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Quantifying the frequency of synchronous carbon and nitrogen export to the river network

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Abstract The biogeochemical cycles of carbon (C) and nitrogen (N) are inextricably linked for a range of reactions. For coupled reactions such as denitrification to occur, however, solutes must be found together in space and time. Using the framework of concentration-discharge (c-Q) relationships, we examine the frequency of synchronous C and N export (i.e. identical c-Q behavior) across a river network using > 5 years of high-frequency sensor data. We demonstrate that across space and time the export of C and N to a river network is asynchronous 57% of the time. The probability of simultaneous export in largely forested watersheds demonstrates little temporal structure, while in more human-impacted watersheds, we observe the highest frequency of asynchronous c-Q behavior. We discuss the implications of synchronous c-Q behavior for solute flux estimation models and develop a theoretical framework for predicting where within a landscape we expect the probability of coupled C and N reactions to be greatest. By simultaneously comparing the variability in C and N c-Q relationships we develop an integrated framework for predicting synchronous export of solutes.

Keywords Concentration-discharge relationships · Dissolved organic carbon · Nitrate · Sensors · Fluorescent dissolved organic matter · River networks

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Introduction

The biogeochemical cycles of carbon (C) and nitrogen (N) are tightly coupled as many transformations within the N cycle require a carbon source. Coupled reactions such as denitrification have important ecosystem-level consequences including the production of greenhouse gases (Beaulieu et al. 2011) and the maintenance of water quality (Mulholland et al. 2008). For coupled reactions to occur however, solutes must be found simultaneously in both space and time. The probability that any given array of solutes needed to drive a biogeochemical reaction encounter one another is thus a first-order control on reaction rates. Currently we lack a landscape-scale framework to

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predict the likelihood of this co-delivery of C and N to a given point.

Advances in high-frequency water quality sensors are resulting in novel insights into those processes that mobilize solutes by matching the chemograph to the hydrograph. These direct comparisons of variability in concentration and discharge result in new opportunities to quantify spatial and temporal variability in solute fluxes and emergent watershed behavior. Concentration-discharge (c-Q) analyses for example, assume that the variability in solute transport is dominated by the independent variable Q, yet often considerable variation in concentration is left unexplained (i.e. low r^2 values) and high coefficients of variation are often reported for concentrations of solutes (Yanai et al. 2015). Recent sensor-based analyses point to considerable variability in concentration relative to variability in flow in association with multiple types of c-Q behaviors (Zimmer et al. 2019; Fazekas et al. 2020). Understanding the relative contribution of variability in concentration and discharge to subsequent variability in solute export represents a significant knowledge gap (Musolff et al. 2015; Zimmer et al. 2019) while also offering an opportunity to generate mechanistic hypotheses regarding the controls on solute mobilization and flux.

Here we use a network of high-frequency water quality sensors to quantify the temporal and spatial variability in synchronous export of DOC (assessed via the optical proxy of fluorescent dissolved organic matter [fDOM]) and NO_3^- across a heterogenous mixed land-use landscape. c-Q relationships provide insight into the controls and timing of material export from the terrestrial environment to the river network (Chorover et al. 2017; Wymore et al. 2017). When concentrations increase with flow, solute generation is termed transport limited (a "flushing" response); while a decrease in concentration with flow is described as source limited (a "diluting" response). The objective of this study is to identify where in space and time the c-Q behaviors of DOC and NO_3^- are synchronous (i.e. either flushing or diluting together) or asynchronous across a heterogenous river network. We ask two motivating questions: (1) what land-use and seasons are associated with synchronous versus asynchronous c-Q behavior? and (2) are certain c-Q behaviors more associated with variability in flow conditions or variability in concentrations evaluated as the ratio of the coefficients of variation of c and Q (CV_c/CV_q; Musolff et al. 2015; Zimmer et al. 2019)? We hypothesize that asynchronous NO_3^- and fDOM c-Q behavior will be frequent due to different source pools and controls over the transport of C and N (Abbott et al. 2018; Koenig et al. 2017; Musolff et al. 2015). We also expect that season and land use will explain a significant amount of variability in the spatial and temporal variation in c-Q behavior due to the influence of both factors on hydrology and solute availability. We use the results from these analyses to (1) garner new insights into sources of error in solute flux estimation models and (2) to develop a predictive framework for the likelihood of coupled C and N biogeochemical reactions at the river network scale.

Methods

Study sites

We used ten streams and rivers across central and southeastern New Hampshire, USA, instrumented with high-frequency sensors during 2013 and 2014. Data in this study were collected year-round from 2013 to 2017 (Snyder et al. 2018; Wymore et al. 2018); however, data collection intervals varied due to site-specific attributes (e.g. sensors removed in winter at one site due to the absence of flows). The stream network spans a gradient in urban development, percentage of agriculture and forest, and concentrations of surface-water DOC and NO_3^- (Table S1) with mean solute concentrations varying by an order of magnitude (0.05–2.69 mg NO_3-N/L and 2.08–15.5 mg C/L).

