source of preaged tOC to the system (McIntosh et al. 2015). Radiocarbon analysis of n-fatty acids from the POC in the Yellow River found relatively younger short chain fatty acids (from 502 yr BP to modern) suggesting recently fixed OC. In contrast, long chain fatty acids characteristic of soil-derived OC were significantly older (1500–1800 yr BP) and most likely reflected OC that had previously been preserved due to mineral association (Tao et al. 2015). In this system, bulk carbon isotopes suggested little temporal variability in composition, yet CSRA discerned multiple tOC pools cycling at significantly different time scales.

Predicted hydrologic regime shifts (e.g., permafrost thaw) due to climate warming and land use changes will alter both the concentration and age of tOC delivered to aquatic systems (Schiff et al. 1997; Vonk et al. 2015) as well as its chemical signature. Butman et al. (2015) found that anthropogenic alteration of watersheds releases preaged DOC into rivers, and radiocarbon measurements of DOC from drained tropical peatlands indicated the export of ancient C (centuries to millennia) (Evans et al. 2013). One of the enduring conundrums in aquatic biogeochemistry is the fate of tOC in downstream receiving systems. As lignin is unique to vascular plant-derived OC, it is a source-specific tracer of tOC during its lateral export and when coupled with CSRA may provide information on the links and lags between C cycling in the terrestrial system and the contemporary aquatic C cycle.

Martin et al. (2013) found the age of lignin phenols in suspended sediments from the Mekong River to be modern suggesting the export of C with a relatively short residence time of ~ 18 yr most likely derived from the upper soil horizon and litter. The radiocarbon content of lignin phenols isolated from DOC of two arctic river systems distinguished distinct age characteristics for different watershed features. Relative to the Mackenzie, the Kolyma River had an older background component of DOM, likely a result of yedoma occurrence in its watershed that was not discernible from bulk radiocarbon DOC measurements (Feng et al. 2017).

Future directions

We have significantly increased our knowledge of the underlying landscape, hydrologic, climatic, and biogeochemical controls on OC cycling in aquatic systems through the collective employment of optical, isotopic, and molecular analytical approaches. These noteworthy findings discussed above provide a foundational understanding of the aquatic C cycle and serves as a platform to address, for example, the following unresolved specific research questions.

How have anthropogenic and climatic drivers altered the source and age of OC delivered to aquatic systems?

Many studies of C mobilization, transformation, and metabolic fate reflect the state of the present-day biogeochemical C cycling. In contrast, palaeoreconstruction of OC sources to sediments in lakes, river, and estuaries provides a long-term integrated perspective of OC sources released from terrestrial environments and the associated hydrologic and physiographic drivers (McGowan et al. 2016). For example, using CSRA of long chain *n*-alkanes and alcohols, Schefuß et al. (2016) found that in the geological past the Congo River discharged aged OC of several thousands of years which is in sharp contrast to the relatively young fluvial OC currently exported (Spencer et al. 2012). The authors suggest that changes in radiocarbon age of OC exported may result from alterations in rainfall and associated changes in wetland cover. Radiocarbon examination of wax lipids from estuarine sediments located in the Eurasian Arctic traced an ancient permafrost derived OC pool that was delivered via deeper hydrological conduits and/or thermokarst erosion (Feng et al. 2013). Concurrent radiocarbon analysis of vascular plant-derived lignin phenols revealed significant inputs of young tOC presumably derived from surface sediments and controlled by river discharge. An isotope mixing model found that significant increases in river run off has enhanced the surface delivery of young carbon and concurrently mobilized an amplification in ancient carbon transfer by 3–6% between 1985 and 2004 (Feng et al. 2013). CSRA data from a sediment core collected from Lake Whittington in the lower Mississippi River indicated that the damming of the Missouri River in the 1950s decreased net OC export, and the isotopic data also reflected increased inputs of soil-derived OC dominated by C-3

vegetation resulting from alterations in farming practices and crop distributions (Bianchi et al. 2015). Thus, sediments provide a robust but frequently overlooked historical record of alterations in OC input which has the potential to inform our understanding and prediction of the response of these systems to climatic and anthropogenic change (Bianchi et al. 2015; McGowan et al. 2016).

When do aquatic systems act as pipes vs. reactors?

Aquatic systems are typically supersaturated with CO₂ that is derived from a combination of: (1) in situ respiration of allochthonous OC, (2) the passive export of CO₂ via surface and groundwater of soil derived C, (3) chemical weathering of underlying bedrock, and (4) sewage. In order to develop a process-based understanding of inland waters in regional C budgets, it is critical to determine their role in CO₂ emissions. Do inland waters function merely as a conduit for the release of terrestrially derived CO₂ to the atmosphere or do they actively mineralize tOC? Hotchkiss et al. (2015) proposed a conceptual model depicting the longitudinal variation in the relative importance of internal processing of tDOC (reactor) relative to external sources of CO₂ (passive terrestrially derived CO₂ [tCO₂; Table 1] emissions). Based on compared rates of aquatic net ecosystem production and CO₂ emissions, they determined that tCO₂ dominated the emissions from headwater streams while internal processing and respiration of tOC increased downstream.

In order to empirically examine the physical and biogeochemical factors that regulate active DOC processing vs. passive transport, Casas-Ruiz et al. (2017) employed a reach scale approach where they monitored changes in DOC concentration and composition over a full hydrological year. Optical spectroscopy and PARAFAC modeling were used to assess the along river dynamics of different DOC sources. They found during high residence times the system functioned as a bioreactor, which supports the Pulse-Shunt Concept (Raymond et al. 2016). PARAFAC modeling determined that protein-like DOC was actively consumed whereas humic-like DOC was passively transported suggesting DOC molecular composition influences its metabolic fate.

