Year-round measurements reveal seasonal drivers of nutrient uptake in a snowmelt-driven headwater stream

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Abstract: Climate change may affect export of essential nutrients to downstream ecosystems in regions that host substantial winter snowpack, yet nutrient uptake is rarely quantified in snow- and ice-covered streams and rivers. To evaluate how nutrient uptake varied year-round in relation to commonly identified drivers of uptake (nutrient concentrations, biological activity, organic matter supply and composition), we measured uptake of ammonium (NH_4) and soluble reactive phosphorus (SRP) at 2 to 4 week intervals for 3 years and dissolved organic carbon (DOC) for 2 years in a forested headwater stream in Michigan's Upper Peninsula. Uptake velocities (V_f) did not vary significantly among seasons for NH_4 or SRP, but a much greater range in V_f was observed for NH_4 in spring and fall. DOC V_f was only detectable in summer and fall and never in winter or spring. Key biological and chemical drivers of uptake also exhibited seasonal differences: benthic algal biomass was greater in winter than all other seasons, dissolved organic matter was more aromatic in spring than fall or winter, and DOC concentrations were higher in spring than winter. NH_4 V_f was nonlinearly related to temperature, with the greatest uptake observed at moderate temperatures in spring and fall, which coincided with seasonal transitions that provide optimal light conditions for autotrophs or organic matter for heterotrophs. Our results suggest that alteration to the timing of key environmental variables will affect the magnitude of nutrient uptake, and that in-stream processing during winter cannot be ignored.
Key words: nutrient uptake, ammonium, phosphate, dissolved organic carbon, dissolved organic matter, headwater, seasonality, Upper Peninsula of Michigan

Understanding controls on the uptake, transformations, and export of nutrients or carbon is essential because excess nutrient loading in aquatic ecosystems can cause harmful algal blooms, dissolved oxygen depletion, or fish die-off (Vitousek et al. 1997), whereas in less altered systems low concentrations of nitrogen (N) or phosphorus (P) can limit primary production (Vitousek and Howarth 1991). In-stream retention and transformation controls N, P, and carbon (C) export to aquatic ecosystems across climatic regions (Peterson et al. 2001, Withers and Jarvie 2008, Hall et al. 2009b, Mineau et al. 2016). Multiple physical (stream size, light availability, temperature, discharge), biological (autotrophic and heterotrophic activity, riparian vegetation), and chemical (C : N stoichiometry, dissolved organic matter [DOM] composition) factors can interact to control spatial variation in nutrient uptake rates in streams (Peterson et al. 2001, Hall et al. 2009b, Coble et al. 2016a, Wymore et al. 2016).

Across fine temporal scales, the timing of optimal environmental conditions relative to nutrient and energy availability is likely a critical determinant of nutrient uptake rates (Hoellein et al. 2007). Increased light availability can increase primary production, which can increase nutrient demand. Likewise, with increased organic matter inputs heterotrophs may require more nutrients to break down DOM (Coble et al. 2016a). Biotic activity can also be controlled by temperature (Kirchman and Rich 1997, Gillooly et al. 2001, Pomeroy and Wiebe 2001), which is an important predictor of uptake in some streams but not others (Martí and Sabater 1996, Butturini and Sabater 1998, Simon et al. 2005, Hoellein et al. 2007, Coble et al. 2016a). Rapid or abrupt seasonal changes, such as the transition from winter to spring (Contosta et al. 2016), may create brief time periods when optimal conditions are met, resulting in high rates of nutrient uptake (Hoellein et al. 2007, Roberts and Mulholland 2007). For

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example, leaf-fall introduces terrestrial organic matter into the stream and reduces shading at a time when temperatures are moderate. Similarly, in spring, snowmelt delivers pulses of organic matter and nutrients when residence time is reduced and light availability is high prior to leaf emergence. However, elucidating multivariate controls on uptake can be difficult because seasonal changes in environmental conditions and uptake rates may exhibit linear, nonlinear, or interactive responses. Capturing the natural complexity of uptake responses to a myriad of shifting environmental conditions thus requires frequent sampling and appropriate statistical modeling.

Globally, autotrophic and heterotrophic compartments are responsible for equal proportions of ammonium (NH_4) uptake (Tank et al. 2018), but little is known about autotrophic biomass and organic matter composition in streams beneath ice and snow, or how their seasonal variability may influence uptake rates. Beneath ice, large quantities of biofilm and phytoplankton biomass have been observed in marine and freshwater ecosystems, and the variability in algal biomass has been attributed to differences in environmental conditions including N availability, light penetration, pH, and discharge (Uehlinger et al. 1998, Whiteford et al. 2016, Mendoza et al. 2017). Distinct DOM composition, with a greater relative proportion of tryptophan-like fluorescence linked to autotrophic production, has also been observed in winter beneath ice and snow relative to other times of the year (Mann et al. 2012, Wickland et al. 2012, Coble et al. 2016b). Therefore, autotrophic and heterotrophic activity are likely equally important for controlling nutrient uptake rates during winter as during other seasons (Hoellein et al. 2007, Roberts and Mulholland 2007). Most measurements of nutrient cycling are made in summer, but measurements of nutrient uptake across seasons not typically sampled can provide new insights on stream nutrient uptake (Mulholland et al. 1985, Simon et al. 2005, Hanafi et al. 2006, Hoellein et al. 2007; Roberts and Mulholland 2007, von Schiller et al. 2008, Hall et al. 2009a). Winter sampling is particularly lacking in watersheds where snow represents a significant portion of annual precipitation because heavy snow and ice cover often make sampling logistically difficult (Hoellein et al. 2007, Marcarelli et al. 2019).

The timing of key environmental variables that control nutrient or C export in snow-dominated regions is likely to shift with climate change because declining snowpack and increasing temperatures have led to earlier initiation of snowmelt but slower rates of melting, lengthening of the vernal window (time between snowmelt and leaf-on), lengthening of the growing season, and shifting of the timing of discharge during spring and fall (Brown and Robinson 2011, Creed et al. 2015, Contosta et al. 2016, Musselman et al. 2017). Thus, if future shifts in hydrology do not accompany concomitant light, temperature, and nutrient conditions necessary to meet biotic demands, the timing and magnitude of nutrient or C uptake is likely to change. There is a large gap in our understanding of within-stream processes beneath snowpack and during spring runoff, and temporally-detailed measurements of in-stream uptake are needed in snow-dominated regions to better understand and predict how climate change will affect in-stream processing.

To understand how climate change may affect freshwater nutrient cycling in northern climates it is necessary to identify how in-stream processing varies across the full annual cycle including during snow/ice cover and spring runoff. Here, we address the research questions: 1) Does uptake of N, P, and C occur in winter when ice/snow often covers the stream?; 2) How does uptake of N, P, and C vary intra-annually in a stream with a snow-dominated hydrograph?; 3) How do the environmental conditions that control uptake rates vary intra-annually?; and 4) Which physical, chemical, and biological variables are related to intra-annual variability in nutrient uptake? To address these questions, we measured in-stream nutrient and C uptake along with environmental covariates at 2 to 4 week intervals for 3 years in a forested headwater stream located on Michigan's Upper Peninsula. We hypothesized that seasonal and intra-annual variation in nutrient spiraling would be driven by increased light availability and nutrient and organic matter pulses in spring and fall, relative to low light and nutrient availability in winter and summer.