Sensors and data collection

Detailed methods associated with this data set can be found in Snyder et al. (2018) and we briefly summarize them here. We used daily mean concentrations of NO_3^- and fDOM derived from data collected at 15-min intervals. We used an EXO2 multiparameter sonde (YSI, Xylem Inc., Yellow Springs, OH, USA) to measure fDOM and a Submersible Ultra-Violet Nitrate Analyzer (SUNA, Satlantic LP, Seabird Scientific, Halifax, NS, Canada) with a 10-mm path length and wavelength range of 190–370 nm to measure NO_3^- concentration. Both the EXO2 and SUNA sensors were inspected, cleaned and

recalibrated every six weeks during deployment and are each equipped with hydro-wipers to prevent biofouling during extended deployments. To avoid erroneous data caused by sensor drift or malfunction, sensors were rotated among sites (Campbell et al. 2013). Data were transmitted via cellular telemetry to servers at the University of New Hampshire from Campbell Scientific CR1000 dataloggers (Logan, Utah, USA). QA/QC procedures followed standard practices (Campbell et al. 2013) and were typically performed daily. FDOM values were corrected using sensor measured temperature, absorbance and turbidity (Snyder et al. 2018). We used predictive mean matching procedures from the aregImpute function in the Hmisc package in R to gap-fill the sensor data set (Harrell Jr 2018; Fazekas et al. 2020).

Average daily discharge at mainstem river sites was obtained from USGS records (LMP: 01073500; GOF: 01092000). Daily discharge was derived from 15-min stage records and rating curves developed for each site. Discharge measurements used to develop a rating curve were made at the two smallest streams using tracer-dilution gauging (BDC and SBM). A combination of a FlowTracker Handheld ADV (SonTek, Xylem Inc., San Diego, CA, USA) and an IQ Plus Acoustic Doppler Velocimeter (ADV) were used at the remaining sites (TPB, DCF, HBF, BEF, WHB, and MCQ).

Windowed analysis of concentration-discharge relationships

To examine variability in c-Q relationships we used moving window linear regression which iteratively advances a fixed bandwidth window (30-days) across a dataset at a fixed interval (= 1 day). For each day we derived a daily average from the 15-min records of NO_3^- and fDOM concentrations, and then determined the c-Q relationship in each 30-day window with a power-law function ($log(c) - log(Q)$). The resultant regression coefficients allow for the quantification of changes in the underlying spatial and temporal structure of c-Q relationships over time (Zimmer et al. 2019). For each 30-day interval we derived the slope b ($c = aQ^b$) where a is the intercept in units of concentration, and b is a unit-less exponent representing the slope of the log-transformed c-Q relationship. To describe variations in NO_3^- and fDOM with discharge, we classified c-Q slopes as either flushing when concentration increased with flow ($b > 0$) or as diluting when concentration decreased with flow ($b < 0$).

To assess the relationship between variation in hydrology or concentration as drivers of change in the c-Q relationship, we evaluated variability in concentration relative to discharge. We calculated the ratio of the coefficients of variation ($CV_c/CV_q = \mu_q\sigma_c/\mu_c\sigma_q$) where c is solute concentration and q is discharge (Musolff et al. 2015; Thompson et al. 2011), for each 30-day window. A $CV_c/CV_q < 1$ reflects little variability in c compared to q and variation in exported solutes is driven primarily by variations in flow. A $CV_c/CV_q > 1$ indicates greater variability in c than q during the 30-day window, representing conditions where variability is driven by biogeochemical processes within the watershed, timing of inputs, or variability in source pools.

Classification of export behavior

To address the main objective and quantify the frequency of synchronous versus asynchronous export behavior, we classified the b term from each individual 30-day windowed regression that fell within each quadrant (I, II, III, IV) intersecting at x = 0 and y = 0 for biplots of NO_3^- versus fDOM c-Q (Fig. 1, upper-right inset). Points within quadrants I and III exhibit synchronous fDOM and NO_3^- export behavior and those within quadrants II and IV exhibit asynchronous fDOM and NO_3^- c-Q behavior. We use the following nomenclature to describe these different carbon and nitrogen export typologies: quadrant I = dual enrichment; quadrant II = NO_3^- enrichment; quadrant III = dual dilution; quadrant IV: DOC enrichment. Quadrants I and III represent synchronous export behaviors while quadrants II and IV represent asynchronous export behaviors.

