Ecosystems (2013) 16: 604–616
DOI: 10.1007/s10021-013-9635-6

ECOSYSTEMS

Hydrologic Drivers and Seasonality of Dissolved Organic Carbon Concentration, Nitrogen Content, Bioavailability, and Export in a Forested New England Stream

Henry F. Wilson,[1,2]* James E. Saiers,[1] Peter A. Raymond,[1] and William V. Sobczak[3]

[1]*School of Forestry and Environmental Studies, Yale University, New Haven, Connecticut, USA;* [2]*Brandon Research Centre, Agriculture and Agri-Food Canada, Brandon, Manitoba, Canada;* [3]*Biology Department, Holy Cross College, Worcester, Massachusetts, USA*

Abstract

We present the results of a full year of high-resolution monitoring of hydrologic event-driven export of stream dissolved organic matter (DOM) from the forested Bigelow Brook watershed in Harvard Forest, Massachusetts, USA. A combination of *in situ* fluorescent dissolved organic matter (FDOM) measurement, grab samples, and bioassays was utilized. FDOM was identified as a strong indicator of concentration for dissolved organic carbon (DOC, $r^2 = 0.96$), dissolved organic nitrogen (DON, $r^2 = 0.81$), and bioavailable DOC (BDOC, $r^2 = 0.81$). Relationships between FDOM and concentration were utilized to improve characterization of patterns of hydrological event-driven export and the quantification of annual export. This characterization was possible because DOM composition remained relatively consistent seasonally; however, a subtle shift to increased fluorescence per unit absorbance was observed for summer and fall seasons and percent BDOC did increase slightly with increasing concentrations. The majority of export occurred during pulsed hydrological events, so the greatest impact of bioavailable exports may be on downstream aquatic ecosystems. Export from individual events was highly seasonal in nature with the highest flow weighted mean concentrations (DOC_{FW}) being observed in late summer and fall months, but the highest total export being observed for larger winter storms. Seasonal trends in DOC export coincide with weather driven changes in surface and subsurface flow paths, potential for depletion and rebuilding of a flushable soil organic matter pool, and the availability of terrestrial carbon sources such as leaf litter. Our approach and findings demonstrate the utility of high frequency FDOM measurement to improve estimates of intra-annual temporal trends of DOM export.

Key words: dissolved organic carbon (DOC); dissolved organic nitrogen (DON); bioavailable organic carbon (BDOC); fluorescent dissolved organic matter (FDOM); forested; stream carbon export; hydrologic event; seasonal; annual.

Received 30 July 2012; accepted 27 November 2012;
published online 30 January 2013

Electronic supplementary material: The online version of this article (doi:10.1007/s10021-013-9635-6) contains supplementary material, which is available to authorized users.

Author contributions: HFW conceived of or designed study, performed research, analyzed data, contributed new methods or models, and wrote the paper; JES and PAR conceived of or designed study, and wrote the paper; and WVS conceived of or designed study, performed research, and wrote the paper.

**Corresponding author; e-mail:* henry.wilson@agr.gc.ca

Introduction

Small headwater streams have been estimated to make up more than 60% of the total linear distance covered by flowing waters in the United States (Leopold and others 1964; Butman and Raymond 2011). These streams represent the interface between the terrestrial and aquatic components of the watershed and play an ecologically critical role in the delivery of energy and nutrients to downstream aquatic ecosystems (Lowe and Likens 2005; Kaplan and others 2008). Headwater streams are common features of many upland-forested watersheds in the United States, yet are not explicitly factored into forest carbon and nutrient budgets. An understanding is emerging from terrestrial studies that the net metabolism of many ecosystems is often very close to being balanced on annual and decadal time scales (Cole and others 2007; Gielen and others 2011); therefore, the fluvial loss of materials to streams may be an important, yet overlooked term in carbon and nutrient budgets. Annual aqueous exports of organic matter are often small in comparison to terrestrial respiration of carbon, but over longer timescales this loss may be important in quantifying regional carbon balances. Regardless of this difference in magnitude, aqueous organic matter fluxes are particularly important in shaping the ecology of downstream aquatic environments (Aitkenhead-Peterson and others 2003). A growing body of research indicates that in forested watersheds the transport of dissolved organic matter (DOM) represents a large and ecologically essential flux of carbon and organically bound nutrients from terrestrial to aquatic environments (Qualls and Haines 1991; Hedin and others 1995; Aitkenhead-Peterson and others 2003; Neff and others 2003).

It is well recognized that the timing and magnitude of DOM flux from forested watersheds is strongly controlled by storms and snowmelt events (Boyer and others 1996; Mulholland 2003; Fellman and others 2009; Sebestyen and others 2009; Raymond and Saiers 2010; Pellerin and others 2012), with recent meta-data analyses indicating that around 86% of the export of dissolved organic carbon (DOC) from forested watersheds in the eastern United States occurs during hydrological events (Raymond and Saiers 2010). In addition to increased DOC export and concentration with hydrological events, increases in the amount of dissolved organic nitrogen (DON) and bioavailable DOC (BDOC) are observed (Buffam and others 2001; Inamdar and Mitchell 2007; Fellman and others 2009). Both DON and BDOC can be associated with the reactivity of DOM (Wiegner and others 2006), highlighting the importance of hydrological events as ‘‘hot moments’’ of both energy and organic nutrient movement from forested watersheds (McClain and others 2003).

Given that rapidly changing discharge patterns often characterize ‘‘flashy’’ headwater streams (Baker and others 2004), accurate characterization of changes in export rates and concentrations of DOM during hydrological events can be particularly challenging (Pellerin and others 2012). In flashy systems with quick response times and short event lengths, it becomes increasingly challenging to utilize traditional sampling programs to characterize event patterns of DOC concentration with changing discharge. Quantification and characterization of these fine scale changes provides insight into watershed physiochemical processes controlling DOC export patterns, improves estimates of export over longer time scales, and can be used to improve a variety of watershed modeling efforts (for example, Pellerin and others 2012; Raymond and Saiers 2010; Buffam and others 2001).

Here we couple high-resolution hydrology and organic carbon biogeochemistry data to examine seasonal differences in hydrologic event-driven export of stream DOM, its N-composition, and its bioavailability. We use a combination of an *in situ* fluorescent dissolved organic matter (FDOM) sensor, grab samples, and conventional bioassays to monitor DOC, DON, and BDOC dynamics in a forested headwater stream and couple this data with high-resolution meteorological and discharge datasets. We report on seasonal variations in the hydrologic phenomena that influence DOC, DON, and BDOC export in a highly studied forested watershed. We also provide estimates of annual DOC, DON, and BDOC export that integrate the varying influence of seasonal, episodic storm events. We observe the greatest potential for high export subsidies of DOC, DON, and BDOC to downstream ecosystems for large storm events occurring during summer and fall months. Seasonal variations in concentration correlate not only with event size, but also with history of flushing, likely changes in flow path, and terrestrial carbon source availability.