METHODS

Study Area

This research was conducted at the 1st order (1.76 km^2) Calumet watershed located on the southern shore of Lake Superior (47°17′N, 88°34′W). Calumet watershed has been the site of long-term monitoring beginning in 1979 and is one of only 2 long-term observational study watersheds that discharge directly into Lake Superior (Stottlemyer and Toczydlowski 2006). Mean annual precipitation at Calumet watershed is 80 cm (Stottlemyer and Toczydlowski 1996) with up to 50% occurring as snowfall (Stottlemyer and Toczydlowski 2006). This headwater stream experiences seasonal changes in snow cover, light availability, nutrient concentrations, and organic matter availability (Fig. 1). Overstory vegetation primarily includes sugar maple (*Acer saccharum* Marsh) and white birch (*Betula papyrifera* March). A Parshall flume equipped with a Stevens pressure transducer (Stevens Water Monitoring Systems Inc., Portland, Oregon) and Li-Cor datalogger (Li-Cor™, Lincoln, Nebraska) continuously monitors discharge. The hydrograph of this stream is characterized by a large peak in spring discharge. The timing and magnitude of this peak varied among the 3 years of our study (Fig. 1), and were consistent with measurements of mean snow water equivalent, which increased from 12.1 to 17.4 to 22.4 cm in water years 2012, 2013, 2014, respectively (R. Stottlemyer, Michigan Technological University, unpublished data).

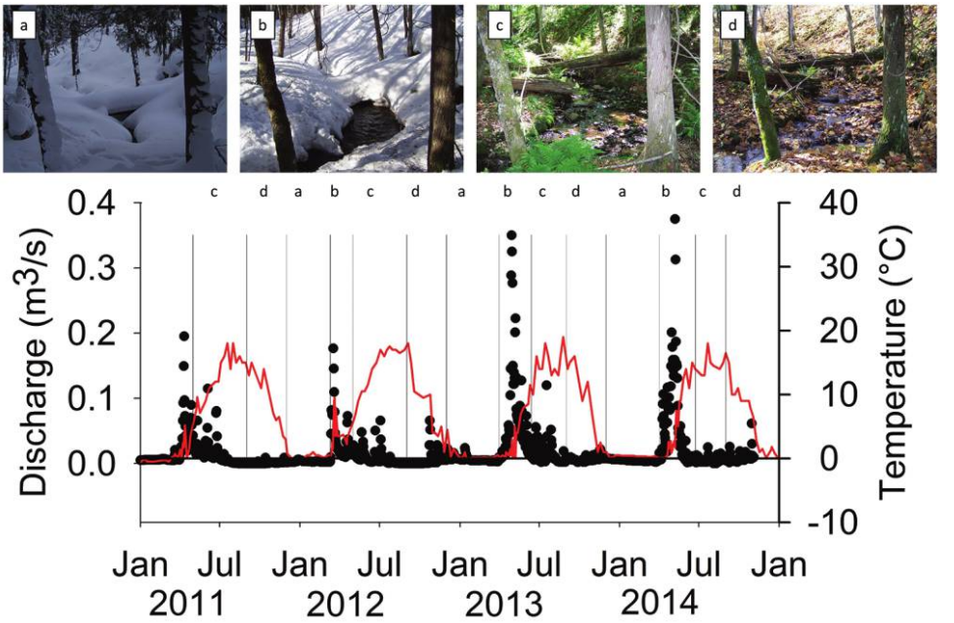

Figure 1. Seasonal variation in conditions at Calumet Watershed as depicted in selected photographs taken from the same location in a) winter, b) spring, c) summer, and d) fall. The hydrograph depicts temporal variation in mean daily discharge (black dots) and weekly spot water temperatures (red line). Vertical lines represent the start/end dates of seasons as defined by environmental conditions. The letters displayed above each line correspond to the seasonal photographs a–d.

Seasonal nutrient spiraling

We measured in-stream spiraling of NH_4 and soluble reactive phosphorus (SRP) across all 4 seasons for 3 y (40 dates) and DOC for 2 y (23 dates). We defined seasons based on major hydrologic and phenological events in the region, rather than defaulting to calendar-based seasons (Fig. 1). Winter extended from December until the first sign of spring snowmelt (identified by a rise in the hydrograph); spring extended until the beginning of leaf-out (identified by increasing canopy cover, which occurred on average 43 days following peak spring discharge); summer extended from end of spring through August; and fall extended through November (identified by leaf-fall). These seasonal transition dates varied from year to year. For example, peak snowmelt runoff ranged between early March and late April within the 3-y duration of our study (Fig. 1). Spring measurements were conducted at 2-wk intervals because of rapidly changing environmental conditions during and following snowmelt. Otherwise, measurements were made monthly. We measured nutrient uptake on 11 dates in spring, 11 in summer, 10 in winter, and 8 in fall for NH_4 and SRP (Fig. S1). We measured DOC uptake on 5 dates each in spring, summer, and fall, and 8 dates in winter.

We used short-term continuous injections of NH_4 (NH_4Cl), phosphate (KH_2PO_4), and glucose ($C_6H_{12}O_6$) with NaCl or Rhodamine WT dye as conservative tracers and standard field and calculation methods to calculate nutrient spiraling metrics (Tank et al. 2017). NH_4 and PO_4 were injected together, while a separate glucose injection was introduced after at least two h or often more than one d later. We set up a 200-m stream reach to achieve a target travel time of 30 min to 1 h, although travel time varied depending on season and discharge. We targeted an enrichment of 10 to 12 μg/L above background for NH_4 and SRP and 5 mg/L above background for DOC. Background water samples were collected and filtered (0.45 μm) immediately before nutrient addition at 7 locations downstream of the nutrient injection point, and water samples were again collected when conservative tracer concentrations at the downstream end of the reach plateaued. To measure uptake in extremely cold conditions, we used a variety of modifications: 1) adding salt to mixing solutions to lower the freezing point, 2) insulating lines to and from the pump with foam pipe insulation, 3) covering the pump and bucket of solution to insulate and prevent falling snow/ice from entering, and 4) performing the addition when air temperatures were highest.

Specific analysis methods varied with the constituent. Ammonium was analyzed using the fluorometric method (Holmes et al. 1999, modified by Taylor et al. 2007) on an Aquafluor handheld fluorometer (Turner Designs, Sunnyvale, CA, USA) on the date of collection. Rhodamine WT concentrations were determined within 5 h of collection also with the Aquafluor handheld fluorometer. SRP samples were frozen until analyzed following the ascorbic acid method (APHA 2005) on a GENESYS™ 10s UV-Vis spectrophotometer (Thermo Fisher Scientific, Waltham, Massa-

chusetts). To determine DOC and total dissolved nitrogen (TDN) concentrations, samples were acidified and analyzed with a Shimadzu TOC-V_{CSN} with a total nitrogen module TNM-1 (Shimadzu Scientific Instruments, Columbia, Maryland). Chloride was determined with ion chromatography (Dionex ICS-900 Ion Chromatograph, Sunnyvale, California).