To visualize the spatial and temporal distribution of concentration-versus discharge-induced variability on c-Q slopes, we plotted the c-Q slope of each solute against its paired CV_c/CV_q value for each 30-day period in our windowed regression approach. We classified any slope b with a $CV_c/CV_q < 1$ as hydrologically influenced export and b with a $CV_c/CV_q > 1$ as concentration influenced export (Zimmer et al. 2019; Thompson et al. 2011; Figs. 2 and 3, upper right inset). The quadrants in these plots occur at the intersect x = 1 and y = 0. In quadrants I and IV the

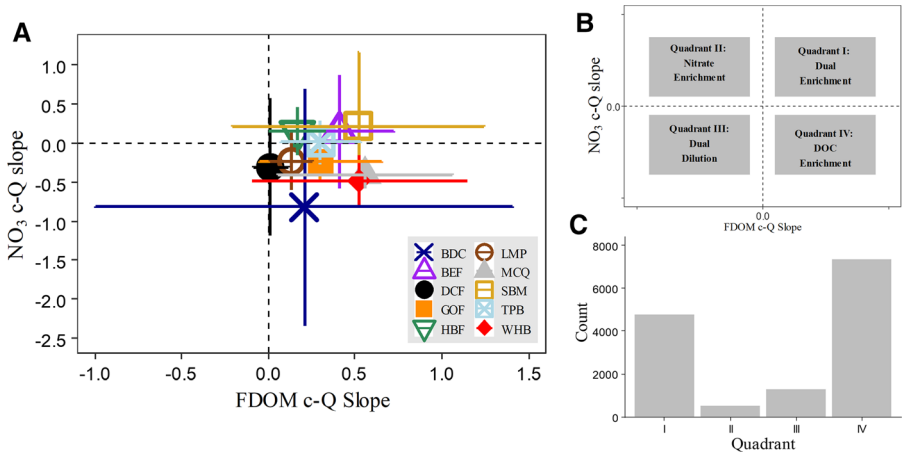

Fig. 1 **a** Biplot of nitrate (NO_3^-) concentration-discharge (c-Q) behavior versus fluorescent Dissolved Organic Matter (FDOM) c-Q behavior. Data points are median slopes from a 30-day windowed regression. Error bars represent standard deviation. Colors and shapes represent the ten sites. **b** Interpretation of quadrants with respect to synchronous and asynchronous c-Q behavior. **c** The count of occurrences of synchronous and asynchronous c-Q relationships from 30-day window regressions as distributed across the four quadrants

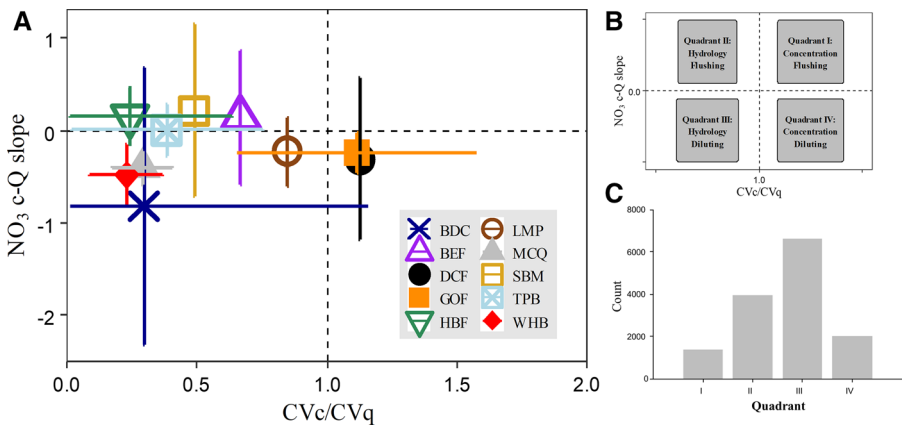

Fig. 2 **a** Nitrate (NO_3^-) concentration-discharge (c-Q) slope versus coefficient of variation for c over the coefficient of variation for Q (CV_c/CV_q). NO_3^- c-Q data are median slopes from a 30-day windowed regression. Color and shape represent the ten study sites. Error bars represent the standard deviation and cutoff above zero. **b** Interpretation of quadrants with respect to the primary driver of variability in the c-Q relationship (hydrology or concentration). **c** The count of occurrences of hydrology- versus concentration-controlled variation in NO_3^- c-Q behavior

variability in concentration is greater than discharge and has either flushing (I) or diluting (IV) c-Q behavior and concentration influenced export. In quadrants II and III the variability in discharge is greater than concentration. Quadrant II has flushing while quadrant III has diluting c-Q behavior. We refer to these different quadrants (I–IV) generally by which term (c or Q) accounts for the greater amount of variation in export behavior.