Methods

Site Description

This study focused on a 1st order section of Bigelow Brook, which is located in the Prospect Hill Tract of the Harvard Forest Long-Term Ecological Research (LTER) Site in north central Massachusetts, USA (lat 42.5°N, long 72°W). The catchment area is 24 ha and is currently under forest cover that has regrown since land clearance and use for pasture throughout the 1800s (Foster 1992). Currently, Eastern Hemlock (*Tsuga canadensis* L.) is common in the vegetation of the catchment and dominates riparian areas, creating a relatively cool and shaded environment (Willacker and others 2009). Surface slope ranges from 15 to 45% in upland areas, but tends to be lower in riparian areas. The catchment is well drained with rocky glacial till derived soil deposits. Monthly mean temperatures range from −7°C in January to 19°C in July (Currie and others 1996). Mean annual precipitation at the Harvard Forest LTER is 110 cm, distributed relatively evenly throughout the year (Harvard Forest 2011).

Hydrological and Meteorological Data

The Fisher Meteorological Station is located 1.5 km from the outlet of the study catchment and is maintained by the Harvard Forest LTER study. In this analysis we utilized measurements of air temperature, precipitation, and soil temperature at 10-cm depth that were recorded every 15 min at the station. In-stream instrumentation was located to coincide with stream discharge measurements being monitored on the Upper Bigelow Brook through the Harvard Forest LTER study. Discharge is calculated at a 15-min interval from depth measurements recorded using a pressure transducer (Druck 1830) and a stage-rating curve. Stream temperature was measured at this same 15-min interval by a Campbell CS547A-L conductivity/temperature sensor connected to a Campbell CR-1000 data logger.

Collection and Analysis of Water Samples

Water samples were collected between October 2009 and November 2010, during both baseflow and stormflow periods (n = 125). Intensive collection periods were targeted to major hydrological events that were expected to dominate export fluxes and less intensive sampling continued throughout base flow periods. Storm events in late spring and early summer were relatively small and flashy in nature, making timing of sampling more challenging, but at least one storm event per season was sampled intensively. Samples were collected into acid washed 1 l polyethylene or polycarbonate bottles either by hand or using an automated sampler (ISCO 3700). Samples were kept on ice and filtered within 36 h of collection through prerinsed 0.22 µm pore size Millipore polycarbonate filters. DOC and DON were quantified using standard analytical methods (Appendix 1 in Supplementary material).

Bioavailable DOC (BDOC) was determined as the change in DOC over single 30-day laboratory incubations. For these incubations, 125 ml of filtered water was re-inoculated with 1 ml of stream water inoculum in loosely capped polycarbonate bottles that were incubated at laboratory temperature under dark conditions. The inoculum was prepared for each sampling event by mixing 50 ml of unfiltered water from each sample collected. Samples were refiltered prior to measurement of final concentration, so we define BDOC as both the DOC lost through complete mineralization to CO_2 and by incorporation into microbial cell material, but not released (Fellman and others 2008).

Generation of High-Temporal Resolution Estimates of DOM Chemistry and Bioavailability Based on FDOM Measures

High-temporal resolution estimates of DOC, DON, and BDOC were generated based on the relationship between laboratory measures of concentration in periodic grab samples and corresponding measurements of FDOM made in-stream (Appendix 1 in Supplementary material). FDOM measures were collected every 15 min (as a single reading) between October 2009 and November 2010 using a Turner Designs Cyclops-7 colored dissolved organic matter (CDOM) fluorometer sensor connected to a Campbell CR-1000 data logger. Of the 365 days over which the instrument was installed, measurements were not collected on 57 days due to low water level and ice formation (44 days), leaf litter accumulation over the sensor (5 days), programming errors (7 days), and debris disturbance caused by the autosampler rinsing process during low flow (1 day). All other missing data occurred during periods without flow and did not impact export measurement. For a detailed description of resulting data gaps see Appendix 1 (Supplementary material). The position of the FDOM sensor was shifted seasonally to maintain submergence with changing water levels (Appendix 1 in Supplementary material). With all placements the sensor was situated under an overhanging boulder to provide constant shading. The sensor was removed for inspection at least monthly, was inspected before each discrete sampling event, and showed noticeable biofouling only after a prolonged period of no flow and standing water in early August.

A strong linear relationship was observed between DOC and *in situ* measurements of FDOM ($r^2 = 0.85$, $P < 0.0001$). However, as compared to check measurements of FDOM made systematically using a laboratory fluorometer (Varian Cary Eclipse) and corrected for inner filter effects (absorbance; Beckman DU 520 spectrophotometer), Cyclops-7 (*in situ*) measures of FDOM were observed to vary with temperature, concentration, and DOM composition. Therefore, FDOM measures were calibrated by comparison with a laboratory fluorometer under controlled conditions and corrected (as described in Appendix 1 in Supplementary material) for temperature impact on fluorescence intensity (using methods similar to Watras and others 2011), concentration driven inner filter effects (similar to Downing and others 2012), and seasonal changes in DOM character. Corrected data were calibrated to Raman Units (R.U.), which can be converted to quinine sulfate units (Q.S.U.; ppb) using the formula Q.S.U. = R.U./0.0767 (Lawaetz and Stedmon 2009).

Hydrologic Event Identification and the Influence of Changing Ecosystem Conditions on Event Driven DOM Export Patterns

Export was estimated for each 15-min measurement interval as the product of Q (L per 15 min) and DOC, DON, or BDOC concentrations (mg l^{-1}) as modeled using the FDOM measurement made at the beginning of the interval as a predictor for concentration (Appendix 1in Supplementary material). Total export for each event was calculated as the sum of export for all 15-min measurement intervals in the event. Because DOC, DON, and BDOC exports and concentrations were highly correlated and because FDOM was used to calculate all of these variables, our analysis of environmental controls on export focused on a single component as a response variable, DOC. Total volume of flow drives export, so flow weighted mean DOC (DOC_{FW}; DOC export/event volume) was utilized to identify influences on export as driven by concentration changes, rather than event volume.

Hydrologic events were identified by analysis of the time series of stream discharge (Q) using the HYSEP method (Sloto and Crouse 1996; Raymond and Saiers 2010) to identify those periods with total discharge in excess of baseflow. Baseflow is steady and continues after the event, whereas the discharge in excess of baseflow that begins with event onset is identified as quickflow (Qq). Excluding those events where FDOM measures could not be collected due to stream conditions, a total of 21 well-defined events were identified between October 30, 2009 and October 30, 2010.

The following measures were derived from hydrological and meteorological datasets to describe temporal changes in environmental conditions: total volume of water exported during the event, volume of water exported during the preceding storm, starting Q (last Q measurement before the onset of Qq), magnitude (maximum Q-starting Q), magnitude of the preceding event, the 1, 3, and 5 day antecedent volumes of water exported, event duration, time since last event, time to peak Q, mean air temperature, soil temperature at 10 cm, and stream water temperature. If any pair of environmental variables was highly correlated and had similar physical meaning, only one of these variables was included in further analysis. To meet the assumption of normality, input data were transformed prior to multiple factor regression (Appendix 1 in Supplementary material).

