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Uptake of ammonium and soluble reactive phosphorus in forested streams: influence of dissolved organic matter composition

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Abstract Many microbes responsible for inorganic nutrient uptake and transformation utilize dissolved organic matter (DOM) as a nutrient or energy source, but little is known about whether DOM composition is an important driver of nutrient uptake in streams. Our goal was to determine whether incorporating DOM composition metrics with other more commonly considered biological, physical, and chemical variables improved our ability to explain patterns of ammonium (NH_4^+–N) and soluble reactive phosphorus (SRP) uptake across 11 Lake Superior tributaries. Nutrient uptake velocities (V_f) ranged from undetectable to 14.6 mm min^{-1} for NH_4^+–N and undetectable to 7.2 mm min^{-1} for SRP. Logistic regressions suggested that DOM composition was a useful predictor of where SRP uptake occurred (4/11 sites) and NH_4^+–N concentration was a useful predictor of where NH_4^+–N uptake occurred (9/11 sites). Multiple regression analysis revealed that the best models included temperature, specific discharge, and canopy cover, and DOM composition as significant predictors of NH_4^+–N V_f. Partial least squares revealed fluorescence index (describing the source of aquatic fulvic acids), specific ultraviolet absorbance at 254 nm (an indicator of DOM aromaticity), temperature, and conductivity were highly influential predictors of NH_4^+–N V_f. Therefore, streams with higher temperatures, lower solute concentrations, more terrestrial DOM signal and greater aromaticity had greater NH_4^+–N V_f. Our results suggest that DOM composition may be an important, yet often overlooked, predictor of NH_4^+–N and SRP uptake in deciduous forest streams that should be considered along with commonly measured predictors.

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Introduction

In aquatic ecosystems dissolved organic matter (DOM) exerts a strong influence on biological, physical, and chemical characteristics, all of which are essential in biogeochemical cycling (Maranger and Pullen 2003; Prairie 2008; Webster et al. 2008). For example, DOM can bind trace elements (Kalbitz and Wennrich 1998), affect water transparency (Kostoglidis et al. 2005), and alter rates of microbial metabolism (Lennon and Pfaff 2005). DOM is comprised of a complex mixture of organic acids and molecules and these components originate from a variety of aquatic and terrestrial organic matter pools, each of which can undergo biogeochemical transformations and photodegradation, further contributing to the complexity of DOM found within aquatic ecosystems (Thurman 1985; McKnight et al. 2003). For example, bacterial processing significantly reduced dissolved organic carbon (DOC) concentrations and depleted ^{13}C in the Tana River in Kenya after 8 days of incubation, presumably due to differences in lability of C_4 herbaceous vegetation versus C_3 woody vegetation (Geeraert et al. 2016). Furthermore, in inland waters autochthonous DOM can be more rapidly utilized by microorganisms than allochthonous DOM (Toming et al. 2013).

In streams and rivers, watershed characteristics can also influence variability in DOM concentration and composition (Gergel et al. 1999; Frost et al. 2006; Yamashita et al. 2011). For example, in streams of Michigan's Upper Peninsula, Frost et al. (2006) found that stream DOM concentration was related to watershed morphology, land cover, and wetland type, but DOM physicochemical properties (e.g., molecular weight, spectral indices) were related to upstream lake and wetland area. In another study of tributaries and the near shore region of the Laurentian Great Lakes, land cover and nutrient concentrations were more strongly related to DOC concentration than DOM composition, supporting the concept that watershed characteristics may differentially influence the concentration and composition of dissolved organics (Larson et al. 2014). Clearly, watershed characteristics serve an important role in determining DOC concentration and DOM composition in streams and rivers, but the effects of this variation in DOM on in-stream ecosystem processes are not well understood.

Cycling of carbon (C) and other limiting nutrients, particularly nitrogen (N) and phosphorus (P), are known to be linked through organismal activity and stoichiometric composition across a variety of ecosystems (Sterner and Elser 2002; Schlesinger et al. 2011), including streams and rivers (Sobczak et al. 2003; Bechtold et al. 2012; Oviedo-Vargas et al. 2013), yet studies have rarely considered DOM composition as a potential predictor of nutrient uptake rates. Bioavailable DOM, which has been linked to DOM composition (Marschner and Kalbitz 2003; Benner 2003), is a substrate for heterotrophic bacteria (Kaplan and Newbold 2003). Heterotrophic bacteria, in turn, require inorganic N and P to build biomass. The availability of these essential nutrients can limit growth and heterotrophic bacteria differ in their metabolic capabilities and utilization of DOM and N, thereby affecting the rates of nutrient uptake (Reed and Martiny 2007; Ghosh and Leff 2013). For example, Ghosh and Leff (2013) found that the bacterial community composition changed relative to DOM and N (quality and concentration) treatments, likely in response to manipulations in the chemical conditions, that in turn altered rates of N uptake (as dissolved organic N and inorganic N). Therefore, DOM composition may be a potentially important, yet overlooked, predictor of nutrient uptake. Previous studies have found that respiration and N assimilation can increase when labile C is added to temperate streams, suggesting that an increase in bioavailable C increases demand for inorganic N (Bernhardt and Likens 2002; Johnson et al. 2009, 2012). Others have found that seasonal variation in N availability may limit C mineralization rates in streams and rivers (Wickland et al. 2012; Coble et al. 2015). Another study found an increase in P uptake occurred following additions of DOC and N, suggesting coupling of C and P uptake in streams (Oviedo-Vargas et al. 2013). Given the potential role of C quantity and quality in controlling inorganic nutrient uptake, simultaneous measurements of both DOM composition and nutrient uptake metrics could, collectively, provide insight into mechanisms responsible for spatial variability in nutrient uptake among watersheds.

Studies of in-stream uptake of N and P have found that processing rates are influenced by a variety of characteristics in and among watersheds including: stream size (Peterson et al. 2001), riparian vegetation (Sabater et al. 2000), climate variability (Mulholland 1992), autotrophic activity (Hall and Tank 2003), and

nutrient inputs (point source or eutrophication; Alexander et al. 2000; Marti et al. 2004), but DOM composition is not typically considered in this context. Here, we applied short-term releases of ammonium (NH_4^+) and phosphate (PO_4^{3-}) into 11 forested tributaries of Lake Superior where we also characterized DOM composition, quantified watershed characteristics, and measured a variety of stream biological, physical, and chemical characteristics. Our goal was to evaluate whether including DOM composition metrics along with a suite of previously studied characteristics would improve our ability to predict patterns of nutrient uptake velocities in these forested streams.