Isotopic analysis ($\delta^{13}\text{C}$) of DIC or CO₂ is an alternative method to assess the sources contributing to stream pCO₂ efflux as the $\delta^{13}\text{C}$ signature is an imprint of the relative biogenic and geogenic sources and in stream biogeochemical processing. Campeau et al. (2017) measured the $\delta^{13}\text{C}$ -DIC across boreal streams and found that passive transport of tCO₂ was the main DIC source although in-stream metabolism and anaerobic processes also made significant contributions. Not surprisingly, these data suggest that fluvial systems may act as both active and passive pipes. We suggest that reach scale HFM measurements consisting of fDOM (DOC proxy) and CRDS coupled with discharge data would provide a platform to gain a predictive understanding of the hydrological and biogeochemical processes influencing the active or passive

role of these systems in regional C cycles and in addition, how they might respond to forecasted increases in DOC export.

Where is “preaged” C lost to the atmosphere along the terrestrial-aquatic landscape and what is the internally derived aquatic contribution?

This particular question is not independent from those above; however, the focus here is specifically on the age and source (see above) of CO₂ released to the atmosphere, which is particularly relevant for Arctic ecosystems. Arctic systems are currently demonstrating climate-induced mobilization of preaged permafrost OC whose metabolic fate is critical to determining the contribution of aquatic systems to the permafrost carbon feedback (PCF; Table 1) (Vonk et al. 2015). The aquatic metabolism of young C with a short residence time in the terrestrial environment will have little long term impact on atmospheric CO₂; however, climate- and human-induced mobilization and subsequent respiration of ancient, previously sequestered OC acts as a positive feedback and a CO₂ source analogous to the burning of gasoline. The role of aquatic systems in regional and global C budgets and its potential contribution to PCF hinges on where preaged OC is mineralized along the terrestrial-aquatic continuum.

Aquatic respiration of climate-mobilized permafrost DOC in fluvial systems represents a positive PCF; however, the passive release of preaged tCO₂, laterally transported from soils, signifies a neutral role for aquatic systems in regional Arctic C budgets. Consequently, to incorporate inland waters in arctic C budgets, it is crucial to identify their active contribution to CO₂ supersaturation.

The radiocarbon age of CO₂ lost by evasion may be calculated indirectly from DI¹⁴C, directly from floating chambers that capture the evaded CO₂ on a molecular sieve (Billett and Garnett 2010), or from headspace equilibration and syringe injection into molecular sieve traps (Garnett et al. 2016). Dean et al. (2018) used the later method to directly measure the ¹⁴C content of aquatic CO₂ coupled with DO¹⁴C from headwater systems of the Canadian Arctic. They found the mobilization of older CO₂ and DOC coincident with seasonal deepening of the active thaw front. However, isotopic disconnects between DOC and CO₂ suggests that the old tCO₂ was transported laterally from thawing soils and not derived from internal aquatic respiration suggesting a neutral role in PCF for these Arctic headwaters. This is in stark contrast to areas with ice-rich yedoma deposits where old DOC was rapidly consumed in bioassays (Mann et al. 2015) suggesting region-specific drivers of the source and metabolic fate of mobilized DOC.

An alternative approach to resolve the internal role of bacterial metabolism in the aquatic-atmospheric CO₂ efflux entails direct determination of bacterial respiration and the corresponding age of C catabolized. Generally, there has been a marked absence of direct isotopic measurements of the age of C respired in aquatic ecosystems as a result of

comparatively lower respiration rates superimposed on a much greater inorganic carbon background relative to terrestrial systems (McCallister et al. 2006). To overcome these difficulties, recent approaches removed the initial background DIC before initiating bioavailability incubations. Respired C was then isolated directly as CO₂ gas or precipitated as carbonates and analyzed by AMS (McCallister et al. 2006; Beaupré et al. 2016; Morling et al. 2016). Without exception, these studies from ponds, lakes, and reservoirs found that the respired C was generally radiocarbon depleted relative to bulk OC sources suggesting a selective respiration of an older OC component. Coupling these respiratory bioassays with measurements of in situ bacterial respiration and an isotopic evaluation of CO₂ efflux (see above) provides a separate means to discern the active aquatic contribution to preaged C loss to the atmosphere (McCallister and del Giorgio 2012).

How is OC biologically and photochemically transformed along its longitudinal transport?