The potential influences of hydrological and meteorological conditions on DOC_{FW} were examined through univariate correlation and multiple factor regression. In regression analysis for DOC_{FW} we generated all possible multiple factor regression models using JMP statistical software (SAS Institute, 2010) and present the three models with highest probability of best fit for discussion (as identified by Akaike weights (w_i); Westphal and others 2003; see Appendix 1 (Supplementary material) for additional information on w_i calculation). The best forward stepwise linear regression model for DOC_{FW} was also identified (probability to enter and leave were 0.15 and 0.10) and was among the top three Akaike weights models. All models presented as best fits were evaluated for collinearity among predictor variables using the variance inflation factor (VIF) and no collinearity was detected (VIF < 10).

For DOC, DON, and BDOC export, forward stepwise regression was utilized to identify models for prediction of export for events with missing concentration data and for comparison with models for DOC_{FW}. The best stepwise models for DOC, DON, and BDOC export were utilized for prediction in gap filling (Appendix 1 in Supplementary material).

Calculation of Annual DOC, BDOC, and DON Export

Total export for the year from October 30, 2009 to October 30, 2010 was calculated similarly to export for individual events, although a gap filling procedure was used where FDOM measures were missing. The predictive models used for gap filling export values for hydrological events with missing FDOM data were developed based on observed influences of environmental conditions on export and are described in the results. This procedure was utilized for four small events in December without measurement due to ice formation, a small event in July where debris disturbed by the autopsampler rinse process impacted the FDOM signal, and one very short event in mid-September where water levels did not increase sufficiently from dry conditions to allow for sensor measurement. Where FDOM data were missing during base flow, a linear change in concentration between known values at the beginning and end of the period was assumed to fill missing values. Gap filled export values for hydrological events and during base flow account for 1.4 and 6% of total modeled export, respectively. Total annual export and estimates of uncertainty were calculated as the 50th, 5th, and 95th percentiles of a randomization procedure to incorporate error from flow measurement, concentration measurement, and gap filling relationships (Appendix 1 in Supplementary material).

Results

Seasonal Changes in DOM Composition

Four seasons were defined based on dominant environmental conditions: (1) from leaf fall through to ice formation (fall), (2) ice formation to snowmelt onset (winter), (3) snowmelt onset to return to baseflow after freshet recession, and (4) from spring to leaf fall (summer). Despite a relatively consistent DOC:DON stoichiometry of DOM for samples collected over the course of the year at our study site (mean DOC:DON = 52, standard deviation of 19), subtle C:N changes were observed seasonally (Figure 1). In addition, changes in the amount of fluorescence per unit absorbance were observed (Figure 1) with seasonal increases from fall through to summer. Across all seasons, DOC and DON relate linearly (Figure 1) with a similar slope in the relationship and concurrent increase with flow; however, fall and snowmelt periods exhibit slightly reduced DON content. DOC:DON is most variable at low concentrations, but these differences are likely amplified by decreasing detectability of DOC at very low concentrations and less variability was observed at higher concentrations.

The relationship between BDOC and DOC did not show clear temporal variation and remained relatively consistent over the course of the year (Figure 1). As DOC concentrations increased during higher discharge conditions, BDOC content increased more rapidly (Figure 1) and as a result, percent bioavailability also increased with DOC concentration (r^2 = 0.32, $P < 0.001$).

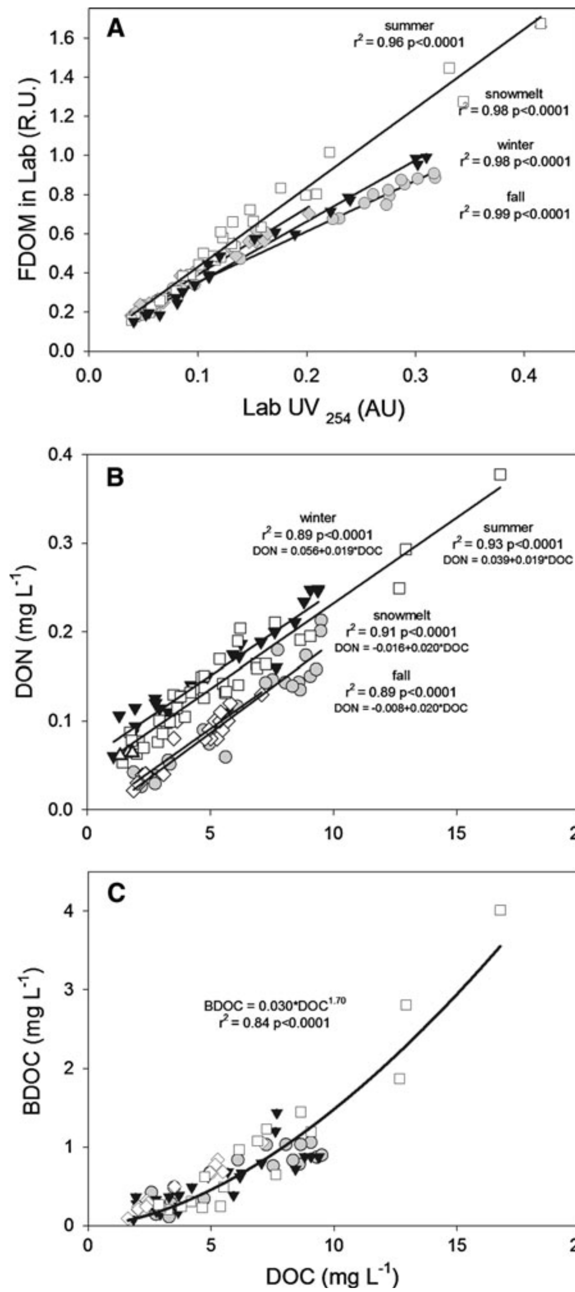

Figure 1. Seasonal FDOM:UV_{254} (**A**), DOC/DON (**B**), and DOC/BDOC (**C**) relationships for samples collected over a range of flow conditions in Bigelow Brook. *Gray circles* indicate samples collected in fall of 2009, downward facing *filled triangles* indicate samples collected in winter of 2009, *gray diamonds* indicate samples collected during snowmelt in 2010, and *white rectangles* indicate samples collected in summer of 2010. No significant seasonal difference was evident in the BDOC/DOC relationship so a single regression was utilized to characterize the overall annual pattern. Time periods for seasonal characterization were defined based on changing climatic conditions and observed seasonal variation in fluorescence intensity per unit absorbance (Appendix 1 in Supplementary material).

Characterization of FDOM and Hydrological Patterns, and Associated Relationships with DOC, DON, and BDOC Concentrations

During the period from October 30, 2009 to October 30, 2010, modeled concentrations (and 95% prediction intervals) ranged from $1.07^{(-0.09–2.23)}$ to $16.71^{(15.47–17.94)}$ mg l^{-1} for DOC, from $0.040^{(-0.02–0.098)}$ to $0.36^{(0.29-0.42)}$ mg l^{-1} for DON, and from $0.12^{(0.005–0.39)}$ to $3.20^{(2.23–4.35)}$ mg l^{-1} for BDOC. A strong linear relationship for each DOM component was observed with FDOM across the full year of sampling. For DOC, DON, and BDOC, r^2 values were 0.96, 0.79, and 0.81, respectively ($P < 0.001$; Appendix 1 in Supplementary material).