Methods

Study sites and sampling design

We selected 11 small to mid-size tributaries situated near the south shoreline of Lake Superior in Michigan's Upper Peninsula with a range in watershed area from 1.7 to 124.2 km^2 and wetland cover from 1 to 30% of total watershed area (Fig. 1; Table 1). Among these watersheds mean slope ranged from 3.4 to 12.9% and agricultural extent ranged from 0 to 0.003% (see Supplementary Material for detailed methods of watershed characteristics). Bedrock among these sites consists of Precambrian sedimentary and volcanic formations (Michigan Department of Environmental Quality 1987; Table 1) and overstory vegetation is predominately comprised of northern hardwood forests. Mean annual precipitation (1983–2013) in the region is ~80 cm (National Atmospheric Deposition Program, Station MI99, Chassell Michigan, available from: http://nadp.sws.uiuc.edu/data/) with up to 50% of annual precipitation received as snow (Stottlemyer 1997). Nutrient concentrations are generally low in streams across this region; background NH_4^+–N concentrations exceeded 10 $\mu g\ L^{-1}$ at only two of the study sites on our sampling dates (Little Huron and Salmon Trout), while soluble reactive P (SRP) concentrations were below 4.2 $\mu g\ L^{-1}$ (Supplemental Table 1) and background DOC concentrations ranged from 2.0 to 9.3 mg L^{-1} (Table 2). Selected study reaches had little to no tributary input and were located 0.2–22.6 river km from Lake Superior (Table 1). Reach lengths ranged from 200 to 1000 m to target a travel time of 40 min to 1 h. Along the study reaches, seven–eight sampling locations were established every 25–100 m, with distance between the sampling locations varying based on total reach length. All sampling was conducted during summer; six study sites (Calumet, Big Pup, Little Huron, Little Garlic, East Branch Huron, Salmon Trout) were sampled in July 2012 and all 11 study sites were sampled in July or August 2013. The five additional sites (Black, Hills, Gratiot, Mountain, Pine) were added in 2013 to increase the spatial extent of our study.

Dissolved organic matter character

For determination of DOM character, 0.45 μm-filtered (Fisherbrand membrane filters) water samples were collected on each study date and stored in HDPE containers. All filters were rinsed three times with stream water and all bottles were rinsed three times with filtered water before samples were collected. Samples were immediately placed in a cooler and then refrigerated (1.6 °C) until analyzed using several spectrophotometric and fluorescent techniques within 3 days of collection. Specific ultraviolet absorbance at a wavelength of 254 nm ($SUVA_{254}$) provides a measure of the amount of chromophoric C, and is correlated with percent aromaticity of DOM (Weishaar et al. 2003). $SUVA_{254}$ was determined by measuring the UV absorbance at 254 nm on room temperature water samples for all study dates using a Thermo Scientific 10s UV–vis spectrophotometer (Thermo Scientific, Waltham, Massachusetts), and dividing this value by the DOC concentration (Weishaar et al. 2003), which was quantified as described below. To characterize the chemical composition of DOM we determined fluorescence excitation–emission matrices (EEMs) with a Horiba Fluoromax-3™ fluorometer (Jobin–Yvon Horiba, France) in 2013 only. EEMs were collected on room-temperature water samples every 3 nm over excitation wavelength intervals between 240 and 600 nm, and every 3.28 nm over emission wavelength intervals between 212 and 620 nm. Fluorescence spectra were corrected for inner filter effects then normalized to the integral of the Milli-Q water Raman peak (Ex 350/Em 371–428) of the sample, and the resulting fluorescence spectra were normalized to an
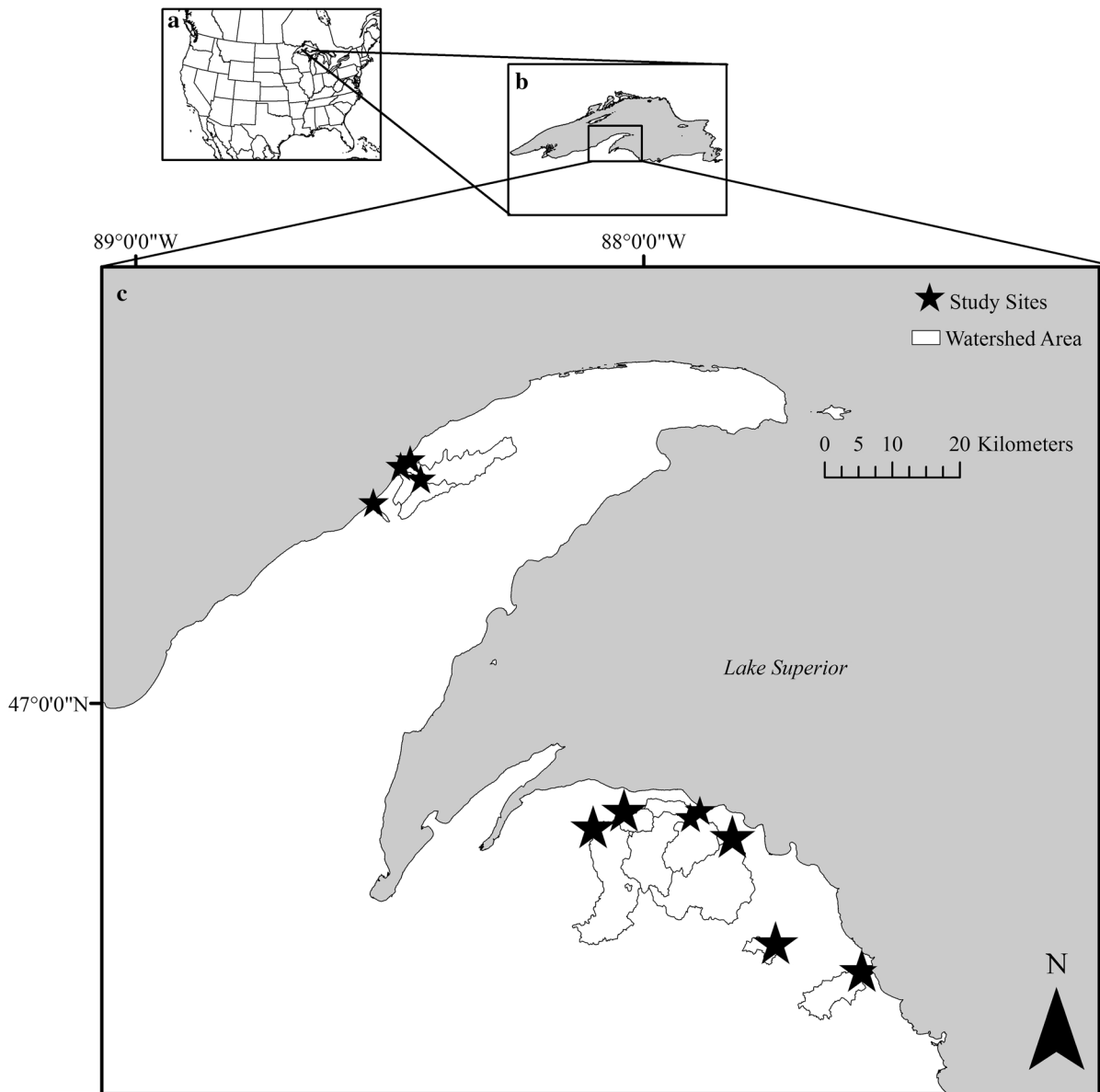
Fig. 1 **a** Location of Laurentian Great Lakes in the contiguous USA. **b** Lake Superior with the study area framed by the *black box*. **c** Location of study sites (*stars*) and their delineated watersheds

external Starna Cells ultrapure Raman peak water standard. Because we were interested in distinguishing the source of DOM, we calculated the fluorescence index (FI) from corrected sample EEMs as the ratio of the emission intensity at 450 to 550 nm acquired with an excitation of 370 nm (McKnight et al. 2001). FI can be used to distinguish sources of aquatic fulvic acids, with high values of FI (above 1.7) indicative of microbially-derived and low values (below 1.2) indicative of terrestrially-derived material (McKnight

et al. 2001). FI is also strongly correlated with aromaticity (McKnight et al. 2001).

