

DECOMPOSITION OF DISSOLVED ORGANIC MATTER IN ARCTIC AND BOREAL
STREAMS

By

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Abstract

Arctic and boreal rivers connect terrestrial, oceanic, and atmospheric carbon (C) pools by transporting and processing dissolved organic matter (DOM). DOM composition influences its susceptibility to decomposition (biolability), which in turn determines whether the associated C is respired, stored, or exported. High-latitude ecosystems are changing rapidly due to processes such as permafrost thaw, shifts in vegetative communities, and increasing discharge, and each of these processes can influence the composition of DOM reaching rivers. The eventual fate of riverine DOM, whether it is mineralized or exported, shifts the balance of global C pools. Therefore, to understand how changes to high-latitude ecosystems influence the global C cycle, we must be able to connect patterns in DOM composition to its biolability and subsequent fate within the C cycle.

The objectives of this study were to describe spatial and temporal patterns in DOM composition and biolability, and to determine links between the composition and biolability of DOM. I sampled DOM from streams along an arctic-boreal gradient in interior Alaska throughout the year. I measured DOM biolability and nutrient limitation of decomposition in laboratory incubations and characterized DOM composition using optical properties and chemical analysis. I found that temporal patterns in DOM composition corresponded to seasonal trends in the hydrology of high-latitude catchments, linking DOM source to shallow, organic-rich flowpaths in spring and deeper groundwater flows in winter. Biolability was low, indicating that the majority of riverine DOM is recalcitrant to biological decomposition. I observed increased biolability in response to phosphorus (P) addition, particularly during spring, indicating that phosphorus limits DOM decomposition.

To further examine the mechanisms driving C processing in streams, I also conducted a series of whole-stream experiments to compare the relative influence of molecular composition and nutrient content of DOM. I added leaf leachate to boreal streams and measured C retention, which represents both biological uptake and sorption. The leachates varied by molecular composition, due to differences in tissue chemistry of plant species, and in nutrient content, because the leaves were collected from plots with different fertilization regimes. Retention was greatest for leachates derived from trees that had been fertilized with P, indicating P-limitation of biological uptake of C or preferential sorption of P-containing organic molecules. Although leachates varied in molecular composition as determined by optical properties, these differences did not correspond to a difference in uptake rates by species. These patterns in DOM retention indicate that nutrient content is a greater constraint on C uptake than molecular composition.

Together, the two studies suggest that export is the primary fate of ambient DOM in high-latitude streams, but that C processing is highly sensitive to inputs of bioavailable DOM. The coupling between the P and C cycles observed in both studies highlights the potential for nutrient availability to constrain or promote CO₂ emissions from C-rich, high-latitude catchments.

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General Introduction

Streams link terrestrial, oceanic, and atmospheric carbon (C) pools. In addition to exporting large quantities of terrestrially derived C to the oceans, streams are also sites of C transformation. In streams, dissolved organic carbon (DOC) is mineralized to carbon dioxide (CO₂) by exposure to sunlight (Cory et al. 2014), as well as by microbial metabolism (Kling et al. 1991). Although streams and rivers occupy a relatively small amount of global surface area, they can serve as hotspots of CO₂ evasion (Jones et al. 2003, Raymond et al. 2013). Streams in high-latitude regions are particularly influential because high-latitude regions contain vast stores of C, with up to 50% of soil organic carbon stored globally (Tarnocai et al. 2009). The fates of DOC in high-latitude regions, which include export to the Arctic Ocean or release as CO₂ to the atmosphere, have the potential to drive or suppress feedbacks within the global C cycle (Schuur et al. 2015).

The composition of DOC influences its fate within the global C cycle. DOC is bound with other elements in a complex mixture known as dissolved organic matter (DOM). DOM composition and reactivity vary depending on its source materials (McKnight et al. 2001, Guerard et al. 2009), and a given sample of DOM can contain thousands of molecules of varying molecular weight (Sleighter and Hatcher 2007). In streams, DOM serves as a substrate for heterotrophic bacteria. By decomposing DOM, microbes obtain energy as well as essential nutrients such as nitrogen (N) and phosphorus (P). The biolability of DOM, its susceptibility to decomposition, is driven by the structure of C-containing molecules as well as by nutrient content.

Flux of CO₂ to the atmosphere from rivers may increase as a result of changes in the composition of DOM received from catchments. Many aspects of high-latitude ecosystems are

changing rapidly, including permafrost condition, such as increased permafrost temperature (Romanovsky et al. 2010), vegetation patterns, such as increased deciduous trees in the boreal forest and increased shrubs in the arctic (Chapin et al. 2000, Johnstone et al. 2010, Fraser et al. 2014), and hydrology, such as increased discharge (Peterson et al. 2002, Walvoord and Striegl 2007). DOM composition is linked to catchment characteristics such as permafrost extent and vegetation type (Harms et al. 2016, O'Donnell et al. 2016), and therefore such changes to high latitude ecosystems will likely influence DOM processing in rivers. Understanding the characteristics of DOM that influence its biolability in high-latitude rivers will enhance predictions of the role of DOC within a changing C cycle.

The central question of this thesis is: Which characteristics of DOM influence its decomposition? The thesis includes an observational study and a manipulative experiment to examine the drivers of DOM biolability in arctic and boreal streams. Chapter 1 addresses how intra-annual and spatial patterns in DOM composition influence its biolability. I sampled DOM from streams along a gradient of discontinuous permafrost in boreal forest to continuous permafrost in arctic tundra. Samples were collected throughout the year, including winter, a time point for which DOM dynamics are vastly understudied. Chapter 2 summarizes an investigation into the mechanisms driving C retention in streams by comparing the relative influence of molecular composition and nutrient content of DOM. I added leaf leachate that varied by molecular composition and nutrient content to streams and measured C uptake. Both chapters compare molecular composition using parallel-factor analysis (PARAFAC) of DOM fluorescence.

Chapter 1 Regional and intra-annual stability of dissolved organic matter composition and biolability in high-latitude rivers¹

1.1 Abstract

Dissolved organic matter (DOM) composition in streams influences its susceptibility to decomposition, which in turn determines whether the associated carbon is respired, stored, or exported. High-latitude ecosystems are changing rapidly through processes such as permafrost thaw, shifts in vegetation communities, and increasing discharge, and to understand the consequences of these changes for the global carbon cycle, we must be able to draw connections between DOM composition and biolability. In this study, we collected samples throughout the year from up to eight streams along an arctic-boreal gradient in Alaska, ranging from discontinuous to continuous permafrost. We measured DOM biolability and nutrient limitation of decomposition through laboratory incubations of streamwater, and characterized DOM composition through optical properties and chemical analysis. We found phosphorus limitation of decomposition, particularly during spring freshet. The biolability of DOM during summer and winter baseflow conditions appears to be limited by a recalcitrant molecular structure rather than nutrient availability, emphasizing the sensitivity of high-latitude streams to increases in biolabile DOM.

1.2 Introduction

Arctic and boreal regions store vast quantities of carbon (C), accounting for up to 50% of the global pool of soil organic carbon (Tarnocai et al. 2009). The riverine transport and

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processing of dissolved organic C (DOC) from these ecosystems represent a key flux between terrestrial, oceanic, and atmospheric C pools (Striegl et al. 2005, McClelland et al. 2007, Raymond et al. 2007, Battin et al. 2009). The composition of dissolved organic matter (DOM) influences its biolability, which determines whether the associated organic C is susceptible to decomposition by heterotrophic microorganisms, where it may be respired to the atmosphere as carbon dioxide, or whether it is recalcitrant to decomposition and is more likely to be exported to the ocean. Projected changes to C and nutrient cycles in high-latitude ecosystems will affect the sources, quantities, and composition of dissolved organic matter (DOM) that reaches rivers (Jones et al. 2005, Abbott et al. 2014), and therefore influence the biolability of DOM and its fate within the global C cycle.

The biolability of DOM in streams is influenced by both nutrient availability and molecular composition. In this study we define molecular composition as differences in aromaticity, molecular weight, and functional groups. Although molecular composition is, by definition, altered when nutrient content changes, we characterized molecular composition at a broader-scale resolution through optical properties. Decomposition is influenced by nutrient content because microbial decomposers require nutrients such as nitrogen (N) and phosphorus (P) for cellular function (Sinsabaugh et al. 2013). Decomposition rate slows when nutrients are limiting, as decomposers shift resource allocation from C acquisition to nutrient acquisition. Nutrient inputs to high-latitude streams are increasing. Between 1991 and 2001, nitrate flux from the Kuparuk River in arctic Alaska increased nearly fivefold (McClelland et al. 2007). There is also evidence that P concentration may be increasing in the Yukon River basin (Toohey et al. 2016). The sensitivity of high-latitude ecosystems to changing nutrient availability remains

unclear (Shaver et al. 1992). Increasing nutrient availability has the potential to increase DOM biolability, and therefore shift the fate of riverine C from export to respiration.

The molecular composition of DOM also influences biolability. Larger molecules and those with a more aromatic structure require microbial decomposers to allocate more energy towards extracellular enzymes to break bonds, slowing the rate of decomposition (Sinsabaugh et al. 2013). However, experimentally derived links between DOM composition and lability remain ambiguous. Humic-like components tend to resist decomposition (Qualls and Haines 1992, Fellman et al. 2009), but can also be somewhat labile (Cory and Kaplan 2012, Kellerman et al. 2015). Protein-like components are associated with greater biolability (Balcarczyk et al. 2009, Fellman et al. 2009). The composition of DOM in high-latitude rivers may change due to thawing permafrost, which releases aliphatic (non-aromatic), highly labile DOM (Spencer et al. 2015). Estimates of the isotopic age of C indicate that permafrost-derived C is readily decomposed in streams (Mann et al. 2015). Increased microbial processing in response to permafrost thaw could lead to increased respiration of CO₂, potentially accelerating the rate of climate change through a positive feedback loop with the atmosphere (Schuur et al. 2015).

Temporal variation in DOM composition can be used to examine links between hydrology and DOM composition. High-latitude ecosystems exhibit strong seasonality in hydrology, in both discharge and depth of groundwater flow (flowpath depth) contributing to streams, which influences the quantity and composition of DOM reaching streams (Finlay et al. 2006, O'Donnell et al. 2010, 2012, Mann et al. 2012, Wickland et al. 2012). DOM exported from arctic rivers tends to be most labile during spring freshet (Holmes et al. 2008, Mann et al. 2012). During freshet, frozen soil forces runoff to flow through organic-rich upper layers causing the DOM that reaches streams to be enriched in terrestrially-based aromatic C (Striegl et

al. 2007, Spencer et al. 2008, Mann et al. 2012). Over half of annual DOC export from high-latitude rivers may occur during freshet, so a lack of estimates of biolability during this time can lead to underestimates of annual DOC flux from terrestrial to aquatic ecosystems (Finlay et al. 2006). Few data are available on the composition and biolability of DOM in winter, but available observations indicate that in winter the DOM pool contains a higher proportion of hydrophilic compounds and a lower ratio of DOC to dissolved organic nitrogen (DON) (O'Donnell et al. 2012), characteristics positively correlated with lability in some studies (Wickland et al. 2007, 2012, Kiikkilä et al. 2013), and some estimates indicate that biolability may be higher in winter (Wickland et al. 2012). The lack of seasonal data limits our ability to predict how the processing of C in streams will respond to climate-related changes such as permafrost thaw, earlier snowmelt, and reduced ice cover, and therefore restricts models of the C budget in high-latitude ecosystems.

Spatial gradients in permafrost extent and ecosystem type can be used as predictive tools to study how climate-related changes such as permafrost thaw and shifts in vegetation communities will influence DOM composition and lability. Spatial variation in DOM composition and lability is influenced by attributes of catchments such as permafrost condition, active layer depth, parent material, catchment slope, and vegetation type (Frey and McClelland 2009, Spencer et al. 2015, Harms et al. 2016, O'Donnell et al. 2016). For example, although wetlands (mires) export more DOC per unit area, the DOC exported from forested catchments can be more labile (Ågren et al. 2008), highlighting the influence of catchment slope and vegetation type. Spatial extent of permafrost in the contributing area of streams may influence DOM composition because permafrost restricts water flow to shallow, organic-rich soil horizons (Petrone et al. 2006, Balcarczyk et al. 2009), and aromaticity of DOM tends to be greater for

watersheds with a greater extent of ice-rich permafrost (O'Donnell et al. 2016). In contrast, DOM released from permafrost itself tends to be aliphatic, rather than aromatic, and highly biolabile (Abbott et al. 2014, Mann et al. 2015, Spencer et al. 2015). By using existing gradients to establish relationships between attributes of catchments and DOM characteristics, we can better predict how changes in these attributes might influence DOM composition and biolability.

The objectives of this study were to describe spatial and temporal patterns in DOM composition and biolability, and to determine links between composition of DOM and its biolability. A composition-based proxy for biolability would be less time-consuming, resource-intensive, and prone to variability than biolability assays, and could be measured over broader spatial and temporal scales, allowing us to monitor and predict changes in hydrologic and gaseous fluxes of C. To address these objectives, we measured the composition and biolability of DOM from streams along an arctic-boreal gradient in interior Alaska. Samples were collected throughout the year, including winter, for which there are very few available estimates of DOM biolability. DOM composition was characterized by optical properties and chemical analysis. Samples were incubated to determine the fraction of DOC that is susceptible to microbial decomposition and response to nutrient fertilization. We hypothesized that nutrient availability constrains the decomposition rate when microbial decomposers are nutrient-limited and alternatively, that the molecular composition of DOM constrains the decomposition rate due to the high energy input required to break down large and complex molecules.

1.3 Methods

1.3.1 Site description and sampling

We sampled streams spanning an arctic-boreal gradient in interior Alaska eight times between April 2015 and May 2016. Permafrost underlying the watersheds ranges from spatially discontinuous in the south, to continuous in the north (Fig. 1, Ferrians 1998). Vegetation types include boreal forest south of the Brooks Range (cover dominated by black spruce (*Picea mariana*), white spruce (*Picea glauca*), paper birch (*Betula neoalaskana*), and quaking aspen (*Populus tremuloides*)) and arctic tundra north of the Brooks Range (dominant species including tussock-forming sedges (*Eriophorum* spp.), mosses (*Sphagnum* spp.), and shrubs (*Betula nana* and *Salix lanata*)). Refer to Harms et al. (2016) for further detail on physical and vegetative attributes of the catchments. Average annual air temperature ranges from -6 °C for the southern sampling locations to -10°C for the northern-most sampling location and average annual precipitation ranges from 460 mm for the southern sites to 320 mm for the northern sites (Bonanza Creek LTER Climate Database 2016, Toolik Environmental Data Center Team 2016).

Streams in this region exhibit strong seasonal patterns in discharge with low flow or bedfast ice during winter and maximum annual discharge during spring snowmelt (Brabets et al. 2000). Streams were sampled up to eight times from April 2015 to May 2016, with collections timed to encompass the full annual range in hydrologic variation (Supplementary Table 1.1). Sampling dates included April, May, June, August/early September, and November 2015 and February, April, and late April/early May 2016. An ice auger was used to collect under-ice samples in winter, and overflow, water discharged above bedfast ice, was collected when present. Samples were collected in acid-washed 1-L bottles, transported on ice, filtered to 0.7 µm (Whatman GF/F) within 24 hours of collection, and filtered to 0.45 µm (Pall GN-6) and 0.2

μm (Whatman Nuclepore Polycarbonate Membrane Filter) within 48 hours of collection.

Analysis of dissolved solute concentrations were performed on 0.7 μm -filtered samples, optical properties were determined on 0.45 μm -filtered subsamples, and 0.2 μm -filtered samples were incubated to determine biolability of DOM.

1.3.2 Chemical analysis

Each sample was analyzed for concentration of DOC, total dissolved N (TDN), ammonium (NH_4^+), total dissolved P (TDP), and major anions and cations. DOC concentration was measured as non-purgeable organic C by non-dispersive infrared gas analysis on a total organic C analyzer (TOC-L CPH, Shimadzu Scientific Instruments, limit of quantification (LOQ) = 0.1 mg C/L) connected to a nitrogen monoxide chemiluminescence analyzer (TNM-L, LOQ = 0.02 mg N/L). NH_4^+ was measured by automated colorimetry (Smartchem 170, Westco Scientific Instruments, LOQ = 0.01 mg N/L) using the phenol hypochlorite method (Solórzano 1969). Anion and cation concentrations were measured on an ion chromatograph (Dionex ICS 2100) equipped with AS18 columns for anions (LOQ : 0.5 $\mu\text{g NO}_3^-/\text{L}$, 8.8 $\mu\text{g Cl}^-/\text{L}$, 0.7 $\mu\text{g SO}_4^{2-}/\text{L}$) and CS16 columns for cations (LOQ: 12 $\mu\text{g Na}^+/\text{L}$, 13 $\mu\text{g K}^+/\text{L}$, 29 $\mu\text{g Mg}^{2+}/\text{L}$, 100 $\mu\text{g Ca}^{2+}/\text{L}$). TDP was measured following persulfate digestion using the molybdate blue method (Murphy and Riley 1962) on a spectrophotometer with a 5-cm cell (Shimadzu UVmini 1240, Shimadzu Scientific Instruments, LOQ = 0.6 $\mu\text{g P}/\text{L}$). Soluble reactive phosphorus (SRP) was measured for a subset of the sampled streams and found to be at or near the detection limit, indicating that at most sites and times, TDP is largely organic. Nitrite is not detectable in the study streams and concentration of DON was therefore determined as $\text{DON} = \text{TDN} - [\text{NO}_3^- - \text{N} + \text{NH}_4^+ - \text{N}]$. A proxy for groundwater inputs was calculated as the molar sum of Na^+ , Mg^{2+} , Cl^- ,

and SO_4^{-2} , all of which are present in high concentration in local groundwater and behave conservatively in this region (O'Donnell et al. 2010, Toohey et al. 2016). When measured values were below the LOQ, the average of the LOQ and zero was used in data analysis.