Statistical analysis

We explored the relationship between synchronous c-Q behavior and seasonal and landscape drivers using multiple logistic regression. We used logistic regression to determine whether the odds of having a synchronous c-Q export classification varied with season and landscape drivers. We used the classification of synchronous (= 1) and asynchronous (= 0) as the binary response. Landscape metrics included the proportion of catchment agriculture, development,

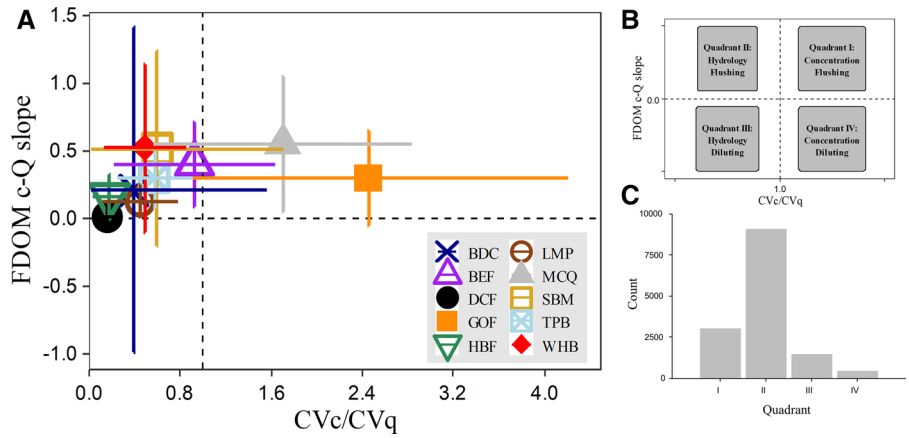

Fig. 3 **a** Fluorescent dissolved organic matter (FDOM) concentration-discharge (c-Q) slope versus coefficient of variation for c over the coefficient of variation for Q (CV_c/CV_q). FDOM c-Q data are median slopes from a 30-day windowed regression. Color and shape represent the ten study sites. Error bars represent the standard deviation and cutoff above zero. **b** Interpretation of quadrants with respect to the primary driver of variability in the c-Q relationship (hydrology or concentration). **c** The count of occurrences of hydrology- versus concentration-controlled variation in NO_3^- c-Q behavior

forest and wetland, stream order, and watershed area (Table S1). We defined seasons according to the water year with October-December as fall, January-March as winter, April-June as spring, and July–September as summer. The logistic regression model was fitted using the glm function in the *stats* package in R (R Core Team 2019). Due to the large sample size, nearly all predictor variables were statistically significant (Table S2, S3). We thus focus on model outputs of the odds ratio and the reduction in deviance resulting from each predictor variable to interpret their effect on the likelihood of synchronous NO_3^- and fDOM c-Q behavior.

Results

Classification of export behavior

Across the stream network, asynchronous NO_3^- and fDOM c-Q export behavior (57%) was more common than synchronous behavior (Table 1). Synchronous c-Q behavior (43%) was primarily comprised of dual enrichment (34%) whereas dual dilution occurred only 9% of the time (Fig. 1). Across the stream network asynchronous transport was most often described as DOC enrichment (93%). Asynchronous export behavior, described as NO_3^- enrichment, was rare, occurring only 7% of the time.

Drivers of synchronous C-Q behavior

Contrary to our expectation, there was not a strong effect of season on synchronous or asynchronous solute export (Tables S2, S3); however, there was considerable inter-site variability in the distribution of 30-day c-Q slopes across quadrants (Figs. 1, S1) where human-impacted sites were more often asynchronous compared to forested sites. While the logistic regression model performed better when season was included, seasonal effects explained little variability relative to land-use based on the reduction in deviance (Table S3). We observed synchronous behavior 73% of the time in forested catchments compared to 15% of the time in impacted stream and larger river catchments. For every unit increase in forest coverage it was 68% more likely to have synchronous NO_3^- and fDOM c-Q behavior. In contrast, for every unit increase in agriculture or developed land use, it was 65–68% more likely that a stream had asynchronous NO_3^- and fDOM c-Q behavior.

The majority of NO_3^- and fDOM c-Q behavior was driven by variability in discharge rather than variability in concentration (Figs. 2 and 3, S2 and S3) occurring ~ 75% of the time (Table 1). We did observe inter-site variability in the occurrence of discharge- vs concentration-induced variability. Across sites, discharge-influenced variability in NO_3^- c-Q behavior ranged from 40 to 100% of the time. The most human-

Table 1 Percentages of synchronous versus asynchronous concentration-discharge (c-Q) behavior and percentages of hydrology- versus concentration-dominated variability in c-Q behavior for each solute across the ten experimental watersheds

	Synchronous	Asynchronous	Nitrate		FDOM		Impact
			Hydrology	Concentration	Hydrology	Concentration	
Overall	43	57	76	24	75	25	–
Hubbard Brook (HBF)	79	21	92	8	100	0	0
Saddleback Mountain (SBM)	87	13	80	20	75	25	0
Bartlett Experimental Forest (BEF)	81	19	65	35	54	46	0
Trout Pond Brook (TPB)	53	47	93	7	90	10	1
Dowst Cate Forest (DCF)	51	49	45	55	100	0	7
Merrimack River (GOF)	16	84	40	60	3	97	9
Lamprey River (LMP)	23	77	58	42	91	9	12
Wednesday Hill Brook (WHB)	2	98	99	1	89	11	30
Burley Demerritt Creek (BDC)	23	77	95	5	74	26	63
McQuesten Brook (MCQ)	9	91	100	0	24	76	88