Parallel factor analysis (PARAFAC) was used to further identify fluorescing components detected using EEMs (Stedmon et al. 2003; Cory and McKnight 2005), and was completed using the PLS-toolbox for MATLAB (Eigenvector Research, Inc.). We used a dataset of 374 samples to develop a robust PARAFAC model (e.g., Stedmon and Bro 2008), and the details of this PARAFAC model have been previously published

Table 1 Watershed characteristics determined using GIS for each site

Stream names	Watershed areas (km^2)	Wetland (% of watershed)	Slope (%)	Distance from Lake Superior (km)	Bedrock
Calumet	1.7	30.0	4.4	0.2	Freda sandstone, Copper Harbor conglomerate, nonesuch formation
Black	10.1	10.6	6.2	0.4	Copper Harbor conglomerate, Freda sandstone
Big Pup	11.1	1.1	11.9	22.6	Archean granitic and gneissic, Jacobsville sandstone
Little Huron	15.7	2.2	12.9	3.1	Archean granitic and gneissic, Jacobsville sandstone
Hills	18.3	13.7	4.1	5.0	Portage Lake Volcanics, Copper Harbor conglomerate, Freda sandstone
Little Garlic	34.8	4.8	8.4	3.1	Archean volcanic and sedimentary, Jacobsville sandstone
Gratiot	45.2	23.3	3.4	0.8	Portage Lake Volcanics, Copper Harbor conglomerate, Freda sandstone
Mountain	62.1	15.0	9.1	4.9	Archean granitic and gneissic, Michigamme formation
East Branch Huron	95.4	10.9	5.2	12.0	Michigamme formation, Jacobsville sandstone
Salmon Trout	107.5	9.9	8.8	5.3	Archean granitic and gneissic, Michigamme formation, Jacobsville sandstone
Pine	124.2	18.8	10.2	2.7	Archean granitic and gneissic, Michigamme formation, Jacobsville sandstone

Watersheds were delineated based on the furthest downstream sampling location within our reach, thus watershed area includes only the area upstream of our study sites. The percent wetland area in the watershed was determined with the National Wetlands Inventory ArcGIS shapefile (US Fish and Wildlife Service, Division of Habitat and Resource Conservation, 26 September 2011, CONUS_wet_poly, Washington, DC, http://www.fws.gov/wetlands/). Bedrock was determined from Michigan Bedrock Geology GIS shapefile (Michigan Department of Environmental Quality 1987)

in Coble et al. (2016). Each of these six components (C1–6) has been previously described in aquatic systems (Coble et al. 2016). C1 was identified as a fulvic-like peak, C2, C3, and C5 were identified as humic-like peaks, C4 was identified as containing both fulvic-like and humic-like peaks, and C6 was identified as the protein-like component tryptophan (Coble et al. 2016). C6 was the only autochthonous-derived component identified. Hereafter, reference to these six components will be expressed as a percentage of all fluorescent DOM.

Predictors of nutrient uptake velocity

To identify whether nutrient uptake velocity could be predicted by other stream characteristics, in addition to DOM composition and the watershed characteristics described above, we measured canopy cover, water temperature, pH, conductivity, background nutrient concentrations, and periphyton biomass on each sampling date. We measured canopy cover as a proxy for light availability in the center of the stream channel at the seven–eight established sampling locations along each stream reach using a densiometer. A YSI multiparameter sonde (YSI Incorporated, Yellow Springs, Ohio) that measured water temperature, dissolved oxygen, conductivity, and pH at 1 min intervals was deployed upon arrival at the downstream end of the reach and was retrieved after all sampling was completed. Background concentrations of NH_4^+–N, SRP, TDN, and DOC were collected as part of nutrient uptake measurements and analyzed as

Table 2 DOC concentration, fluorescence index (FI), and specific ultra-violet absorbance at 254 nm (SUVA$_{254}$) among streams

Site names	Years	DOC (mg C L^{-1})	FI	SUVA$_{254}$ (L mg^{-1} m^{-1})
Calumet	2012	7.92	NA	2.93
Little Huron	2012	4.68	NA	2.65
Big Pup	2012	2.14	NA	2.43
Little Garlic	2012	4.74	NA	3.16
East Branch Huron	2012	6.67	NA	3.75
Salmon Trout	2012	3.29	NA	3.20
Calumet	2013	8.60	1.18	3.37
Little Huron	2013	4.30	1.19	3.89
Big Pup	2013	2.00	1.23	3.07
Little Garlic	2013	5.10	1.23	3.33
Black	2013	7.25	1.17	3.29
Hills	2013	6.07	1.15	3.18
Gratiot	2013	6.54	1.21	3.58
East Branch Huron	2013	9.30	1.17	3.90
Mountain	2013	5.94	1.15	3.18
Salmon Trout	2013	3.79	1.15	3.43
Pine	2013	5.89	1.17	3.46

High values of FI have been associated with microbially-derived and low values have been associated with terrestrially-derived sources of fulvic acids (McKnight et al. 2001)

described below. In addition, on each date we collected background water samples for analysis of common anions and total dissolved P (TDP) as described below.

To determine periphyton biomass as chlorophyll *a*, we collected samples of the dominant substrate as either rocks or fine sediment at each of the seven–eight water sampling locations. For fine sediments, we collected the top 1 cm of sediment using a 2 cm diameter core. For rocks, we selected three rocks haphazardly at each location and scrubbed the attached algae into approximately 150 mL of water. A subsample of the resulting slurry was filtered through pre-ashed GF/F filters and frozen until laboratory analysis. Periphyton chlorophyll a was analyzed using the spectrophotometric method (American Public Health Association, APHA 2005; Nusch 1980). After analysis, the planar rock shape was traced and weighed to determine the surface area of the rocks (Bergey and Getty 2006).

Nutrient uptake and water chemistry

We quantified whole-reach uptake of NH_4^+–N and SRP using nutrient spiraling techniques (Stream Solute Workshop 1990). Discharge was measured on each sampling date at each site except Calumet by measuring depth and velocity at 60% depth at 10 points equally distributed across the wetted width of each reach using a Flow-mate^{TM} model 2000 flow meter (Marsh McBirney, Inc., Frederick, Maryland) and wading rod. At Calumet, an established Parshall flume, Stevens pressure transducer (Stevens Water Monitoring Systems, Inc., Portland, Oregon), and Li-Cor data logger (Li-Cor, Lincoln, Nebraska) with stage–discharge relationship allowed for on-site determination of discharge on each sampling date (Stottle-myer and Toczydlowski 2006). Prior to the addition of nutrients, replicate samples of filtered water (0.45 μm) were collected for analysis of background concentrations of NH_4^+–N and SRP at each sample location within each study reach. At all sites we conducted a short-term (2–4 h) release of combined NH_4^+–N and SRP by pumping a solution of NH_4Cl, KH_2PO_4, and a conservative tracer (rhodamine WT) into the upstream end of the study reach at a constant rate of 100 mL min^{-1}. To ensure that the solution had reached plateau prior to sampling at downstream locations, rhodamine concentrations were monitored by analyzing stream water with a handheld fluorometer (Turner Designs, Palo Alto, California). We targeted an increase in background nutrient concentrations of 12 μg L^{-1} for NH_4^+–N and SRP, and 7 μg L^{-1} for rhodamine WT; because we did not have

background nutrient information prior to conducting injections for some study sites, this resulted in enrichment factors (enrichment/background concentrations) ranging from 1.4 to 6.7 for NH_4^+–N, and 1.0–8.5 for SRP. After the conservative tracer reached a plateau concentration at the downstream end of the reach, we again collected replicate samples from all sampling locations. Additional water samples were collected at the conclusion of the release upstream from the nutrient addition site to confirm that background conditions did not change during the nutrient addition.