1.3.3 DOM absorbance and fluorescence

To characterize DOM composition, we measured absorbance of UV and visible light as well as fluorescence spectra on filtered samples (0.45 μm , Pall GN-6) using a fluorometer (Jobin-Yvon Horiba Aqualog-800-C, Horiba Instruments) with a 1-cm quartz cuvette (Firefly Scientific). Excitation-emission matrices (EEMs) were collected over an excitation range of 240–600 nm every 3 nm and an emission range of 247–847 nm every 2.33 nm with an integration time of 0.1 seconds and a medium gain. Data were instrument-corrected, blank-subtracted, Raman-normalized, and corrected for inter-filter effects. Parallel factor analysis (PARAFAC) was applied to resolve fluorescing components using the DOMFluor toolbox (version 1-7, Stedmon and Bro 2008) in Matlab (version R2015b, MathWorks). The PARAFAC model was based on the 52 samples presented here, as well as additional samples to increase the power of the model, including a set of 45 samples of leachates derived from plant species common to the boreal region included by this study (Mutschlecner et al. n.d.), and 132 stream water samples that had been incubated and/or acidified, for a total of $n=229$ samples supporting the model. A model including five components was best supported by the data as determined by inspection of residuals for random variation and model validation (Supplementary Figure 1.2). Model validation was conducted following the approach of Stedmon et al. (2003), and included split-half analysis, which compares PARAFAC models made on two halves of the data, and random initialization, which performs 10 iterations with different starting estimates to confirm

that the least squares result was not a local minimum (Supplementary Figure 1.3). To identify the chemical attributes of the components identified by PARAFAC analysis, excitation and emission spectra were compared with published data using the OpenFluor database (Table 1.2). PARAFAC components 1-4 were matched to previously described components in the OpenFluor database using a 0.95 Tucker convergence coefficient (Murphy et al. 2014). Component 5 visually appeared to consist of two components, but the model was unable to resolve them separately (Supplementary Figure 1.2). Component 5 did not match components within OpenFluor, even when the two portions of the component were compared separately, but was instead visually matched to previously reported excitation and emission curves that were not present in the OpenFluor database (Table 1.2, Cory and McKnight 2005).

In addition to the PARAFAC analysis of EEMs, several indices were calculated from both EEMs and absorbance of light in the UV-visible range. Fluorescence index (FI) describes the contribution of terrestrial and microbial sources to the DOM pool and is calculated as the ratio of emission intensities at 470 nm and 520 nm at excitation 370 nm, with values of 1.4 indicating terrestrially-derived fulvic acids and 1.9 indicating microbially-derived fulvic acids (McKnight et al. 2001, Cory et al. 2010). Humification index (HIX) is calculated as the area under the emission spectra 435-480 nm divided by the sum of the areas under the 300-345 nm and 435-480 nm regions at excitation at 254 nm, and ranges from 0 – 1, with higher values indicating a greater degree of humic substance content (Ohno 2002). Biological index (BIX) indicates autotrophic productivity and is calculated as the ratio of emission intensities at 380 nm and 430 nm at excitation 310 nm, with values >1 indicating recently produced DOM of autochthonous origin (Huguet et al. 2009). Freshness index ($\beta:\alpha$) indicates the relative contribution of recently produced DOM and is calculated as the ratio of emission intensity at 380

nm divided by the maximum emission intensity between 420-435 nm at excitation 310 nm, with higher values indicating a greater proportion of recently produced DOM (Parlanti et al. 2000, Wilson and Xenopoulos 2009). From absorbance of light in the UV-visible range, we calculated specific ultraviolet absorbance at 254 nm (SUVA), which is associated with greater aromatic content, by dividing the absorbance at 254 nm by DOC concentration (Weishaar et al. 2003), and spectral slope ratio (SR), which is negatively correlated with DOM molecular weight and aromaticity, as the ratio of the slope from 275 to 295 nm to the slope from 350 to 400nm with log-linear fits (Helms et al. 2008).

1.3.4 DOC biolability

Laboratory incubations were conducted to measure biolability of DOC, defined as the fraction of DOC that is susceptible to microbial decomposition. Stream water from each site was filtered to 0.2 μm (Whatman Nuclepore Polycarbonate Membrane Filter) to remove the majority of microorganisms and 120 mL stream water was incubated in a glass jar that had been ashed for 4 hours at 450°C. For each stream and sampling date, three replicates each of control, N, P, and N+P treatments were incubated. The concentration of N in the N treatment was 1 mM greater than ambient (50:50 NH_4Cl and KNO_3). The concentration of P in the P treatment was 0.0625 mM greater than ambient (added as KH_2PO_4). The N + P treatment contained both nutrients and the control treatment had an equivalent volume of Nanopure water added in place of a nutrient solution. Nutrient concentrations were selected to relieve possible nutrient limitation of decomposition and to match the Redfield ratio of 16:1 for N:P.

Microbial inoculum added to the incubated streamwater included both water column and sediment-derived communities. Preliminary tests indicated that DOC loss was greater when

inoculum was derived from stream sediment (mean loss = 0.3 mg/L, standard deviation = 0.1 mg/L) than from the water column (mean loss = 0.1 mg/L, standard deviation 0.3 mg/L). The sediment-derived inoculum for all incubations was collected from the Chena River. At each sampling date, we collected a slurry of sediment and water, except in November and February, when ice cover prevented collection of sediment; for those dates we used sediment collected in October 2015 and frozen until use. To produce the inoculum, the sediment slurry was shaken, filtered to 0.7 μm , and then to 0.2 μm until each filter clogged. The 0.2- μm filters were placed in a beaker of Nanopure water at a ratio of 1 filter to 10 mL water. We also added a matching number of clogged 0.2 μm filters following filtration of the stream samples collected on each sampling date to include microbes present in the water column in the inoculum. The resulting composite inoculum was added to each water sample at a ratio of 1 mL inoculum:100 mL sample water. Preliminary tests indicated that reproducibility of measured DOC biolability was greater using inocula prepared as 1 clogged, 0.2- μm filter:10 mL water than for more dilute inocula. Additionally, incubation of 10 mg/L glucose in stream water without added nutrients lost $55 \pm 9.98\%$ DOC, on average within 21 days, indicating that the inoculum was viable. Samples were incubated in the dark, at 25°C.

To measure the loss of DOC from stream water, samples were collected from the incubated samples on day 0 following inoculation and addition of nutrients and on day 21. All samples were filtered to 0.2 μm and were immediately acidified and refrigerated until analysis. Samples were analyzed for DOC concentration as previously described. Absolute loss of DOC was calculated as the change in DOC concentration between day 0 and day 21, and percent loss was calculated by dividing absolute loss by the initial DOC concentration. Biolability was not measured in April 2015 or for Slate Creek and Kugaruk River in June 2015.

1.3.5 Statistical analysis

All statistical analyses with the exception of PARAFAC analysis (described above) were conducted in R (version 3.3.1, R Core Team 2016). We examined the effects of nutrient treatment (control, N, P, and N+P) and sampling date on DOC loss using linear mixed-effects models with the lme4 package (Bates et al. 2015). Separate means (based on laboratory replicates) for each site and date were incorporated as a random effect. We contrasted models that included fixed effects of season, nutrient treatment, and their interaction using Akaike's information criterion corrected for small sample size (AICc) to select a final model that was most parsimonious and best supported by the data. Residuals were visually inspected for normality and constant variance using normal probability plots and plots of predicted vs. observed values. We estimated marginal R^2 , the variance explained by fixed effects and conditional R^2 , variance explained by random effects, as metrics of model fit using the MuMIn package (Barton 2016).

We assessed temporal patterns in stream chemistry using mixed-effects models that included site as a random effect and sampling event as a fixed effect. Tukey's honestly significance difference was used post hoc to evaluate differences among sampling dates ($\alpha = 0.05$). We evaluated temporal patterns in bulk carbon and nutrient concentrations, as well as optical properties of DOM. Temporal patterns in DOM composition were also assessed using a composite index quantified by principal components analysis using the five components from PARAFAC analysis as well as FI, HIX, BIX, $\beta:\alpha$, SUVA, and SR (function *princomp*). Principal Component 1 resulting from this analysis explained 56% of the total variance and was used in the mixed-effects model.

We used multiple regression analyses to explore the chemical attributes correlated with biolability. Candidate predictor variables included bulk nutrient concentrations (DOC, TN, DON, NO_3^- , NH_4^+ , TDP), a proxy for groundwater inputs (GW), optical indices of DOM composition (SUVA, SR, FI, HIX, BIX, $\beta:\alpha$), and the five components from the PARAFAC model. Each model included site as a random effect. Predictor variables were centered and standardized by subtracting the mean and dividing by standard deviation prior to analysis. Initial models indicated that ambient DOC concentration was the dominant correlate of both absolute DOC loss and total percent DOC loss. To further explore the effects of other potential predictors of DOC biolability, we modeled the residuals of the relationships between biolability and DOC concentration. R^2 values for these models were <0.1 and they are not reported. Multiple logistic regression analysis was similarly performed to explore the factors correlated with an enhancement of biolability in nutrient fertilization assays. Logistic models included site as a random effect, and the binary response represented whether DOC loss was greater in the amended assay compared to unamended control. All models including a single predictor were analyzed and the predictor variables from the eight models with the lowest AICc values were chosen for inclusion in models with up to three predictors. Predictors with correlation coefficients $>|0.5|$ were not allowed in the same model.

1.4 Results

1.4.1 Spatial and temporal patterns in DOM composition

DOC concentration was greatest for the sampling events in May, which occurred during spring freshet, and lowest during February, when samples were collected from beneath ice or as overflow (Figure 1.2). A second peak in DOC concentration occurred for most sites in August

coinciding with precipitation and increased discharge (Supplemental Figure 1.1). DON concentration generally followed patterns in DOC. Average TDP concentration was approximately four times greater during spring freshet in May than during summer baseflow in June or winter baseflow in November and February. The proxy for groundwater inputs (GW) increased throughout the winter and declined during freshet, decreasing nearly 60% between April and May 2016. A nutrient-rich sample was collected from overflow on the still ice-covered Kuparuk River in April 2016 and was withheld from statistical analysis due to concentrations of labile DOC and nutrients that were nearly ten standard deviations greater than mean values.

Molecular composition also varied temporally, as characterized by optical properties (Figure 1.3). Overall, all sampling dates showed a strong signature of terrestrially derived DOM, with FI values ranging between 1.4-1.6, with the exception of an overflow sample collected in April from Slate Creek with an FI of 2.0, indicating microbially derived DOM (McKnight et al. 2001). Across all sites and sampling events, low BIX values (<1) indicate little contribution of autotrophic productivity to the DOM pool (Huguet et al. 2009). BIX was significantly lower during May, indicating that less autochthonous DOM was present during snowmelt than during other sampling periods (Figure 1.2). SUVA significantly decreased between May and June, and SR significantly increased, indicating that both aromaticity and average molecular weight decreased between freshet and the onset of summer baseflow. Five fluorescing components of DOM were identified from PARAFAC analysis of EEMs. Through comparisons with the OpenFluor database, Component 1 (Kothawala et al. 2014) and Component 2 (Shutova et al. 2014) were identified as humic-like (Table 1.2). Component 3 was identified as a high molecular weight fraction of terrestrially derived DOM (Kothawala et al. 2014). Component 4

was identified as protein-like (Dainard et al. 2015) and Component 5 was visually matched to the fluorescence spectra of an uncharacterized component previously observed by Cory and McKnight (2005) in samples from a range of aquatic environments including arctic lakes. Humic-like components (C1, C2, and C3) represented 88% of fluorescent component loadings, on average. The loading of Component 4 tended to be largest during April of both years of the study (Figure 1.3), indicating a higher proportion of protein-like DOM at or just before the onset of snowmelt. Overall, the optical properties of DOM were most distinct during May, as shown by temporal patterns in Principal Component 1, which summarized the optical indices and was positively related to indicators of humic content such as HIX and the humic-like PARAFAC components C1 and C3, and negatively related to indicators of autochthonous DOM such as BIX, $\beta:\alpha$, and the protein-like PARAFAC component C4 (Supplementary Table 1.2). The significant differences in Principal Component 1 between April and May of both years indicate a change in molecular composition, specifically a shift towards a greater proportion of humic-like DOM in May. Despite these temporal patterns, intra-annual variation was lower for metrics of DOM composition than for bulk concentrations of solutes, shown by the coefficient of variation (CV) estimated across sampling events (Table 1.1).

Spatial variation also resulted in patterns in DOM composition. DOC concentration varied four-fold across sites spanning the arctic-boreal gradient but there was no latitudinal trend. HIX tended to be lower for the arctic sites (Bonanza, Slate, and Kuparuk), and nitrate concentration was approximately three times higher for the southernmost sites (Chena, Chatanika, and Poker) than the other sites (Table 1.1). Spatial variation, quantified by the CV of annual means across sites, was greater for bulk concentrations than for optical properties of DOM (Table 1.1). Spatial CVs were greater than average temporal CVs for DOC, DON, TDP

and NO_3^- concentrations, whereas temporal variation exceeded spatial variation for all other measured attributes (Table 1.1).

1.4.2 Spatial and temporal patterns in DOM biolability

Biologically labile DOC represented a small fraction of the total DOC pool in nearly all samples measured. Total loss of DOC in unfertilized incubations ranged from 0 – 42% of the DOC pool, with an average of 4% (Figure 1.4). The effects of nutrient fertilization and sampling date on both mass and fractional loss of DOC were determined by comparing models containing fixed effects of nutrient treatment, sampling event, and their interaction, with a random mean for each site and sampling date combination included in all models, and AICc values indicated strongest support for a model including nutrient treatment as a fixed effect (Supplementary Table 1.3). Nutrient treatment had a significant effect on both percent and absolute loss, as the difference in AICc values was greater than 2 units for models including a nutrient effect compared to models without the nutrient effect (Supplementary Table 1.3). However, the explanatory value of the nutrient-only model was low (marginal $R^2 < 0.1$), and therefore the model including nutrient treatment, sampling event, and their interaction was further analyzed for both response variables. Overall, P addition increased DOC loss by $3.0 \pm 0.5\%$ or 0.3 ± 0.1 mg/L over unfertilized controls (Supplementary Table 1.4). Examination of the interaction of sampling event and nutrient treatment indicated that P increased decomposition most during April and May, with significant increases in DOC loss from the P and N+P treatments compared to control, as determined by Bonferroni-corrected multiple comparisons of amended assays to unamended controls (Figure 1.4). There was no significant effect of the N treatment at any time.

Although biolability was nearly five times greater in April and May than for the rest of the year, Tukey's post hoc comparisons indicated that this effect was not significant.

The primary driver of biolability was ambient DOC concentration, which explained greater than 65% variation in biolability in terms of fractional loss of DOC (Figure 1.5). DOC concentration similarly explained absolute loss of DOC (Absolute loss (mg C/L) = $0.44 \cdot \text{DOC} + 0.20$, marginal $R^2 = 0.68$ and conditional $R^2 = 0.79$). Site was included as a random effect in these models. Multiple regression models using the residuals from the DOC model with the predictor variables (Table 1) and a random effect of site explained little of the variability in biolability ($R^2 < 0.1$). We also determined the chemical attributes of streamwater that contributed to enhancement of decomposition in nutrient-amended assays. Logistic regression models were used to identify predictors of N response, a binary indicator of enhanced biolability in N-amended assays compared to controls. In these models, the random effect of site did not explain any variance and was therefore dropped from models. The best models of N response contained DOC concentration and measures of molecular composition such as the loading of C1 and C4. We report the modeled relationship with DOC concentration, and the top model without DOC concentration due to multicollinearity of predictors. Enhancement of biolability in N-amended samples compared to controls was positively related to DOC concentration and negatively related to C:P and the protein-like PARAFAC component C4 (Table 1.3). However, other models containing variables describing composition of the DOM pool performed similarly ($\Delta\text{AICc} \sim 2$ for models containing C1, C:N, and DON). Logistic models describing the response of biolability to P addition explained little variance ($R^2 < 0.2$) and are not reported.

1.5 Discussion

We examined the characteristics of DOM that influence its decomposition in high-latitude streams, and quantified regional and intra-annual variation in these characteristics to improve our understanding of the mechanisms that drive biolability. Humic-like components dominated fluorescent loading in the DOM pool across an arctic-boreal gradient and throughout the year, indicating strong hydrologic connection between streams and a large pool of organic matter. P addition significantly increased biolability of DOC, supporting our hypothesis that nutrient limitation constrains decomposition in high-latitude streams. Although molecular composition varied intra-annually, optical properties of DOM were only weakly associated with biolability, suggesting that the compounds that drive biolability may not be optically detectable. Ambient DOC concentration was the strongest predictor of biolability, indicating that the size of the DOM pool influences its overall biolability. Lower DOC concentrations may reflect DOM pools that have already undergone C processing.

1.5.1 Links between DOM composition and hydrology

Temporal patterns in DOM composition corresponded to seasonal trends in the hydrology of high-latitude catchments. Greater SUVA values and lower SR, BIX, and FI values observed during freshet compared to during baseflow represent a shift in DOM source towards more aromatic, high-molecular weight, terrestrially based DOM. Other high-latitude studies have found similar trends in composition of DOM (Spencer et al. 2008, Mann et al. 2012), and have hypothesized that the composition of DOM in high-latitude catchments shifts with the depth of dominant flowpaths connecting catchments to streams. Shallow soil horizons in high-latitude regions are rich in organic C (Tarnocai et al. 2009, Ping et al. 2010, DeLuca and Boisvenue

2012), and DOM in pore water from these soils is highly aromatic (Wickland et al. 2007). Further support for the contribution of DOM from a shallow flowpath during freshet comes from the greater proportion of Component 5 during May of both years of the study (Figure 1.3). Component 5 may indicate the influence of leaf litter from surface layers, because although the precise identity of Component 5 is uncharacterized, its relative loading was greater for leachates from boreal plant species (mean = 41%, Mutschlecner et al. n.d.) than for stream samples in this study (mean = 2%). Molecular composition, as summarized by Principal Component 1, was only distinct during the May sampling event, and was not significantly different between winter and summer. In winter, when organic soil horizons are frozen, deeper flowpaths contribute more towards riverine DOM. This is evidenced by higher concentrations of conservative ions (Figure 1.2), which are sourced from deeper mineral soils (Keller et al. 2010). Although depth of dominant flowpaths connecting catchments to streams contrasts between winter and summer, composition of DOM in streams indicated that DOM is sourced largely from soil organic matter throughout the year. Both spatial and temporal variation tended to be lower for measures of DOM composition, such as the PARAFAC components and optical indices, than for concentration or nutrient content, further indicating the contribution of a large terrestrial source of DOM to the stability in the composition of DOM exported by rivers.