Understanding the role of C in fluvial systems requires knowledge of the inputs, losses, and transformations of OC along its longitudinal transit. Advanced approaches are confirming the highly reactive nature of DOM at the soil-water interface, for example, with MS (Mosher et al. 2015) and in situ sensors (Pollard et al., 2013). There is also mounting evidence that water residence time (WRT; Table 1) is of paramount importance in predicting the molecular composition (Kothawala et al. 2014; Kellerman et al. 2015) and reactivity of DOM (Catalán et al. 2016; Evans et al. 2017). Early conceptualizations of the river continuum concept (RCC; Table 1) (Vannote et al. 1980) suggested rapidly changing molecular composition of DOM with movement downstream. Mosher et al. (2015) tested the RCC by assessing the diversity of DOM molecular formulae with stream order and observed the greatest chemodiversity in headwaters streams and the ubiquitous presence of CRAM across all sites. Further, it has been suggested that the degree of downstream reactivity of DOM is a direct legacy of upstream source and in stream biogeochemical processing (Lambert et al. (2016a). Hutchins et al. (2017) monitored the optical, chemical, and molecular properties of DOM across a boreal soil-stream-river continuum and noted a hot spot of biopolymer and low molecular weight DOM degradation at the soil-stream interface and selective removal of humic-like aromatic DOM likely driven by photolysis in the stream channel. The evolution of DOM composition along longitudinal transport in the Congo River, led Lambert et al. (2016b) to speculate that photodegradative relative to biological pathways tend to be more sensitive to alterations in WRT. With increasing stream order, DOM appears to progress to a state of chemostasis, whereby the balance of biogeochemical processes diminish variation in concentration and composition (Creed et al. 2015). Thus, we increasingly understand that DOM reactivity is most severe at headwaters, and becomes more stable with increasing stream order. Yet, what

remains unclear is the relative importance of key processes, namely sedimentation and photochemical and biological degradation on DOM losses as its reactivity shifts with time.

Conclusions

Inland waters occupy a unique landscape position whereby the internal transformations and fate of aquatic OC has significant implications for the C budget of the three primary CO₂ reservoirs (atmospheric, terrestrial, and marine). We stress the importance of combined molecular, optical, and isotopic approaches as a proponent to widen the OC analytical window in the future and better enable the abiotic and biotic understanding of the complex dynamics of aquatic OC cycling. In addition, we emphasize the need to better resolve the hydrological and biogeochemical drivers of DOM chemo and structural diversity and corresponding links to its bio- and photo-lability. We further underscore the need to elucidate the active role of inland waters in the C cycle particularly with respect to lateral transfer of preaged OC and whether the C is returned to the atmosphere, enters the trophic food web, is sequestered in sediments or is injected into the marine biosphere. Future studies should seek to better identify the environmental, geographic, climatic, and anthropogenic factors regulating the delivery of preaged tOC to aquatic systems as well as its fate and transformation along its seaward transit.

References

- Beaupré, S. R., N. Mahmoudi, and A. Pearson. 2016. IsoCaRB: A novel bioreactor system to characterize the lability and natural carbon isotopic (14C, 13C) signatures of microbially respired organic matter. *Limnol. Oceanogr.: Methods* **14**(10): 668–681. <https://doi.org/10.1002/lom3.10121>.
- Benner, R., and others. (1992). Bulk chemical characteristics of dissolved organic matter in the ocean. *Science*, **255**(5051), 1561–1564. <https://doi.org/10.1126/science.255.5051.1561>.