For each date, we corrected plateau nutrient concentrations for background concentrations and normalized these values to the conservative tracer. We then applied a natural log transformation to the normalized background-corrected concentrations, plotted those values versus distance (m) from the location of the injection, and fit a linear model to calculate 1st-order uptake rate coefficient (k). We used a threshold of $p < 0.10$ to determine whether linear regressions were significant. Non-significant regressions were further evaluated to identify whether the added nutrient or DOC was 1) rapidly removed (e.g., prior to the 3rd sampling station), or 2) whether there was no decline in the solute over the length of the reach. When the added nutrient or DOC was removed rapidly, we estimated uptake length based on when the solute was depleted and used this shorter reach length as an estimate for uptake length. This situation occurred only on two fall dates for NH_4 (29 September 2012 and 20 October 2012). When no decline in the solute occurred over the length of the reach, uptake was considered undetectable. For all analyses, we assigned undetectable uptake a value of $V_f = 0$, but the true value is likely a value between 0 and the lowest uptake we detected.

Uptake length (S_w; m) is the mean distance a nutrient molecule travels downstream in inorganic form before it is taken up and is calculated as:

$$S_w = k^{-1} \quad \text{(Eq. 1)}$$

From these values other nutrient spiraling metrics can be calculated including uptake velocity (V_f; m/min) and uptake rate (U; mg m^{-2} min^{-1}) (Tank et al. 2017):

$$V_f = \frac{(u \times z)}{S_w} \quad \text{(Eq. 2)}$$

$$U = V_f \times C \quad \text{(Eq. 3)}$$

where u = velocity (m^2/min), depth = z (m), and C = concentration (mg/m^3) (Table S1–S2). Cross-sectional widths and depths were measured at each of the 7 sampling locations within the study reach on each sampling date. Water velocity was calculated by dividing discharge (m^3/s) by the mean cross-sectional area (m^2) measured on each sampling date.

Here, we report nutrient uptake velocity (V_f), the rate of demand by stream biota and abiotic processes (Tank et al. 2017) because it standardizes for discharge, which allows for comparisons among dates with variable flow conditions (Davis and Minshall 1999).

Environmental covariates

We characterized physical, chemical, and biological factors that could control nutrient or carbon uptake. Physical factors characterized were discharge (Q), water temperature, and % canopy cover as a proxy for light availability. Chemical factors characterized were dissolved oxygen, turbidity, specific conductivity, pH, NH_4, SRP, DOC, and TDN concentrations. We characterized DOM composition as specific ultraviolet absorbance at 254 nm ($SUVA_{254}$, an indicator of C aromaticity), fluorescence index (FI) values, and parallel factor analysis (PARAFAC) scores derived from Excitation Emission Matrices (EEMs). We characterized biological factors as standing crops of allochthonous and autochthonous materials: chlorophyll *a* (Chl *a*) as an estimate of algal biomass (Steinman et al. 2007) and biofilm ash-free dry mass (AFDM) to represent the total living and dead organic material. Most predictors were measured on all study dates, but a subset were added as the study progressed. Canopy cover measurements began in March 2012; Chl *a* and AFDM characterization began in April 2012; DOC, TDN and $SUVA_{254}$ measurements began in July 2012; and all other DOM characterization began in March 2013 (Figs S2–S5).

Physical parameters of discharge and canopy cover were measured on each sampling date. We estimated discharge from the continuous data record for the stream as described above. We used a concave spherical densiometer (Model-C, Forestry Suppliers, Jackson, Mississippi) to estimate canopy cover on each date at each of the 7 sampling locations along the reach. In winter we determined the combined % of ice and snow covering the stream rather than canopy cover, therefore, estimates represent shading from both ice/snow and the tree canopy.

To quantify water temperature and water chemistry metrics (dissolved oxygen, turbidity, conductivity, and pH), we used a YSI 6920 V2 multiparameter sonde equipped with 6560 conductivity/temperature, 6150 optical dissolved oxygen (ODO), 6136 turbidity, and 6565 pH probes (YSI Incorporated, Yellow Springs, Ohio). We took measurements at the downstream end of the reach during each injection and determined background concentrations of NH_4, SRP, DOC, and TDN as part of the nutrient uptake measurement as described above.

DOM composition has been previously shown to be an important predictor of nutrient uptake in streams in Michigan's Upper Peninsula, likely due to inorganic nutrient demands of the heterotrophic bacteria that break down structurally complex DOM, or via abiotic sorption of nutrients onto DOM (Coble et al. 2016a). To estimate DOM composition, we collected filtered (0.45 μm) water samples at least monthly in 2013 and 2014, with more frequent collection in spring and fall. DOM composition samples were kept at 4°C and analyzed within 24 hours of collection for $SUVA_{254}$ and EEMs. EEMs were characterized on a Jobin–Yvon Horiba Fluoromax-TM fluorometer (Jobin Yvon

Horiba, Longjumeau, France) following the methods of Coble et al. (2016b). FI was identified from sample EEMs corrected for inner filter effects as the ratio of the emission intensity at 450 to 550 nm acquired at an excitation of 370 nm (McKnight et al. 2001). We calculated $SUVA_{254}$ by dividing the UV absorbance at 254 nm wavelength by the DOC concentration (Weishaar et al. 2003). Terrestrially-derived DOM is often associated with greater $SUVA_{254}$ and lower FI values, both of which indicate greater C aromaticity (McKnight et al. 2001, Weishaar et al. 2003). To identify fluorescing components, we used a PARAFAC that identified 6 components, all of which were previously identified in the literature (see Coble et al. 2016b for details). All components were quantified and analyzed as the percentage of total fluorescent DOM and will hereafter be referred to as C1, C2, C3, C4, C5, and C6. C1 is a fulvic-like fluorophore; C2, C3, and C5 are humic-like fluorophores; C4 is a fulvic-like and humic-like fluorophore; and C6 is a tryptophan-like fluorophore. Of these components, C1 may be of both terrestrial or autochthonous origin, C2–C5 are all associated with terrestrially-derived DOM, and C6 is typically assumed to originate from autochthonous production (Coble et al. 2016b).

Biofilms can be responsible for the removal of nutrients from the water column and, therefore, we estimated biofilm standing crops as both Chl *a* and AFDM. Three rocks or sediment cores (1-cm depth) were collected monthly from each of the 7 locations in the stream reach. All biofilm was removed from the rock surface by scrubbing with a firm brush and rinsing into ~150 mL of water. Subsamples from the resulting slurry were filtered onto pre-ashed 0.7-μm glass fiber filters and frozen until analysis. We traced planar rock shapes on paper and weighed the cutouts to determine rock surface areas (Bergey and Getty 2006). Concentrations of periphyton Chl *a* were estimated with the spectrophotometric method after extracting chlorophyll pigments in ethanol (Nusch 1980, APHA 2005). Following analysis, we estimated AFDM as the difference between the mass of samples following oxidization in a muffle furnace at 550°C for 4 h and the initial dry mass of those samples.