1.5.2 Low biolability across sites and seasons

Overall, we found that the majority of riverine DOM is recalcitrant to biological decomposition. Although the range in lability for this study (0-42%) agrees with other estimates for arctic and boreal streams (0-53%; Kawahigashi et al. 2004, Balcarczyk et al. 2009, Mann et al. 2012, Wickland et al. 2012, Vonk et al. 2013, Larouche et al. 2015), mean lability measured

in this study was 4%, in the lower range of previously reported values. Balcarczyk et al. (2009) include estimates of lability from a major tributary of one of the streams reported here (Poker), and yet those estimates of biolability (6-17%) were greater than for this study (<1%) in the same season. For headwater streams within the same watershed, White et al. (2008) found maximum absolute loss of DOC of nearly 7 mg C/L, whereas maximum absolute loss for this study was 2.4 mg C/L. The estimates reported in the present study may be lower because they represent larger-order streams and rivers, compared to the headwaters previously studied in the Poker Creek catchment. Estimates of biolability decrease with increasing stream size (Vonk et al. 2015), likely due to longer exposure to biological and photochemical processes which remove biolabile DOM from the DOM pool. However, some estimates of lability were also greater for the Yukon River (Wickland et al. 2012) than we report for smaller rivers. The Yukon River includes glacial influence (Wickland et al. 2012), and glaciated watersheds tend to export DOM of higher biolability than non-glaciated watersheds (Hood et al. 2009), whereas the streams sampled in the present study are sourced from precipitation and groundwater. Discrepancies in biolability estimates among studies may also be due to differences in incubation time, temperature, filter pore size, inoculum type, and method of analysis, and a standardized protocol will enable more direct comparisons between studies (Vonk et al. 2015).

In this study, DOC loss remained low even when nutrient limitation was relieved through fertilization, suggesting that biolability in these streams is primarily limited by molecular composition rather than nutrients. Despite the large temporal and spatial range of samples, variation in biolability estimates and composition of DOM was low, limiting detection of correlations between lability and optical metrics of DOM composition. Microbial respiration in boreal streams can be limited by labile C (Burrows et al. 2017), and rates of C processing in

this region would therefore increase in response to inputs of labile carbon. DOM from actively degrading permafrost is highly labile (Abbott et al. 2014, Spencer et al. 2015), and warmer temperatures and longer thaw seasons may increase the release of permafrost-derived C throughout the thaw period. Shifting the fate of riverine DOM from export to respiration can have a substantial effect on regional and even global C budgets (Kling et al. 1991, Butman et al. 2015). Low biolability indicates that export, rather than respiration, is the dominant fate of DOM in these streams.

Although we found low biolability of DOM, photolability can also result in DOC mineralization (Osburn et al. 2009, Cory et al. 2014). Photochemical processes are the dominant pathway for DOC processing in some arctic rivers (Cory et al. 2014), and the relative importance of these mechanisms for boreal rivers is a critical direction for further study. Photolability may be less influential in boreal rivers due to turbidity and shaded upper portions of stream networks. Low photolability for DOC derived from permafrost (Stubbins et al. 2017) emphasizes the importance of biological decomposition to the fate of permafrost DOC.

1.5.3 Nutrient limitation of biolability

Despite low biolability overall, the addition of P increased DOC loss, particularly during spring (Figure 1.4). In addition, increased biolability in response to N addition was more likely when ambient P was more available (i.e., when C:P ratios were lower), demonstrating P-limitation of the N response (Table 1.3). Low P concentration in high-latitude rivers (Peterson et al. 1985, Fellman et al. 2008, Holmes et al. 2012) represent a potential constraint on CO₂ derived from in-stream respiration. Few data are available on P limitation of heterotrophs in high-latitude streams, with the exception of a fertilization study for the Kuparuk River (Peterson et al.

1993). Peterson et al. (1993) observed increased heterotrophic activity through two mechanisms, a direct response through P addition, and an indirect response in which P increased autochthonous production of labile C, which subsequently increased heterotrophic activity, and found that the second mechanism was the chief process. In this study, the effect of P was through the first mechanism (direct response), and may have been relatively muted (3% increase in DOC loss for P treatment relative to control) because a lack of labile C exerted a stronger constraint on decomposition.

P limitation of biolability indicates coupling between C and P cycles. C and nutrient cycles tend to be coupled because organisms require elements in specific proportions (Sterner and Elser 2002). The constraints of coupled cycles are often not accounted for in ecosystem modeling and climate change predictions (Finzi et al. 2011). The coupling of C and P cycles could alter C budgets in high-latitude ecosystems as less C may be respired (and more C may be exported) than predicted based on C availability alone. Some estimates indicate that P availability in the Yukon River Basin may be increasing (Toohey et al. 2016), and our study indicates that this would increase DOM processing in streams.

1.5.4 Conclusions

We found that DOM composition of high-latitude streams changes with seasonal patterns in catchment hydrology, but that these changes in composition do not correspond directly to changes in biolability. Over a regional spatial extent and an annual time period, the overall molecular composition of DOM is remarkably stable. Our study indicates that increased P availability has the potential to increase rates of C processing. Nutrient-stimulated increases to

DOM biolability have the potential to greatly influence high-latitude C budgets by shifting proportions of respired and exported DOC.

1.6 References

- Abbott, B., J. R. Larouche, J. B. Jones, W. B. Bowden, and A. W. Balsler. 2014. Elevated dissolved organic carbon biodegradability from thawing and collapsing permafrost. *Journal of Geophysical Research: Biogeosciences* 119:2049–2063.
- Ågren, A., M. Berggren, H. Laudon, and M. Jansson. 2008. Terrestrial export of highly bioavailable carbon from small boreal catchments in spring floods. *Freshwater Biology* 53:964–972.
- Arp, C. D., and S. Stuefer. 2016. Climate data from the Terrestrial Environmental Observation Network (TEON), Arctic Landscape Conservation Cooperative. University of Alaska Fairbanks, Water and Environmental Research Center. URL: <http://ine.uaf.edu/werc/research> Fairbanks, Alaska.
- Balcarczyk, K. L., J. B. Jones, R. Jaffé, and N. Maie. 2009. Stream dissolved organic matter bioavailability and composition in watersheds underlain with discontinuous permafrost. *Biogeochemistry* 94:255–270.
- Barton, K. 2016. MuMIn: Multi-Model Inference. R package version 1.15.6. <https://CRAN.R-project.org/package=MuMIn>.
- Bates, D., M. Mächler, B. Bolker, and S. C. Walker. 2015. Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*:1–51.
- Battin, T. J., L. a. Kaplan, S. Findlay, C. S. Hopkinson, E. Marti, A. I. Packman, J. D. Newbold, and F. Sabater. 2009. Biophysical controls on organic carbon fluxes in fluvial networks. *Nature Geoscience* 2:595–595.
- Bonanza Creek LTER Climate Database. 2016. Bonanza Creek LTER - University of Alaska Fairbanks (<http://www.lter.uaf.edu>). National Science Foundation Long-Term Ecological Research program grant number DEB-1026415 and USDA Forest Service, Pacific Northwest Research Station Agreement # RJVA-PNW-01-JV-11261952-231.
- Brabets, T. B., B. Wang, and R. M. Meade. 2000. Environmental and hydrologic overview of the Yukon River Basin, Alaska and Canada. U.S. Geological Survey: Water-Resources Investigations Report 99-4204.
- Burrows, R. M., H. Laudon, B. G. McKie, and R. A. Sponseller. 2017. Seasonal resource limitation of heterotrophic biofilms in boreal streams. *Limnology and Oceanography* 62:164–176.
- Butman, D., S. Stackpoole, E. Stets, C. P. McDonald, D. W. Clow, and R. G. Striegl. 2015. Aquatic carbon cycling in the conterminous United States and implications for terrestrial carbon accounting. *Proceedings of the National Academy of Sciences of the United States of America* 113:58–63.
- Cory, R. M., and L. A. Kaplan. 2012. Biological lability of streamwater fluorescent dissolved organic matter. *Limnology and Oceanography* 57:1347–1360.

- Cory, R. M., and D. M. McKnight. 2005. Fluorescence spectroscopy reveals ubiquitous presence of oxidized and reduced quinones in dissolved organic matter. *Environmental Science and Technology* 39:8142–8149.
- Cory, R. M., J. M. Purcell, and A. G. Marshall. 2010. Singlet oxygen in the coupled photochemical and biochemical oxidation of dissolved organic matter 44:3683–3689.
- Cory, R. M., C. P. Ward, B. C. Crump, and G. W. Kling. 2014. Sunlight controls water column processing of carbon in arctic fresh waters. *Science* 345:925–928.
- Dainard, P. G., C. Guéguen, N. McDonald, and W. J. Williams. 2015. Photobleaching of fluorescent dissolved organic matter in Beaufort Sea and North Atlantic Subtropical Gyre. *Marine Chemistry* 177:630–637.
- DeLuca, T. H., and C. Boisvenue. 2012. Boreal forest soil carbon: Distribution, function and modelling. *Forestry* 85:161–184.
- Environmental Data Center Team. 2016. Environmental Data Center Team. Meteorological monitoring program at Toolik, Alaska. Toolik Field Station, Institute of Arctic Biology, University of Alaska Fairbanks, Fairbanks, AK 99775.
http://toolik.alaska.edu/edc/abiotic_monitoring/data_query.php.
- Fellman, J. B., D. V. D'Amore, E. Hood, and R. D. Boone. 2008. Fluorescence characteristics and biodegradability of dissolved organic matter in forest and wetland soils from coastal temperate watersheds in southeast Alaska. *Biogeochemistry* 88:169–184.
- Fellman, J. B., E. Hood, R. T. Edwards, and J. B. Jones. 2009. Uptake of allochthonous dissolved organic matter from soil and salmon in coastal temperate rainforest streams. *Ecosystems* 12:747–759.
- Ferrians, O. 1998. Permafrost Map of Alaska, USA, Version 1. Boulder, Colorado USA. NSIDC: National Snow and Ice Data Center.
- Finlay, J., J. Neff, S. Zimov, A. Davydova, and S. Davydov. 2006. Snowmelt dominance of dissolved organic carbon in high-latitude watersheds : implications for characterization and flux of river DOC. *Geophysical Research Letters* 33:1–5.
- Finzi, A. C., A. T. Austin, E. E. Cleland, S. D. Frey, B. Z. Houlton, and M. D. Wallenstein. 2011. Responses and feedbacks of coupled biogeochemical cycles to climate change: examples from terrestrial ecosystems. *Frontiers in Ecology and the Environment* 9:61–67.
- Frey, K. E., and J. W. McClelland. 2009. Impacts of permafrost degradation on arctic river biogeochemistry. *Hydrological Processes* 23:169–182.
- Harms, T. K., J. W. Edmonds, H. Genet, I. F. Creed, D. Aldred, A. Balsler, and J. B. Jones. 2016. Catchment influence on nitrate and dissolved organic matter in Alaskan streams across a latitudinal gradient. *Journal of Geophysical Research: Biogeosciences* 121:350–369.

- Helms, J. R., A. Stubbins, J. D. Ritchie, E. C. Minor, D. J. Kieber, and K. Mopper. 2008. Absorption spectral slopes and slope ratios as indicators of molecular weight, source, and photobleaching of chromophoric dissolved organic matter. *Limnology and Oceanography* 53:955–969.
- Holmes, R. M., J. W. McClelland, B. J. Peterson, S. E. Tank, E. Bulygina, T. I. Eglinton, V. V. Gordeev, T. Y. Gurtovaya, P. A. Raymond, D. J. Repeta, R. Staples, R. G. Striegl, A. V. Zhulidov, and S. A. Zimov. 2012. Seasonal and annual fluxes of nutrients and organic matter from large rivers to the arctic ocean and surrounding seas. *Estuaries and Coasts* 35:369–382.
- Holmes, R. M., J. W. McClelland, P. A. Raymond, B. B. Frazer, B. J. Peterson, and M. Stieglitz. 2008. Lability of DOC transported by Alaskan rivers to the Arctic Ocean. *Geophysical Research Letters* 35:L03402.
- Hood, E., J. Fellman, R. G. M. Spencer, P. J. Hernes, R. Edwards, D. D'Amore, and D. Scott. 2009. Glaciers as a source of ancient and labile organic matter to the marine environment. *Nature* 462:1044–1047.
- Huguet, A., L. Vacher, S. Relexans, S. Saubusse, J. M. Froidefond, and E. Parlanti. 2009. Properties of fluorescent dissolved organic matter in the Gironde Estuary. *Organic Geochemistry* 40:706–719.
- Jones, J. B., K. C. Petrone, J. C. Finlay, L. D. Hinzman, and W. R. Bolton. 2005. Nitrogen loss from watersheds of interior Alaska underlain with discontinuous permafrost. *Geophysical Research Letters* 32:1–4.
- Kawahigashi, M., K. Kaiser, K. Kalbitz, A. Rodionov, and G. Guggenberger. 2004. Dissolved organic matter in small streams along a gradient from discontinuous to continuous permafrost. *Global Change Biology* 10:1576–1586.
- Keller, K., J. D. Blum, and G. W. Kling. 2010. Stream geochemistry as an indicator of increasing permafrost thaw depth in an arctic watershed. *Chemical Geology* 273:76–81.
- Kellerman, A. M., D. N. Kothawala, T. Dittmar, and L. J. Tranvik. 2015. Persistence of dissolved organic matter in lakes related to its molecular characteristics. *Nature Geoscience* 8:454–459.
- Kiikkilä, O., A. Smolander, and V. Kitunen. 2013. Degradability, molecular weight and adsorption properties of dissolved organic carbon and nitrogen leached from different types of decomposing litter. *Plant and Soil* 373:787–798.
- Kling, G. W., G. W. Kipphut, and M. C. Miller. 1991. Arctic lakes and streams as gas conduits to the atmosphere: implications for tundra carbon budgets. *Science* 251:298–301.
- Kothawala, D. N., C. A. Stedmon, R. A. Muller, G. A. Weyhenmeyer, S. J. Kohler, and L. J. Tranvik. 2014. Controls of dissolved organic matter quality: evidence from a large-scale boreal lake survey. *Global Change Biology* 20:1101–1114.

- Larouche, J. R., B. W. Abbott, W. B. Bowden, and J. B. Jones. 2015. The role of watershed characteristics, permafrost thaw, and wildfire on dissolved organic carbon biodegradability and water chemistry in Arctic headwater streams. *Biogeosciences* 12:4221–4233.
- Mann, P. J., A. Davydova, N. Zimov, R. G. M. Spencer, S. Davydov, E. Bulygina, S. Zimov, and R. M. Holmes. 2012. Controls on the composition and lability of dissolved organic matter in Siberia's Kolyma River basin. *Journal of Geophysical Research: Biogeosciences* 117:1–15.
- Mann, P. J., T. I. Eglinton, C. P. McIntyre, N. Zimov, A. Davydova, J. E. Vonk, R. M. Holmes, and R. G. M. Spencer. 2015. Utilization of ancient permafrost carbon in headwaters of Arctic fluvial networks. *Nature Communications* 6:1–7.
- McClelland, J. W., M. Stieglitz, F. Pan, R. M. Holmes, and B. J. Peterson. 2007. Recent changes in nitrate and dissolved organic carbon export from the upper Kuparuk River, North Slope, Alaska. *Journal of Geophysical Research* 112:1–13.
- McKnight, D. M., E. W. Boyer, P. K. Westerhoff, P. T. Doran, T. Kulbe, and D. T. Andersen. 2001. Spectrofluorometric characterization of dissolved organic matter for indication of precursor organic material and aromaticity. *Limnology and Oceanography* 46:38–48.
- Murphy, J., and J. P. Riley. 1962. A modified single solution method for the determination of phosphate in natural waters. *Analytica Chimica Acta* 27:31–36.
- Murphy, K. R., C. A. Stedmon, P. Wenig, and R. Bro. 2014. OpenFluor- an online spectral library of auto-fluorescence by organic compounds in the environment. *Analytical Methods* 6:658–661.
- Mutschlecner, A., J. J. Guerard, J. B. Jones, and T. K. Harms. (n.d.). Phosphorus constrains uptake of dissolved organic matter in boreal streams. Manuscript in preparation.
- O'Donnell, J. A., G. R. Aiken, E. S. Kane, and J. B. Jones. 2010. Source water controls on the character and origin of dissolved organic matter in streams of the Yukon River basin, Alaska. *Journal of Geophysical Research: Biogeosciences* 115:1–12.
- O'Donnell, J. A., G. R. Aiken, D. K. Swanson, S. Panda, K. D. Butler, and A. P. Baltensperger. 2016. Dissolved organic matter composition of Arctic rivers: linking permafrost and parent material to riverine carbon. *Global Biogeochemical Cycles*.
- O'Donnell, J. A., G. R. Aiken, M. A. Walvoord, and K. D. Butler. 2012. Dissolved organic matter composition of winter flow in the Yukon River basin: Implications of permafrost thaw and increased groundwater discharge. *Global Biogeochemical Cycles* 26:1–18.
- Ohno, T. 2002. Fluorescence inner-filtering correction for determining the humification index of dissolved organic matter. *Environmental Science & Technology* 36:742–746.
- Parlanti, E., K. Wörz, L. Geoffroy, and M. Lamotte. 2000. Dissolved organic matter fluorescence spectroscopy as a tool to estimate biological activity in a coastal zone submitted to anthropogenic inputs. *Organic Geochemistry* 31:1765–1781.