Ammonium was analyzed on the day of sample collection using a fluorometric method (Holmes et al. 1999, adapted by Taylor et al. 2007) and analyzed with either a Turner Aquafluor (Turner Designs, Palo Alto, California) handheld fluorometer or a Trilogy laboratory fluorometer (Turner Designs Palo Alto, California). To account for any interference of rhodamine in our plateau NH_4^+–N measurements we compared rhodamine-spiked and rhodamine-free standard curves of NH_4^+–N, determined a correction factor, and applied this correction factor to all plateau concentrations. All SRP samples were frozen until laboratory analysis using the ascorbic acid method (APHA 2005) and analyzed on a Thermo Scientific Gensys10s UV–vis spectrophotometer (Thermo Scientific, Waltham, Massachusetts). DOC samples were kept cool (4 °C) until laboratory analysis, when they were acidified with hydrochloric acid for quantification of DOC and TDN concentrations using a Shimadzu TOC-V_{CSN} with a total N module TNM-1 (Shimadzu Scientific Instruments, Columbia, Maryland). Rhodamine WT concentrations were analyzed within 5 h of collection with an Aquafluor handheld fluorometer. We also analyzed fluoride (F^-), chloride (Cl^-), nitrite (NO_2^-), bromide (Br^-), nitrate (NO_3^-), phosphate (PO_4^{3-}), and sulfate (SO_4^{2-}) on a Dionex ICS-900 ion chromatograph (Dionex, Sunnyvale, California) and TDP by performing an ammonium persulfate digestion method followed by the ascorbic acid method (APHA 2005). When nutrient concentrations were below detection limits they were replaced with a value of 1/2 the detection limit for inclusion in statistical analyses (Supplemental Table 1).

To determine nutrient uptake lengths (S_w), background-corrected nutrient concentrations (plateau minus background concentration) were normalized by dividing by the conservative tracer concentrations,

and the natural log of the normalized values were plotted against distance from the nutrient addition site (Stream Solute Workshop 1990). If a linear regression fit to this plot was significant ($\alpha = 0.10$), S_w (m) was then calculated as the inverse slope of the regression line (k_w; Stream Solute Workshop 1990):

$$S_w = \frac{1}{k_w}.$$

From S_w we calculated uptake velocity (V_f; m s^{-1}):

$$V_f = \frac{(u \times z)}{S_w},$$

where u is the velocity (m s^{-1}), and z is the depth (m).

We used S_w to assess how far a nutrient travels before it is taken up in relation to the total stream distance prior to entering Lake Superior. Because S_w is highly influenced by discharge (Davis and Minshall 1999; Hall et al. 2002), we used V_f (converted to mm s^{-1}) for all analyses comparing nutrient uptake among sites and years.

Statistical analyses

We used a principal components analysis (PCA) to compare DOM characteristics among watersheds and to reduce the number of DOM composition variables included in later analyses. DOM characteristics included in this analysis were the six fluorescent components identified by PARAFAC (C1–6; expressed as a percentage of fluorescent DOM), DOC concentration, SUVA$_{254}$, and FI.

Due to the large amount of nutrient uptake measurements that resulted in undetectable uptake (3 for NH_4^+, 10 for SRP; see "Nutrient uptake metrics" section) we also sought to determine if any of the predictor variables explained the likelihood of significant uptake of NH_4^+–N or SRP. We used binary logistic regression with a categorical response variable of yes or no to describe whether uptake occurred. The DOM PC axes 1 and 2 (PC1 and PC2) derived from the PCA analysis, which reduced the DOM character variables into two predictors, were used in place of individual DOM character variables. All other variables measured across the study sites were also included in this logistic regression analysis. Logistic regression analyses were performed using JMP Pro (version 12.1.0, SAS Institute, Inc.)

Following the logistic regression analysis, multiple linear regression was used to identify significant predictors of uptake velocity when it was detectable at the study sites. We only constructed regression models for 2013, when all streams were sampled and DOM was characterized using fluorometric analysis. Prior to model selection, we removed additional predictors due to significant correlations ($p > 0.05$) with other predictor variables: DOC, molar ratio DOC:DIN, TDN, slope, dissolved oxygen, and PC1 (the first component axis from the PCA of DOM composition). Therefore, the multiple regression models included the following predictors: canopy cover, stream water temperature, conductivity, pH, benthic chlorophyll *a*, specific discharge, percent wetland area, concentrations of NH_4^+–N and SRP, and PC2 (the second component axis from the PCA of DOM composition). For variables collected with the multiparameter sonde, we used the mean during the background sampling period. The variables wetland area and SRP did not meet the assumption that residuals were normally distributed and a normal distribution could not be achieved through transformation of the predictor variables, thus they were excluded. We identified the best model(s) based on the smallest Akaike's information criteria (AIC) and only considered models greater than two AIC units from the null model (Burnham and Anderson 2002). Multiple regression analyses were performed using R (version 3.1.2, R Core Development Team). Finally, we used a multivariate partial least squares (PLS) analysis where variance in the predictors is used to explain variance in the response, which is less sensitive than multiple regression to correlation among predictor variables and deviations from normality (Carrascal et al. 2009). The PLS was conducted with uptake velocity as the response variable and specific discharge (discharge/watershed area), percent wetland area, slope, stream temperature, canopy cover, conductivity, pH, benthic chlorophyll a, TDN, NH_4^+–N, NO_3^-–N, SRP, DOC, the molar ratio of DOC:DIN, FI, $SUVA_{254}$, and the six fluorescent components identified by PARAFAC (C1–6, expressed as a percentage of fluorescent DOM) as predictor variables. Note that here we use all individual metrics of DOM composition rather than the PC axes because PLS works well when variables are correlated and PLS performs simultaneous decomposition of predictor and response variables, whereas

PCA only decomposes predictor variables. Given these strengths of the PLS analysis the model was better informed by inclusion of the individual predictors. All data were autoscaled prior to analysis. Within a PLS biplot, the location of the variables indicates the correlation structure such that variables situated near each other are positively correlated and variables situated opposite each other are negatively correlated. Variables situated near the origin have very little influence on the overall model, and variables situated a greater distance from the origin have a greater overall influence on the model (Kothawala et al. 2014). The importance of a variable on the overall model was determined by the variable importance on the projection (VIP) scores. VIP scores ≥ 2 were considered highly influential, between 1 and 2 as moderately influential, and <1 as less influential to the overall model. We identified outliers as samples exceeding the 95% confidence limit in the Hotelling's T^2 analysis, and internal cross validation was performed to test the repeatability of the analysis and validate the model. PCA and PLS analyses were performed using MATLAB (MATLAB®) with the PLS toolbox (Eigenvector Research, Inc.).