Statistical analyses

To determine if V_f and predictor variables varied among seasons, we used a non-parametric Kruskal–Wallis test because the assumptions of normality were not met. All data spanning 3 y and all 4 seasons were included in this analysis. When significant, we used non-parametric multiple comparisons to evaluate differences among seasons.

To examine how V_f responded to variation in multiple predictors, we applied nonparametric multiplicative regression (NPMR) as implemented in HyperNiche Version 2.29 (MjM Software Design, Gleneden Beach, Oregon; Appendix S1). We selected NPMR because it is well-suited for response variables that may exhibit unimodal responses to predictor variables and when interactions among variables are expected, whereas other commonly used regression and multivariate approaches only allow for additive relationships and often assume linear or sigmoid response curves (McCune 2006). Response variables were included as quantitative or categorical variables, and Gaussian weighting functions were used to conduct 'free search' iterations (allowing an indefinite number of predictors rather than a pre-determined number of variables) of combinations of variables and their smoothing parameters ('tolerances' in HyperNiche). Simultaneously in HyperNiche, screening is conducted to remove correlated variables and identify the best fit between the response and each predictor individually using a kernel smoothing function (McCune 2011). To evaluate model fit we used a cross-validated R^2 ($\times R^2$), which compares the residual sum of squares to the total sum of squares of each model run for a cross-validation dataset that omits 1 data point, which reduces overfitting and produces more realistic error estimates (McCune 2006). Each simulation was run 1000× and we calculated a p-value to assess significance of the model as the proportion of randomization runs that resulted in an equal or better fit than the original model (McCune 2006). In contrast to traditional approaches, NPMR is not structured by a fixed model equation and there are no coefficients or slopes to compare (McCune 2006). Rather, to evaluate the relative importance of predictors variables in final NMPR models we used sensitivity analysis (McCune 2006, 2011), where a higher sensitivity value indicates greater influence of that predictor variable on the model and a value of 0 indicates no detectable effect of that predictor on the response variable (McCune 2006). For example, a sensitivity value of 1 indicates that if the predictor changes 10%, then the response would change 10% (McCune 2006). NPMR has been used to assess nonlinear multiplicative relationships of community and climate data (e.g., Reisner et al. 2013, Pilliod et al. 2017). We could not include all predictors in NPMR models because all environmental covariates were not measured on all dates where uptake was measured. Nine predictors were included in NH_4 V_f and SRP V_f NPMR models (Q, NH_4, SRP, temperature, canopy cover, conductivity, pH, benthic Chl *a*, AFDM), and 12 predictors were included in DOC V_f NPMR models (Q, NH_4, SRP, temperature, canopy cover, conductivity, pH, benthic Chl *a*, AFDM, DOC, TDN, $SUVA_{254}$).

Few studies have examined whether DOM composition may be an important predictor of uptake, but metrics of DOM composition could not be included in our NPMR analyses because of the shorter time series of DOM composition data (25 dates over 2 y for DOC and $SUVA_{254}$ and 18 dates over 1.5 y for FI and PARAFAC components). Moreover, many of these metrics are autocorrelated and, thus, were not well-suited for NPMR analyses or multivariate regression. Therefore, we used individual regressions to evaluate the relationship between V_f and each DOM characteristic (DOC concentration, FI, $SUVA_{254}$, or PARAFAC

components C1–C6) across this subset of our data. For each regression we selected the best-fit model (linear or nonlinear) based on the variance explained.

For SRP and DOC, a large proportion of the dataset included 0 values for uptake and values for these variables could not be transformed to meet the assumption of normality. Therefore, we used nonparametric logistic regression individually for each DOM composition characteristic (DOC, FI, $SUVA_{254}$, or PARAFAC components C1–C6) to determine which predicted the probability of uptake occurring, with uptake as a binary response variable. Models were evaluated based on the variance explained.

RESULTS

Intra-annual variability in nutrient uptake velocity and comparisons among seasons

Seasonal patterns in nutrient uptake were observed for DOC V_f, but not for NH_4 or SRP V_f, each of which demonstrated a high degree of within-season variability. We found no significant seasonal variability in NH_4 V_f(Kruskal–Wallis: $X^2 = 7.24$, df = 3, $p = 0.06$) (Fig. 2A) or SRP V_f (Kruskal–Wallis, $X^2 = 2.48$, df = 3, $p = 0.48$) (Fig. 2B). The greatest range in NH_4 V_f was observed in fall and spring, with rapid uptake observed on some dates and negligible uptake on others (Fig. 2A). NH_4 uptake was undetectable on 1 of 10 dates in winter and 1 of 11 dates in spring. For SRP, uptake was undetectable on 8 of 11 dates in summer, 7 of 8 dates in fall, 8 of 10 dates in winter, and 7 of 11 dates in spring. DOC V_f was strongly seasonal with greater uptake observed in summer and fall than winter and spring, when uptake was below detection on all sampling dates (Kruskal–Wallis: $X^2 = 12.43$, df = 3, $p = 0.006$; Fig. 2C). DOC uptake was undetectable on 1 of 5 dates in summer, 1 of 5 dates in fall, 8 of 8 dates in winter, and 5 of 5 dates in spring. For the entire period of study, the greatest V_f was observed in fall for NH_4 and DOC (November 2012 and October 2013, respectively), and in spring for SRP (May 2014). Despite low temperatures and the occasional presence of ice cover, uptake was frequently detectable in winter for NH_4 (Fig. 2A), but only occasionally for SRP (Fig. 2B) and never for DOC (Fig. 2C).

Ten environmental parameters also varied seasonality. Temperature ($X^2 = 23.7$, df = 3, $p < 0.0001$) and canopy cover ($X^2 = 14.3$, df = 3, $p = 0.003$) showed significant seasonal variation with the lowest values in the winter and spring, respectively (Fig. 3A, B). Specific conductivity was lowest in the spring ($X^2 = 24.5$, df = 3, $p < 0.0001$; Fig. 3C) when discharge was highest ($X^2 = 19.1$, df = 3, $p = 0.0003$; Fig. 3D). Chl *a* standing crop was significantly greater in winter than all other seasons ($X^2 = 11.6$, df = 3, $p = 0.009$; Fig. 3E). Background concentrations of NH_4 ($X^2 = 13.6$, df = 3, $p = 0.004$; Fig. 4A) and DOC ($X^2 = 8.8$, df = 3, $p = 0.03$; Fig. 4C), differed significantly among seasons, with 2× higher concentrations of ammonium in summer relative to the other seasons, and significantly higher DOC concentrations in spring than winter. DOM also differed distinctly across seasons with a reduced terrestrial DOM signature observed in winter relative to summer and spring (Fig. 4D–H). SUVA was lowest (Fig. 4D) and FI was highest (Fig. 4E) in fall and winter compared with the spring, both of which suggest decreased DOM aromaticity and more microbial-like fulvic acids (SUVA $X^2 = 8.6$, df = 3, $p = 0.04$; FI $X^2 = 9.6$, df = 3, $p = 0.02$) (Fig. 4D, E). The relative percentage of the humic-