- Peterson, B. J., L. Deegan, J. Helfrich, J. E. Hobbie, B. Moller, T. E. Ford, A. Hershey, A. Hiltner, G. Kipphut, M. A. Lock, D. M. Fiebig, V. Mckinley, M. C. Miller, J. R. Vestal, B. J. Peterson, L. Deegan, J. Helfrich, J. E. Hobbie, M. Hullar, B. Moller, T. E. Ford, M. C. Miller, and J. R. Vestal. 1993. Biological responses of a tundra river to fertilization. *Ecology* 74:653–672.
- Peterson, B. J., J. E. Hobbie, A. E. Hershey, M. A. Lock, T. E. Ford, J. R. Vestal, V. L. McKinley, M. A. Hullar, M. C. Miller, R. M. Ventullo, and G. S. Volk. 1985. Transformation of a tundra river from heterotrophy to autotrophy by addition of phosphorus. *Science* 229:1383–1386.
- Petrone, K. C., J. B. Jones, L. D. Hinzman, and R. D. Boone. 2006. Seasonal export of carbon, nitrogen, and major solutes from Alaskan catchments with discontinuous permafrost. *Journal of Geophysical Research: Biogeosciences* 111:1–13.
- Ping, C. L., G. J. Michaelson, E. S. Kane, E. C. Packee, C. A. Stiles, D. K. Swanson, and N. D. Zaman. 2010. Carbon stores and biogeochemical properties of soils under black spruce forest, Alaska. *Soil Science Society of America Journal* 74:969–978.
- Qualls, R. G., and B. L. Haines. 1992. Biodegradability of dissolved organic matter in forest throughfall, soil solution, and stream water. *Soil Science Society of America Journal* 56:578–586.
- R Core Team. 2016. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. www.r-project.org.
- Raymond, P. A., J. W. McClelland, R. M. Holmes, A. V. Zhulidov, K. Mull, B. J. Peterson, R. G. Striegl, G. R. Aiken, and T. Y. Gurtovaya. 2007. Flux and age of dissolved organic carbon exported to the Arctic Ocean: a carbon isotopic study of the five largest arctic rivers. *Global Biogeochemical Cycles* 21:1–9.
- Schuur, E. A. G., A. D. McGuire, G. Grosse, J. W. Harden, D. J. Hayes, G. Hugelius, C. D. Koven, and P. Kuhry. 2015. Climate change and the permafrost carbon feedback. *Nature* 520:171–179.
- Shaver, G., W. Billings, F. Chapin III, A. Giblin, and K. Nadelhoffer. 1992. Global change and the carbon balance of arctic ecosystems. *BioScience* 42:433–441.
- Shutova, Y., A. Baker, J. Bridgeman, and R. K. Henderson. 2014. Spectroscopic characterisation of dissolved organic matter changes in drinking water treatment: from PARAFAC analysis to online monitoring wavelengths. *Water Research* 54:159–169.
- Sinsabaugh, R. L., S. Manzoni, D. L. Moorhead, and A. Richter. 2013. Carbon use efficiency of microbial communities: stoichiometry, methodology and modelling. *Ecology Letters* 16:930–939.
- Solórzano, L. 1969. Determination of ammonia in natural waters by the phenylhypochlorite method. *Limnology and Oceanography* 14:799–801.

- Spencer, R. G. M., G. R. Aiken, K. P. Wickland, R. G. Striegl, and P. J. Hernes. 2008. Seasonal and spatial variability in dissolved organic matter quantity and composition from the Yukon River basin, Alaska. *Global Biogeochemical Cycles* 22:1–13.
- Spencer, R. G. M., P. J. Mann, T. Dittmar, T. I. Eglinton, C. McIntyre, R. M. Holmes, N. Zimov, and A. Stubbins. 2015. Detecting the signature of permafrost thaw in Arctic rivers. *Geophysical Research Letters* 42:2830–2835.
- Stedmon, C. A., and R. Bro. 2008. Characterizing dissolved organic matter fluorescence with parallel factor analysis: a tutorial. *Limnology and Oceanography: Methods* 6:572–579.
- Stedmon, C. A., S. Markager, and R. Bro. 2003. Tracing dissolved organic matter in aquatic environments using a new approach to fluorescence spectroscopy 82:239–254.
- Striegl, R. G., G. R. Aiken, M. M. Dornblaser, P. A. Raymond, and K. P. Wickland. 2005. A decrease in discharge-normalized DOC export by the Yukon River during summer through autumn. *Geophysical Research Letters* 32:1–4.
- Striegl, R. G., M. M. Dornblaser, G. R. Aiken, K. P. Wickland, and P. A. Raymond. 2007. Carbon export and cycling by the Yukon, Tanana, and Porcupine rivers, Alaska, 2001–2005. *Water Resources Research* 43.
- Tarnocai, C., J. G. Canadell, E. A. G. Schuur, P. Kuhry, G. Mazhitova, and S. Zimov. 2009. Soil organic carbon pools in the northern circumpolar permafrost region. *Global Biogeochemical Cycles* 23:1–11.
- Toohey, R. C., N. M. Herman-Mercer, P. F. Schuster, E. Mutter, and J. C. Koch. 2016. Multi-decadal increases in the Yukon River Basin of chemical fluxes as indicators of changing flowpaths, groundwater, and permafrost. *Geophysical Research Letters* 43:12,120–12,130.
- Vonk, J. E., P. J. Mann, S. Davydov, A. Davydova, R. G. M. Spencer, J. Schade, W. V. Sobczak, N. Zimov, S. Zimov, E. Bulygina, T. I. Eglinton, and R. M. Holmes. 2013. High biolability of ancient permafrost carbon upon thaw. *Geophysical Research Letters* 40:2689–2693.
- Vonk, J. E., S. E. Tank, P. J. Mann, R. G. M. Spencer, C. C. Treat, R. G. Striegl, B. W. Abbott, and K. P. Wickland. 2015. Biodegradability of dissolved organic carbon in permafrost soils and aquatic systems: a meta-analysis. *Biogeosciences* 12:6915–6930.
- Weishaar, J., G. Aiken, B. Bergamaschi, M. Fram, R. Fujii, and K. Mopper. 2003. Evaluation of specific ultra-violet absorbance as an indicator of the chemical content of dissolved organic carbon. *Environmental Science and Technology* 37:4702–4708.
- White, D., V. Autier, K. Yoshikawa, J. Jones, and S. Seelen. 2008. Using DOC to better understand local hydrology in a subarctic watershed. *Cold Regions Science and Technology* 51:68–75.
- Wickland, K. P., G. R. Aiken, K. Butler, M. M. Dornblaser, R. G. M. Spencer, and R. G. Striegl. 2012. Biodegradability of dissolved organic carbon in the Yukon River and its tributaries: Seasonality and importance of inorganic nitrogen. *Global Biogeochemical Cycles* 26:1–14.

- Wickland, K. P., J. C. Neff, and G. R. Aiken. 2007. Dissolved organic carbon in Alaskan boreal forest: sources, chemical characteristics, and biodegradability. *Ecosystems* 10:1323–1340.
- Wilson, H. F., and M. A. Xenopoulos. 2009. Effects of agricultural land use on the composition of fluvial dissolved organic matter. *Nature Geoscience* 2:37–41.

1.7 Tables

Table 1.1. DOC lability, bulk chemistry, and optical properties of DOM in the 8 streams included in this study, listed from south to north. Values are reported as mean and coefficient of variation (CV) summarized across the sampling events at each site, with number of sampling events indicated for each site. Average temporal CV is summarized across all sites, and the CV of means across the study period indicates spatial variance. CVs are not available for Slate Creek as no DOC loss was observed. Included are the proxy for groundwater inputs (GW), which represents a sum of conservative ions, fluorescence index (FI), humification index (HIX), biological index (BIX), freshness index ($\beta:\alpha$), specific ultraviolet absorbance (SUVA), and spectral slope ratio (SR). Stoichiometric ratios are given in molar units.

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	DOC loss %	DOC loss mg/L	DOC mg/L	DON $\mu\text{g/L}$	NH_4^+ $\mu\text{g/L}$	NO_3^- $\mu\text{g/L}$	TDP $\mu\text{g/L}$	C:N	C:P
Chena (n=8)	2.2 (119)	0.1 (77)	4.7 (67)	89 (83)	32 (204)	259 (40)	3 (67)	14 (63)	4274 (38)
Chatanika (n=8)	4.7 (76)	0.4 (87)	5.0 (71)	115 (70)	15 (110)	245 (50)	3 (83)	16 (72)	6463 (90)
Poker (n=8)	0.9 (85)	0.1 (101)	5.5 (79)	105 (75)	15 (74)	347 (31)	4 (87)	14 (82)	3918 (47)
Tatalina (n=7)	4.7 (72)	1.0 (93)	14.4 (59)	352 (73)	36 (109)	62 (56)	6 (76)	36 (25)	7407 (35)
No Name (n=3)	1.8 (169)	0.3 (161)	16.0 (19)	443 (15)	23 (122)	18 (62)	16 (46)	39 (3)	2869 (41)
Bonanza (n=5)	3.5 (40)	0.2 (27)	5.1 (50)	129 (39)	7 (0)	87 (103)	3 (76)	27 (49)	5253 (54)
Slate (n=6)	0.0	0.0	3.9 (85)	102 (66)	10 (56)	90 (28)	3 (95)	20 (59)	4909 (86)
Kuparuk (n=7)	14.4 (130)	1.2 (88)	7.5 (72)	328 (97)	41 (133)	45 (96)	17 (141)	27 (60)	2873 (68)
Average temporal CV (%)	99	90	63	65	101	58	84	52	57
Average spatial CV (%)	110	129	70	88	74	74	97	59	50

Table 1.1 continued.

	GW μM	FI	HIX	BIX	$\beta:\alpha$	SUVA $\text{L mg}^{-1} \text{m}^{-1}$	SR
Chena	404 (23)	1.53 (5)	0.90 (11)	0.57 (13)	0.55 (12)	3.16 (18)	0.82 (10)
Chatanika	366 (26)	1.51 (4)	0.94 (14)	0.53 (15)	0.51 (13)	3.34 (23)	0.81 (11)
Poker	278 (40)	1.48 (2)	0.94 (15)	0.51 (15)	0.49 (14)	3.38 (18)	0.78 (12)
Tatalina	660 (55)	1.53 (3)	0.95 (4)	0.54 (11)	0.53 (11)	3.60 (12)	0.75 (5)
No Name	429 (45)	1.52 (2)	0.94 (1)	0.56 (8)	0.55 (8)	3.96 (7)	0.78 (4)
Bonanza	216 (42)	1.46 (4)	0.87 (10)	0.51 (19)	0.49 (17)	3.72 (19)	0.82 (17)
Slate	419 (33)	1.59 (12)	0.82 (15)	0.58 (24)	0.54 (22)	3.17 (27)	0.89 (20)
Kuparuk	299 (71)	1.54 (4)	0.84 (12)	0.57 (17)	0.55 (17)	3.37 (48)	0.82 (27)
Average temporal CV (%)	42	5	10	16	14	22	13
Average spatial CV (%)	42	4	7	10	9	18	7

Table 1.2. Fluorescent components in streamwater DOM as identified through PARAFAC analysis of EEMs.

Component	Excitation Max nm	Emission Max nm	Description	Reference
C1	340	460	Humic-like	C1, Kothawala et al. 2014
C2	300	390	Humic-like	C2, Shutova et al. 2014
C3	290/400	500	High molecular weight fraction of terrestrially derived humic-like material	C3, Kothawala et al. 2014
C4	275	320	Protein-like	C4, Dainard et al. 2015
C5	315	450	Characteristics unknown	C10, Cory and McKnight 2005

Table 1.3. Multiple regression models for predictors of binary response in biolability to N addition. AICc indicates Akaike's information criterion, corrected for small sample size.

Response	Predictors	Model	R ²	AICc
N response	C4, CP	$y = -1.71 * C4 - 0.92 * CP - 1.75$	0.21	45.23
N response	DOC	$y = 1.02 * DOC - 1.24$	0.16	45.38

1.8 Figure legends

Figure 1.1. Locations of sampled streams in northern Alaska. Red points indicate sampling locations with catchment areas indicated. Stream area upstream of the sampling point is shown in dark blue and major roads are indicated by black lines. Permafrost data are from Ferrians (1998).

Figure 1.2. Temporal patterns in DOM and bulk chemistry of streams. Plots display a proxy for groundwater contribution based on the sum of conservative ions (GW), fluorescence index (FI), humification index (HIX), biological index (BIX), freshness index ($\beta:\alpha$), specific ultraviolet absorbance (SUVA), and spectral slope ratio (SR). Principal Component 1 summarizes the five components from PARAFAC analysis as well as FI, HIX, BIX, $\beta:\alpha$, SUVA, and SR. Points are means across all sampled streams with error bars indicating one standard error (see Supplementary Table 1.1 for number of streams sampled on each date). Points with arrows indicate an outlier sampled as overflow from the Kuparuk River. Letters indicate significant differences between sampling events based on Tukey's honestly significance difference, following linear mixed effects models that included site as a random effect. NS indicates not significant.

Figure 1.3. Temporal patterns in DOM composition indicated by components from PARAFAC analysis of EEMs. Fmax represents fluorescence intensity, the fluorescence emission at the wavelength of maximum intensity, and error bars indicate one standard error.

Figure 1.4. DOC lability in streamwater incubated under ambient and nutrient-amended conditions. Significant effects of nutrient addition, as determined through comparisons to the control treatment within each sampling event, are indicated by *.

Figure 1.5. Regression of ambient DOC concentration by total percent loss of DOC during incubations of streamwater. Line represents fitted relationship from a mixed effects model including site as a random effect.