Results

DOM characteristics

Differences in $SUVA_{254}$, FI, and fluorescing components identified by PARAFAC indicate variability in DOM chemical characteristics among sites. $SUVA_{254}$, an indicator of DOM aromaticity, ranged from 2.43 to 3.75 L mg^{-1} m^{-1} in 2012 and from 3.07 to 3.90 L mg^{-1} m^{-1} in 2013 (Table 2). In both 2012 and 2013, the lowest $SUVA_{254}$ values were observed at Big Pup and the greatest (greater aromaticity) at East Branch of the Huron (Table 2). FI ranged from 1.15 to 1.23 among sites (Table 2); FI values below 1.2 are characteristic of terrestrially derived fulvic acids and FI values above 1.7 are characteristic of microbially-derived fulvic acids (McKnight et al. 2001).

The PCA model of DOM characteristics identified four PCs that collectively explained 97% of the variability in the model. The first PC axis explained 63.4% of the variability in the model and included strong positive loadings from components with longer

Fig. 2 Principal components analysis loading plot of the first two principal components (PCs) axes displaying DOM characteristics (*open circles*) and sites (*closed circles*). DOM characteristics were abbreviated as: fluorescence index (FI), dissolved organic carbon concentration (DOC), specific ultraviolet absorbance at 254 nm (SUVA$_{254}$), and the six DOM components identified by PARAFAC analysis (*C1–6*)
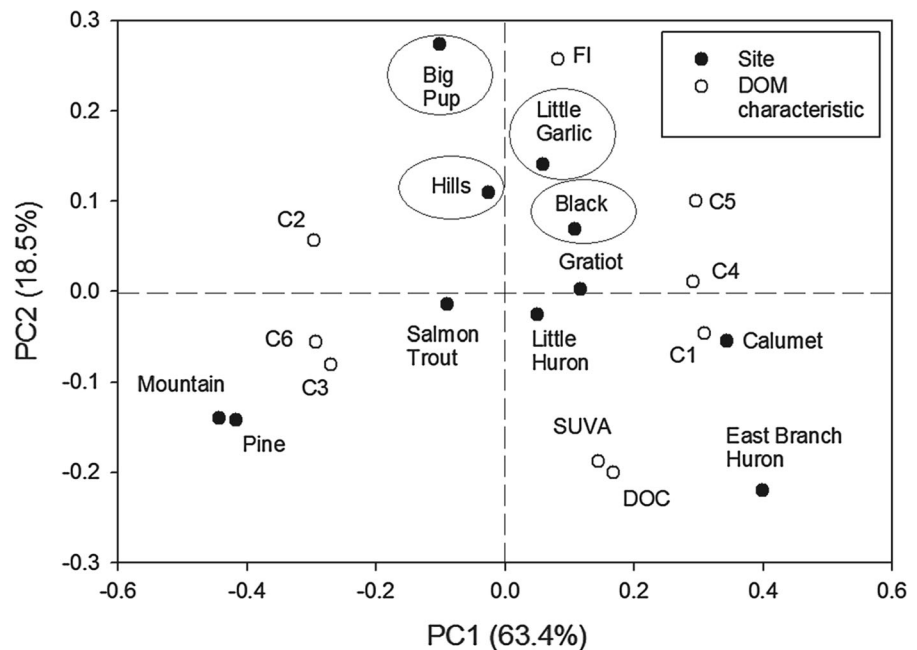
emission wavelengths (C4, C5) and strong negative loadings from components with shorter emission wavelengths (C3, C6; Fig. 2). The exception to this was that C1 and C2, which were located on opposite ends of PC1, had similar, mid-range emission wavelengths. The second PC axis explained 18.5% of the variability in the model, with FI with positive loadings and DOC concentration and SUVA$_{254}$ with strong negative loading (Fig. 2). The third and fourth PC axes explained 9% of the variability in the model with positive DOC loadings and 6% of the variability in the model with positive SUVA$_{254}$ loadings.

Nutrient uptake metrics

Across our study sites, NH_4^+–N V_f ranged from undetectable to 8.8 mm min^{-1} in 2012 and from undetectable to 14.6 mm min^{-1} in 2013 (Fig. 3). We observed significant uptake of NH_4^+–N at least 1 year (2012 or 2013) in all of our study sites, except Little Huron (Fig. 3). There were similarities in NH_4^+–N V_f among years at each site (Fig. 3). For example, the East Branch of the Huron had the fastest NH_4^+–N V_f measured among study sites in both years, while there was no significant uptake in the Little Huron in either year. The Salmon Trout was the only site where uptake of NH_4^+–N was observed in 2012, but not 2013 (Fig. 3). NH_4^+–N S_w ranged from 128 to 385 m in

2012 and from 313 to 1667 m in 2013 (Supplemental Table 3).

Across sites, SRP V_f ranged from undetectable to 7.2 mm min^{-1} in 2012 and from undetectable to 1.2 mm min^{-1} in 2013. Significant uptake ($\alpha = 0.10$) of SRP was observed at three sites in 2012 and four sites in 2013. Only one site, Big Pup Creek, had significant uptake of SRP in both years, and SRP V_f was 11× greater at this site in 2012 than in 2013 (Fig. 3). The four sites with significant SRP uptake (Big Pup, Hills, Little Garlic, Black) all expressed similarities in DOM characteristics as identified from the PCA analysis (Fig. 2), including greater FI, C5 (humic-like), and C2 (humic-like) than any of the other sites. SRP S_w ranged from 313 to 1667 m in 2012 and from 833 to 3333 m in 2013 (Supplemental Table 3).

Predictors of nutrient uptake velocity

Logistic regressions to determine whether biological, physical, or chemical characteristics could explain where significant uptake occurred revealed that NH_4^+–N had a significant effect on NH_4^+–N V_f, but the parameter estimates were considered unstable. Unstable parameter estimates occur when the model perfectly predicts the response or if there are more parameters than can be estimated by the data (JMP Statistical Software user notes). Such models are
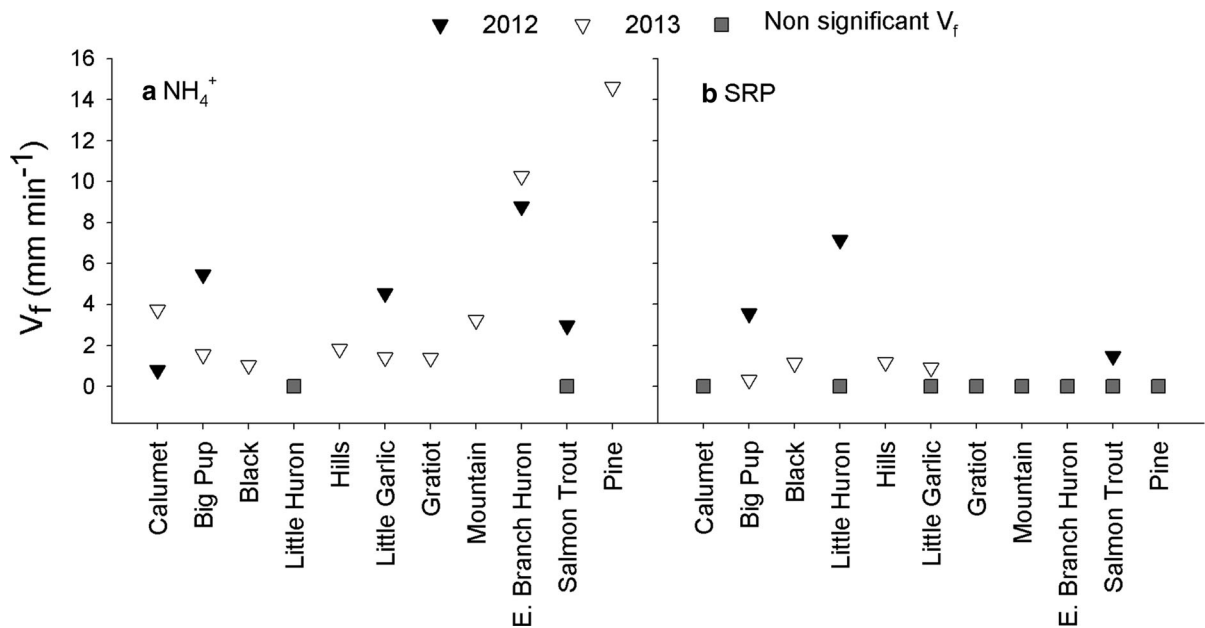
Fig. 3 Uptake velocity (V_f) of **a** NH_4^+–N, and **b** SRP measured at each of the 11 study sites in 2012 (*closed triangle*) and 2013 (*open triangle*). The *square symbol* indicates undetectable measurements of uptake, which for display purposes only were placed at a value of 0.0 mm min^{-1}