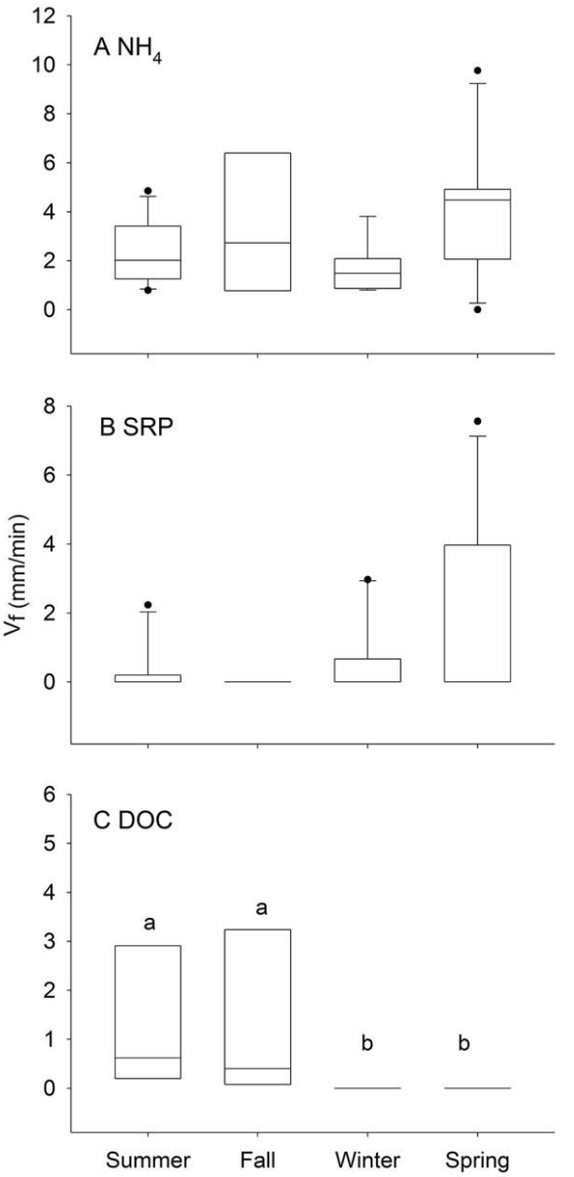

Figure 2. Box plots showing within and across season variation in NH_4 V_f (A), SRP V_f (B), and DOC V_f (C). Different lower case letters denote significant differences between seasons as determined by Kruskal–Wallis tests. Box plots display the 10th, 25th, 50th, 75th, and 90th percentiles of values. Black dots represent outliers beyond the 10th and 90th percentiles.

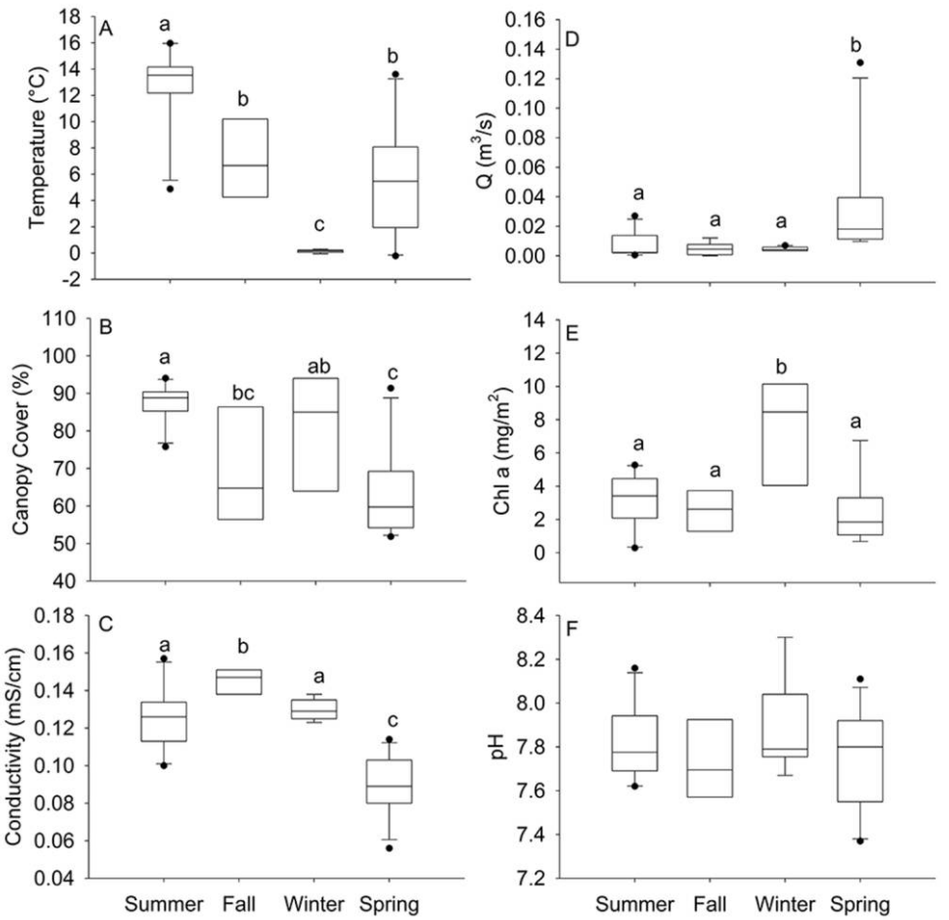

Figure 3. Box plots showing within and across season variation in temperature (A), canopy cover (B), conductivity (C), discharge (D), chlorophyll *a* (E) and pH (F). Box plots display the 10th, 25th, 50th, 75th, and 90th percentiles of values. Black dots represent outliers beyond the 10th and 90th percentiles. Different lower case letters denote significant differences between seasons as determined by Kruskal–Wallis tests.

like fluorophore C3 was greater in winter than spring or summer ($X^2 = 11.5$, df = 3, $p = 0.01$) (Fig. 4F), and the relative percentage of the fulvic/humic-like fluorophore C4 was greater in spring than fall or winter ($X^2 = 9.4$, df = 3, $p = 0.02$) (Fig. 4G), both of which suggest a seasonal shift in the relative fraction of these terrestrial components. The greatest relative percentage of tryptophan-like fluorophores (C6), which is an autochthonous DOM signature, were observed in winter and spring ($X^2 = 10.1$, df = 3, $p = 0.02$) (Fig. 4H). No significant differences were observed among seasons for SRP, AFDM, pH, TDN, C1, C2, and C5 (Figs 4B, S6).