1.9 Figures

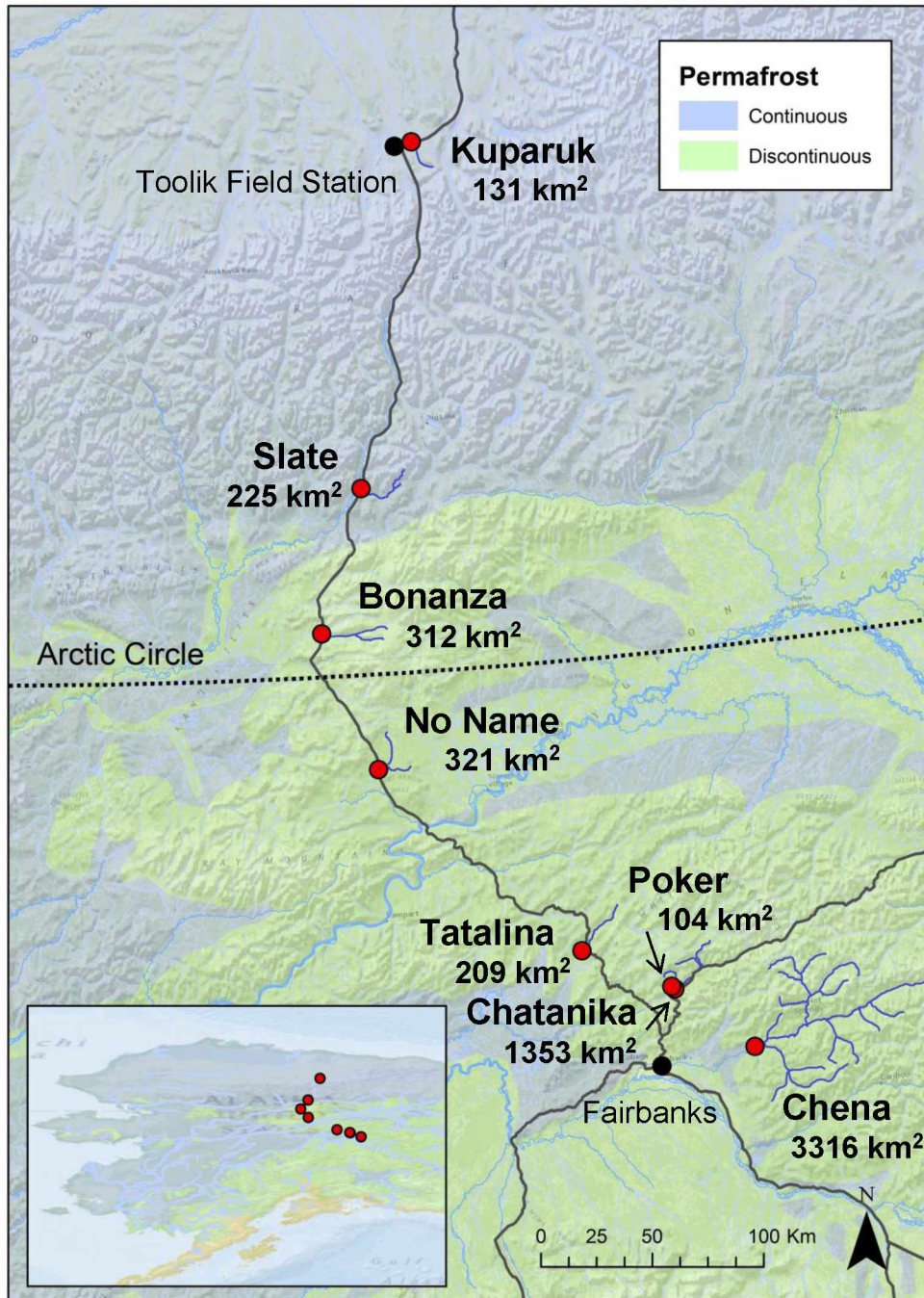


Figure 1.1

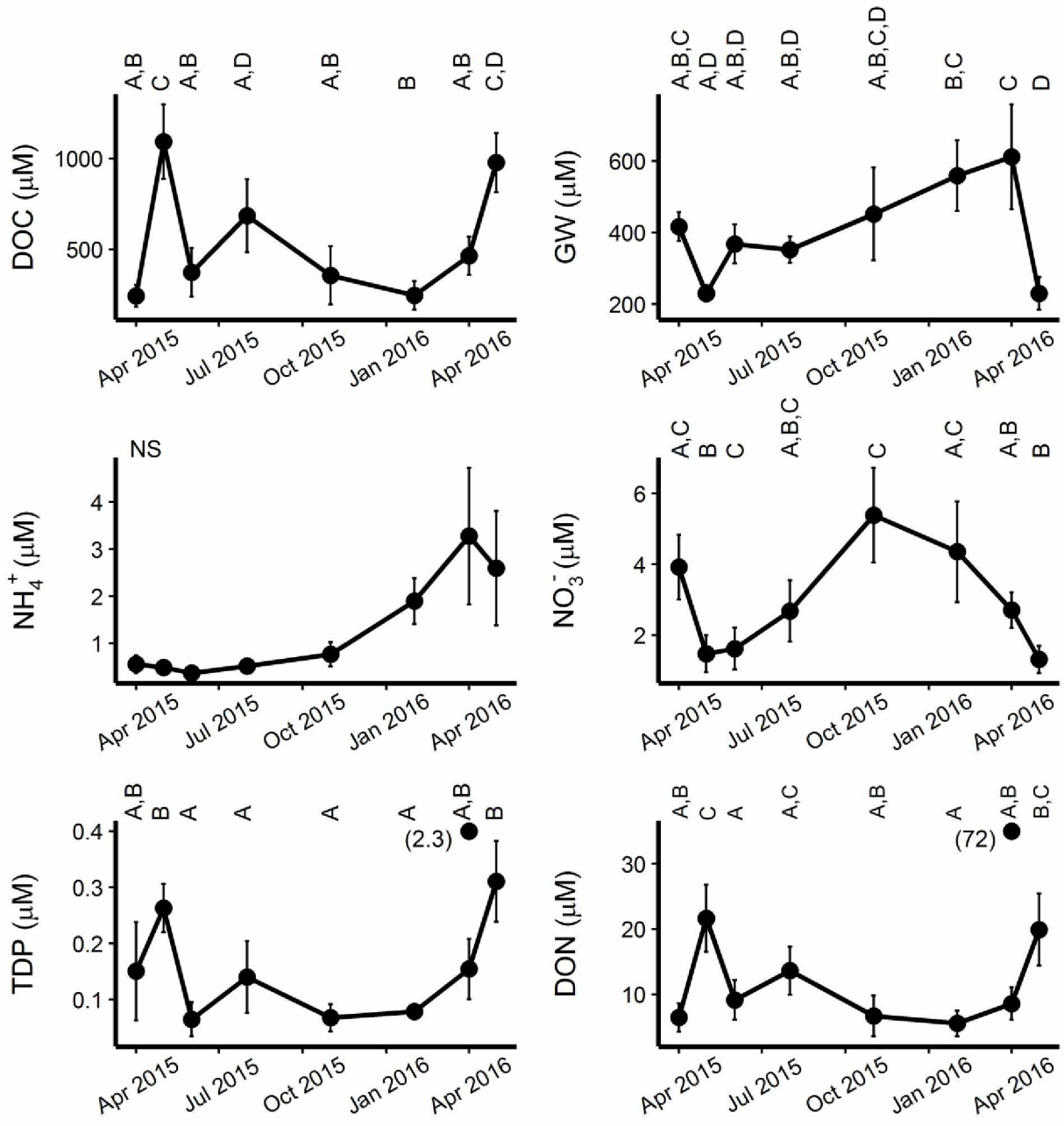


Figure 1.2

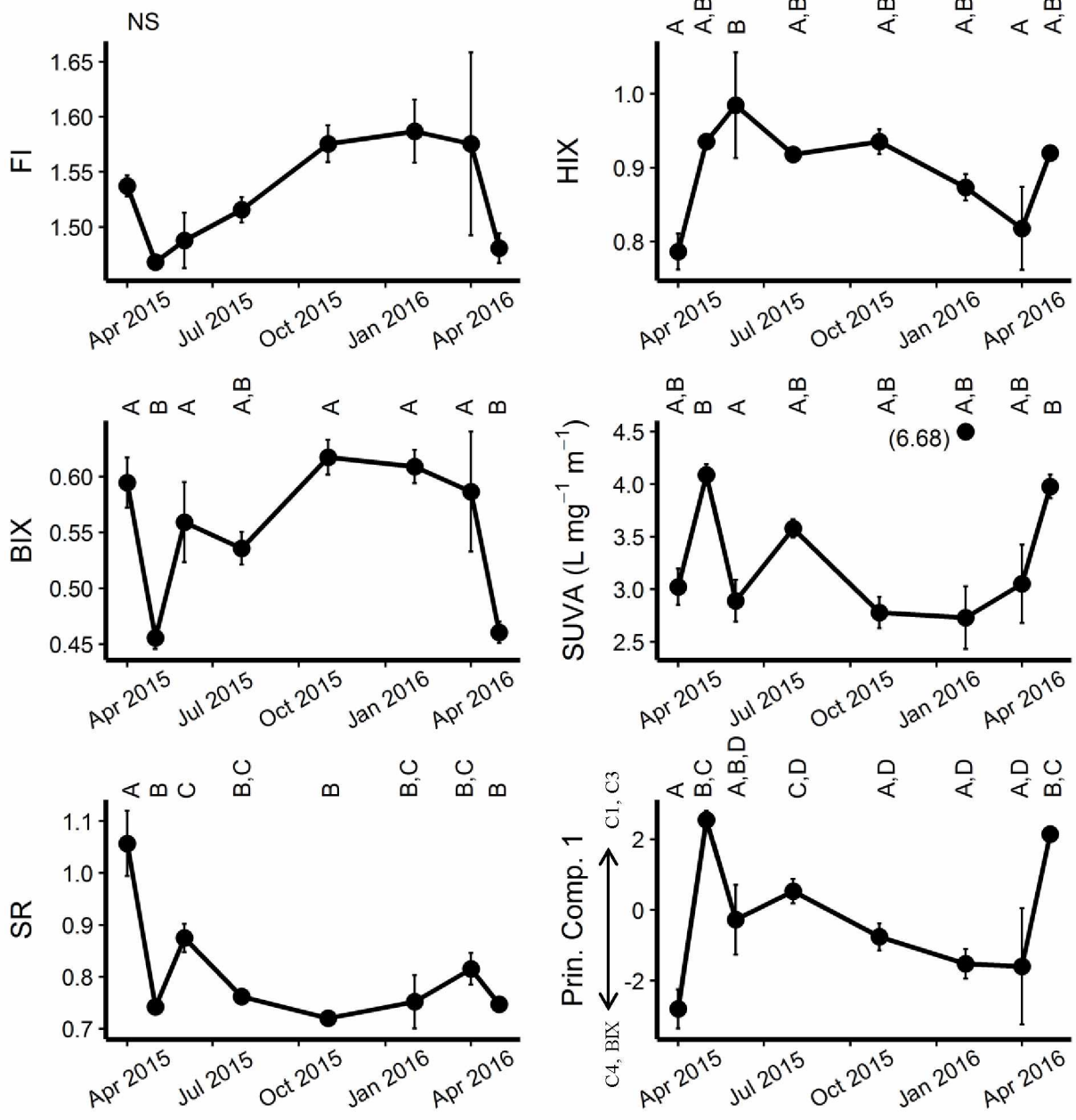


Figure 1.2 continued

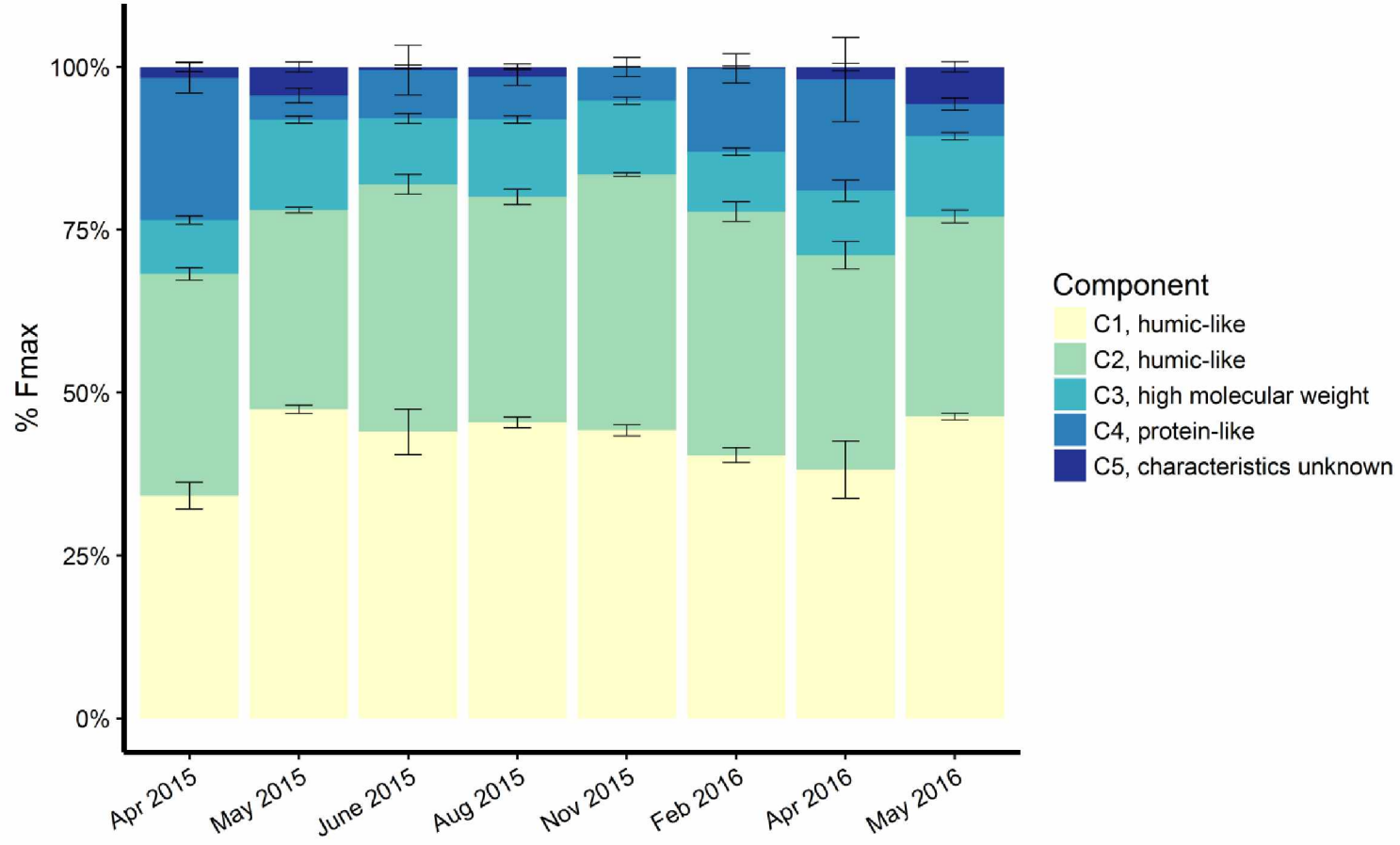


Figure 1.3

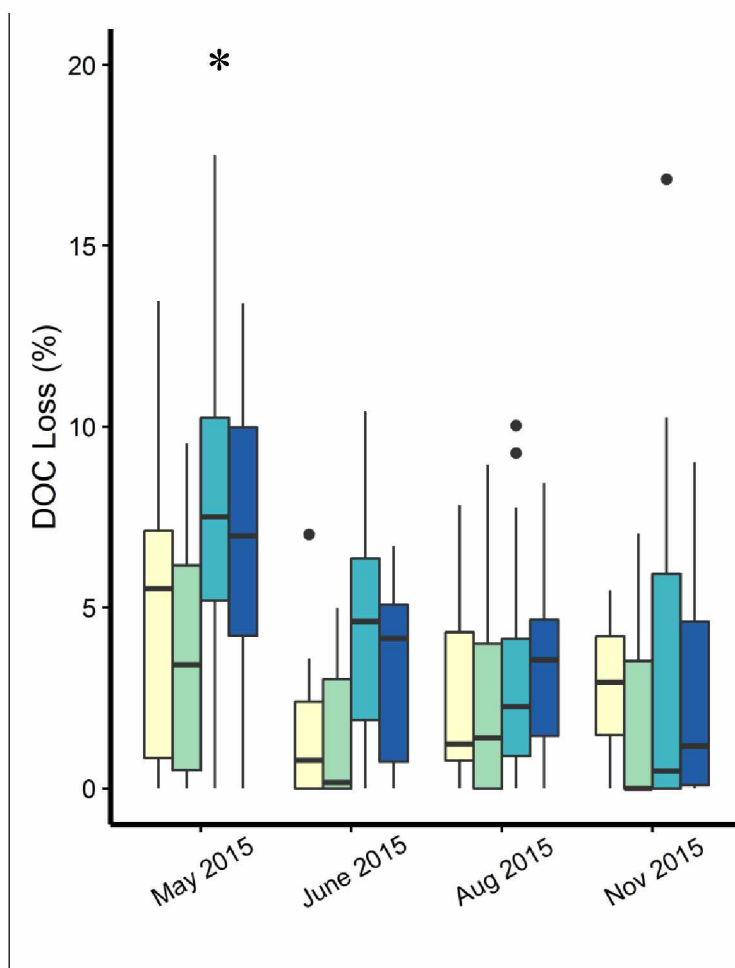
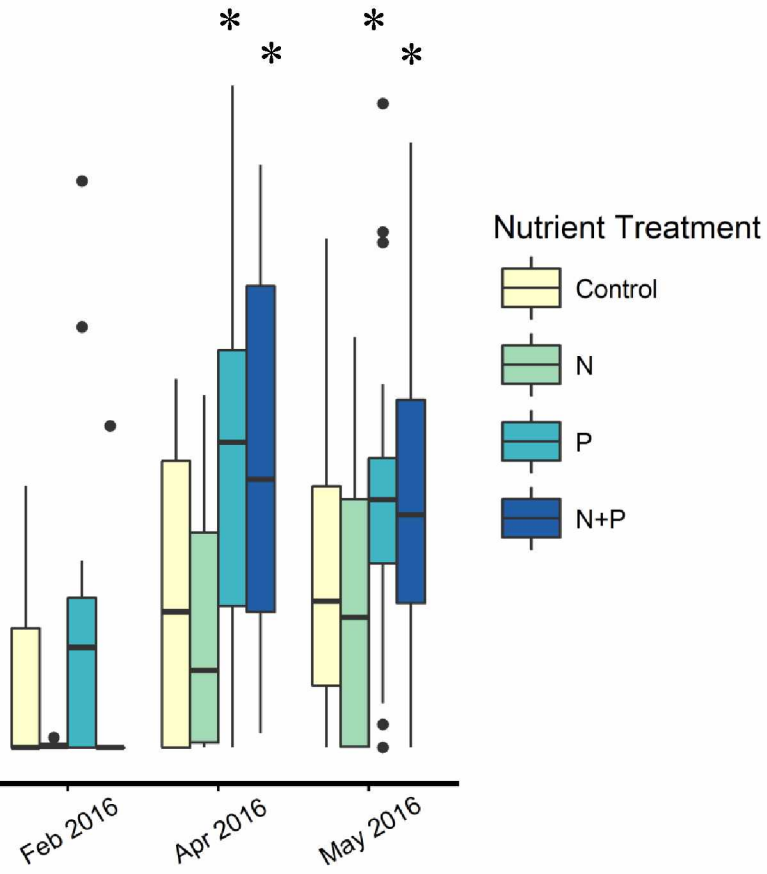


Figure 1.4



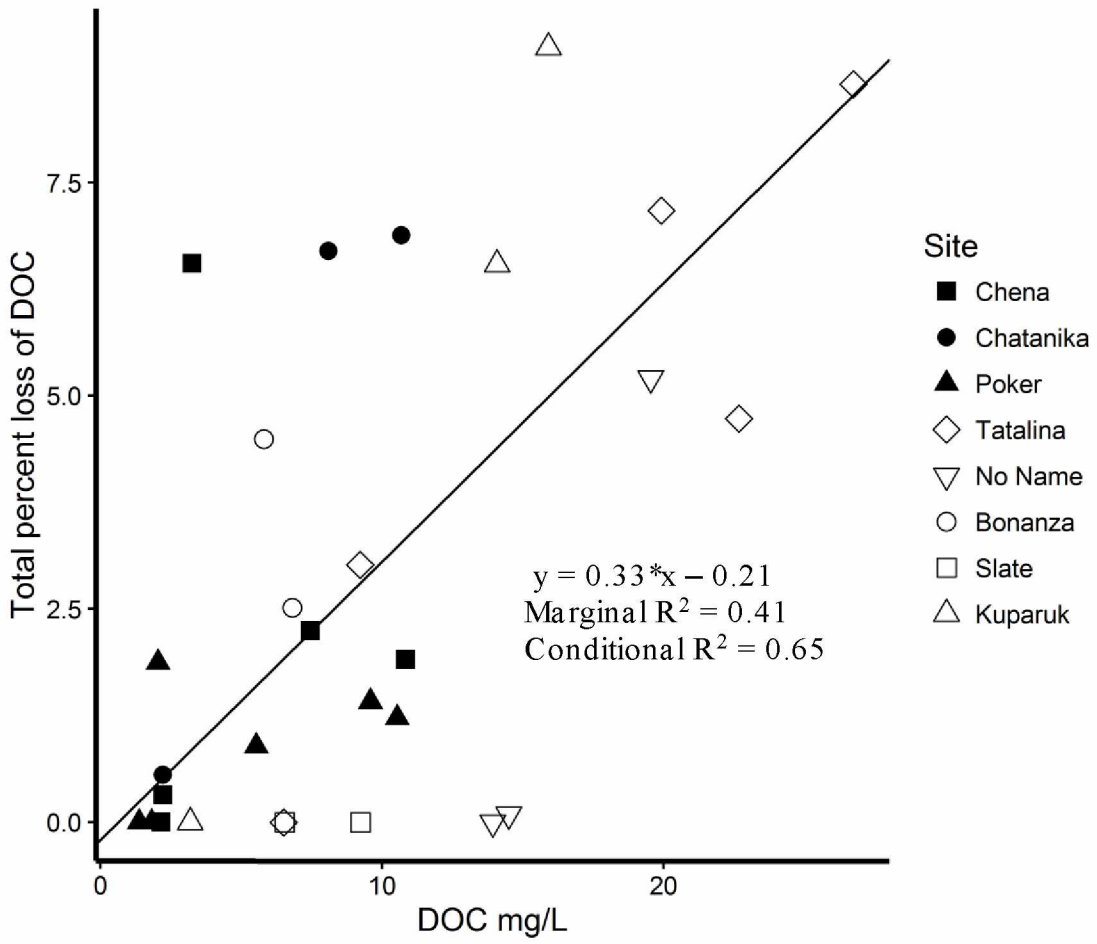


Figure 1.5

1.9 Supplementary information

Supplementary Table 1.1. Sampling dates. Under-ice samples are indicated with * and overflow samples with °.