Sites are organized by level of anthropogenic impact estimated by the sum of watershed area that is characterized as percent developed and agriculture

impacted headwater catchments, WHB, MCQ, and BDC, had > 95% of the variability in NO_3^- c-Q slopes explained by discharge. In contrast, the highest occurrence of concentration-influenced variability for NO_3^- occurred in the mainstem rivers (LMP and GOF, 42 and 60%, respectively) and in the wetland-influenced DCF (55%).

Across sites, discharge accounted for the variability in fDOM c-Q, as little as 3% (GOF) to as much as 100% (HBF and DCF) of the time. In forested catchments, fDOM had discharge- influenced variability between 54 and 100% of the time. The two larger rivers, GOF and LMP, showed opposite patterns with respect to discharge-influenced fDOM c-Q variability at 3 and 91%, respectively. At the landscape scale, synchronous and asynchronous c-Q behavior can be influenced by the variation in concentration or discharge for both NO_3^- and fDOM as every characterization of c-Q behavior exists in every quadrant in Figs. 2 and 3 (supplemental Fig. 2 and 3).

Discussion

Synchrony of DOC and NO_3^- transport

At the landscape scale we more frequently observed asynchronous transport of NO_3^- and DOC delivery to the river network, with the greatest asynchrony observed in human-impacted watersheds. The most frequent form of asynchrony occurs with NO_3^- dilution and DOC flushing (Fig. 1), conditions which can be described as a high DOC: NO_3^- ratio and that solutes delivered are relatively nutrient limited. Asynchronous export is likely a product of how source processes and the production rates and location of DOC and NO_3^- pools interact with hydrologic connectivity within the catchment. By parsing patterns of c-Q using a moving window analysis we can describe intra-annual variation not captured at more coarse resolutions (e.g. Coble et al. 2018) while also integrating intervals of time between storm events. Finer-scale analyses are necessary to predict the timing of solute delivery and the co-delivery of solutes to the river network.

Contrary to our expectations, we did not find an influence of season on the likelihood that DOC and NO_3^- c-Q behavior is asynchronous. This expectation was based on the distinct seasonality associated with northern temperate forests including periods of snowmelt runoff associated with pulses of NO_3^- (Creed et al. 1996), vernal windows (Contosta et al. 2017), and pulses of DOC with leaf fall (McDowell and Fisher 1976). This result contrasts with previous work in these watersheds showing seasonal variation in asynchronous c-Q behavior during individual high

flow events (Koenig et al. 2017). Our work differs from Koenig et al. (2017) in our fundamental approach to examining c-Q relationships. Koenig et al. (2017) primarily focused on individual storm events, whereas this study took advantage of a more integrative moving window approach to assess c-Q behavior. This allowed us to quantify the proportion of synchronous versus asynchronous export behavior across the entire time series of concentration and runoff data. Our use of logistic regression further allowed us to partition the variation in synchronous c-Q behavior attributable to season versus land-use.

The potential insights provided by different c-Q analyses are realized when they are appropriately matched to the timescale of the question being asked. The inherent premise of c-Q analyses requires concentration data across a range of flow values which are used to inform regression-based analyses and to determine the direction of the relationship between c and Q. This leads to a focus on watershed behavior at high flows. And while sensors are enabling high-frequency measurements during events (e.g. Koenig et al. 2017; Vaughan et al. 2017; Wymore et al. 2019), they are also revealing a high degree of variability in c-Q behavior at the lower range of discharge (Fazekas et al. 2020; Kincaid et al. 2020). c-Q analyses based on long-term weekly grab samples can provide insights into overarching central tendencies (e.g. Wymore et al. 2017; Zarnetske et al. 2018); yet, analyses at this scale tend to smooth over intra-annual variability and can mischaracterize overall c-Q behavior (Fazekas et al. 2020). Generalizations from these lower-resolution analyses are often limited by a high proportion of unexplained variation. In contrast, the analysis of hysteresis is appropriate to understand event-based dynamics and the potentially disproportionate yield of solutes during high-flow events (Inamdar et al. 2006). If research questions, however, are focused on how variable the behavior of a system is across the range of all flow conditions, rolling window analyses (Zimmer et al. 2019; Fazekas et al. 2020), may be appropriate as they integrate timesteps between those at the event and annual scale.