- Bianchi, T. S., V. Galy, B. E. Rosenheim, M. Shields, X. Cui, and P. VanMetre. 2015. Paleoreconstruction of organic carbon inputs to an oxbow lake in the Mississippi River watershed: Effects of dam construction and land use change on regional inputs. *Geophys. Res. Lett.* **42**(19): 7983–7991. <https://doi.org/10.1002/2015GL065595>.
- Billett, M. F., and M. H. Garnett. 2010. Isotopic composition of carbon dioxide lost by evasion from surface water to the atmosphere: Methodological comparison of a direct and indirect approach. *Limnol. Oceanogr.: Methods* **8**: 45–53. <https://doi.org/10.4319/lom.2010.8.0045>.
- Bour, A. L., B. D. Walker, T. A. B. Broek, and M. D. McCarthy. 2016. Radiocarbon analysis of individual amino acids: Carbon blank quantification for a small-sample high-pressure liquid chromatography purification method. *Anal. Chem.* **88**(7): 3521–3528. <https://doi.org/10.1021/acs.analchem.5b03619>.
- Bro, R., and M. Vidal. 2011. EEMizer: Automated modeling of fluorescence EEM data. *Chemom. Intell. Lab. Syst.* **106**(1): 86–92. <https://doi.org/10.1016/j.chemolab.2010.06.005>.
- Butman, D. E., H. F. Wilson, R. T. Barnes, M. A. Xenopoulos, and P. A. Raymond. 2015. Increased mobilization of aged carbon to rivers by human disturbance. *Nat. Geosci.* **8**(2): 112–116. <https://doi.org/10.1038/NGEO2322>.
- Campeau, A., and others. (2017). Multiple sources and sinks of dissolved inorganic carbon across Swedish streams, refocusing the lens of stable C isotopes. *Sci. Rep.*, **7**(1), 1–14. <http://doi.org/10.1038/s41598-017-09049-9>.
- Caraco, N., J. E. Bauer, J. J. Cole, S. Petsch, and P. Raymond. 2010. Millennial-aged organic carbon subsidies to a modern river food web. *Ecology* **91**(8): 2385–2393. <https://doi.org/10.1890/09-0330.1>.
- Casas-Ruiz, J. P., and others. 2017. A tale of pipes and reactors: Controls on the in-stream dynamics of dissolved organic matter in rivers. *Limnol. Oceanogr.* **62**: S85–S94. <http://doi.org/10.1002/lno.10471>.
- Catalán, N., R. Marcé, D. N. Kothawala, and L. J. Tranvik. 2016. Organic carbon decomposition rates controlled by water retention time across inland waters. *Nat. Geosci.* **9**(7): 501–504. <http://doi.org/10.1038/ngeo2720>.
- Cherrier, J., J. E. Bauer, E. R. M. M. Druffel, R. B. Coffin, and J. P. Chanton. 1999. Radiocarbon in marine bacteria: Evidence for the ages of assimilated carbon. *Limnol. Oceanogr.* **44**(3): 730–736. <http://doi.org/10.4319/lo.1999.44.3.0730>.
- Cloern, J. E., E. A. Canuel, and D. Harris. 2002. Stable carbon and nitrogen isotope composition of aquatic and terrestrial plants of the San Francisco Bay estuarine system. *Limnol. Oceanogr.* **47**(3): 713–729. <http://doi.org/10.4319/lo.2002.47.3.0713>.
- Cole, J. J., and others (2007). Plumbing the global carbon cycle: Integrating inland waters into the terrestrial carbon budget. *Ecosystems*, **10**(1), 171–184. <https://doi.org/10.1007/s10021-006-9013-8>.
- Cory, R. M., C. P. Ward, B. C. Crump, and G. W. Kling. 2014. Sunlight controls water column processing of carbon in arctic fresh waters. *Science* **345**(6199): 925–928. <https://doi.org/10.1126/science.1253119>.
- Cottrell, B. A., and others (2013). A regional study of the seasonal variation in the molecular composition of rainwater. *Atmos. Environ.*, **77**, 588–597. <https://doi.org/10.1016/j.atmosenv.2013.05.027>.
- Crawford, J. T., and others. (2015). High-speed limnology: Using advanced sensors to investigate spatial variability in biogeochemistry and hydrology. *Environ. Sci. Technol.*, **49**(1), 442–450. <https://doi.org/10.1021/es504773x>.
- Creed, I. F., and others. (2015). The river as a chemostat: Fresh perspectives on dissolved organic matter flowing down the river continuum. *Can. J. Fish. Aquat. Sci.*, **72**(8): 1272–1285. <http://doi.org/10.1139/cjfas-2014-0400>.
- Dean, J., and others. (2018). Abundant pre-industrial carbon detected in Canadian Arctic headwaters—implications for