Site	Sampling dates							
Chena	4/17/2015	5/12/2015	6/16/2015	9/1/2015	11/11/2015	2/8/2016*	4/10/2016	4/26/2016
Chatanika	4/17/2015*	5/12/2015	6/16/2015	9/1/2015	11/10/2015	2/9/2016*	4/12/2016	4/26/2016
Poker	4/17/2015*	5/12/2015	6/16/2015	9/1/2015	11/10/2015	2/9/2016*	4/12/2016*	4/26/2016
Tatalina		5/12/2015	6/16/2015	9/1/2015	11/11/2015*	2/8/2016*	4/10/2016°	5/12/2016
No Name			6/24/2015	8/22/2015				5/13/2016
Bonanza	4/24/2015°	5/15/2015	6/24/2015	8/22/2015				5/13/2016
Slate	4/24/2015°	5/14/2015	6/23/2015	8/21/2015			4/26/2016°	5/12/2016
Kuparuk	4/23/2015°	5/15/2015	6/23/2015	8/21/2015		2/25/2016°	4/26/2016°	5/12/2016

Supplementary Table 1.2. Loadings of optical measures included in the principal component analysis. C1–C5 were determined by PARAFAC analysis of EEMS. Also included are specific ultraviolet absorbance (SUVA), spectral slope ratio (SR), fluorescence index (FI), humification index (HIX), biological index (BIX), and freshness index ($\beta:\alpha$).

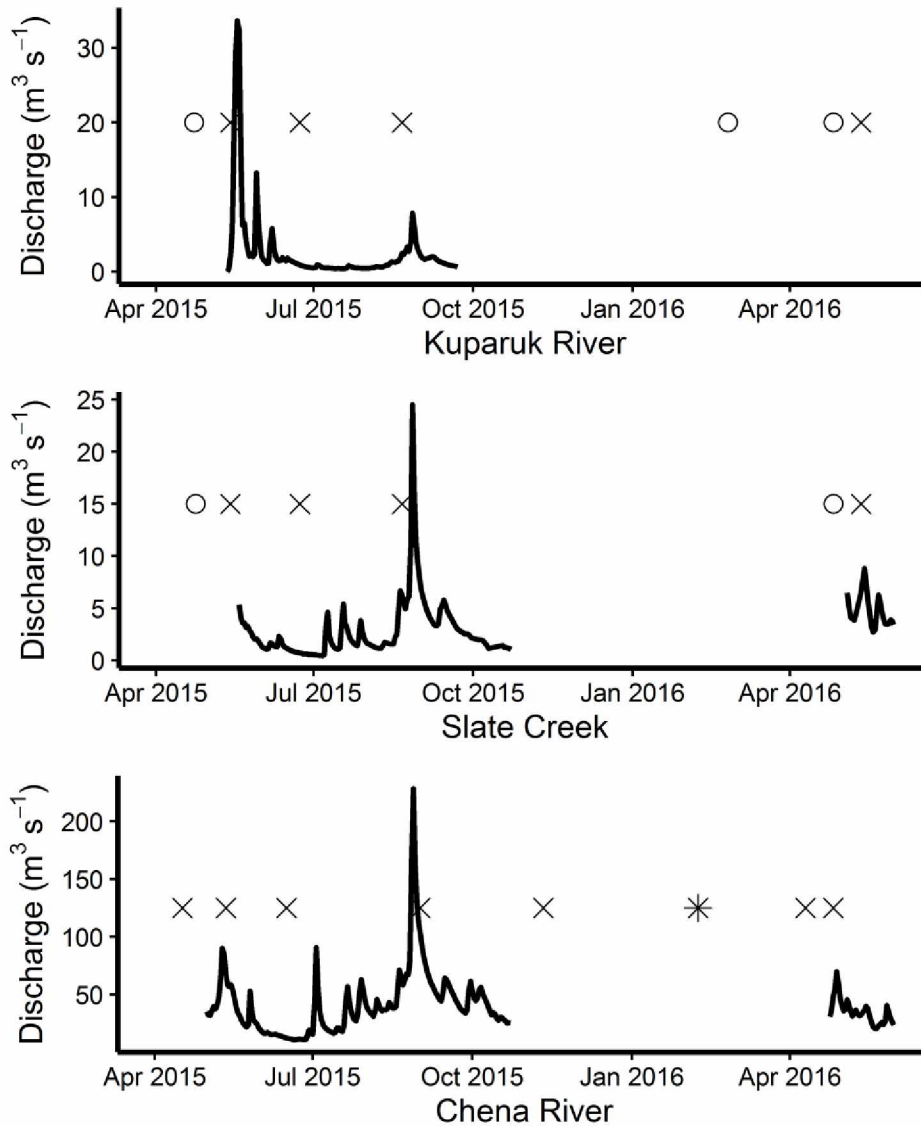
	Component 1 loading
C1	0.371
C2	-0.157
C3	0.362
C4	-0.351
C5	0.195
SUVA	0.236
SR	-0.220
FI	-0.261
HIX	0.308
BIX	-0.377
$\beta:\alpha$	-0.370

Supplementary Table 1.3. Models compared during multi-model inference to select a model to test for an effect of nutrient addition on DOC loss. Percent loss and absolute loss (mg) represent the change in DOC through 21 day incubations of streamwater. The model including the interaction effect was chosen for both percent loss and absolute loss due to the higher R^2 .

Response	Fixed Effects	Random Effects	AIC	Marginal R^2	Conditional R^2
Percent loss	Date	Date, Site	2252	0.11	0.41
Percent loss	Nutrient	Date, Site	2188	0.09	0.55
Percent loss	Date*Nutrient	Date, Site	2210	0.22	0.61
Absolute loss	Date	Date, Site	475	0.2	0.71
Absolute loss	Nutrient	Date, Site	423	0.03	0.82
Absolute loss	Date*Nutrient	Date, Site	436	0.24	0.84

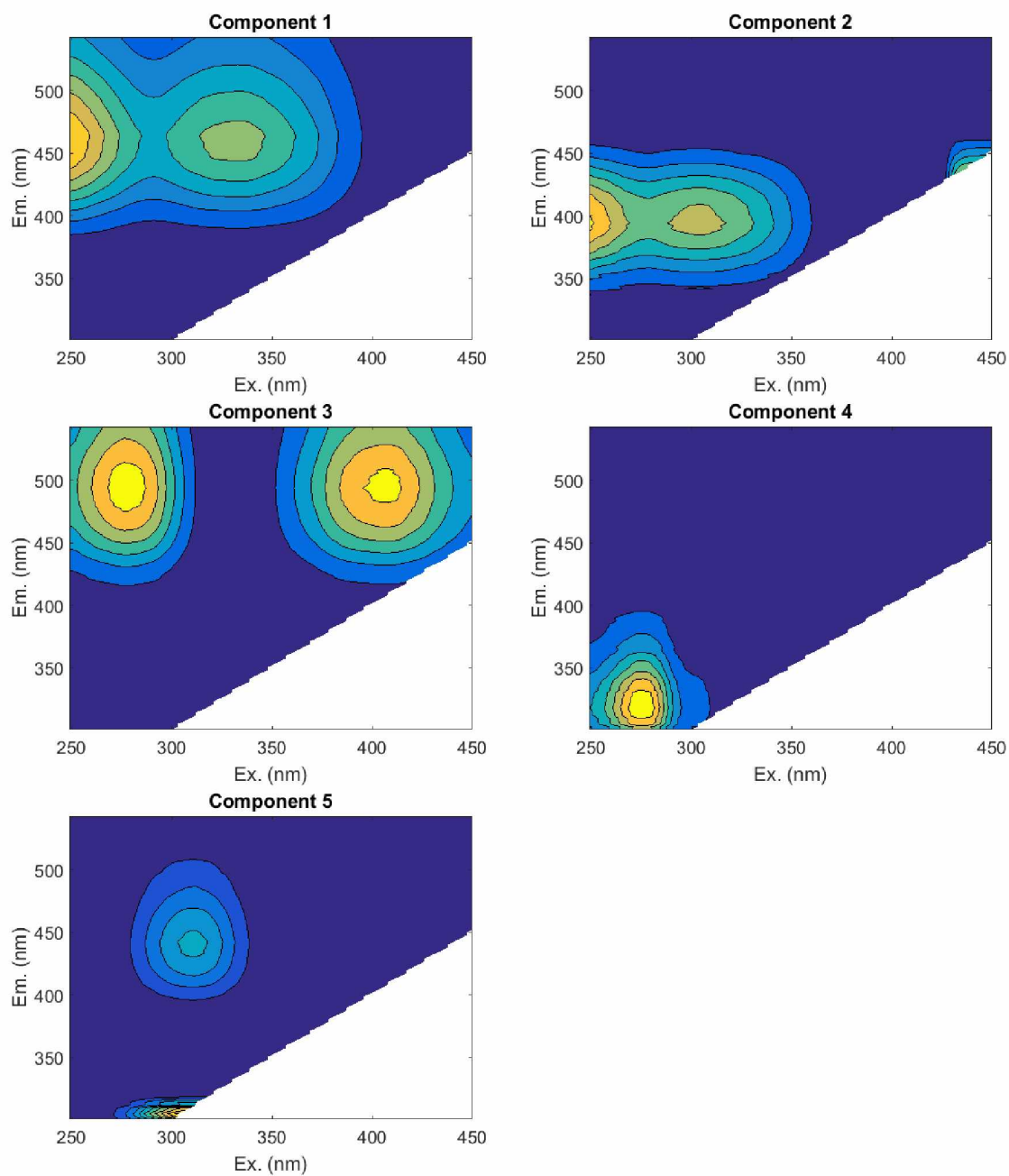
Supplementary Table 1.4. Effect of added nutrients on lability of DOC. Values, with standard errors, represent the difference in DOC loss in response to nutrient addition relative to the control treatment within each sampling event. Bold values indicate a significant effect of added nutrients as determined through Bonferroni corrected multiple comparisons between nutrient treatment and control for each sampling event. The N treatment was not significant during any month.

	Percent loss		Absolute loss (mg/L)	
	P	N+P	P	N+P
May 2015 (n=7)	3.8 ± 1.1	3.0 ± 1.1	0.4 ± 0.1	0.4 ± 0.1
June (n=6)	2.9 ± 1.3	2.1 ± 1.3	0.3 ± 0.1	0.2 ± 0.1
August (n=8)	1.5 ± 1.2	1.6 ± 1.2	0.2 ± 0.1	0.2 ± 0.1
November (n=4)	4.6 ± 2.3	3.5 ± 2.3	0.2 ± 0.1	0.2 ± 0.1
February 2016 (n=5)	0.9 ± 1.5	-0.2 ± 1.9	0.0 ± 0.1	0.0 ± 0.2
April (n=6)	4.1 ± 1.4	4.7 ± 1.4	0.3 ± 0.1	0.3 ± 0.1
May (n=8)	3.7 ± 1.1	5.5 ± 1.1	0.4 ± 0.1	0.6 ± 0.1

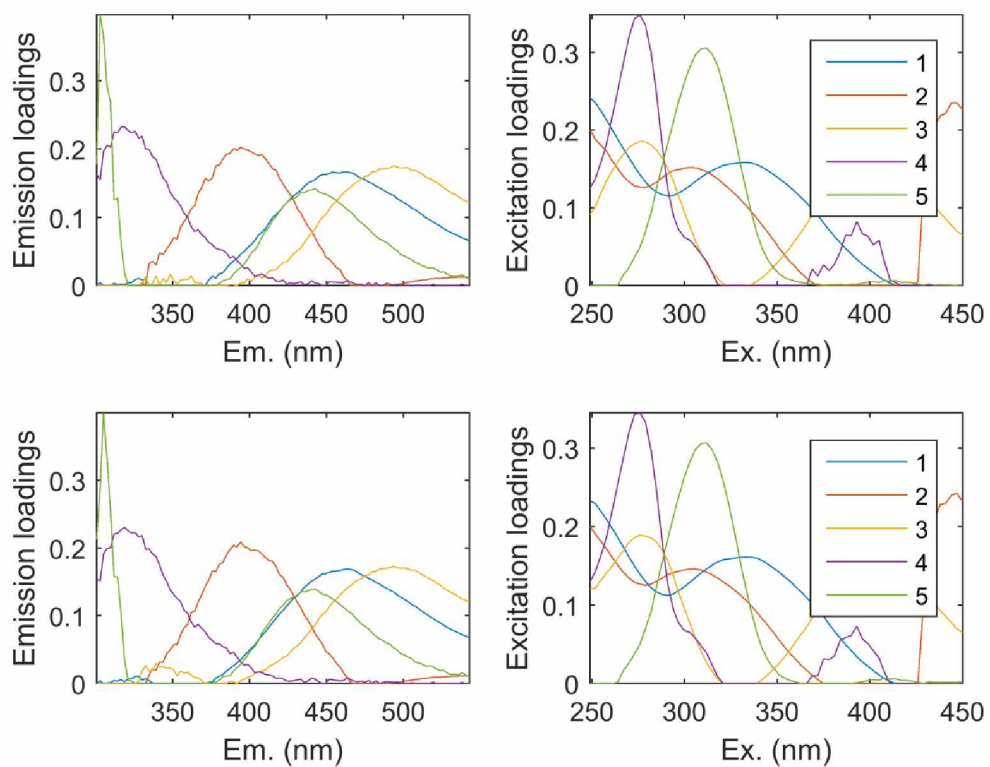


Supplementary Figure 1.1. Hydrograph of gauged streams within the set of sampled streams.

Sampling dates are indicated by × for open water samples, * for under-ice samples, and ○ for overflow samples. Data for the Chena River (15493000) and Slate Creek (15564879) are from the US Geological Survey (USGS) gauging stations. Data for the Kuparuk River are collected by the Water and Environmental Research Center at the University of Alaska Fairbanks (Arp and Stuefer 2016).



Supplementary Figure 1.2. Excitation-emission matrices of components identified during PARAFAC analysis.



Supplementary Figure 1.3. Split-half validation of the five component PARAFAC model. The same five components were resolved on both halves of the dataset, validating the model as a good fit to the data. The top two panels show emission and excitation spectra for the five components resolved in the first half of the dataset and the bottom two panels for the second half of the dataset.

Chapter 2 Influence of molecular composition and nutrient availability on uptake of dissolved organic matter in boreal streams¹

2.1 Abstract

Uptake of carbon (C), either by physical mechanisms or the biological process of decomposition, is a key driver of the transformation and storage of C and nutrients within ecosystems. Both the molecular composition and nutrient content of organic matter influence the rate at which it is retained in streams, but the relative influence of these characteristics remains unclear. We estimated the effects of nutrient content and molecular composition of dissolved organic C (DOC) on uptake in boreal streams by measuring *in situ* rates of C retention following introduction of leachates derived from of alder (*Alnus incana* ssp. *temuifolia*), poplar (*Populus balsamifera*), and white spruce (*Picea glauca*) trees subject to long-term fertilization with nitrogen (N) or phosphorus (P). Leachate C:N varied approximately two-fold, and C:P varied nearly 20-fold across species and nutrient treatments. Uptake of DOC was greatest for leachates derived from trees that had been fertilized with P, a finding consistent with P-limitation of decomposition and/or preferential sorption of P-containing molecules. Optical measures indicated that leachates derived from the three tree species varied in molecular composition, but uptake of DOC did not differ across species, suggesting weak constraints on retention imposed by molecular composition relative to nutrient limitation. Observed coupling between P and C cycles highlights the potential for increased P availability to enhance DOC retention in headwater streams.

¹ Mutschlecner, A., J. J. Guerard, J. B. Jones, and T. K. Harms. 2017. Phosphorus constrains uptake of dissolved organic matter in boreal streams. Preparation for submission to Ecosystems.

2.2 Introduction

Retention of organic matter recycles essential elements within ecosystems and regulates the global carbon (C) cycle (Schlesinger and Andrews 2000, Cole et al. 2007). In both terrestrial and aquatic ecosystems, retention is influenced by nutrient availability as well as the molecular composition of the organic matter (Berg and Staaf 1980, Elwood et al. 1981, Qualls and Haines 1992, Mineau et al. 2013). Nutrient availability is changing on a global scale due to processes such as permafrost thaw (Jones et al. 2005, McClelland et al. 2007), nitrogen (N) deposition (Galloway et al. 2008, Mineau et al. 2013), and eutrophication (Conley et al. 2009). Global change also influences the molecular composition of detritus through the effects of atmospheric CO₂ concentration on plant tissue chemistry (Peñuelas et al. 1997, Lindroth et al. 2001), and shifts in plant species composition (Chapin et al. 2000, Johnstone et al. 2010, Fraser et al. 2014). Understanding how these factors regulate retention of DOC is thus critical to projecting changes in the global C cycle, but the relative influences of molecular composition and nutrient content on retention remain unclear.

Nutrients influence DOM retention through both biotic and abiotic mechanisms. In terms of biological retention, nutrient content influences organic matter retention because microbial decomposers require essential nutrients for cellular function (Sinsabaugh et al. 2013). When nutrients are limiting, decomposers shift resource allocation from C acquisition to nutrient acquisition, slowing the retention rate (Sinsabaugh et al. 1997). N content of organic matter typically increases retention of leaf litter in N-limited terrestrial ecosystems (Berg and Staaf 1980, Taylor et al. 1989), and phosphorus (P) availability constrains retention of litter and derived leachates in P-limited terrestrial ecosystems (Hobbie and Vitousek 2000, Wieder et al. 2008). In streams, N-rich, protein-based dissolved compounds readily decompose (Balcarczyk et

al. 2009, Fellman et al. 2009) and co-limitation of retention by N and P in some streams demonstrates the strong constraints of nutrients on decomposers (Mineau et al. 2013). When nutrient limitation is relieved, accelerated retention results in higher rates of CO₂ evasion from streams (Rosemond et al. 2015). Nutrients also influence physical retention of DOM. P is highly sorptive to streambed sediments (Reddy et al. 1999, Schade et al. 2016) and N-rich DOM may be preferably sorbed to mineral surfaces in rivers (Aufdenkampe et al. 2001).