Stream discharge over the hydrological year ranged from 1.72 mm h^{-1} observed during a rain on snow event in January of 2009 to 0 mm h^{-1} during a dry period in late August of 2010 (Figure 2). The total precipitation for the year was 1,139 mm, near the long-term mean of 1,100 mm as measured at the Fisher Meteorological Station.

FDOM, DOC, DON, and BDOC consistently showed increases during periods of higher Q; however, maximum concentration did not occur at maximum Q (Figures 2, 3). The highest concentrations were observed during a storm event in October of 2010, whereas maximum concentrations for higher discharge events in January and March were lower, highlighting the differential nature of export patterns observed under contrasting environmental conditions.

Despite the existence of minor hysteresis in the relationship between concentration and Q, the nature of concentration changes with changing discharge could be summarized well for each event using a power law equation of $DOC = k(Q)^n$ (r^2 values ranged from 0.40 to 0.92 with an average best fit of 0.79, all P values < 0.001) (Figure 3). In comparing the resulting modeled DOC versus Q relationships for storm events over the course of the year, a consistent seasonal trend is evident. During fall and summer months, higher discharge normalized concentrations were observed (higher k) and concentrations tended to increase rapidly with event onset, approach a peak concentration quickly, maintain elevated concentration for longer, but decrease rapidly toward the end of an event (lower n). In spring and winter months, slower rates of increase toward a lower maximum concentration and a slower decrease with event recession were observed (Figure 3).

DOC, BDOC, and DON Export

On an event basis, modeled DOC, BDOC, and DON exports were strongly controlled by event volume, but residual variations in export and DOC_{FW} were influenced by other environmental conditions. A high degree of correlation was observed among these variables due to common underlying climate and weather driven controls (Table 1). Volume of the last event and event magnitude were important predictors of residual variation in models for export and were included in two of the top three models for DOC_{FW} (Table 2); however, starting Q, event length, time since last event, and event volume were also identified as potential predictors of DOC_{FW} (Table 1).

Utilizing measurements of DOC, DON, and BDOC as modeled every 15 min based on FDOM measurements, 50th, 5th, and 95th percentiles for annual exports from the Upper Bigelow Brook over the period from October 2009 to November 2010 were 17.2 kg ha^{-1} (17.1–17.3), 0.448 kg ha^{-1} (0.444–0.451), and 2.17 kg ha^{-1} (2.15–2.19), respectively. The majority of export occurred during hydrological events (63%) or during the non-event snowmelt conditions between March 1 and May 1, 2010 (20%). The overall period of snowmelt runoff in late March was particularly important in defining total annual export due to high discharge rates, but large events in January, February, and October were also important (Figure 2).

Discussion

Utilization of FDOM to Improve Measurement of Trends in Concentration and Export of DOM Components

Here we demonstrate the utility of high frequency FDOM measurement to improve estimates of temporal trends in concentration and annual export estimates of DOC, DON, and BDOC in a forested stream. Our study is among the first to utilize high-resolution FDOM measurements over a full year in exploring DOM dynamics and our results indicate that with adequate calibration, FDOM can offer a useful indicator of DOC, DON, and BDOC concentrations at Bigelow Brook and can significantly improve estimates of annual and event-based export.

However, our results also indicate that variation in DON and BDOC content of DOM is more variable than for DOC, as are relationships with FDOM. Further research is required to more clearly define expected DON variation with season and changing

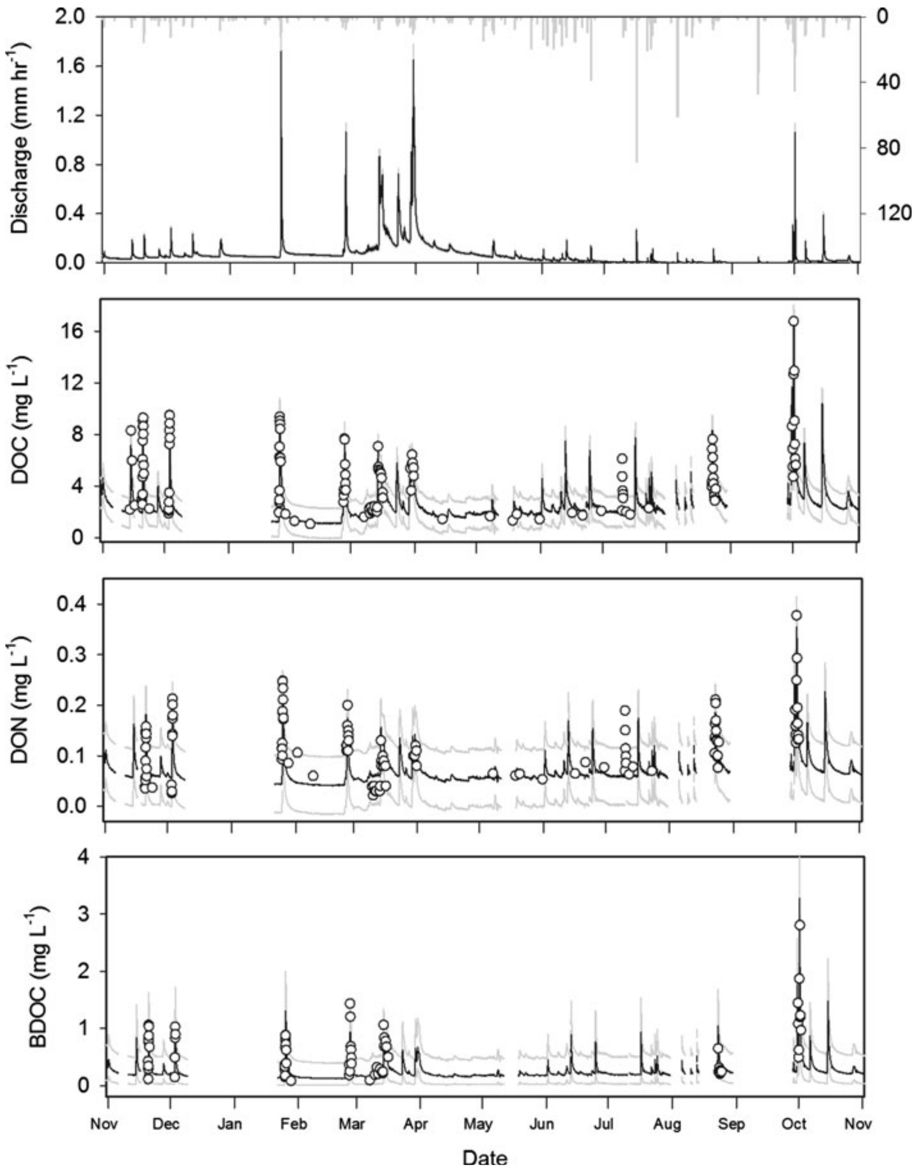

Figure 2. Time series of precipitation, discharge, and modeled DOC, DON, and BDOC concentrations for Bigelow Brook. The 95% prediction intervals for modeled concentrations are based on regression of measured values with FDOM and are indicated by *light gray lines*. The 95% prediction intervals for discharge measurement based on water level are also indicated with *light gray lines*. *Open circles* show measured concentration values. *Gray bars* in the panel showing discharges show hourly precipitation. Gaps in the time series occur where FDOM data were missing.

hydrology. DON content was modeled based on the overall annual relationship with DOC (as modeled using FDOM). This results in slight underestimations during fall and snowmelt periods, that are relatively subtle in the context of calculation of export, but that deserve further attention in defining temporal changes in the potential reactivity (as indicated by DOC:DON) of DOM in headwater streams.