- the permafrost carbon feedback. *Environ. Res. Lett.*, **13**: 034024. <http://doi.org/10.1088/1748-9326/aaa1fe>.
- Evans, C. D., and others. (2013). Deep instability of deforested tropical peatlands revealed by fluvial organic carbon fluxes. *Nature*, **493**(7434), 660–3. <http://doi.org/10.1038/nature11818>.
- Evans, C. D., M. F. Futter, F. Moldan, S. Valinia, Z. Frogbrook, and D. Kothawala. 2017. Variability in organic carbon reactivity across lake residence time and trophic gradients. *Nat. Geosci.* **10**(11): 832. <https://doi.org/10.1038/ngeo3051>.
- Fellman, J. B., E. Hood, and R. G. M. Spencer. 2010. Fluorescence spectroscopy opens new windows into dissolved organic matter dynamics in freshwater ecosystems: A review. *Limnol. Oceanogr.* **55**(6): 2452–2462. <http://doi.org/10.4319/lo.2010.55.6.2452>.
- Fellman, J. B., E. Hood, P. A. Raymond, J. Hudson, M. Bozeman, and M. Arimitsu. 2015. Evidence for the assimilation of ancient glacier organic carbon in a proglacial stream food web. *Limnol. Oceanogr.* **60**(4): 1118–1128. <https://doi.org/10.1002/lno.10088>.
- Feng, X., and others. (2013). Differential mobilization of terrestrial carbon pools in Eurasian Arctic river basins. *Proc. Natl. Acad. Sci. USA*, **110**(35), 14168–14173. <https://doi.org/10.1073/pnas.1307031110>.
- Feng, X., J. E. Vonk, C. Griffin, N. Zimov, D. B. Montluçon, L. Wacker, and T. I. Eglinton. 2017. ¹⁴C variation of dissolved lignin in arctic river systems. *ACS Earth Space Chem.* **1**(6): 334–344. <http://doi.org/10.1021/acsearthspacechem.7b00055>.
- Fernandes, R., A. Dreves, M.-J. Nadeau, and P. M. Grootes. 2013. A freshwater lake saga: Carbon routing within the aquatic food web of lake Schwerin. *Radiocarbon* **55**(2–3): 1102–1113. https://doi.org/10.2458/azu_js_rc.55.16357.
- Findlay, S., R. L. Sinsabaugh, D. T. Fischer, and P. Franchini. 1998. Sources of dissolved organic carbon supporting planktonic bacterial production in the tidal freshwater Hudson River. *Ecosystems* **1**(3): 227–239. <https://doi.org/10.1007/s100219900018>.
- Finlay, J. C., M. E. Power, and G. Cabana. 1999. Effects of water velocity on algal carbon isotope ratios: implications for river food web studies. *Limnol. Oceanogr.* **44**(5): 1198–1203.
- Follett, C. L., D. J. Repeta, D. H. Rothman, L. Xu, and C. Santinelli. 2014. Hidden cycle of dissolved organic carbon in the deep ocean. *Proc. Natl. Acad. Sci. USA* **111**(47): 16706–16711. <http://doi.org/10.1073/pnas.1407445111>.
- Fujibayashi, M., M. Ogino, and O. Nishimura. 2016. Fractionation of the stable carbon isotope ratio of essential fatty acids in zebrafish *Danio rerio* and mud snails *Bellamya chinensis*. *Oecologia* **180**(2): 589–600. <https://doi.org/10.1007/s00442-015-3486-0>.
- Garnett, M. H., M. F. Billett, P. Gulliver, and J. F. Dean. 2016. A new field approach for the collection of samples for aquatic ¹⁴CO₂ analysis using headspace equilibration and molecular sieve traps: The super headspace method. *Ecohydrology* **9**(8): 1630–1638. <http://doi.org/10.1002/eco.1754>.
- Guillemette, F., T. S. Bianchi, and R. G. M. Spencer. 2017. Old before your time: Ancient carbon incorporation in contemporary aquatic foodwebs. *Limnol. Oceanogr.* **62**(4), 1682–1700. <https://doi.org/10.1002/lno.10525>.
- Hertkorn, N., and others (2006). Characterization of a major refractory component of marine dissolved organic matter. *Geochim. Cosmochim. Acta*, **70**(12), 2990–3010. <https://doi.org/10.1016/j.gca.2006.03.021>.
- Hertkorn, N., M. Harir, B. P. Koch, B. Michalke, and P. Schmitt-Kopplin. 2013. High-field NMR spectroscopy and FTICR mass spectrometry: Powerful discovery tools for the molecular level characterization of marine dissolved organic matter. *Biogeosciences* **10**(3): 1583–1624. <https://doi.org/10.5194/bg-10-1583-2013>.
- Hotchkiss, E. R., R. O. Hall Jr., R. A. Sponseller, D. Butman, J. Klaminder, H. Laudon, and J. Karlsson. 2015. Sources of and processes controlling CO₂ emissions change with the size of streams and rivers. *Nat. Geosci.* **8**(9): 696–699. <http://doi.org/10.1038/ngeo2507>.
- Hutchins, R. H. S., and others. (2017). The optical, chemical, and molecular dissolved organic matter succession along a boreal soil-stream-river continuum. *J. Geophys. Res. Biogeosci.*, **122**(11), 2892–2908. <http://doi.org/10.1002/2017JG004094>.
- Inamdar, S., and others. (2011). Fluorescence characteristics and sources of dissolved organic matter for stream water during storm events in a forested mid-Atlantic watershed. *J. Geophys. Res. Biogeosci.*, **116**(3): G03043. <http://doi.org/10.1029/2011JG001735>.
- Ingalls, A., and A. Pearson. 2005. Ten years of compound-specific radiocarbon analysis. *Oceanography* **18**(3): 18–31. <http://doi.org/10.5670/oceanog.2005.22>.
- Ishikawa, N. F., M. Uchida, Y. Shibata, and I. Tayasu. 2012. Natural C-14 provides new data for stream food-web studies: A comparison with C-13 in multiple stream habitats. *Mar. Freshw. Res.* **63**(3): 210–217. <https://doi.org/10.1071/MF11141>.
- Ishikawa, N. F., M. Uchida, Y. Shibata, and I. Tayasu. 2014. Carbon storage reservoirs in watersheds support stream food webs via periphyton production. *Ecology* **95**(5): 1264–1271. <https://doi.org/10.1890/13-0976.1>.
- Ishikawa, N. F., M. Yamane, H. Suga, N. O. Ogawa, Y. Yokoyama, and N. Ohkouchi. 2015. Chlorophyll a-specific $\Delta^{14}\text{C}$, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in stream periphyton: Implications for aquatic food web studies. *Biogeosciences* **12**(22): 6781–6789. <https://doi.org/10.5194/bg-12-6781-2015>.
- Ishikawa, N. F., and others. (2016). Terrestrial-aquatic linkage in stream food webs along a forest chronosequence: Multi-isotopic evidence. *Ecology*, **97**(5), 1146–1158. <https://doi.org/10.1890/15-1133.1>.
- Jaffé, R. and others 2013. Global charcoal mobilization from soils via dissolution and riverine transport to the oceans.