Assessments which incorporate multiple timescales of analysis can help explain seemingly contradictory results by accounting for processes which operate at different scales. For example, contradictory results regarding the effects that season has on asynchronous export (i.e. results reported here vs. Koenig et al. [2017]) may result from the fact that analyses of hysteresis do not quantitatively incorporate antecedent conditions. In contrast, the rolling-window analyses conducted here does incorporate c-Q behavior during the previous 30 days potentially masking the effects of seasonality. It is unlikely that the differences in seasonal effects are the result of using daily means in our analysis as opposed to the 15-min record as the overall patterns are similar and robust to these differences in the time-scale of analysis (Supplemental Fig. 4 and 5). These variable conclusions from the same study system and data set suggest that the activation of particular reservoirs of solutes can vary as a function of an interaction between flow regimes (i.e. baseflow vs. storm flows) and seasons. Rolling-window analyses are flexible as the time-steps, window size, and resolution of the input data can be adjusted depending on the temporal scale of the question being addressed. As such, rolling window regression analyses may be particularly useful for understanding the scale at which certain processes operate to influence variability in c-Q behavior.

Enhanced asynchrony of c-Q processes with land-use is consistent with other studies that document how solute export changes under anthropogenic forcing (Basu et al. 2011; Creed et al. 2015). The relatively low CVc/CVq ratios for NO_3^- in the impacted catchments signify that concentrations and sources of NO_3^- do not vary significantly in time (Fig. 2). This stationarity suggests that NO_3^- export can be estimated as a simple function of discharge (Basu et al. 2010; Thompson et al. 2011). One exception to this general pattern occurs in the agricultural site BDC which shows relatively greater variability in the degree of synchronous vs. asynchronous c-Q behavior (Fig. 1). While BDC's median response is best described as DOC enrichment (quadrant IV), variability extends into quadrants of dual enrichment and dual dilution. Although development and agriculture are often associated with NO_3^- dilution due to changes in source pools, DOC consistently flushes across watersheds (Zarnetske et al. 2018) indicating less sensitivity in DOC c-Q behavior to changes in watershed land-use and land-cover.

Variability in NO_3^- and fDOM c-Q behavior was primarily associated with variability in discharge (Fig. 2 and 3; Zimmer et al. 2019; Thompson et al. 2011). The central tendency of CV_c/CV_q values < 1 indicates that the mobilization of solutes is strongly

controlled by hydrological connectivity rather than the ability of biotic and biogeochemical processes to exert control over the export of these solutes. There were exceptions to this general pattern across the network and CV_c/CV_q values > 1 are reported in the literature (Zimmer et al. 2019). Values > 1 may provide insights into the variability in watershed function and processes dictating solute availability across space and time. Low CV_c/CV_q ratios for both fDOM and NO_3^- at HBF suggest that this site has more consistent concentrations relative to discharge. In contrast at BEF, which like HBF also drains steep mountainous terrain, other processes contribute to variability in c-Q rather than discharge alone (represented by CV_c/CV_q standard deviation > 1). This increase in concentration-based variation may point to biological activity along the flow path and within the stream channel. At the two mainstem rivers (LMP and GOF), as well as the wetland-influenced DCF, NO_3^- export behavior had no dominant pattern with CV_c/CV_q ratios ranging from effectively zero to over 10 across individual 30-day windowed regressions. It is likely that the NO_3^- 30-day c-Q slopes at DCF, which range from -5 to 3, are driven by a suite of different processes that vary over space and time such as the seasonality of the wetland outflow, the activation of various catchment source pools, or variability in biological activity and the stratification of the water column and hyporheic zone. We do not think that highly variable CV_c/CV_q values are the result of using daily mean data rather than the 15-min sensor record. A subset of analyses to address this potential artefact show a similar degree of variability in the range of c-Q slopes and CV_c/CV_q values (supplemental Fig. 4 and 5).

As with average nutrient and organic matter concentrations, the c-Q signals observed in larger rivers are a product of the processes occurring upstream as well as catchment land-cover and land-use. Consistent with the patterns observed in their tributaries (MCQ; and DCF, WHB and BDC, respectively), GOF and LMP demonstrate flushing export regimes; however, the larger GOF spans a much larger range of CV_c/CV_q values including values $>> 1$. In GOF, the variability in hydrology in a given 30-day window is relatively stable compared to variation in concentration, reflecting the importance of biogeochemical processing of DOC in the landscape and upstream portions of the network. LMP is a 6th order river embedded within a system of wetlands, which can be a substantial source of DOC to adjacent fluvial systems (Raymond and Hopkinson 2003). These larger rivers have different sources and mechanisms regulating DOC transport reflected in the more variable CV_c/CV_q signal of LMP likely resulting from the temporal variability in the integration of the proximate wetland signal and other catchment sources. While we do not anticipate these patterns in GOF and LMP to be generalizable across all large rivers, this pattern is consistent with other high-order rivers (e.g. Mississippi River; Zimmer et al. 2019).