As was illustrated by Pellerin and others (2012), high-resolution FDOM datasets in forested streams can significantly improve snowmelt estimates of export and temporal DOC dynamics over daily or weekly sampling regimes (Appendix 2 in Supplementary material). Our results indicate that the greatest deviation from actual values of export estimates as a result of utilizing reduced sampling frequency is likely to occur during lower flow seasons (Appendix 2 in Supplementary material), when storms tend to be smaller and flashier in nature (Figure 2) and concentrations change more rapidly with event onset, reaching higher maximum concentrations at peak flows (Figure 3). This observation, in combination with observed temporal variability in DON, highlights the need for the design of sampling regimes to account not only for the strong links between concentrations and discharge, but also for the seasonally variable nature of these relationships.

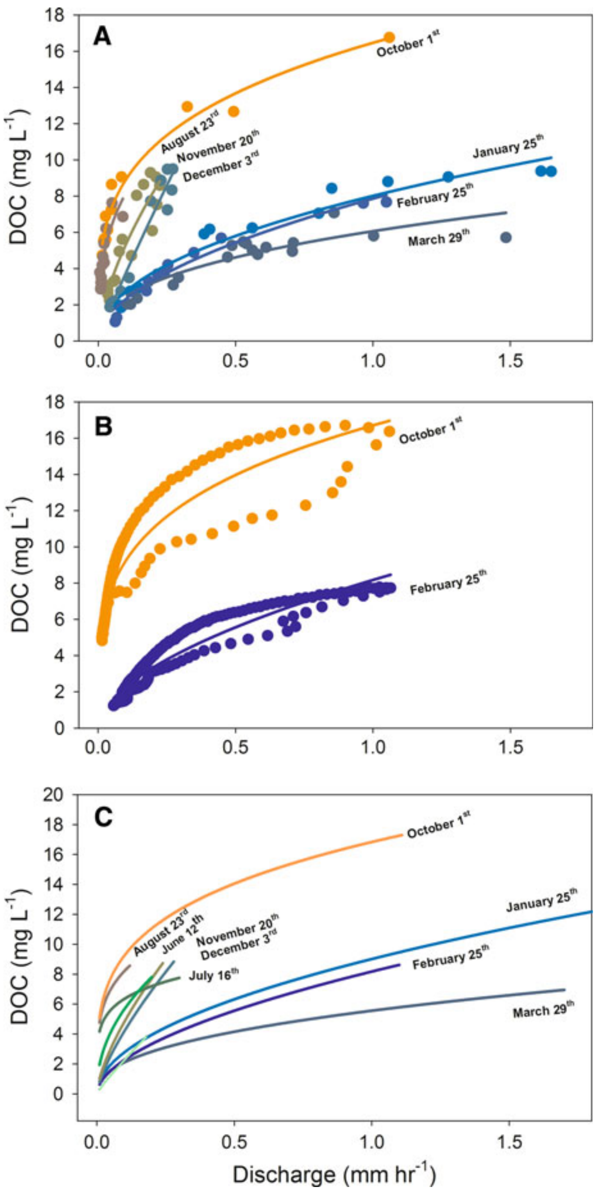

Figure 3. Best fit power law relationships (DOC = $k(Q)^n$) to characterize seasonal changes in concentration–discharge relationships in Bigelow Brook. (**A**) DOC concentrations measured for grab samples collected during targeted events. (**B**) and (**C**) DOC concentrations were modeled based on FDOM. Examples of DOC concentration as modeled every 15 min are shown for contrasting events in October (*orange*) and February (*blue*) (**B**). Modeled best fit regression lines are shown only for the largest hydrological event recorded in each month of observation (**A**). The period of observation was from October 30, 2009 to October 30, 2010. DOC concentrations were modeled based on FDOM. R^2 values for each event ranged from 0.40 to 0.92 with an average best fit of 0.79 with all *P* values being less than 0.001 (Color figure online).

The Importance of DOC Export for Headwater Watershed Carbon Biogeochemistry

Few studies have concurrently examined organic matter fluxes in water with measurement of net ecosystem exchange with the atmosphere, even though the role of inland waters has been hypothesized to be an important component of terrestrial carbon cycling (Cole and others 2007; Battin and others 2008). In some temperate coniferous systems DOC losses up to 100 kg ha^{-1} y^{-1} may represent as much as 12% of net ecosystem productivity (NEP) and represent an important flux in defining net ecosystem carbon balance (NECB) or net biome production (NBP) (Gielen and others 2011); however, in other forested systems DOC export fluxes have only a minor impact on NECB (Shibata and others 2005; 6 kg ha^{-1} y^{-1}, Kindler and others 2010; 35 ± 13 kg ha^{-1} y^{-1}). For the Bigelow Brook watershed the total annual export of DOC (17.2 kg ha^{-1} y^{-1}) is small (<0.56%) in comparison to values of carbon storage (as NEP) measured by Hadley and others (2008) for a hemlock dominated section of Harvard Forest (3,100–3,800 kg ha^{-1} y^{-1}). However, carbon uptake has continued to increase as the forest has matured and eventual decline with aging (Barford and others 2001) may increase the importance of annual DOC export.

Those studies utilizing an indirect, plot-based approach where DOC export is a function of indirectly measured drainage (drainage = precipitation − evapotranspiration − soil water content) and concentration of DOC measured below the root zone (Kindler and others 2010; Gielen and others 2011) measured significantly higher annual DOC fluxes than the current study or Shibata and others (2005), where DOC was measured at the watershed outlet. The plot-based approach also includes the flux to groundwater and deeper soil horizons in export estimates, but when measured at the stream outlet a part of this flux will either become a part of watershed C storage through sorption en-route to the stream (Schwesig and others 2003) or is lost through microbial processing (Schindler and Krabbenhoft 1998) before reaching the stream. Accurate characterization of these transformations will be critical in improving the measurement of both lateral and vertical carbon fluxes in forested headwater watersheds like that of Bigelow Brook. Measurement of lateral flux out of a soil monolith and vertical flux with the

Table 1. Correlations Among Hydrological and Meteorological Variables Used to Characterize Environmental Conditions at Bigelow Brook in Harvard Forest and With Flow Weighted Mean DOC Concentration Modeled for Each Event

	ln magnitude of last event	ln event magnitude	ln event volume	√ min Q	ln volume of last event	√ time since last event	ln event length	ln length of rise	ln flow weighted mean DOC
Water temperature	−0.44	−0.48	−0.86	−0.83	−0.76	−0.04	−0.86	−0.71	0.46
ln magnitude of last event		0.30	0.49	0.48	0.75	0.05	0.55	0.53	−0.24
ln event magnitude			0.79	0.43	0.29	0.01	0.59	0.52	0.37
ln event volume				0.85	0.70	−0.10	0.90	0.75	−0.13
√ min Q					0.85	−0.19	0.79	0.58	−0.49
ln volume of last event						0.00	0.69	0.49	−0.58
√ time since last event							−0.20	−0.05	−0.10
ln event length								0.88	−0.32
ln length of rise									−0.23

atmosphere may underestimate watershed carbon storage by not accounting for retention and storage that occurs en-route to stream, but outside the experimental plot area. Alternatively, if lateral export is measured only in-stream, storage may be overestimated and the importance of soil water DOC losses may be underestimated where transformation occurs en-route to stream and in riparian areas outside the footprint of vertical flux measurements (Cole and others 2007).