The molecular composition of organic matter may present an additional constraint on physical retention because hydrophobic compounds in DOM are preferentially sorbed to soils (Kaiser et al. 1996, Ussiri and Johnson 2004). In terms of biological retention, this pattern is reversed, as hydrophilic components of DOM are more susceptible to decomposition (Kiikkilä et al. 2013). Molecular composition may constrain decomposition because when molecules are larger and aromatic, microbial decomposers must expend more energy to produce extracellular enzymes that break diverse and high-energy bonds, slowing the rate of decomposition (Sinsabaugh et al. 2013). Among bacteria, the genetic traits linked to the use of high-molecular weight molecules are less common than those linked to the use of low-molecular weight molecules (Logue et al. 2016), indicating that C complexity limits decomposition. Lignin, a phenolic biopolymer with many aromatic groups, represents a relatively complex molecular composition that tends to resist decomposition (Meentemeyer 1978), whereas monomeric carbohydrates decompose quickly (Bernhardt and McDowell 2008). Leachates from different plant species vary in content of lignin, cellulose, and bound proteins, which influences their susceptibility to retention (Cleveland et al. 2004). Due in part to variation in molecular composition, species-based variation in leaf litter chemistry explains more variation in retention than does temperature across biomes globally (Cornwell et al. 2008).

The relative effects of nutrients and molecular composition on retention of C remain unresolved, in part because nutrient content and molecular composition of organic matter are often tightly linked. Many N-rich substances, such as amino acids, also have a relatively simple molecular composition, making it difficult to determine which is the proximate influence on the rate of retention (Brookshire et al. 2005, Lutz et al. 2011). Molecular composition of *in situ* DOM, as characterized via optical properties, influences uptake of N and P in streams (Coble et al. 2016). In terrestrial studies, the ratio of lignin:N is often the best predictor of decomposition (Melillo et al. 1982, Prescott et al. 2004). Separating the relative influence of lignin and N has proven difficult because the roles of lignin and N may change over time during decomposition (Berg and Staaf 1980), and the influence of N may depend on the initial lignin concentration (Taylor et al. 1989). High ratios of lignin:P can limit leaf litter retention in streams (Hladysz et al. 2009). Separating the effects of molecular composition and nutrient content on retention is critical to predicting whether C is stored in ecosystems or respired as CO₂, and how global change will alter the fates of C.

We performed experiments to determine the relative influence of molecular composition and nutrient content of organic matter on the retention of dissolved organic matter (DOM) in boreal streams. DOM composition and reactivity vary depending on its source materials (McKnight et al. 2001, Guerard et al. 2009). Terrestrially derived DOM provides the primary energy source in many stream ecosystems (Fisher and Likens 1973, Neff and Asner 2001) and streams play an active role in the processing of these C inputs (Cole et al. 2007). Retention in streams can offset terrestrial storage in C budgets (Butman et al. 2015), and contribute to hot spots of CO₂ evasion within the landscape (Jones et al. 2003, Raymond et al. 2013). We hypothesized that the rate of uptake of DOC is influenced by molecular composition, which

determines its susceptibility to microbial attack and preferential sorption. Alternatively, we hypothesized that the nutrient content of DOM constrains uptake when microbial decomposers are nutrient-limited. Further, preferential sorption of N or P may contribute to more rapid retention of N or P containing molecules. To test these hypotheses, we measured uptake of DOM derived from leaf leachates from three plant species that were known to vary in molecular composition due to inherent species differences (Van Cleve et al. 1993) and in nutrient content due to growth under long-term fertilization with N or P (Ruess et al. 2013). We compared molecular composition using differences derived from the optical properties of DOM, such as aromaticity, molecular weight, and fluorescence. The novelty of this approach is the factorial study design manipulating *both* molecular composition and nutrient content.

2.3 Methods

2.3.1 Site description

This study was conducted at Caribou-Poker Creeks Research Watershed (65.15°N, 147.50°W), which is located approximately 50 km northeast of Fairbanks, Alaska. The climate is continental, with warm summers (mean = 15.1°C in July), cold winters (mean = - 21°C in January), and low precipitation (460 mm, of which approximately 38% falls as snow; 2010 - 2016; Bonanza Creek LTER Climate Database 2016). The vegetation is typical of interior Alaska, including hardwood forests (*Betula papyrifera*, *Populus tremuloides*) on south-facing slopes, and black spruce (*Picea mariana*) on north-facing slopes. Experiments were carried out in five first-order streams: C1, C2, C3, C4, and P6, with watershed areas of 6.7, 5.2, 5.7, 10.4, and 7 km² respectively (Table 2.1). Stream temperature ranged 2–5°C.

2.3.2 DOM uptake experiments

To determine whether DOM retention is driven by nutrient content or molecular composition, we measured C uptake in streams following addition of DOM derived from leaf leachates produced from three species of trees subject to long-term nutrient fertilization. Leaves were collected from the Bonanza Creek Experimental Forest (64.82°N, 147.87°W), located approximately 35 km southwest of Fairbanks, AK. The Bonanza Creek Long-Term Ecological Research program maintains plots (20x20 m) of alder (*Alnus incana* ssp. *temuifolia*), poplar (*Populus balsamifera*), and white spruce (*Picea glauca*) fertilized with N (100 kg N · ha⁻¹ · yr⁻¹ as NH₄NO₃) or P (80 kg P · ha⁻¹ · yr⁻¹ as P₂O₅) as well as unfertilized control plots (30x30 m). Fertilization has occurred annually since 1997 for N and since 2004 for P (Ruess et al. 2013). Leaves were harvested in September 2013 and 2014 from nine plots: alder control (AC), alder N (AN), alder P (AP), poplar control (PC), poplar N (PN), poplar P (PP), spruce control (SC), spruce N (SN), and spruce P (SP) (Supplementary Table 2.1). Leaves were clipped from branches after senescence had begun, so that resorption would have occurred and chemical composition of leaves would include leachable compounds that could be present in streams. Freshly fallen leaves were also collected from the forest floor. Leaves were dried at 60°C to constant mass and stored in paper bags.

Leachates were prepared by soaking crushed leaves in deionized water. Alder and poplar leaves were crushed by hand to roughly 1-cm fragments and spruce needles were crushed to the same size using a hammer. Leaves were placed in nylon bags (approximately 200 μm mesh size), mixed with deionized water (18 MΩ) at a ratio of 100 g leaves to 1 L water, and continuously stirred at 4°C for 36–48 hours. Resulting leachates were filtered through 0.7 μm glass fiber filters (Whatman GF/F) and frozen until use (≤ 7 days).

We measured uptake of dissolved organic C (DOC) following addition of leachates to streams in July 2015. The nine types of leachates were added to each of the five streams, resulting in 45 total experiments. Experiments at each stream were performed over one to two days to maintain constant discharge and antecedent conditions across experiments within each stream, and the leachates were added to each stream in a random order. Reach length at each stream was chosen such that travel time was approximately 30 minutes. We estimated travel time on the day prior to leachate additions by releasing a 300-g slug of sodium chloride (NaCl) and monitoring the resulting change in conductivity.

We measured retention of DOC in streams following addition of a slug of each leachate. Prior to the start of experiments, we collected three water samples along the study reach for analysis of ambient stream chemistry. We also measured stream width at 15 transects along the reach. Discharge was measured by NaCl dilution gauging at both the top and bottom of the study reach prior to the first and following the last experiment each day. To measure C uptake, we added a slug containing NaCl as a conservative tracer and one of the nine leachate types to the top of the reach and collected samples through the resulting breakthrough curve at the bottom of the study reach. The mass of NaCl added was adjusted to achieve a target concentration of 10 mg Cl⁻/L above background concentration at the peak of the breakthrough curve, and the volume of leachate in each slug was adjusted to yield an increase of 1.5 mg DOC/L above background concentration. The target concentration of DOC was selected so that added DOC could be reliably detected above background without elevating DOC concentration such that reaction kinetics would differ significantly from ambient conditions. Arrival of the tracer was monitored using a conductivity probe at the downstream station, and 25 water samples were taken throughout the breakthrough curve. Water samples were filtered in the field (0.7 μm, Whatman

GF/F), placed on ice, and frozen until analysis. The next leachate was added after specific conductivity declined to a stable background value.

2.3.3 Chemical analysis and fluorescence

Samples from the uptake experiments were analyzed for concentration of Cl^- and DOC. Samples of ambient stream water as well as diluted aliquots of each leachate were analyzed for DOC, total dissolved N (TDN), ammonium (NH_4^+), nitrate (NO_3^-), and total dissolved P (TDP). Chloride and NO_3^- concentrations were measured on an ion chromatograph (Dionex ICS 2100, AS18 column, Thermo Fisher Scientific) with limits of quantification (LOQ) of 0.03 mg Cl^-/L and 0.5 $\mu\text{g NO}_3^- \text{-N/L}$. DOC concentration was measured as non-purgeable organic C by non-dispersive, infrared gas analysis following combustion on a total organic C analyzer (TOC-L CPH, Shimadzu Scientific Instruments, LOQ = 0.1 mg C/L) connected to a TN module with detection of N by chemiluminescence (TNM-L, LOQ = 0.02 mg N/L). NH_4^+ was measured by automated colorimetry (Smartchem 170, Westco Scientific Instruments, LOQ = 0.01 mg N/L) using the phenol hypochlorite method (Solórzano 1969). TDP was measured following persulfate digestion using the molybdate blue method (Murphy and Riley 1962) on a spectrophotometer with a 5-cm cell (Shimadzu UVmini 1240, Shimadzu Scientific Instruments, LOQ = 0.6 $\mu\text{g P/L}$). Nitrite is not detectable in the study streams and concentration of dissolved organic N (DON) was therefore determined by difference of TDN and $\text{NO}_3^- \text{-N} + \text{NH}_4^+ \text{-N}$. C and N content of dry leaf tissue was determined using an elemental analyzer (CHNS-O, Costech Analytical Technologies Inc., LOQ = 0.03 mg C and 0.01 mg N). P content of dry leaf tissue was measured as for water samples described above. When measured values were below the LOQ, one half of LOQ was used in data analysis.

To characterize the molecular composition of the leachates, we measured absorbance of UV and visible light as well as fluorescence spectra on filtered samples (0.45 μm , Pall GN-6) using a fluorometer (Jobin-Yvon Horiba Aqualog-800-C, Horiba Instruments) with a 1-cm quartz cuvette (Firefly Scientific). Excitation-emission matrices (EEMs) were collected over an excitation range of 240–600 nm every 3 nm and an emission range of 247–847 nm every 2.33 nm with an integration time of 0.1 seconds and a medium gain. Data were instrument-corrected, blank-subtracted, Raman-normalized, and corrected for inner-filter effects. The dataset included a total of 229 samples including the 45 leachates samples as well as 184 stream samples from boreal and arctic Alaska (Mutschlecner et al. n.d.). Parallel factor analysis (PARAFAC) was applied to resolve fluorescing components using the DOMFluor toolbox (version 1-7, Stedmon and Bro 2008) in Matlab (version R2015b, MathWorks). PARAFAC assumes statistically independent components with non-negativity constraints. A model including five components was best supported by the data. Model validation was conducted following the approach of Stedmon et al. (2003), and included split-half analysis, which compares PARAFAC models made on two halves of the data, inspection of residuals, and random initialization, which performs 10 iterations with different starting estimates to confirm that the least squares result is not a local minimum. To identify the chemical attributes of the components, excitation and emission spectra were compared with published data using the OpenFluor database (Supplementary Table 2.2). PARAFAC components 1-4 were matched to previously described components in the OpenFluor database using a 0.95 Tucker convergence coefficient (Murphy et al. 2014). Component 5 appeared to consist of two components, but the model was unable to resolve them separately. Component 5 did not match those present in OpenFluor, but visually matched previously reported excitation and emission curves (Supplementary Table 2.2, Cory and

McKnight 2005). From the UV-vis scans, we calculated specific ultraviolet absorbance at 254 nm (SUVA), which is associated with greater aromatic content, by dividing the absorbance at 254 nm by DOC concentration (Weishaar et al. 2003), and spectral slope ratio (SR), which is negatively correlated with DOM molecular weight, as the ratio of the slope from 275 to 295 nm to the slope from 350 to 400nm with log linear fits (Helms et al. 2008).

2.3.4 DOC uptake

Biogeochemical retention of DOC in the streams, which can be attributed to biological uptake, photochemical reactions, and sorption, was calculated by mass balance using the method described by Covino et al. (2010). First, the mass of tracer recovered (T_{MR}) was calculated for both the conservative (Cl^-) and reactive (DOC) tracers by multiplying the time-integrated tracer concentration (T_C) by discharge (Q):

$$T_{MR} = Q \int_0^t T_C(t) dt$$

Next, total tracer retention (TR) was calculated as the difference between the mass of tracer added and the mass of tracer recovered (T_{MR}):

$$TR = \text{Mass DOC added} - T_{MR}(\text{DOC})$$

Chloride was assumed to be biologically and chemically inactive in the stream, so the proportion of chloride not recovered represents physical retention in slower flowpaths. This proportion ($T_{MR}(Cl\%)$) was applied to the mass of the added reactive tracer (DOC) to account for hydrologic storage of C (HS):

$$HS = (100 - T_{MR}(Cl\%)) * \text{Mass of DOC added}$$

Finally, biogeochemical retention ($R, \%$) was calculated as the difference between total tracer retention and hydrologic retention:

$$R = TR - HS$$

We calculated metrics of nutrient spiraling following the Stream Solute Workshop (1990) to allow comparison with previous estimates of DOC uptake. These metrics describe the simultaneous transport and retention of solutes as they travel downstream. Uptake length (S_w , m), which describes the mean distance a molecule of C travels in dissolved form in the water column before being taken up, was calculated as the negative inverse of the slope of a regression between the natural logarithm of the DOC:Cl⁻ ratios of the injectate and tracer masses recovered (background-corrected) against the reach length (D). Uptake velocity (V_f , mm/min), the vertical rate at which DOC moved from the water column to the benthos due to biological demand, was calculated as stream discharge divided by the uptake length times average stream width. Finally, the areal uptake rate (U , mg m⁻² day⁻¹) was calculated by multiplying V_f by the geometric mean of conservative (based on Cl⁻ recovery) and observed background-corrected DOC concentrations throughout the breakthrough curve.

2.3.5 Statistical methods

We examined the effects of nutrient treatment (control, N, and P) and plant species (alder, poplar, and spruce) on DOC retention in streams using linear mixed-effects models in R (version 3.3.1, R Core Team 2016), with the lme4 package (Bates et al. 2015). All models included a random intercept to account for variation in DOC uptake among the five streams. We contrasted models including the effects of nutrient, plant species, and both effects plus their interaction using Akaike's information criterion corrected for small sample size (AICc) and

likelihood ratio tests as metrics to select a final model that was most parsimonious and best supported by the data. Residuals were visually inspected for normality and constant variance using normal probability plots and plots of predicted vs. observed values. We estimated marginal and conditional R^2 as metrics of model fit using the MuMIn package (Barton 2016). Finally, we applied post hoc tests to determine differences in DOC uptake among species from unfertilized control plots, and between nutrient treatments within each species using the `glht` function in the `multcomp` package (Hothorn et al. 2008), and adjusting p-values for multiple comparisons using the single-step method. A similar statistical approach was used to determine differences in chemical attributes of the leachates.

We conducted a principal components analysis using Components 1, 2, 4 and 5 from PARAFAC analysis as well as SUVA and SR to summarize differences in molecular composition of leachates across species and fertilization treatments (function *princomp*). Principal component 1 explained 75% of variance and was used in models as described above to test for species and nutrient effects on leachate chemistry.

2.4 Results

2.4.1 Litter and leachate chemistry

Leaf tissue chemistry varied by species and fertilization treatment, and the relative abundance of C, N, and P in solid tissues differed from that of leachates (Table 2.2). C:N of leaves was significantly lower for alder grown in control plots than for poplar and spruce control plots, but was similar to alder leaves grown under fertilization (Table 2.2). In contrast, poplar leaves and spruce needles were more N-rich when fertilized with N. C:P of leaves was significantly different between species as well as between nutrient treatments within a species

with significant P-enrichment of P-fertilized trees relative to the control and N treatments (Table 2.2). The nine leachates varied in nutrient concentrations but not in concentration of DOC (Table 2.2). DON concentration for PN and SN leachates was approximately twice that of leachates from control and P-fertilized leaves of the same species whereas leachates from alder had similar concentrations of DON regardless of fertilization (Table 2.2). TDP concentration was approximately 7 times, 20 times, and 2 times higher for leachates from P-fertilized leaves compared to controls for alder, poplar, and spruce, respectively, and post hoc tests revealed that these differences were significant (Table 2.2). Soluble C, the percent of total leaf C that was recovered as DOC in leachates, varied little across species and fertilization treatments (Table 2.2). Soluble N was lowest for alder compared to the other species, and soluble P was lowest for spruce (Table 2.2).

The leachates from different species varied in molecular composition based on optical measures (Figure 2.1). These measures only pertain to optically active fractions of the DOM pool, but they revealed strong species-based differences. Spruce leachates had the highest average molecular mass, as indicated by SR and the highest aromaticity, as indicated by SUVA (Supplementary Table 2.3), though it should be noted that absorbance spectra for spruce leachates indicated a peak near 275 nm rather than a monotonic trend. PARAFAC modeling of EEMs distinguished five significant components, and the relative loadings of these components varied by species (Figure 2.1). Through comparisons with OpenFluor, Component 1 (Kothawala et al. 2014) and Component 2 (Shutova et al. 2014) were identified as humic-like (Table 2.2). Component 3 was present in stream water samples included in the PARAFAC model, but not in leachates, and is therefore not reported here. Component 4 was identified as protein-like (Dainard et al. 2015) and Component 5 was visually matched with Component 10 from Cory and

McKnight (2005), which is uncharacterized. Although the fluorescence signature of Component 4 matched that of protein-like molecules, it is possible that Component 4 may represent small aromatic molecules that fluoresce similarly to proteins. Component 4 and Component 5 differed most strongly between species with spruce leachates having the greatest relative loading of Component 4 and the lowest relative loading of Component 5 (Figure 2.1). The first component of the principal components analysis conducted on the four PARAFAC components as well as SUVA and SR was positively related to SR and Component 5, and negatively related to Component 4 (protein-like) from the PARAFAC analysis (Supplementary Table 2.4). Post hoc tests revealed that the metrics of molecular composition separated by this principal component differed by species, with pronounced differences between alder, poplar, and spruce, but not by nutrient treatment within a species (Figure 2.1).