acceptable at classifying observations, but not for making inferences about the parameters (JMP Statistical Software user notes). In this case, higher background NH_4^+–N concentrations were consistent with lower odds of detecting significant NH_4^+–N uptake. Similarly, PC2, derived from a PCA of DOM composition, had a significant effect on SRP V_f with unstable parameter estimates. This complete separation of the data suggests that greater PC2 values were consistent with greater odds of observing significant SRP uptake compared to lower PC2 values, a finding that is consistent with visual interpretation of the PCA analysis (Fig. 2). Conductivity was also a significant predictor of when SRP uptake was observed, such that an increase in conductivity indicated an increased odds of significant SRP uptake [logit(π) = −6.50 (se = 3.81) + 41.95 (se = 26.60) × conductivity; p = 0.03]. Only sites with significant uptake (α = 0.10) were included in the PLS and multiple regression models to explore relationships among DOM composition along with the other measured stream characteristics (Tables 1, 2; Supplemental Table 1) and nutrient V_f. For NH_4^+–N, this resulted in excluding two sites (Little Huron and the Salmon Trout) from both analyses. For SRP, we did not conduct PLS analysis because V_f was

undetectable at 7 of 11 sites. We did conduct linear regression analysis to identify significant predictors of SRP V_f when it was detectable. Due to our limited sample size we a priori limited multiple regression models to a maximum of three predictors for NH_4^+–N V_f and one predictor for SRP V_f. Therefore, all predictor variables were considered in SRP V_f models, while some predictor variables were excluded from multiple regression NH_4^+–N V_f models because they were correlated with other predictor variables.

Multiple regression model selection resulted in six significant models that predicted NH_4^+–N uptake velocity (Table 3). The best model explained 87% of the variance, had an Akaike weight of 0.69, and included temperature, specific discharge, and percent canopy cover as predictor variables (Table 3). The second best model included the predictors temperature, specific discharge, and pH, while the third best included temperature, pH, and conductivity (Table 3). The only significant single predictor model included PC2, which predicted 49% of the variability and was negatively related to NH_4^+–N V_f, suggesting that NH_4^+–N V_f increased with increasing DOC concentration, SUVA$_{254}$, and lower FI (the main variables described by PC2).

Table 3 Multiple regression model results of uptake velocity of ammonium (NH_4^+–N) and soluble reactive phosphorus (SRP) as response variables and a variety of biological, physical, and chemical variables as predictors

Responses	Models	AIC	p values	R^2	ω	ΔAIC
NH_4^+–N V_f	Temp + Q/w − canopy cover	44.05	0.01	0.87	0.69	0
	Temp + Q/w − pH	47.35	0.03	0.82	0.13	3.30
	Temp − pH − Cond	47.92	0.03	0.81	0.10	3.87
	Temp − Chl$a$ − canopy cover	49.48	0.05	0.77	0.05	5.43
	Temp − pH	51.51	0.05	0.64	0.02	7.46
	−PC2	52.70	0.04	0.49	0.01	8.65
	Null	56.71	–	–	–	–
SRP V_f	DOC:DIN	−5.12	0.01	0.97	0.66	0
	−PC2	−2.35	0.03	0.94	0.16	2.77
	DOC	−1.31	0.04	0.92	−0.10	3.81
	−Slope	−1.07	0.04	0.92	0.08	4.05
	Null	6.91	–	–	–	–

Only sites with significant uptake ($p \leq 0.05$) were included in the models (NH_4^+–N V_f model, $n = 9$, SRP V_f model, $n = 4$). We selected models with AIC lower than two units below that of the Null model of complete randomness, and >0.01 Akaike weight (ω; Burnham and Anderson 2002). All of the models are based on the following covariates, except for the Null model which assumes a constant rate: *DOC* dissolved organic carbon concentration (mg L^{-1}), *Temp* temperature (°C), *canopy cover* canopy cover (%), *Chla* benthic chlorophyll *a* (mg m^{-2}), *Cond* specific conductivity (μS cm^{-1}), *DOC:DIN* (molar ratio), *pH* mean pH, *Q/w* specific discharge (m^2 s^{-1}), *slope* watershed slope (%), *PC2* PCA component PC2 value

Four significant single linear regression models explaining variability in SRP uptake velocity were identified, and included: DOC:DIN, PC2, DOC concentration, and watershed slope as predictor variables and explained between 92 and 97% of the variability in SRP V_f (Table 3). However, some streams with high DOC concentrations did not have detectable uptake, and thus were not included in this analysis. Given that only four sites could be included in regression analyses, the interpretation of these results is limited.

The PLS model for NH_4^+–N V_f identified four latent variables (LVs), which collectively explained 95.5% of the variance in NH_4^+–N V_f (R^{2}Y = 0.955) and 75.0% of the variance in the predictor variables (R^{2}X = 0.750). PLS analysis identified FI, temperature, SUVA$_{254}$, and conductivity as highly influential predictors; canopy cover, pH, SRP, C5, and C6 as moderately influential predictors; and the remaining variables as less influential predictors (Fig. 4). Here, we report the two LVs that explained the most variability and the highly and moderately influential predictors. The first LV axis (LV1) explained 19% of the variability in the X variables and 77% of the variability in the Y variable (NH_4^+–N V_f). LV1 was a function of FI, conductivity, and C5 with negative loadings, and temperature and SRP with positive loadings (Fig. 4). The second LV axis explained ∼34%

of the variability in the X variables and 9% of the variability in the Y variable. Along the LV2 axis SUVA$_{254}$ had positive loadings and pH had negative loadings (Fig. 4). Two of the most influential predictors, FI and conductivity, were situated on the opposite end of the LV1 axis from NH_4^+–N V_f (Fig. 4) suggesting a negative correlation between these variables. Therefore, as FI and conductivity decreased NH_4^+–N V_f increased among these streams. The other two highly influential predictors, temperature and SUVA$_{254}$, were situated on the positive LV1 axis the same direction as NH_4^+–N V_f suggesting a positive correlation between these variables. These results suggest that streams with a strong terrestrial FI signal (lower FI value), high degree of aromatic DOM, low conductivity, and high water temperatures exhibited higher uptake velocities than streams without these characteristics.