- Science **340**(6130): 345–347. <http://doi.org/10.1126/science.1231476>.
- Jeong, J. J. and others 2012. Differential storm responses of dissolved and particulate organic carbon in a mountainous headwater stream, investigated by high-frequency, in situ optical measurements. *J. Geophys. Res. Biogeosci.* **117**(3): 1–13. <http://doi.org/10.1029/2012JG001999>.
- Keaveney, E., P. Reimer, and R. Foy. 2015. Young, old, and weathered carbon—part 1: Using radiocarbon and stable isotopes to identify carbon sources in an alkaline, humic lake. *Radiocarbon* **57**(3): 407–423. https://doi.org/10.2458/azu_rc.57.18354.
- Kellerman, A. M., D. N. Kothawala, T. Dittmar, and L. J. Tranvik. 2015. Persistence of dissolved organic matter in lakes related to its molecular characteristics. *Nat. Geosci.* **8**: 454–459. <https://doi.org/10.1038/NGEO2440>.
- Koch, B. P., T. Dittmar, M. Witt, and G. Kattner. 2007. Fundamentals of molecular formula assignment to ultrahigh resolution mass data of natural organic matter. *Anal. Chem.* **79**(4): 1758–1763. <http://doi.org/10.1021/ac061949s>.
- Kothawala, D. N., C. A. Stedmon, R. A. Müller, G. A. Weyhenmeyer, S. J. Köhler, and L. J. Tranvik. 2014. Controls of dissolved organic matter quality: Evidence from a large-scale boreal lake survey. *Glob. Chang. Biol.* **20**(4): 1101–1114. <http://doi.org/10.1111/gcb.12488>.
- Lambert, T., S. Bouillon, F. Darchambeau, P. Massicotte, and A. V. Borges. 2016a. Shift in the chemical composition of dissolved organic matter in The Congo River network. *Biogeosciences* **13**(18): 5405–5420. <http://doi.org/10.5194/bg-13-5405-2016>.
- Lambert, T., C. R. Teodoru, F. C. Nyoni, S. Bouillon, F. Darchambeau, P. Massicotte, and A. V. Borges. 2016b. Along-stream transport and transformation of dissolved organic matter in a large tropical river. *Biogeosciences* **13**(9): 2727–2741. <http://doi.org/10.5194/bg-13-2727-2016>.
- Lang, S. Q., C. P. McIntyre, S. M. Bernasconi, G. L. Früh-Green, B. M. Voss, T. I. Eglinton, and L. Wacker. 2016. Rapid ^{14}C analysis of dissolved organic carbon in non-saline waters. *Radiocarbon* **58**(03): 505–515. <http://doi.org/10.1017/RDC.2016.17>.
- Larsen, T., M. Ventura, N. Andersen, D. M. O'Brien, U. Piatkowski, and M. D. McCarthy. 2013. Tracing carbon sources through aquatic and terrestrial food webs using amino acid stable isotope fingerprinting. *PLoS One* **8**(9): e73441. <https://doi.org/10.1371/journal.pone.0073441>.
- Maher, D. T., I. R. Santos, J. R. F. W. Leuven, J. M. Oakes, D. V. Erler, M. C. Carvalho, and B. D. Eyre. 2013. Novel use of cavity ring-down spectroscopy to investigate aquatic carbon cycling from microbial to ecosystem scales. *Environ. Sci. Technol.* **47**(22): 12938–12945. <https://doi.org/10.1021/es402776>.
- Maher, D. T., K. Cowley, I. R. Santos, P. Macklin, and B. D. Eyre. 2015. Methane and carbon dioxide dynamics in a subtropical estuary over a diel cycle: Insights from automated in situ radioactive and stable isotope measurements. *Mar. Chem.* **168**: 69–79. <http://doi.org/10.1016/j.marchem.2014.10.017>.
- Maie, N., C. Y. Yang, T. Miyoshi, K. Parish, and R. F. Jaffe. 2005. Chemical characteristics of dissolved organic matter in an oligotrophic subtropical wetland/estuarine ecosystem. *Limnol. Oceanogr.* **50**(1): 23–35. <https://doi.org/10.4319/lo.2005.50.1.0023>.
- Mann, P. J., and others. (2015). Utilization of ancient permafrost carbon in headwaters of Arctic fluvial networks. *Nat. Commun.*, **6**(7856), 1–7. <https://doi.org/10.1038/ncomms8856>.
- Marom, A., J. S. O. McCullagh, T. F. G. Higham, and R. E. M. Hedges. 2013. Hydroxyproline dating: Experiments on the ^{14}C analysis of contaminated and low-collagen bones. *Radiocarbon* **55**(2): 698–708. <https://doi.org/10.1017/S0033822200057854>.
- Martin, E. E., and others. (2013). Age of riverine carbon suggests rapid export of terrestrial primary production in tropics. *Geophys. Res. Lett.*, **40**(21), 5687–5691. <https://doi.org/10.1002/2013GL057450>.
- Mayorga, E., and others. (2005). Young organic matter as a source of carbon dioxide outgassing from Amazonian rivers. *Nature*, **436**(7050), 538–541. <https://doi.org/10.1038/nature03880>.
- McCallister, S. L., J. E. Bauer, J. E. Cherrier, and H. W. Ducklow. 2004. Assessing sources and ages of organic matter supporting river and estuarine bacterial production: A multiple-isotope ($\Delta^{14}\text{C}$, $\delta^{13}\text{C}$, and $\delta^{15}\text{N}$) approach. *Limnol. Oceanogr.* **49**(5): 1687–1702. <https://doi.org/10.4319/lo.2004.49.5.1687>.
- McCallister, S. L., F. Guillemette, and P. A. del Giorgio. 2006. A system to quantitatively recover bacterioplankton respiratory CO_2 for isotopic analysis to trace sources and ages of organic matter consumed in freshwaters. *Limnol. Oceanogr. Methods* **4**: 406–415. <https://doi.org/10.4319/lom.2006.4.406>.
- McCallister, S. L., and P. A. del Giorgio. 2008. Direct measurement of the $\delta^{13}\text{C}$ signature of carbon respired by bacteria in lakes: Linkages to potential carbon sources, ecosystem baseline metabolism, and CO_2 fluxes. *Limnol. Oceanogr.* **53**(4): 1204–1216. <http://doi.org/10.4319/lo.2008.53.4.1204>.
- McCallister, S. L., and P. A. del Giorgio. 2012. Evidence for the respiration of ancient terrestrial organic C in northern temperate lakes and streams. *Proc. Natl. Acad. Sci. USA* **109**(42): 16963–16968. <https://doi.org/10.1073/pnas.1207305109>.
- McIntosh, H. A., A. P. McNichol, L. Xu, and E. A. Canuel. 2015. Source-age dynamics of estuarine particulate organic matter using fatty acid $\delta^{13}\text{C}$ and $\Delta^{14}\text{C}$ composition. *Limnol. Oceanogr.* **60**(2): 611–628. <https://doi.org/10.1002/lno.10053>.