Patterns of DOM Bioavailability and N Content

The consistent trend in fluorescence per unit carbon and nitrogen that we observed (Appendix 1 in Supplementary material) and the moderately high C:N ratio of the DOM exported does support the assumption of a mainly terrestrial DOM source. However, the patterns reported here also stress the importance of focusing on hydrologic events when trying to characterize bioavailable DOM export, and, to date, measuring patterns of bioavailability during events has been done only a handful of times (Buffam and others 2001; Fellman and others 2009).

DON content may also act as an indicator of the reactivity of DOM (Wiegner and others 2006) with high benthic DON demand in headwater streams and the potential for rapid in-stream transformation (Brookshire and others 2005). Bigelow Brook's annual DON flux of 0.448 kg ha^{-1} y^{-1} is small in comparison to rates of soil N mineralization (80–100 kg ha^{-1} y^{-1}) (Melillo 1981), but represents about 5.6% of modeled annual N-deposition rates (8 kg ha^{-1} y^{-1}) and exceeds measured rates of N from deposition assimilated into vegetative tissues (<5%) at the forest (Nadelhoffer and others 1999). Also, it is evident that DON represents the dominant form of nitrogen in aqueous export from the watershed with average concentrations being 2.2 times those measured for total inorganic nitrogen in stream water (0.12 and 0.055 mg l^{-1}, respectively).

Due to disproportionate increases in BDOC with increasing DOC (Figure 1), percent bioavailability increases with DOC concentration ($r^2 = 0.32$, $P < 0.001$). Buffam and others (2001) identified a similar trend of increasing bioavailability with hydrological events and together these results further illustrate the importance of events to reactive DOM transport. The 30-day laboratory incubations we utilize act as an indicator of bioavailable DOC content, but may not fully reflect potential availability under *in situ* conditions. The seasonal

Table 2. Best Regression Models for Flow Weighted Mean DOC Concentration (DOC_{FW}) and DOC, DON, and BDOC Export for Hydrological Events

	Best regression model(s)	r^2	ΔAIC	w_i
Flow weighted mean DOC	+ volume of event − min Q − time since last event − event length	0.84	0.00	0.09
	+ magnitude − volume of last event − event length	0.80	1.01	0.05
	+ magnitude − volume of last event − time since last event − event length	0.83	1.04	0.05

Response variable	Best stepwise model	r^2	P
ln DOC event export	1.07 + 0.87 ln volume of event + 0.35 ln magnitude − 0.17 volume of last event	0.98	<0.0001
ln DON event export	−2.78 + 0.88 ln volume of event + 0.30 ln magnitude − 0.14 volume of last event	0.98	<0.0001
ln BDOC event export	−0.84 + 0.83 ln volume of event + 0.47 ln magnitude − 0.22 volume of last event	0.96	<0.0001

Based on Akaike Information Criterion (AICc), the three best candidate models for DOC_{FW} are shown. Best models to predict export were identified by forward stepwise regression and best stepwise model for DOC_{FW} was among the top 3 models selected by AICc (indicated in italic font). Export units are total in kg for the 24 ha watershed, event volumes are expressed in m^3, relative peak discharge as mm h^{-1}, and mean water temperature was measured in °C. Analysis was completed utilizing data collected for 21 hydrological events between October 2009 and November 2010.

consistency of the BDOC to DOC relationship reflects consistency in the dominant and more slowly mineralizable components of the DOM pool; however, temporal differences in the amount of more rapidly bioavailable DOC might be expected with the subtle differences in DOM composition that we observed across seasons (DOC:DON, FDOM:UV_{254}). In particular, humic fractions of lower molecular weight tend to show increased FDOM:UV_{254} (Stewart and Wetzel 1980) as we observed during summer (Figure 1).

Changes in bioavailability are possibly driven by the changes in aromaticity and humicity (Vidon and others 2008; Inamdar and others 2011), protein like fluorescence (Fellman and others 2009; Inamdar and others 2011), and source (Dalzell and others 2007) that have been observed to occur in stream systems with storm events; however, the consistency of the relationship between FDOM and DOC indicates that any influence of compositional changes on fluorescence per unit carbon is not easily detectable using traditional FDOM sensors. Because shifts in bioavailability are generally accompanied by structural changes that may be detectable at excitation, emission wavelength combinations other than those we measure, it may be possible to develop new FDOM sensor based systems that target fluorophores most linked to bioavailability in a particular stream system.

Overall, DOC, DON, BDOC, and %BDOC export all follow a similar temporal pattern, with flushing being strongly connected to hydrological events (Figure 2). The clear increases in concentrations that we observe with increasing discharges highlights the importance of hydrological events as ''hot moments'' for the export of bioavailable DOM (McClain and others 2003). The mass exported is tightly controlled by flushing events and as such, is clearly episodic (Figure 2). It can be expected that with higher rates of discharge and more rapid transport time, this bioavailable material may have the greatest influence on ecosystems well downstream of source headwaters and soils.

Seasonal and Hydrologic Drivers of DOM Export Patterns

The nature of the relationships between event-based DOC concentration and discharge can be expected to differ seasonally, with higher export per unit discharge occurring during summer months (Butturini and Sabater 2000; Sebestyen and others 2009; Inamdar and others 2011; Pellerin and others 2012) (Figure 3). Our results show the temporal consistency of this seasonal response with an unprecedented level of detail and over a full annual time period. We show not only that there is greater export per unit discharge in summer as compared to winter in a forested headwater watershed, but also that there is a highly consistent temporal progression in this response with decreases from late summer through winter and increases through spring to fall (Figure 3). In addition, due to consistent seasonal variation in DON and BDOC with increasing DOC, a similar pattern of seasonality of export can be expected (Figures 1, 2). The high degree of correlation among environmental predictor variables makes it challenging to identify causality of the observed seasonal changes in export rate. However, the results presented here highlight a number of plausible, but non-exclusive hypotheses requiring further evaluation. In addition, we clearly demonstrate the utility of high-resolution FDOM measurement as a tool in future research to help elucidate specific mechanisms controlling the flux and fate of terrestrial DOM in headwater streams.

One hypothesis for the observed temporal pattern of DOC export is that concentration is controlled by changes in watershed organic matter storage as influenced by flushing history, where a large pool of DOC exists within the watershed and rebuilds between events. The volume of the preceding event was strongly correlated with DOC_{FW} and was a consistent predictor of residual variation in export response (Table 2). This predictor may reflect both the amount of DOM flushed from the watershed recently and recent inputs to watershed soil pore-water storage. Similarly, time since last event was also identified as predictor of residual variation in DOC_{FW} (Table 2). Other studies have speculated on similar controls of DOC based on correlation with antecedent flows (Raymond and Saiers 2010) or potential for accumulation of a flushable pool (Boyer and others 1996), but the correlation between volume of the previous event and temperature (−0.76) highlights the fact that storm size is a seasonal phenomenon and that additional explanations are plausible.