Implications of estimations of elemental fluxes

While sensors offer clear advantages in their ability to capture the true range of solute variability, they also highlight challenges associated with signal deconvolution and parsing out short-term noise versus-long term signal. For example, modeled monthly flux estimates are often more accurate than estimates based on modeled 15-min data due to a smoothing of noise generated in high-frequency records (Appling et al. 2015). Data presented here provide two important insights for the estimation of fluxes. First, model selection (e.g. composite methods, regression model-methods) is often informed by the strength of the c-Q relationship (i.e. r^2 values; Preston et al. 1989; Aulenbach et al. 2016). The assumption of a single c-Q behavior for a watershed, based on weekly grab-sample data likely introduces uncertainty into flux estimates. The use of a windowed-regression approach to evaluate c-Q behavior demonstrates how dynamic c-Q relationships can be within a single watershed. Annual-scale assessment of c-Q behavior frequently mischaracterizes watershed c-Q behavior (Fazekas et al. 2020) and storm responses (i.e. directionality of hysteresis) are often not uniform throughout a year (Koenig et al. 2017; Knapp et al. 2020). The assumption that Q can be used universally as a predictor of concentration within a single study system (Yanai et al. 2015) likely needs to be revisited. Insights from the use of a moving-window regression analysis of c-Q behavior highlight the difficulty of LOADEST and LOADFLEX modeling approaches using weekly samples that only capture a fraction of the variability in solute concentration.

Second, flux models assume that variability in c-Q relationships and flux are dominated by discharge

(Appling et al. 2015; Diamond and Cohen 2018). Across the watersheds studied here, approximately 25% of the time the variability in c-Q relationships was assignable to variability in concentration, while within watershed percentages varied from 0–60 and 0–97% of the time for NO_3^- and fDOM, respectively. Only one out of the ten watersheds had all CV_c/CV_q values < 1 (MCQ) and many watersheds had values $>> 1$, including the two large rivers. Similar assessment of variability in solute concentration, across watersheds of highly variable size, points to similar dynamics (Yanai et al. 2015; Zimmer et al. 2019). While multiple sources of error are recognized in flux estimates, the use of CV ratios at a sub-weekly scale offers an effective way to parse the variation between c and Q and their relative contributions to error in models of flux estimates. Assessing the variability in both the numerator and denominator of the c-Q relationship as well as variability in c-Q behavior itself, provides insights into the delivery of solutes to a point in the river network.

Implications for coupled biogeochemical reactions at the river network scale

The concept of collision theory (Trautz 1916; Lewis 1918) can be broadly applied to understanding the distribution of biogeochemical reactions at the landscape scale. For example, because many freshwater ecosystems receive significant quantities of solutes from the surrounding landscape via hydrological connectively, the probability of certain reactions occurring will increase when solutes move through space and time synchronously and when concentrations of both are high. In the case of a reaction such as denitrification, microorganisms must be able to simultaneously access an oxidizable source of dissolved organic carbon (DOC) and nitrate (NO_3^-). If, however, one solute increases in concentration while the concentration of the other solute dilutes during transport, then the probability of a reaction occurring should decrease. Among various solutes, heterogeneity in synchronous versus asynchronous responses to flow should manifest throughout the river network creating a mosaic of biogeochemical activity at the landscape scale. Developing landscape scale models that predict heterogeneity in biogeochemical processes remains a challenge due to the lack of integration of both the space and time dimensions.

Using the data presented in Fig. 1 (see also supplemental Fig. 1), and the framework of synchronous solute delivery, we create a landscape-scale heatmap that predicts when (time dimension) and where (spatial dimension) the potential for coupled C and N reactions is greatest (Fig. 4). This heatmap is generated by quantifying the frequency in which 30-day moving window C-Q relationships can be described as either dual enrichment and dual dilution (quadrants I and III, respectively, in Fig. 1). For example, dual enrichment reflects those times of year and those sites where conditions would facilitate coupled C and N reactions since both solutes are flushing together and simultaneously increasing in concentration. And while hydrologic synchrony can occur as dual dilution, the probability of reactions in the stream is reduced as concentrations of both solutes are diluting in response to flow. In this collision theory framework, quadrants describing either NO_3^- or DOC enrichment have the lowest probability of reactions since one solute is always decreasing in concentration.