Discussion

Nutrient uptake velocity related to temperature, water chemistry, and DOM quality

Our results demonstrate that the occurrence and/or rates of inorganic nutrient uptake can be predicted by
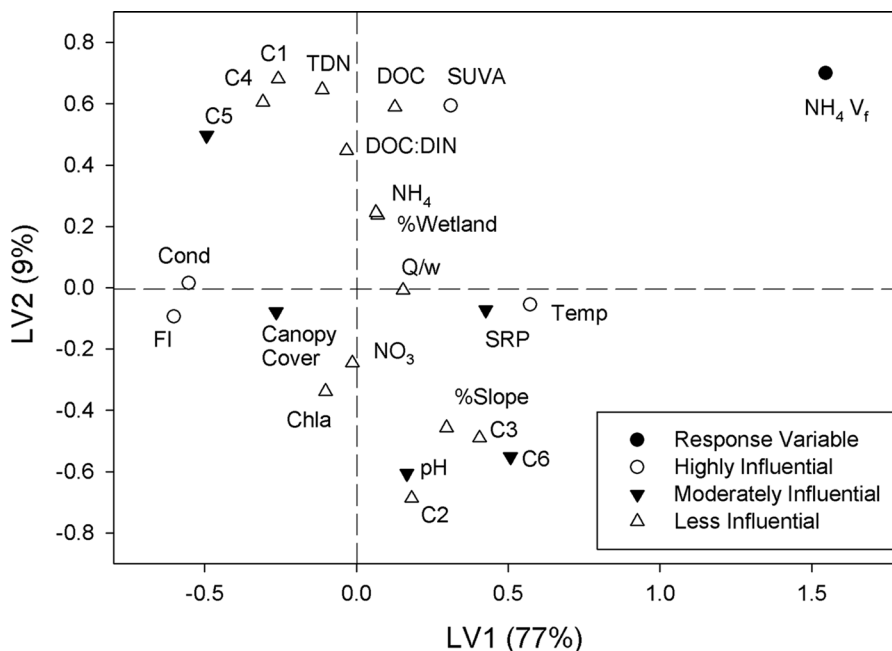
Fig. 4 Partial least squares loading plot predicting the variability of ammonium uptake velocity (NH₄–N V$_f$; response variable; *closed circle*) with the following predictor variables abbreviated as conductivity (Cond), temperature (Temp), and fluorescence index (FI), specific ultraviolet absorbance (SUVA), soluble reactive phosphorus concentration (SRP), ammonium concentration (NH$_4^+$–N), dissolved organic carbon concentration (DOC), the ratio of DOC concentration–NH$_4^+$–N concentration (DOC:NH₄) specific discharge (Q/w), benthic chlorophyll *a* (Chl*a*). Only the first two latent variables (LVs) are shown. Highly influential predictors (*open circles*) were determined identified by having a variable importance on the projection (VIP) score >2, and moderately influential predictors (*closed triangles*) were identified by having a VIP score between 1 and 2, less influential predictors (*open triangles*) were identified by having a VIP score less than 1. Little Huron and the Salmon Trout River were excluded from analysis because NH$_4^+$–N V$_f$ was undetectable in 2013

DOM quality in forested streams, along with other more commonly measured variables like water chemistry (conductivity) and water temperature. We found that DOM quality (FI and SUVA$_{254}$) was a very important predictor of the magnitude of NH$_4^+$–N V$_f$ and where SRP uptake was observed. Although the specific predictors of NH$_4^+$–N V$_f$ identified by the PLS and regression analyses differed, we rely primarily on the PLS results when interpreting controls of NH$_4^+$–N uptake because the PLS permitted inclusion of all predictor variables, even if they were highly correlated (Carrascal et al. 2009). Collectively, our results suggest that streams with higher temperatures, lower solute concentrations (as indicated by conductivity), a more terrestrial DOM signal, and greater DOM aromaticity had greater NH$_4^+$–N V$_f$, a result that is consistent with previous hypotheses that resource availability controls NH$_4^+$–N uptake (Hoellein et al. 2007).

Temperature, which was positively related to NH$_4^+$–N V$_f$, likely exerts an important control on DOM composition by affecting rates of microbial metabolism (e.g., Kirchman and Rich 1997; Pomeroy and Wiebe 2001). According to Pomeroy and Wiebe (2001), temperature affects all chemical and biochemical processes and should always be considered a potentially limiting factor that interacts with essential nutrient and organic matter availability. In our study, streams with lower solute concentrations (conductivity) had greater NH$_4^+$–N demand, suggesting an interactive role of element availability and temperature. Previous studies have found positive (Butturini and Sabater 1998; Simon et al. 2005) and negative (Marti and Sabater 1996; Hoellein et al. 2007) relationships or no relationship (Hoellein et al. 2007) between temperature and NH$_4^+$–N uptake. In a study examining temporal variation of nutrient uptake in Michigan's Upper Peninsula Hoellein et al. (2007)

found temperature was negatively related to NH_4^+–N uptake at only one of three sites and they hypothesized that these inconsistencies were due to the timing of resource availability of DOM and light, which were highest in colder months when NH_4^+–N demand was greatest.

FI is a key measure of DOM quality that was identified as a negatively related, highly influential predictor of NH_4^+–N uptake and as an indicator of where SRP uptake occurred. The relatively low values and tight range in FI (1.15–1.23) reveal a strong terrestrial signal in our study streams compared with other streams (FI = 1.25–1.85 ; McKnight et al. 2001; Hood et al. 2006; Belmont et al. 2009). SUVA$_{254}$, another indicator of relative aromaticity, was also a positively related highly influential predictor of NH_4^+–N V_f and was an indicator of where SRP uptake occurred. Photochemical processing has been found to reduce absorbance of DOM and reduce SUVA$_{254}$ values (Helms et al. 2013; Logvinova et al. 2015), indicating a decrease in aromaticity. Although our FI values suggest that DOM in all of our streams is largely terrestrially-derived, we posit that the small changes we detected reflect differences in DOM composition such that lower values indicated higher susceptibility of the DOM pool to heterotrophic biodegradation, creating greater demand for NH_4^+–N. This mechanism is consistent with previous studies that have shown that DOM biodegradability in Arctic rivers increased with increasing lignin contributions to the DOM pool (Holmes et al. 2008; Spencer et al. 2008). Our proposed mechanism diverges from the traditional paradigm that terrestrial DOM is recalcitrant (Hedges et al. 1988; Gough et al. 1993; Opsahl and Benner 1998), but is consistent with more recent studies that have challenged such paradigms (Ward et al. 2013; McCallister and del Giorgio 2012; Marín-Spiotta et al. 2014). There is mounting evidence from terrestrial and aquatic ecosystems that biological and environmental characteristics, rather than molecular structure alone, determine organic matter stability (Schmidt et al. 2011; Marín-Spiotta et al. 2014). Alternatively, our results could suggest that microbes require more inorganic N to breakdown the more structurally complex DOM. This interpretation is supported by previous work conducted at Calumet watershed, where we found that NH_4^+–N limited the biodegradability of DOC in October, when

aromaticity was greatest, but not at other times of the year (Coble et al. 2015).