- McGowan, S., and others. (2016). Long-term perspectives on terrestrial and aquatic carbon cycling from palaeolimnology. *Wiley Interdiscip. Rev. Water*, **3**(2), 211–234. <https://doi.org/10.1002/wat2.1130>.
- Meinson, P., A. Idrizaj, P. Nöges, T. Nöges, and A. Laas. 2016. Continuous and high-frequency measurements in limnology: history, applications, and future challenges. *Environ. Rev.* **24**(1): 52–62. <https://doi.org/10.1139/er-2015-0030>.
- Miller, M. P., and D. M. McKnight. 2010. Comparison of seasonal changes in fluorescent dissolved organic matter among aquatic lake and stream sites in the Green Lakes Valley. *J. Geophys. Res.* **115**: G00F12. <http://doi.org/10.1029/2009JG000985>.
- Mopper, K., A. Stubbins, J. D. Ritchie, H. M. Bialk, and P. G. Hatcher. 2007. Advanced instrumental approaches for characterization of marine dissolved organic matter: Extraction techniques, mass spectrometry, and nuclear magnetic resonance spectroscopy. *Chem. Rev.* **107**(2): 419–442. <http://doi.org/10.1021/cr050359b>.
- Morling, K., N. Kamjunke, and J. Tittel. 2016. A simplified method of recovering CO₂ from bacterioplankton respiration for isotopic analysis. *J. Microbiol. Methods* **121**: 8–10. <https://doi.org/10.1016/j.mimet.2015.12.008>.
- Mosher, J. J., Kaplan, L. A., Podgorski, D. C., McKenna, A. M., & Marshall, A. G. (2015). Longitudinal shifts in dissolved organic matter chemogeography and chemodiversity within headwater streams: A river continuum reprise. *Biogeochemistry*, **124**(1–3), 371–385. <https://doi.org/10.1007/s10533-015-0103-6>.
- Murphy, K. R., K. D. Butler, R. G. M. Spencer, C. A. Stedmon, J. R. Boehme, and G. R. Aiken. 2010. Measurement of dissolved organic matter fluorescence in aquatic environments: An interlaboratory comparison. *Environ. Sci. Technol.* **44**(24): 9405–9412. <https://doi.org/10.1021/es102362t>.
- Murphy, K. R., C. A. Stedmon, D. Graeber, and R. Bro. 2013. Fluorescence spectroscopy and multi-way techniques. PARAFAC. *Anal. Methods* **5**(23): 6557–6566. <https://doi.org/10.1039/c3ay41160e>.
- Murphy, K. R., C. A. Stedmon, P. Wenig, and R. Bro. 2014. OpenFluor—an online spectral library of auto-fluorescence by organic compounds in the environment. *Anal. Methods* **6**(3): 658–661. <https://doi.org/10.1039/c3ay41935e>.
- Ohkouchi, N., Y. Nakajima, H. Okada, N. O. Ogawa, H. Suga, K. Oguri, and H. Kitazato. 2005. Biogeochemical processes in the saline meromictic Lake Kaiike, Japan: Implications from molecular isotopic evidences of photosynthetic pigments. *Environ. Microbiol.* **7**(7): 1009–1016. <https://doi.org/10.1111/j.1462-2920.2005.00772.x>.
- Petrone, K. C., J. B. Fellman, E. Hood, M. J. Donn, and P. F. Grierson. 2011. The origin and function of dissolved organic matter in agro-urban coastal streams. *J. Geophys. Res. Biogeosci.* **116**(1): G01028. <http://doi.org/10.1029/2010JG001537>.
- Pollard, P. C. 2013. In situ rapid measures of total respiration rate capture the super labile doc bacterial substrates of freshwater. *Limnol. Oceanogr. Methods* **11**: 584–593. <https://doi.org/10.4319/lom.2013.11.584>.
- Raymond, P. A., and J. E. Bauer. 2001. Use of ¹⁴C and ¹³C natural abundances for evaluating riverine, estuarine, and coastal DOC and POC sources and cycling: A review and synthesis. *Org. Geochem.* **32**(4): 469–485. [https://doi.org/10.1016/S0146-6380\(00\)00190-X](https://doi.org/10.1016/S0146-6380(00)00190-X).
- Raymond, P. A., and others. (2007). Flux and age of dissolved organic carbon exported to the Arctic Ocean: A carbon isotopic study of the five largest arctic rivers. *Glob. Biogeochem. Cycles*, **21**(4), 1–9. <https://doi.org/10.1029/2007GB002934>.
- Raymond, P. A., and others. (2013). Global carbon dioxide emissions from inland waters. *Nature*, **503**(7476), 355–359. <https://doi.org/10.1038/nature12760>.
- Raymond, P. A., J. E. Saiers, and W. V. Sobczak. 2016. Hydrological and biogeochemical controls on watershed dissolved organic matter transport: Pulse-shunt concept. *Ecology* **97**(1): 5–16. <http://doi.org/10.1890/14-1684.1>.
- Repeta, D. J., T. M. Quan, L. I. Aluwihare, and A. Accardi. 2002. Chemical characterization of high molecular weight dissolved organic matter in fresh and marine waters. *Geochim. Cosmochim. Acta* **66**(6): 955–962. [http://doi.org/10.1016/S0016-7037\(01\)00830-4](http://doi.org/10.1016/S0016-7037(01)00830-4).
- Rosenheim, B. E., and V. Galy. 2012. Direct measurement of riverine particulate organic carbon age structure. *Geophys. Res. Lett.* **39**(19): 1–6. <http://doi.org/10.1029/2012GL052883>.
- Saraceno, J. F., B. A. Pellerin, B. D. Downing, E. Boss, P. A. M. Bachand, and B. A. Bergamaschi. 2009. High-frequency in situ optical measurements during a storm event: Assessing relationships between dissolved organic matter, sediment concentrations, and hydrologic processes. *J. Geophys. Res. Biogeosci.* **114**(4): 1–11. <http://doi.org/10.1029/2009JG000989>.
- Schefuß, E., and others (2016). Hydrologic control of carbon cycling and aged carbon discharge in The Congo River basin. *Nat. Geosci.*, **9**(9), 687–690. <https://doi.org/10.1038/ngeo2778>.
- Schiff, A. S. L., R. Aravena, S. E. Trumbore, M. J. Hinton, R. Elgood, and P. J. Dillon. 1997. Export of DOC from forested catchments on the Precambrian shield of central Ontario: Clues from ¹³C and ¹⁴C source. *Biogeochemistry* **36**(1): 43–65.
- Sessions, A. L., S. P. Sylva, and J. M. Hayes. 2005. Moving-wire device for carbon isotopic analyses of nanogram quantities of nonvolatile organic carbon. *Anal. Chem.* **77**(20): 6519–6527. <http://doi.org/10.1021/ac051251z>.
- Simpson, A. J., and others. (2004). The application of LC-NMR and LC-SPE-NMR to compositional studies of natural organic matter. *Analyst*, **129**(12), 1216–1222. <https://doi.org/10.1039/b408064e>.
- Sobczak, W. V., and P. a. Raymond. 2015. Watershed hydrology and dissolved organic matter export across time scales:

- minute to millennium. *Freshwater Sci.* **34**(1): 392–398. <http://doi.org/10.1086/679747>.
- Spencer, R. G. M., and others. (2007). Diurnal variability in riverine dissolved organic matter composition determined by *in situ* optical measurements in the San Joaquin River (California, USA). *Hydrol. Process.*, **21**, 3181–3189. <https://doi.org/10.1002/hyp.6887>.
- Spencer, R. G. M., and others. (2012). An initial investigation into the organic matter biogeochemistry of The Congo River. *Geochim. Cosmochim. Acta*, **84**, 614–627. <https://doi.org/10.1016/j.gca.2012.01.013>.
- Stedmon, C. A., and R. Bro. 2008. Characterizing dissolved organic matter fluorescence with parallel factor analysis: A tutorial. *Limnol. Oceanogr.: Methods* **6**: 572–579. <https://doi.org/10.4319/lom.2008.6.572>.
- Stubbins, A., and others. (2010). Illuminated darkness: Molecular signatures of Congo River dissolved organic matter and its photochemical alteration as revealed by ultrahigh precision mass spectrometry. *Limnol. Oceanogr.*, **55**(4), 1467–1477. <https://doi.org/10.4319/lo.2010.55.4.1467>.
- Tao, S., T. I. Eglinton, D. B. Montluçon, C. McIntyre, and M. Zhao. 2015. Pre-aged soil organic carbon as a major component of the Yellow River suspended load: Regional significance and global relevance. *Earth Planet. Sci. Lett.* **414**: 77–86. <https://doi.org/10.1016/j.epsl.2015.01.004>.
- Vannote, R. L., G. W. Minshall, K. W. Cummins, J. R. Sedell, and C. E. Cushing. 1980. River continuum concept. *Can. J. Fish. Aquat. Sci.* **3**: 130–137. <https://doi.org/10.1139/f80-017>.
- Vonk, J. E., and others. (2015). Reviews and syntheses: Effects of permafrost thaw on Arctic aquatic ecosystems. *Biogeosciences*, **12**(23), 7129–7167. <http://doi.org/10.5194/bg-12-7129-2015>.
- Webb, J. R., D. T. Maher, and I. R. Santos. 2016. Automated, *in situ* measurements of dissolved CO₂, CH₄, and d¹³C values using cavity enhanced laser absorption spectrometry: Comparing response times of air-water equilibrators. *Limnol. Oceanogr. Methods* **14**(5): 323–337. <https://doi.org/10.1002/lom3.10092>.
- Wickland, K. P., J. C. Neff, and G. R. Aiken. 2007. Dissolved organic carbon in Alaskan boreal forest: Sources, chemical characteristics, and biodegradability. *Ecosystems* **10**(8): 1323–1340. <https://doi.org/10.1007/s10021-007-9101-4>.
- Zhang, Y., and others. (2010). Characteristics and sources of chromophoric dissolved organic matter in lakes of the Yungui Plateau, China, differing in trophic state and altitude. *Limnol. Oceanogr.*, **55**(6), 2645–2659. <http://doi.org/10.4319/lo.2010.55.6.2645>.
- Zigah, P. K., E. C. Minor, J. P. Werne, and S. L. McCallister. 2011. Radiocarbon and stable carbon isotopic insights into provenance and cycling of carbon in Lake Superior. *Limnol. Oceanogr.* **56**(3): 867–886. <https://doi.org/10.4319/lo.2011.56.3.0867>.

Acknowledgments

We are grateful to three anonymous reviewers for their thoughtful and constructive feedback which greatly improved the quality of the manuscript. This work was funded in part by NSF-DEB 1127962 to S.L.M. D.N.K. acknowledges a starting grant from the Swedish National Science Foundation Vetenskapsrådet. N.F.I was a Research Fellow (25-1021) and an Overseas Research Fellow (28-0214) of the Japan Society for the Promotion of Science.

Submitted 24 August 2017

Revised 14 August 2018

Accepted 09 September 2018