Emergent patterns across the heatmap reinforce the role of season versus land-use in controlling synchronous solute delivery as color variation in the heatmap varies vertically with site more than

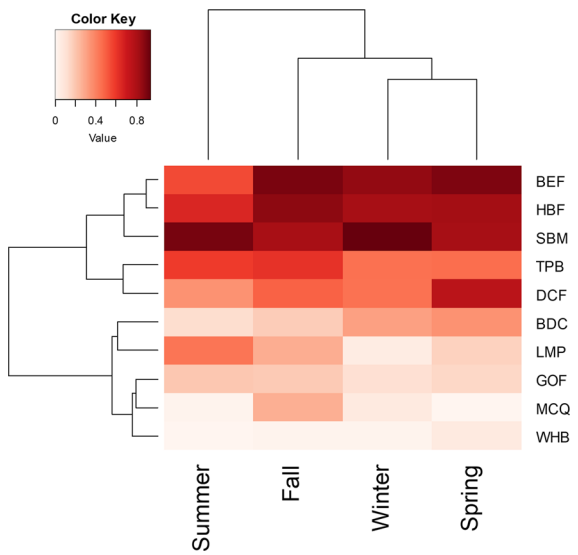

Fig. 4 Heat map of frequencies of synchronous nitrate (NO_3^-) and fluorescent dissolved organic matter (FDOM) concentration-discharge (c-Q) behavior. Matrix is arrayed along seasons and sites. Site abbreviation located in Table 1. Darker red colors represent a greater frequency of synchronous export. Dendrograms connect seasons and sites that are more similar in their frequencies of synchronous c-Q behavior

horizontally with season. We also apply dendrograms to examine related functionality among watersheds. Bifurcations of the dendrogram indicate a clear clustering of sites and the strong role of land-use land-cover in affecting synchronous export and therefore the likelihood of coupled biogeochemical reactions. The first major bifurcation is between forested and the more impacted sites. Within the forested site cluster, steeper more mountainous watersheds (BEF, HBF, SBM) form one sub-group, while wetland-dominated systems (TPB, DCF) form a second cluster. Within the more human impacted cluster, mainstem rivers and their tributaries group together (LMP and BDC; GOF and MCQ), while a septic dominated stream (WHB) forms its own sub-group. The use of dendrograms provides a qualitative metric to examine the regional coherence of watershed function and the ability of anthropogenic impacts to disrupt such patterns. While we acknowledge the difficulties in predicting river network-scale biogeochemical processes, including those associated with the removal of nitrogen (Mulholland et al. 2008; Wollheim et al. 2008), we view this initial predictive framework as an opportunity for hypothesis testing and future modification. By starting with a basic principle of chemistry and reaction kinetics (collision theory) we develop a framework where other factors which influence biogeochemical reaction rates can be introduced to add multivariate predictive power.

Ecological and resource stoichiometry (Sterner and Elser 2002; Cross et al. 2005) is one such concept that will need to be considered to advance the model presented here. While dual enrichment of DOC and NO_3^-, for example, will facilitate reactions based on the first-order principles of reaction kinetics, the stoichiometry of the exported load will need to approximate the stoichiometric demands of the microbial community. Background solute concentrations will also be important for more robust predictive models. Sites characterized by high background concentrations of DOC and low concentrations of NO_3^- (i.e. high DOC: NO_3^- ratio) and which have export regimes described as DOC enrichment and NO_3^- dilution (quadrant IV in Fig. 1) may experience very high rates of coupled C N reactions. DOC:NO_3^- ratios correlate positively with rates of NO_3^- uptake and the increased availability of energy drives the demand for nutrients (Rodríguez-Cardona et al. 2016; Wymore et al. 2016; Plont et al. 2020). The capacity for the microbial community to remove nutrients, however, is finite (Mulholland et al. 2008) and continuous enrichment of DOC may cause the relationship between DOC: NO_3^- ratios and NO_3^- uptake to become asymptotic. In systems characterized by low DOC: NO_3^- ratios (i.e. higher energy demand) the export of DOC to the river network may be the primary process driving supply rates of reactants. In such a case, quadrants associated with dual- and DOC enrichment (quadrant I and IV in Fig. 1, respectively) may offer the strongest predictive capacity. The full integration of the principles of stoichiometry into this type of model development will also require recognizing the stoichiometric flexibility of microbial communities (e.g. Hood and Sterner 2010). A broad assessment of how synchronous and asynchronous export regimes correlate with background concentrations of solutes would be an important next step to understand how to best pair solute export regimes with stoichiometry in order to predict in-stream biogeochemical reactions. Other factors such as the composition of DOM, and DO, pH and temperature, which also respond to changes in flow (O'Donnell and Hotchkiss 2019; Vaughan et al. 2019; Wagner et al. 2019), will also help to determine the distribution of coupled C and N reactions across the landscape.

A robust understanding of in-stream biogeochemical processes and network-scale patterns (Alexander et al. 2009; Wollheim et al. 2017) will come from the integration of multiple c-Q based relationships with a stoichiometry-based description of the stream ecosystem itself. Inferences regarding other biogeochemical processes may also be possible through this approach. For example, flushing of NO_3^- may reflect nitrification and the production of NO_3^- along flow paths especially if flows paths are concomitant with the delivery of oxygen (Creed et al. 1996). Such a scenario would connect soil biogeochemical processes to in-stream biogeochemical reactions across the terrestrial-aquatic interface.

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