Sediment characteristics can also affect NH_4^+ uptake efficiency through abiotic sorption (Triska et al. 1994), and NH_4^+ sorption onto water column DOM or stream sediments may provide an alternate explanation for the observed relationships among the DOM characteristics, FI (negative) and SUVA$_{254}$ (positive), and NH_4^+–N uptake among these forested streams. Although FI and SUVA$_{254}$ measured in our study reflect the composition of DOM in the water column, DOM can form aggregates with colloids into larger particles that eventually sink (i.e., through flocculation), effectively translocating DOM from the water column to the sediment (Tranvik and von Wachenfeldt 2009), and thus these indices may also provide some information about stream sediment composition. Variability in underlying geology across sites (Table 1) likely controls stream sediment composition (e.g., Ankers et al. 2003), hydrology (e.g., Reynolds 1995), and vegetation (e.g., Kruckeberg 2002), which in turn, control characteristics of stream DOM among these watersheds (McKnight et al. 2003; Dalzell et al. 2011). If abiotic sorption of NH_4^+–N is a dominant process explaining differences in NH_4^+–N uptake among streams then our results suggest that in the presence of more structurally complex organic matter (lower FI) more NH_4^+ sorption occurs. We do not know the importance of abiotic sorption in NH_4^+–N uptake among these streams, but likely there is some interaction of biotic and abiotic processes that account for differences in NH_4^+–N uptake.

Some previous studies (Bernhardt and Likens 2002; Johnson et al. 2009), but not all (Bechtold et al. 2012), have found that N uptake can increase as C concentrations increase, but our results suggest that DOM composition is a more important predictor of NH_4–N V_f than DOC concentration. Furthermore, we did not observe a relationship between DOC background concentrations and NH_4^+–N V_f using a single predictor regression model. Apparently natural variability in background DOC concentrations in our study streams was not related to variability in N uptake. It is possible that the form of C added (as acetate) rather than the quantity of C was the mechanism behind greater N uptake in other studies (Bernhardt and Likens 2002; Johnson et al. 2009). We also acknowledge the

relatively narrow range in stream chemistry and DOM composition of our study sites, which were representative of a broad range of tributaries along the south shore of Lake Superior, but did not capture a large agricultural or urban gradient common to other regions. Future work should incorporate streams with a greater range of background N, P, and DOC concentrations and DOM composition.

Our ability to determine predictors of SRP V_f was limited by the relatively small number (4) of sites where uptake was detectable, but our results do show that DOM composition exerts a strong control on where SRP uptake occurs. We found that SRP uptake was observed at sites with greater microbial-like aquatic fulvic acids, less aromaticity, and lower DOC concentrations. Intriguingly, this is the opposite relationship with FI than was observed for NH_4^+–N V_f. The metabolic demands of the microbial community may shift with resource availability and substrate supply (Hill et al. 2006, 2010; Williams et al. 2012), resulting in uptake of SRP only with less aromatic, more microbially-derived DOM, and when demand for NH_4^+ is reduced.

Variability in uptake among nutrients, watersheds and years

NH_4^+ is known to be highly bioreactive (e.g., Peterson et al. 2001), and in our study sites we observed the greatest and most consistently detectable uptake velocities for NH_4^+–N relative to SRP. In Lake Superior tributaries, both N and P can limit biomass of primary producers (Allen and Hershey 1996; Wold and Hershey 1999), yet we generally observed rapid uptake of NH_4^+–N and not SRP. Physical, chemical, and biological processes can remove both NH_4^+–N and SRP from the water column (Triska et al. 1994; Mulholland et al. 1990; Peterson et al. 2001; Withers and Jarvie 2008). Despite multiple pathways for the uptake of P we did not often observe uptake of SRP across these streams. DOM composition appears to constrain uptake of SRP across our study sites, thereby allowing SRP to be exported from these streams to Lake Superior without measurable uptake.

Uptake lengths of NH_4^+–N and SRP are likely overestimated, and uptake velocities are likely underestimated in our study due to saturation of benthic nutrient demand (Mulholland et al. 1990, 2002; Payn et al. 2005). Additionally, we used rhodamine WT as a conservative tracer in this study, but Runkel (2015) suggests that rhodamine WT may not travel conservatively in hyporheic zones, which could also affect our estimates of nutrient uptake. Without corrections for underestimation of V_f (e.g., via method of Payn et al. 2005) or the potential nonconservative transport of rhodamine WT, we found that NH_4^+–N and SRP uptake velocities in our study sites were similar to previous measurements across a variety of streams (NH_4^+ V_f = 2.2–10.4 mm min^{-1}, PO_4^{3-} V_f 0.9–6.0 mm min^{-1}, Ensign and Doyle 2006). Regionally, our study sites on the south shore of Lake Superior (NH_4^+–N V_f 0.8–14.6 mm min^{-1}, SRP V_f 0.3–7.2 mm min^{-1}) revealed lower uptake velocities than in some north shore streams (NH_4^+ V_f 1.1–28.4 mm min^{-1}, PO_4^{3-} V_f 0.75–15.7 mm min^{-1}; Lehto and Hill 2013), but were similar with other measurements of inland Upper Peninsula streams (NH_4^+ V_f 1.9–7.6 mm min^{-1}, SRP V_f 1.1–10.7 mm min^{-1}; Hoellein et al. 2007).

Export of solutes to downstream ecosystems

The relationships we found between DOM composition, conductivity, temperature and NH_4^+–N demand imply that landscape characteristics may have an indirect control on nutrient export to downstream ecosystems by modulating in-stream nutrient uptake rates. Ammonium was quickly taken up and likely cycled multiple times in the study streams and rivers before being exported to Lake Superior. For most of our study sites, the distance NH_4^+–N traveled before being taken up was 30–98% shorter than the distance to Lake Superior, suggesting NH_4^+–N could be cycled up to 50 times before being exported. However, we did not account for the distance NH_4^+–N traveled in organic form after being taken up (Von Schiller et al. 2015). Therefore, the total spiraling length could be much greater in these streams. On the other hand, our results suggest that these small to mid-size streams likely exported SRP directly to Lake Superior without measureable uptake. SRP uptake lengths indicate that SRP was only taken up before reaching Lake Superior in 3 of 6 streams in 2012 and 4 of 11 streams in 2013. Although SRP concentrations are relatively low, riverine export of SRP could be important for fueling primary productivity in near shore regions of Lake Superior, where phytoplankton and bacterioplankton

have been shown to be limited or co-limited by P (Sterner et al. 2004). Riverine export of P from the more than 1500 tributaries of Lake Superior (Coble et al. 2016) may therefore supply important nutrient subsidies to the near-shore region of the largest lake in the world, potentially fueling productivity in this oligotrophic downstream environment (e.g., Biddanda and Cotner 2002).

Conclusions

Our results provide strong support for the inclusion of DOM composition in addition to concentration, when deciphering and quantifying variability in uptake of N and P in aquatic ecosystems. Coupled cycling of C, N, and P occurs across aquatic ecosystems (e.g., marine, lake) and our findings support the concept that DOM composition may also serve as an important control on biogeochemical cycling in these ecosystems. Standardization and application of novel analytical and quantitative approaches to characterize DOM composition (e.g., Stedmon et al. 2003; Stedmon and Bro 2008) have paved the way for DOM composition to be integrated into a wide variety of aquatic biogeochemical studies. Finally, our results highlight the importance of stream–lake linkages in delivering essential nutrients to downstream ecosystems (e.g., Williamson et al. 2008).

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