Environmental predictors of nutrient uptake velocity

The environmental predictors of nutrient and DOC V_f varied among the 3 solutes, but suggest physical, chemical, and biotic variables may each be important predictors. NH_4 V_f was best explained by 4 predictor variables that included temperature, canopy cover, conductivity, and AFDM ($\times R^2 = 0.51$; Table 1). NH_4 V_f was elevated when canopy cover was low, temperature was low to moderate, conductivity was low, and AFDM was moderate to high. We present 3-D graphical representations of the best model for each response to show interactions between the 2 most influential variables (Fig. 5A). SRP V_f was best predicted by a single predictor (conductivity) and was greatest at low to moderate conductivity ($\times R^2 = 0.16$; Fig. 5B; Table 1). When SRP V_f was considered as a categorical response variable (uptake detectable or not) NH_4, SRP, and Chl *a* best predicted when SRP uptake occurred. Variability in the magnitude of DOC V_f was best explained by the combination of discharge, benthic Chl *a*, and $SUVA_{254}$ ($\times R^2 = 0.25$). DOC V_f was greater when Chl *a* was low, $SUVA_{254}$ was high, and discharge was low (Fig. 5C). When DOC V_f was considered as a response variable (uptake detectable or not), temperature, conductivity, and $SUVA_{254}$ were the best predictors (Table 1).

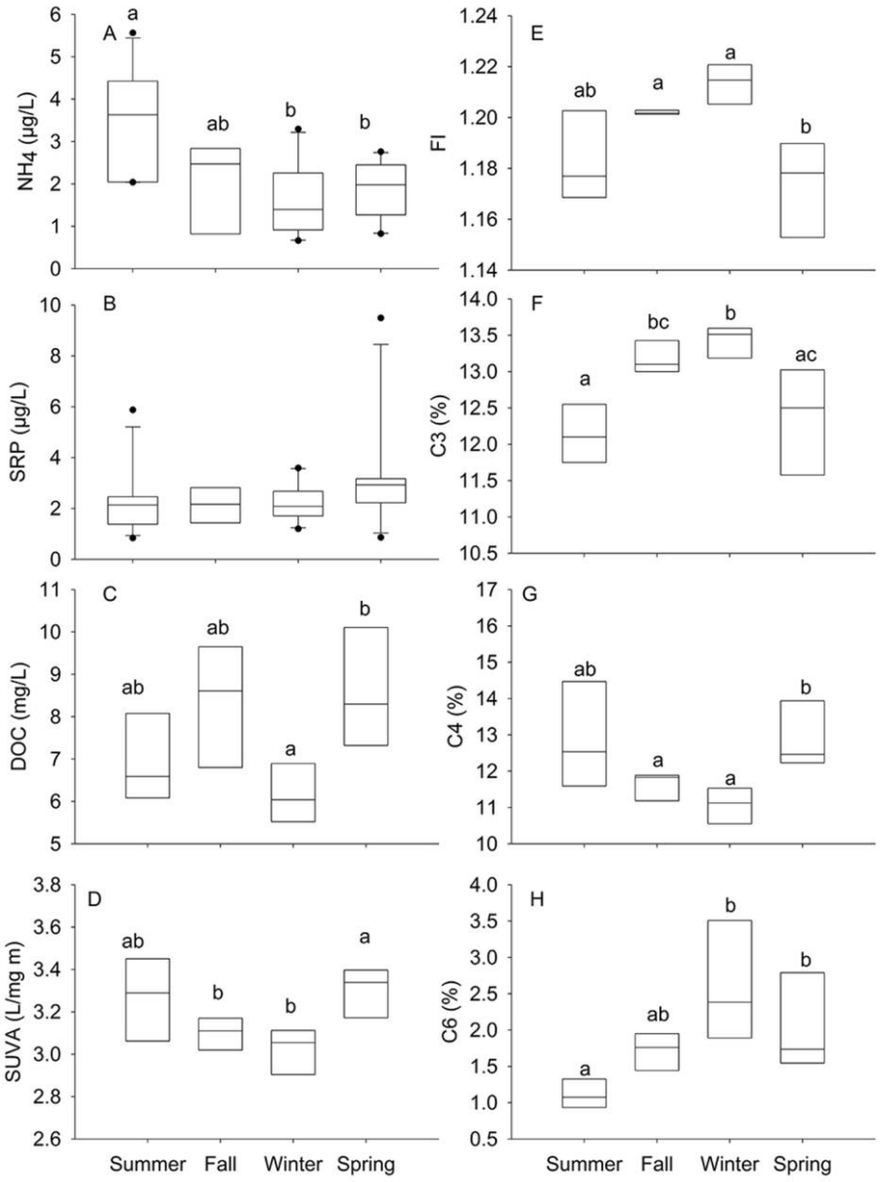

Figure 4. Box plots showing within and across season variation in NH_4 (A) DOC (B), SUVA (C), FI (D), C3 (E), C4 (F), and C6 (G). Note that FI is unitless. C3, C4, and C6 are descriptors of DOM composition identified from PARAFAC modeling of excitation-emission spectra as described in the text. All DOM composition descriptors are expressed as percent of total fluorescence of a sample. C1, C2 and C5 are not included because they were not significantly different among seasons (see Supplemental Fig. S6). Box plots display the 10^{th}, 25^{th}, 50^{th}, 75^{th}, and 90^{th} percentiles of values. Black dots represent outliers beyond the 10^{th} and 90^{th} percentiles. Different lower case letters denote significant differences between seasons as determined by Kruskal–Wallis tests.

DOM concentration or character may also be important predictors of nutrient or C uptake. Individual regressions revealed that $SUVA_{254}$ explained 41%, DOC concentration explained 39%, and FI explained 25% of the variability in NH_4 V_f (Table 2). $SUVA_{254}$ and DOC explained more of the variability in NH_4 uptake than FI. No DOM composition metrics explained when SRP V_f occurred based on logistic regression. For DOC uptake, logistic regression revealed that the probability of DOC uptake increased with increasing C6, a tryptophan-like fluorophore ($X^2 = 6.60$, $p = 0.01$, $n = 15$) and decreased with C5, a humic-like fluorophore ($X^2 = 4.5$, $p = 0.03$, $n = 15$).

DISCUSSION

Nutrient uptake and processing have seldom been measured year-round in streams in regions that receive a significant snowpack (Hoellein et al. 2007), yet transitions be-

Table 1. Nonparametric multiplicative regression (NMPR) model results. Both quantitative (Q) and categorical (C) response variables were used in nonparametric logistic regressions to identify which factors were important in predicting uptake. V_f = uptake velocity; SRP = soluble reactive phosphorus; DOC = dissolved organic carbon; n = sample size.