Seasonal variation in ecosystem productivity may also influence DOC export. Increased forest productivity during warmer summer and fall months may increase both below ground carbon allocation and above ground production of leaf litter, particularly during fall leaf drop. In hardwood stands at Harvard Forest about 15% of soil DOC may be derived from root exudates and a strong correlation exists between annual soil solution DOC and fungal biomass (Aitkenhead-Peterson and others 2003). Therefore, a second hypothesis for the nature of seasonal trends in DOC export is the buildup of a pool of leachable soil carbon in summer and fall. The decreasing level of DOC per unit discharge (Figure 3) through fall and winter months may reflect the depletion of this pool through time.

A second explanation for this depletion in DOC/*Q* from fall through to early spring is seasonally changing flow path and dilution by snowmelt, where potential for flow through soil horizons with higher concentrations of leachable organic matter is reduced in winter (Hope and others 1994; Boyer and others 1996; Aitkenhead-Peterson and others 2003; Hood and others 2006). Although small decreases in DOC per unit discharge are still observed between periods without snowcover (for example, between November and December), additional decreases (Figure 3) correspond to the development of a snowpack and increased potential for dilution and flow over, rather than through surface soils. In addition, the likely importance of changing flowpath in controlling differences in export response at shorter timescales is illustrated by the consistent importance of event magnitude (the peak discharge rate observed during an event) in predicting residual variation in both export and DOC_{FW} (Table 2). This pattern can be related to the strong positive relationship observed between discharge and concentration for all DOM components measured in this study. In other studies, concentration increases with increasing discharge have been attributed to a shift in dominant flowpath from deeper soil horizons (where increased potential exists for contact with sorptive mineral soil) to more shallow flowpaths through soils of higher organic matter content (Hope and others 1994; Boyer and others 1996; Aitkenhead-Peterson and others 2003; Hood and others 2006).

Conclusions

Overall, the largest DOC losses/downstream subsidies of DON and BDOC might be expected for large storm events occurring during summer and fall months (Figure 2), but further clarification of the physiochemical mechanisms controlling mobilization and transport is required before the potential impact of changing event timing and frequency can be fully defined. This improved understanding will be particularly important if predicted increases in the frequency of extreme precipitation events are realized (Palmer and Raisanen 2002) and as there is variation in the annual depth and duration of the winter snowpack. At the Harvard Forest LTER there are indications that storm frequency has increased over the past century from 15 to an average of 25 storms per year (Hayden and Hayden 2003), thus the Bigelow Brook watershed is well suited to serve as a sentinel ecosystem for evaluating long-term changes in forest-stream carbon cycling in response to regional hydrologic change.

Acknowledgments

Harvard Forest staff, particularly Mark Vanscoy and Emery Boose aided in the installation and transport of field equipment and in providing hydrological and meteorological data. Helpful discussions occurred with Na Xu during the development of methods for FDOM temperature correction. Caroline Dewing and Brittni Devlin provided technical assistance during the processing of water samples. We are also grateful to the constructive comments made by two anonymous reviewers and the Associate Editor. This research was supported through a Yale Institute for Biospheric Studies Environmental Fellowship awarded to H.F. Wilson, and a grant to J. Saiers from the Hydrological Sciences Program of the National Science Foundation (EAR-114478). This study was also supported by LTER IV: Integrated Studies of the Drivers, Dynamics, and Consequences of Landscape Change in New England—DEB-0620443.

REFERENCES

Aitkenhead-Peterson JA, McDowell WH, Neff JC. 2003. Sources, production, and regulation of allochthonous dissolved organic matter inputs to surface waters. In: Findlay S, Sinsabaugh RL, Eds. Aquatic ecosystems: interactivity of dissolved organic matter. Burlington: Academic Press. p. 25–70.

Baker DB, Richards RP, Loftus TT, Kramer JW. 2004. A new flahsiness index: characteristics and applications to midwestern rivers and streams. J Am Water Resour Assoc 40:503–22.

Barford CC, Wofsy SC, Goulden ML, Munger JW, Pyle EH, Urbanski SP, Hutyra L, Saleska SR, Fitzjarrald D, Moore K. 2001. Factors controlling long- and short-term sequestration of atmospheric CO_2 in a mid-latitude forest. Science 294:1688–91.

Battin TJ, Kaplan LA, Findlay S, Hopkinson CS, Marti E, Packman AI, Newbold JD, Sabater F. 2008. Biophysical controls on organic carbon fluxes in fluvial networks. Nat Geosci 1:95–100.

Boyer EW, Hornberger GM, Bencala KE, McKnight D. 1996. Overview of a simple model describing variation of dissolved organic carbon in an upland catchment. Ecol Model 86:183–8.

Brookshire ENJ, Valett HM, Thomas SA, Webster JR. 2005. Coupled cycling if dissolved organic nitrogen and carbon in a forest stream. Ecology 86:2487–96.

Buffam I, Galloway JN, Blum LK, McGlathery KJ. 2001. A stormflow/baseflow comparison of dissolved organic matter concentrations and bioavailability in an Appalachian stream. Biogeochemistry 53:269–306.

Butman D, Raymond PA. 2011. Significant efflux of carbon dioxide from streams and rivers in the United States. Nat Geosci 4:839–42.

Butturini A, Sabater F. 2000. Seasonal variability of dissolved organic carbon in a Mediterranean stream. Biogeochemistry 51:303–21.

Cole JJ, Prairie YT, Caraco NF, McDowell WH, Tranvik LJ, Striegl RG, Duarte CM, Kortelainen P, Downing JA, Middelburg JJ, Melack J. 2007. Plumbing the global carbon cycle: integrating inland waters into the terrestrial carbon budget. Ecosystems 10:171–84.

Currie W, Aber J, McDowell W, Boone R, Magill A. 1996. Vertical transport of dissolved organic C and N under long-term N amendments in pine and hardwood forests. Biogeochemistry 35:471–505.

Dalzell BJ, Filley TR, Harbor JM. 2007. The role of hydrology in annual organic carbon loads and terrestrial organic matter export from a midwestern agricultural watershed. Geochim Cosmochim Acta 71:1448–62.

Downing BD, Pellerin BA, Bergamaschi BA, Saraceno JF, Kraus TEC. 2012. Seeing the light: the effects of particles, dissolved materials, and temperature on in situ measurements of DOM fluorescence in rivers and streams. Limnol Oceanogr Methods 10:767–75.

Fellman JB, D'Amore DV, Hood E, Boone RD. 2008. Fluorescence characteristics and biodegradability of dissolved organic matter in forest and wetland soils from coastal temperate watersheds in southeast Alaska. Biogeochemistry 88:169–84.

Fellman JB, Hood E, Edwards RT, D'Amore DV. 2009. Changes in the concentration, biodegradability, and fluorescent properties of dissolved organic matter during stormflows in coastal temperate watersheds. J Geophys Res 114:G01021.

Foster DR. 1992. Land-use history (1730–1990) and vegetation dynamics in central New England, USA. J Ecol 80:753–71.

Gielen B, Neirynck J, Luyssaert S, Janssens IA. 2011. The importance of dissolved organic carbon fluxes for the carbon balance of a temperate Scots pine forest. Agric For Meteorol 151:270–8.

Hadley JL, Kuzeja PS, Daley MJ, Phillips NG, Mulcahy T, Singh S. 2008. Water use and carbon exchange of red oak- and eastern hemlock-dominated forests in the northeastern USA: implications for ecosystem-level effects of hemlock woolly adelgid. Tree Physiol 28:615–27.

Harvard Forest. 2011. Physiological and biological characteristics of Harvard Forest. Petersham: Faculty of Arts and Sciences of Harvard University.

Hayden BP, Hayden NR. 2003. Decadal and century-long changes in storminess at long-term ecological research sites. New York: Oxford University Press.

Hedin LO, Armesto JJ, Johnson AH. 1995. Patterns of nutrient loss from unpolluted, old-growth temperate forests: evaluation of biogeochemical theory. Ecology 76:493–509.

Hood E, Gooseff MN, Johnson SL. 2006. Changes in the character of stream water dissolved organic carbon during flushing in three small watersheds, Oregon. J Geophys Res 111:G01007.

Hope D, Billett MF, Cresser MS. 1994. A review of the export of carbon in river water: fluxes and processes. Environ Pollut 84:301–24.

Inamdar S, Singh S, Dutta S, Levia D, Mitchell M, Scott D, Bais H, McHale P. 2011. Fluorescence characteristics and sources of dissolved organic matter for stream water during storm events in a forested mid-Atlantic watershed. J Geophys Res 116:G03043.

Inamdar SP, Mitchell MJ. 2007. Storm event exports of dissolved organic nitrogen (DON) across multiple catchments in a glaciated forested watershed. J Geophys Res 112:G02014.

Kaplan LA, Bott TL, Jackson JK, Newbold JD, Sweeney BW. 2008. Protecting headwaters: The scientific basis for safeguarding stream and river ecosystems. Avondale: Stround Water Research Centre.

Kindler R, Siemens J, Kaiser K, Walmsley DC, Bernhofer C, Buchmann N, Cellier P, Eugster W, Gleixner G, Grunwald T, Heim A, Ibrom A, Jones SK, Jones M, Klumpp K, Kutsch W, Larsen KS, Lehuger S, Loubet B, McKenzie R, Moors E, Osborne B, Pilegaard K, Rebmann C, Saunders M, Schmidt MWI, Schrumpf M, Seyfferth J, Skiba U, Soussana JF, Sutton MA, Tefs C, Vowinckel B, Zeeman MJ, Kaupenjohann M. 2010. Dissolved carbon leaching from soil is a crucial component of the net ecosystem carbon balance. Glob Change Biol 17:1167–85.

Lawaetz AJ, Stedmon CA. 2009. Fluorescence intensity calibration using the raman scatter peak of water. Appl Spectrosc 63:936–40.

Leopold LB, Wolman MG, Miller JP. 1964. Fluvial processes in geomorphology. San Francisco: W.H Freeman and Company.

Lowe WH, Likens GE. 2005. Moving headwater streams to the head of the class. Bioscience 55:196–7.

McClain ME, Boyer EW, Dent CL, Gergel SE, Grimm NB, Groffman PM, Hart SC, Harvey JW, Johnston CA, Mayorga E, McDowell WH, Pinay G. 2003. Biogeochemical hot spots and hot moments at the interface of terrestrial and aquatic ecosystems. Ecosystems 6:301–12.

Melillo JM. 1981. Nitrogen cycling in deciduous forests. In: Clark FE, Rosswal T, Eds. Nitrogen cycling in terrestrial ecosystems: processes, ecosystem strategies, and management impacts. Stockholm: Ecological Bulletin. p 427–42.

Mulholland PJ. 2003. Large-scale patterns in dissolved organic carbon concentration, flux, and sources. In: Stuart F, Robert LS, Eds. Aquatic ecosystems: interactivity of dissolved organic matter. Burlington: Academic Press. p. 139–59.

Nadelhoffer KJ, Downs MR, Fry B. 1999. Sinks for 15N-enriched additions to an oak forest and a red pine plantation. Ecol Appl 9:72–86.

Neff JC, Chapin FSI, Vitousek PM. 2003. Breaks in the cycle: dissolved organic nitrogen in terrestrial ecosystems. Front Ecol Environ 1:205–11.

Palmer TN, Raisanen J. 2002. Quantifying the risk of extreme seasonal precipitation events in a changing climate. Nature 415:512–14.

Pellerin B, Saraceno J, Shanley J, Sebestyen S, Aiken G, Wollheim W, Bergamaschi B. 2012. Taking the pulse of snowmelt: in situ sensors reveal seasonal, event and diurnal patterns of nitrate and dissolved organic matter variability in an upland forest stream. Biogeochemistry 108:183–98.

Qualls RG, Haines BL. 1991. Geochemistry of dissolved organic nutrients in water percolating through a forest ecosystem. Soil Sci Soc Am J 55:1112–23.

Raymond P, Saiers J. 2010. Event controlled DOC export from forested watersheds. Biogeochemistry 100:197–209.

Schindler JE, Krabbenhoft DP. 1998. The hyporheic zone as a source of dissolved organic carbon and carbon gases to a temperate forested stream. Biogeochemistry 43:157–74.

Schwesig D, Kalbitz K, Matzner E. 2003. Mineralization of dissolved organic carbon in mineral soil solution of two forest soils. J Plant Nutr Soil Sci 166:585–93.

Sebestyen SD, Boyer EW, Shanley JB. 2009. Responses of stream nitrate and DOC loadings to hydrological forcing and climate change in an upland forest of the northeastern United States. J Geophys Res 114:G02002.

Shibata H, Hiura T, Tanaka Y, Takagi K, Koike T. 2005. Carbon cycling and budget in a forested basin of southwestern Hokkaido, northern Japan. Ecol Res 20:325–31.

Sloto RA, Crouse MY. 1996. HYSEP: a computer program for streamflow hydrography separation analysis. Water-resource investigations 96-4040. USGS, Lemonyne.

Stewart AJ, Wetzel RG. 1980. Fluorescence: absorbance ratios-a molecular-weight tracer of dissolved organic matter. Limnol Oceanogr 25:559–64.

Vidon P, Wagner LE, Soyeux E. 2008. Changes in the character of DOC in streams during storms in two Midwestern watersheds with contrasting land uses. Biogeochemistry 88:257–70.

Westphal M, Field SA, Tyre AJ, Paton D, Possingham HP. 2003. Effects of landscape pattern on bird species distribution in the Mt. Lofty Ranges, South Australia. Landsc Ecol 18:413–26.

Wiegner TN, Seitzinger SP, Glibert PM, Bronk DA. 2006. Bioavailability of dissolved organic nitrogen and carbon from nine rivers in the eastern United States. Aquat Microb Ecol 43:277–87.

Willacker JJ, Sobczak WV, Colburn EA. 2009. Stream macroinvertebrate communities in paired hemlock and deciduous watersheds. Northeast Nat 16:101–12.