Response	Response type	Predictor Variables	Sensitivity	$\times R^2$	n
NH_4 V_f	Q	Temperature	0.40	0.51	33
		Canopy cover	0.15		
		Conductivity	0.23		
		Ash-free dry mass	0.52		
SRP V_f	Q	Conductivity	2.30	0.16	33
SRP V_f	C	NH_4	0.38	0.25	33
		SRP	0.48		
		Chlorophyll *a*	0.94		
DOC V_f	Q	Q	0.20	0.25	22
		Chlorophyll *a*	0.72		
		Specific ultraviolet absorbance	0.15		
DOC V_f	C	Temperature	0.99	0.83	22
		Conductivity	0.49		
		Specific ultraviolet absorbance	0.58		

tween winter and its shoulder seasons are vulnerable to climate change (Creed et al. 2015, Contosta et al. 2016). Previous measurements of year-round uptake have predominately been conducted in regions that do not experience consistent winter snowpack (Mulholland et al. 1985, Simon et al. 2005, Hanafi et al. 2006, Roberts and Mulholland 2007, von Schiller et al. 2008) or have avoided the months of heaviest snow and ice cover (Hoellein et al. 2007). Despite extremely low temperatures and ice/snow cover, we observed uptake of NH_4 and occasional uptake of SRP during winter, with rates comparable to those observed during other seasons. In contrast, DOC uptake remained below detection in both winter and spring, indicating DOC uptake is strongly seasonal. We found that SRP uptake velocity was highest and most variable during spring, immediately following winter snowmelt, while NH_4 and DOC uptake were highest in the fall, during leaf-fall, which agrees with the findings of other seasonal studies (Roberts and Mulholland 2007, Hoellein et al. 2007, Hall et al. 2009a). Biofilm Chl *a* and DOM composition were important predictors of DOC uptake rates and occurrence of SRP uptake identified in either the NPMR or regression analyses, and they were also different during winter compared with the other seasons. Chl *a* biomass was greatest in winter and DOM composition had a greater terrestrial DOM signal in spring than in other seasons (high DOC concentration, SUVA and low FI). By measuring uptake across complete annual cycles, our study suggests that the timing of resource availability is likely an important control on the magnitude and occurrence of uptake rates in forested, temperate streams.

Resource availability appears to strongly regulate temporal variability in V_f in this stream. In the only prior study that captured a similar range in seasonal temperature as our study (0–18.1°C), Hoellein et al. (2007) did not observe a consistent relationship between temperature and NH_4 or SRP uptake in 4 streams in Michigan's Upper Peninsula. They hypothesized this lack of temperature-dependence was due to the timing of resource availability, with greater organic matter and light availability occurring at times when temperatures were low. Consistent with their hypothesis, we observed the greatest rates of NH_4 uptake when light availability was high, temperatures were moderate, solute concentrations were low, and biofilm AFDM was higher. These environmental characteristics are often observed together in this northern temperate stream in spring and fall prior to leaf-on and after leaf-off, and are likely important predictors of NH_4 uptake because they influence the biological processes (e.g., gross primary productivity or ecosystem respiration) that drive uptake. For example, light availability is critical for primary production (Mulholland et al. 2001, Bernot et al. 2010, Roberts et al. 2007), temperature is a 1st-order control on the rate of biochemical and physiological processes (Kirchman and Rich 1997, Gillooly et al. 2001, Pomeroy and Wiebe 2001), and terrestrial organic matter fuels heterotrophic respiration in forested streams (Hynes 1975, Marcarelli et al. 2011). Collectively, our results suggest that a suite of abiotic and biotic factors produce optimal conditions for either biological activity or abiotic sorption, both of which can vary temporally.

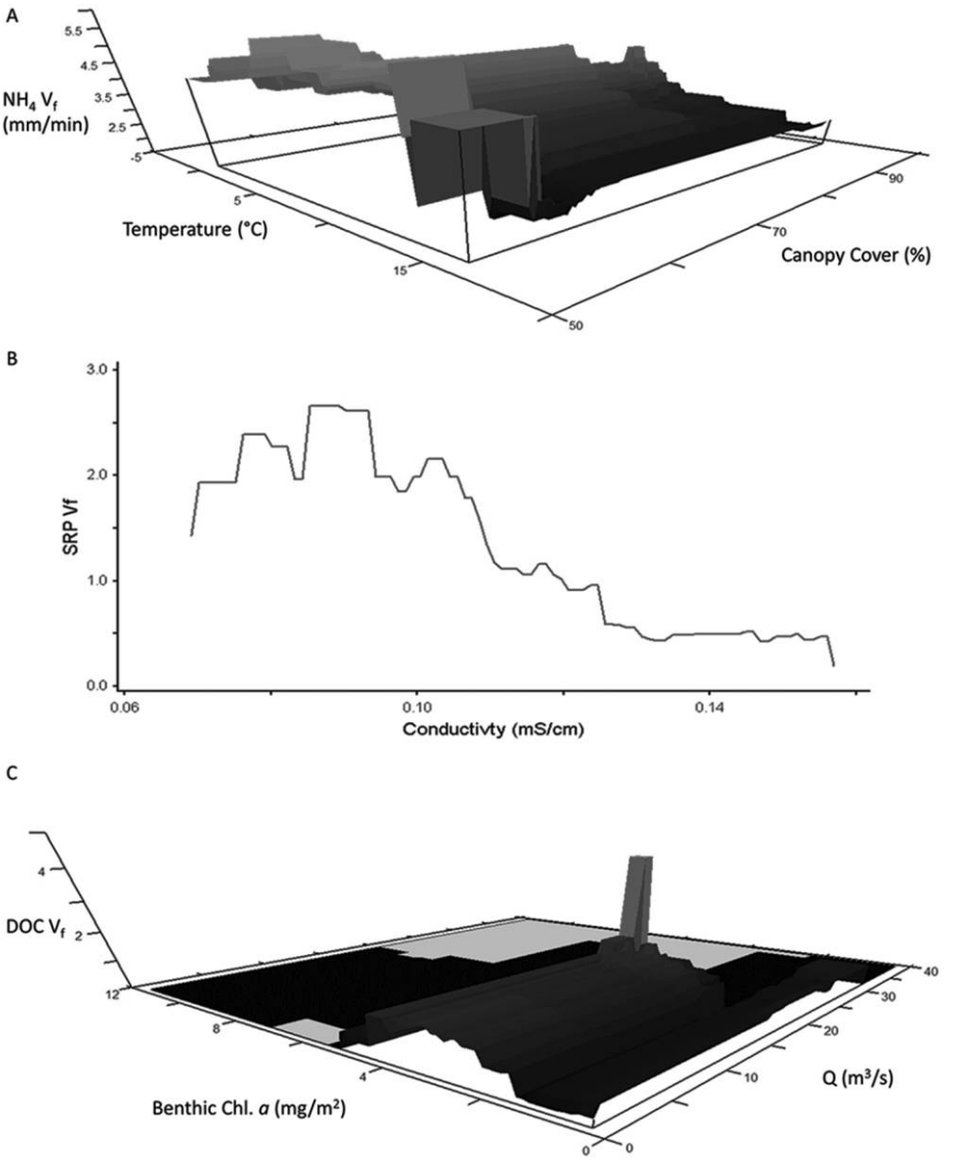

Figure 5. Nonparametric multiplicative regression (NPMR) modeled relationships between environmental predictors and NH_4 V_f (A), SRP V_f (B), and DOC V_f (C). We present three-dimensional graphical representations of the best model for each response to show interactions between the two most influential variables; however, for SRP only a single variable was influential thus a two-dimensional representation is shown.

A notable observation of this study is that SRP uptake was rarely detectable, and that detectable SRP V_f was regulated by SRP concentration, NH_4 concentration, and benthic algal standing stock, whereas solute concentrations (as specific conductivity) best predicted the magnitude of SRP V_f, albeit with low explanatory power. Undetectable uptake of SRP was not reported by Hoellein et al. (2007) in their year-round study of 4 Upper Peninsula study streams, but in a regional analysis of nutrient uptake in 11 Upper Peninsula streams during summer, we found that SRP uptake was only observed at study sites with less aromatic C and lower DOC concentrations than observed at other sites (Coble et al. 2016a). This finding contrasts with the seasonal results described in the current study, where none of the DOM composition metrics were significantly related to the occurrence of SRP V_f. Therefore, the possible abiotic and biotic controls on P uptake in this and other small, snowmelt driven streams are in need of further study.

Few studies have directly examined whether DOM character affects in-stream C uptake, but several studies have found greater rates of C breakdown in the presence of labile C (Guenet et al. 2014, Hotchkiss et al. 2014, Coble et al. 2015), and others have suggested that in-stream DOC demand may be related to DOM complexity (e.g.,

Table 2. Regression models for predicting rates of log NH_4 uptake (V_f) from individual dissolved organic matter (DOM) character variables. Only significant ($p < 0.05$) models are presented. The following predictor variables were considered and were transformed as necessary to meet assumptions of normality: dissolved organic carbon (DOC), specific ultraviolet absorbance (SUVA), fluorescence index (FI), C1, C2, C3, C4, C5, C6.

Predictor	Model	r^2	p	n
SUVA	$-4.33 + 9.28 \times \log$ SUVA	0.41	<0.001	25
DOC	$-2.08 + 2.74 \times \log$ DOC	0.39	<0.001	25
FI	$1.67 - 17.45 \times \log$ FI	0.25	0.043	18

Johnson et al. 2009). We found DOC concentration and DOM composition can be important predictors of temporal variability in uptake of NH_4 and DOC, with greater uptake demand associated with greater C aromaticity. These findings are consistent with previous observations that greater DOC concentrations and more aromatic C were associated with greater NH_4 demand across Upper Peninsula streams (Coble et al. 2016a). C uptake was not examined in the prior study, but here we also found DOC uptake was highly seasonal, occurring only in summer and fall, and the greatest magnitude of uptake was associated with low discharge, low AFDM, and high C aromaticity. C aromaticity was greatest in spring and summer, but this set of optimal discharge and benthic AFDM conditions were met only in summer and fall. Our results suggest that organic matter composition serves an important role in regulation of NH_4 and DOC uptake, and organic matter composition should be integrated into future studies of nutrient and C uptake across space and time.

Benthic Chl *a* was a predictor of DOC V_f, and we found that benthic algal biomass was greater in winter than in all other seasons. Although only a few other studies have described seasonal patterns in algal standing crops across full annual cycles in cold climates, they have similarly described elevated biomasses in streams under ice and snow cover. For example, Uehlinger et al. (1998) also found Chl *a* biomass was greatest in fall and winter in groundwater-fed channels and the main channel in a glacial floodplain in Switzerland, and attributed the low algal biomass observed in summer to high flow that increased shear stress, sediment transport, and turbidity. The high concentrations of benthic algal biomass observed in winter in our study likely occurred because of environmental conditions that either promoted biofilm growth and storage of Chl *a*, or reduced biofilm removal via scour or grazing (e.g., Rounick and Gregory 1981, Uehlinger et al. 1998). In boreal streams, Burrows et al. (2017) found microbial respiration of biofilms, which was strongly related to water temperature, was greatest in summer and least in winter, suggesting reduced microbial activity in winter. Furthermore, others have suggested that Chl *a* may not be indicative of live autotrophic biomass in extremely cold conditions with slow decomposition rates (e.g., Howard-Williams et al. 1989), which may also explain why Chl *a* was not a predictor of NH_4 uptake rates in our study. For example, Tank et al. (2017) found that NH_4 uptake was more strongly associated with living autotrophic biomass than total biomass of the biofilm. Others have demonstrated that biofilms can allocate greater storage of Chl *a* under low light conditions (Hill 1996) or when excess nutrients are available (e.g., Kromkamp 1987), although our background nutrient concentrations in the winter were low. We did not quantify light penetration to the water surface in winter although ice and snow cover suggests low light conditions were likely.

The high degree of variability in uptake within a season reveals that optimal environmental conditions for uptake are only met briefly, resulting in periods of highly efficient uptake in the shoulder seasons (McClain et al. 2003). For example, the greatest uptake in spring occurred approximately 1 month following peak spring discharge for NH_4 (28–40 d) and SRP (22−41 d), but high nutrient demand was not observed on all spring dates. In a Mediterranean mountain stream, a flood reduced benthic standing stock by 65%, changed hydraulic properties, and reduced nutrient retention, but nutrient retention recovered rapidly as the flood receded (Argerich et al. 2008). The rapid uptake observed on some dates in fall in our study could have been caused by leaf litter accumulation increasing water residence time in the stream (sensu Argerich et al. 2008). Collectively our results demonstrate that frequent temporal measurements in seasons not often measured can yield new insights into the controls on nutrient uptake, and that winter remains an equally important period for in-stream nutrient processing and biological activity and should not be ignored.

Our results showed that the greatest within-season variability of uptake rates occurred in spring and fall for NH_4, spring for SRP, and fall and summer for DOC, and that these rates relied on specific suites of environmental conditions. This observation is important because environmental conditions are particularly susceptible to change in the shoulder seasons (spring and fall). Lengthening of the vernal window extends the period between spring discharge and leaf-on (Contosta et al. 2016), a time when uptake of NH_4 and SRP can be most efficient. Thus, changing leaf phenology in spring could increase nutrient retention in streams.

However, the absence of a substantial snowpack could also cause asynchronous changes in physical and biological transitions during the vernal window in northern temperate regions (Contosta et al. 2016), potentially disrupting conditions favorable for efficient uptake. Decreased volume and duration of snowpack (Brown and Robinson 2011) could also decrease the magnitude of spring discharge. Fall, the neglected season in climate change research (Gallinat et al. 2015), is also experiencing changes in phenology with leaf-off occurring later. Yet the overall duration of the autumnal window remains unchanged because photoperiod remains constant relative to climatic change (Creed et al. 2015). Any alteration to the timing of key environmental conditions will cause feedbacks in timing and magnitude of nutrient processing and export, with important implications for downstream productivity spanning oligotrophic to eutrophic ecosystems.

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