2.4.2 Stream characteristics and C retention

The five headwater streams had similar chemical and hydrologic characteristics. In the streams, background DOC concentration ranged from 1.06 – 3.5 mg/L (Table 2.1). Background nutrient concentrations varied little between sites. Stream discharge varied between sites, ranging between 6.4 and 39 L/s. Chloride was used as a conservative tracer to account for physical retention of water and solutes along flowpaths of greater residence time than the duration of each experiment. Recovery of Cl^- ranged from 40–92% of the mass added across all sites. Stream C3 demonstrated the lowest average percent recovery (51%), possibly due to a disproportionately long study reach length relative to discharge.

Retention of DOC ranged from 20–52% of the DOC added as leachate (Figure 2.2). Peak DOC concentration during the leachate addition was 1.09 mg DOC/L above background, on

average (range: 0.42 – 2.27 mg/L). A mixed-effects model including the effects of nutrients and species and their interaction was selected to best explain variation in DOC uptake. AICc values (300.2) for the interaction model, 304.8 and 301.7 for models including only species or nutrient effects) indicated a significant effect of nutrients. The model including the interaction term was selected for further analysis, and these fixed effects explained 25% of the variance in uptake rate (marginal R^2), with fixed and random effects explaining a total of 64% of the variance (conditional R^2). Post hoc tests indicated that retention of DOC was significantly greater for AP relative to AN, and for PP relative to PC, but there was no difference among nutrient treatments for spruce (Figure 2.2). Further, retention of DOC did not differ among tree species grown in control plots (Figure 2.2).

2.5 Discussion

By separating the effects of molecular composition (contributed by species) from nutrient content (manipulating leaf N and P content within species), we found that nutrient content, particularly P, was the primary influence on retention of DOC in boreal headwater streams. Elevated retention of DOC following addition of leachates with the highest P concentrations supports our hypothesis that nutrient content constrains uptake. This conclusion is supported by the lack of an effect of P on DOC uptake from spruce leachates, in which fertilization yielded only a doubling of P-content relative to control (relative to a several-fold difference for P-fertilized alder and poplar compared to control). Leachates from alder, poplar, and spruce differed in molecular composition, as demonstrated by differences in optical measures of molecular compositions, but similar rates of DOC retention across leachates derived from the

three species grown under unfertilized conditions suggested that molecular composition was not a driver of uptake.

Enhanced DOC uptake for leachates with a higher P content suggests two possible mechanisms of retention. P is highly sorptive (Reddy et al. 1999), and greater DOC uptake for leachates with a higher P content could be due to preferential sorption of P-containing molecules. Alternately, this pattern could be attributed to heterotrophic demand for P-containing molecules due to P limitation. Average ambient N:P at our study streams was 546:1, over 30 times the Redfield ratio of 16:1 (Redfield 1958), evidence for P limitation. P limitation has been documented in other high-latitude rivers, as revealed by autotrophic and heterotrophic responses to P fertilization of the Kuparuk River in arctic Alaska (Peterson et al. 1993), and more rapid uptake of P than N in Siberian headwater streams (Schade et al. 2016). Some temperate rivers and streams have similarly shown P-limitation of heterotrophic activity, including the Fraser River in British Columbia (Mohamed et al. 1998) and Walker Branch, a second-order woodland stream in Tennessee (Elwood et al. 1981). Increased phosphatase activity has been documented in response to labile C enrichment (Oviedo-Vargas et al. 2013), supporting our hypothesis that biological demand for P drove the observed DOC uptake patterns. Abiotic and biotic mechanisms for the P effect on retention are not exclusive, as biological uptake can follow sorption (Meals et al. 1999).

In contrast to the effect of P, we did not find evidence of N limitation of DOC uptake. One explanation for this result is that the leachates were relatively more P-enriched than N-enriched (e.g., PP contained 19 times more P than PC, but PN only contained 2 times more N than PC) and thus were more likely to provide P in excess of a limitation threshold than were N-fertilized leachates. The pattern in nutrient content of the leachates can be attributed to the

higher solubility of P from the leaves: 47% for P compared to 8% percent for N, which is similar to solubilities observed in tropical leaves (Schreeg et al. 2013). Lack of an effect of N on retention might also support the notion that P, rather than N, is the primary limiting nutrient to heterotrophs in these streams. The effects of P addition in P-limited ecosystems may be stronger than the effect of N addition in N-limited ecosystems because of larger relative differences between P availability in substrates compared to microbial demand than occur for N (Hobbie and Vitousek 2000). Further, strong sorption capacity for P by the streambed (Reddy et al. 1999) may limit availability of P to decomposers more strongly than N. Finally, Lutz et al. (2011) observed rapid retention of N-rich DOM even in the presence of inorganic N, and hypothesized that DOM molecules with a higher N content may serve as a source of labile C, but we found no support for this hypothesis in boreal streams, where leachates enriched in DON were decomposed at a similar rate to unenriched leachates.

Previous studies in both terrestrial and aquatic ecosystems have conflicted on whether molecular composition (Coq et al. 2010, Hättenschwiler and Jørgensen 2010, Kiikkilä et al. 2013) or nutrient content (Cleveland et al. 2006, Wieder et al. 2008, Mineau et al. 2013) exerts a stronger influence on retention. Despite variation in molecular composition contributed by leachates of different species, we found no support for the hypothesis that molecular composition limits uptake. This result contrasts with previous laboratory-based studies that correlated species or genotype-level variation in molecular composition of leachates with lability of DOC (Wickland et al. 2007, Burrows et al. 2013, Wymore et al. 2015) and *in situ* estimates of DOC uptake that positively correlated with the protein content of soil-derived leachates (Fellman et al. 2009). Lack of an apparent effect of molecular composition might have occurred because the leachates all contained high proportions of readily degradable compounds. An average uptake

velocity (V_f) of 3.2 mm/min for leachates in this study (Table 2.3) compares closely with the average uptake rate of simple organic molecules (2.94 mm/min) based on a review of reach-scale estimates of DOC uptake (Mineau et al. 2016), indicating that leachate-derived DOC is highly bioavailable.

The lack of observed differences in DOC uptake among species may be due to ambiguity in the effects of particular components of DOM on the rate of retention. For example, both humic and nonhumic proportions of leached DOM can be readily biodegraded (Cleveland et al. 2004). Such patterns might result if microorganisms are adapted to the compounds found in leachates (Judd et al. 2006), which is likely in our study because the tree species used to create the leachates are present in the watersheds of the study streams. Strong microbial demand for organic C may result in uptake of molecules with a wide range of molecular compositions, and shaded streams with low productivity, such as our study sites, may be particularly energy-limited (Burrows et al. 2017). Finally, sorption can be an important abiotic mechanism of DOC removal (McDowell 1985) that may obscure differences in uptake between species and provide an alternate explanation for high uptake rates.

The high bioavailability of leachate-derived DOC can lead to overestimates of watershed-scale DOC removal (Mineau et al. 2016). Following the approach of Mineau et al. (2016), we adjusted V_f determined from leachate releases using the rate of DOC loss for laboratory incubations of stream water collected the same summer from Poker Creek, which includes all five study streams as tributaries (Mutschlecner et al. n.d.). We used this adjusted V_f to calculate relative retention of DOC (R) for the headwaters as $R = 1.0 - e^{(-V_f/HL)}$, where hydraulic load (HL) is equal to discharge/width*length (Wollheim et al. 2006). DOC retention within the headwaters ranged from 0.41% using the adjusted V_f for control leachates and 0.71% under P

addition. These rates reflect the average percent retention from one headwater stream, and C retention for the entire headwater portion of the stream network would be approximately 2%. This low removal rate can be attributed to low lability of ambient DOM in these streams during summer, with approximately 1-3% DOC loss over 21 day incubations (Mutschlechner et al. n.d.). Therefore, any changes in DOM source that increase the proportion of biolabile DOM would lead to rapid increases in C processing.

Observed similarity in rates of DOC uptake from deciduous and coniferous trees has important implications for the future trajectory of C cycling in boreal forests. Climate change is expected to increase the abundance of deciduous trees in the boreal forest due to changes in fire regime and permafrost loss (Bonan 2008, Johnstone et al. 2010, Shanin et al. 2013), and these changes would affect the characteristics of DOM reaching streams. Our results indicate that under the ambient nutrient regime, a shift in the relative abundances of conifers and deciduous trees would not alter the processing of terrestrial DOM in streams. However, we demonstrated that species vary in both their capacity to incorporate nutrients into tissues, and in the allocation of nutrients to soluble compounds, which resulted in an interactive effect of species and nutrients on uptake of DOM. Thus, observed and predicted increases in abundance of deciduous species in the boreal forest combined with projected increases in nutrient availability at high latitudes (Frey and McClelland 2009, Toohey et al. 2016) could result in enriched nutrient content of DOM and increased retention of leachate-derived C.

C uptake in streams represents a potentially important part of the global C cycle (Cole et al. 2007, Butman et al. 2015), but a stronger mechanistic understanding of the factors regulating C uptake in streams would better constrain predictions of the fates of C in stream networks (Hotchkiss et al. 2015). We have demonstrated coupling between C and nutrient cycles due to P-

limitation of retention in boreal streams. Accounting for the coupling of C and nutrient cycles will improve estimates of C storage (Finzi et al. 2011), and identifying the nutrients limiting C processing in heterotrophic streams, including headwaters and stream networks of high latitudes, may provide a key refinement to C budgets.

2.6 References

- Aufdenkampe, A. K., J. I. Hedges, J. E. Richey, A. V. Krusche, and C. A. Llerena. 2001. Sorptive fractionation of dissolved organic nitrogen and amino acids onto fine sediments within the Amazon Basin. *Limnology and Oceanography* 46:1921–1935.
- Balcarczyk, K. L., J. B. Jones, R. Jaffé, and N. Maie. 2009. Stream dissolved organic matter bioavailability and composition in watersheds underlain with discontinuous permafrost. *Biogeochemistry* 94:255–270.
- Barton, K. 2016. MuMIn: Multi-Model Inference. R package version 1.15.6. <https://CRAN.R-project.org/package=MuMIn>.
- Bates, D., M. Mächler, B. Bolker, and S. C. Walker. 2015. Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*:1–51.
- Berg, B., and H. Staaf. 1980. Decomposition rate and chemical changes of Scots Pine needle litter. II. Influence of chemical composition. *Ecological Bulletins* 32:373–390.
- Bernhardt, E. S., and W. H. McDowell. 2008. Twenty years apart: comparisons of DOM uptake during leaf leachate releases to Hubbard Brook valley streams in 1979 versus 2000. *Journal of Geophysical Research: Biogeosciences* 113:1–8.
- Bonan, G. B. 2008. Forests and climate change: forcings, feedbacks, and the climate benefits of forests. *Science* 320:1444–1449.
- Bonanza Creek LTER Climate Database. 2016. Bonanza Creek LTER - University of Alaska Fairbanks (<http://www.lter.uaf.edu>). National Science Foundation Long-Term Ecological Research program grant number DEB-1026415 and USDA Forest Service, Pacific Northwest Research Station Agreement # RJVA-PNW-01-JV-11261952-231.
- Brookshire, J. E. N., M. H. Valett, S. A. Thomas, and J. R. Webster. 2005. Coupled cycling of dissolved organic nitrogen and carbon in a forest stream. *Ecology* 86:2487–2496.
- Burrows, R. M., J. B. Fellman, R. H. Magierowski, and L. A. Barmuta. 2013. Allochthonous dissolved organic matter controls bacterial carbon production in old-growth and clearfelled headwater streams. *Freshwater Science* 32:821–836.
- Burrows, R. M., H. Laudon, B. G. McKie, and R. A. Sponseller. 2017. Seasonal resource limitation of heterotrophic biofilms in boreal streams. *Limnology and Oceanography* 62:164–176.
- Butman, D., S. Stackpoole, E. Stets, C. P. McDonald, D. W. Clow, and R. G. Striegl. 2015. Aquatic carbon cycling in the conterminous United States and implications for terrestrial carbon accounting. *Proceedings of the National Academy of Sciences of the United States of America* 113:58–63.

- Chapin, F. S., E. S. Zavaleta, V. T. Eviner, R. L. Naylor, P. M. Vitousek, H. L. Reynolds, D. U. Hooper, S. Lavorel, O. E. Sala, S. E. Hobbie, M. C. Mack, and S. Díaz. 2000. Consequences of changing biodiversity. *Nature* 405:234–42.
- Cleveland, C. C., J. C. Neff, A. R. Townsend, and E. Hood. 2004. Composition, dynamics, and gate of leached dissolved organic matter in terrestrial ecosystems: results from a decomposition experiment. *Ecosystems* 7:275–285.
- Cleveland, C. C., S. C. Reed, and A. R. Townsend. 2006. Nutrient regulation of organic matter decomposition in a tropical rain forest. *Ecology* 87:492–503.
- Coble, A. A., A. M. Marcarelli, E. S. Kane, and C. J. Huckins. 2016. Uptake of ammonium and soluble reactive phosphorus in forested streams: influence of dissolved organic matter composition. *Biogeochemistry* 131:355–372.
- Cole, J. J., Y. T. Prairie, N. F. Caraco, W. H. McDowell, L. J. Tranvik, R. G. Striegl, C. M. Duarte, P. Kortelainen, J. A. Downing, J. J. Middelburg, and J. Melack. 2007. Plumbing the global carbon cycle: integrating inland waters into the terrestrial carbon budget. *Ecosystems* 10:172–185.
- Conley, D. J., H. W. Paerl, R. W. Howarth, D. F. Boesch, S. P. Seitzinger, K. E. Havens, C. Lancelot, and G. E. Likens. 2009. Controlling eutrophication: nitrogen and phosphorus. *Science* 323:1014–1015.
- Coq, S., J.-M. Souquet, E. Meudec, V. Cheyrier, and S. Hättenschwiler. 2010. Interspecific variation in leaf litter tannins drives decomposition in a tropical rain forest of French Guiana. *Ecology* 91:2080–2091.
- Cornwell, W. K., J. H. C. Cornelissen, K. Amatangelo, E. Dorrepaal, V. T. Eviner, O. Godoy, S. E. Hobbie, B. Hoorens, H. Kurokawa, N. Pérez-Harguindeguy, H. M. Quested, L. S. Santiago, D. A. Wardle, I. J. Wright, R. Aerts, S. D. Allison, P. Van Bodegom, V. Brovkin, A. Chatain, T. V. Callaghan, S. Díaz, E. Garnier, D. E. Gurvich, E. Kazakou, J. A. Klein, J. Read, P. B. Reich, N. A. Soudzilovskaia, M. V. Vaieretti, and M. Westoby. 2008. Plant species traits are the predominant control on litter decomposition rates within biomes worldwide. *Ecology Letters* 11:1065–1071.
- Cory, R. M., and D. M. McKnight. 2005. Fluorescence spectroscopy reveals ubiquitous presence of oxidized and reduced quinones in dissolved organic matter. *Environmental Science and Technology* 39:8142–8149.
- Covino, T., B. McGlynn, and M. Baker. 2010. Separating physical and biological nutrient retention and quantifying uptake kinetics from ambient to saturation in successive mountain stream reaches. *Journal of Geophysical Research* 115:G04010.
- Dainard, P. G., C. Guéguen, N. McDonald, and W. J. Williams. 2015. Photobleaching of fluorescent dissolved organic matter in Beaufort Sea and North Atlantic Subtropical Gyre. *Marine Chemistry* 177:630–637.

- Elwood, J. W., J. D. Newbold, A. F. Trimble, and R. W. Stark. 1981. The limiting role of phosphorus in a woodland stream ecosystem: effects of P enrichment on leaf decomposition and primary producers. *Ecology* 62:146–158.
- Fellman, J. B., E. Hood, R. T. Edwards, and J. B. Jones. 2009. Uptake of allochthonous dissolved organic matter from soil and salmon in coastal temperate rainforest streams. *Ecosystems* 12:747–759.
- Finzi, A. C., A. T. Austin, E. E. Cleland, S. D. Frey, B. Z. Houlton, and M. D. Wallenstein. 2011. Responses and feedbacks of coupled biogeochemical cycles to climate change: examples from terrestrial ecosystems. *Frontiers in Ecology and the Environment* 9:61–67.
- Fisher, S. G., and G. E. Likens. 1973. Energy flow in Bear Brook, New Hampshire: an integrative approach to stream ecosystem metabolism. *Ecological Monographs* 43:421–439.
- Fraser, R. H., T. C. Lantz, I. Olthof, S. V. Kokelj, and R. A. Sims. 2014. Warming-induced shrub expansion and lichen decline in the western Canadian Arctic. *Ecosystems*:1151–1168.
- Frey, K. E., and J. W. McClelland. 2009. Impacts of permafrost degradation on arctic river biogeochemistry. *Hydrological Processes* 23:169–182.
- Galloway, J. N., A. R. Townsend, J. W. Erisman, M. Bekunda, Z. Cai, J. R. Freney, L. A. Martinelli, S. P. Seitzinger, and M. A. Sutton. 2008. Transformation of the nitrogen cycle: recent trends, questions, and potential solutions. *Science* 320:889–892.
- Guerard, J. J., P. L. Miller, T. D. Trouts, and Y. Chin. 2009. The role of fulvic acid composition in the photosensitized degradation of aquatic contaminants. *Aquatic Sciences* 71:160–169.
- Hättenschwiler, S., and H. B. Jørgensen. 2010. Carbon quality rather than stoichiometry controls litter decomposition in a tropical rain forest. *Journal of Ecology* 98:754–763.
- Helms, J. R., A. Stubbins, J. D. Ritchie, E. C. Minor, D. J. Kieber, and K. Mopper. 2008. Absorption spectral slopes and slope ratios as indicators of molecular weight, source, and photobleaching of chromophoric dissolved organic matter. *Limnology and Oceanography* 53:955–969.
- Hobbie, S., and P. M. Vitousek. 2000. Nutrient limitation of decomposition in Hawaiian forests. *Ecology* 81:1867–1877.
- Hotchkiss, E. R., R. O. Hall Jr, R. A. Sponseller, D. Butman, J. Klaminder, H. Laudon, M. Rosvall, and J. Karlsson. 2015. Sources of and processes controlling CO₂ emissions change with the size of streams and rivers. *Nature Geoscience* 8:696–699.
- Hothorn, T., F. Bretz, and P. Westfall. 2008. Simultaneous inference in general parametric models. *Biometrical Journal* 50:346–363.

- Johnstone, J. F., T. N. Hollingsworth, F. S. Chapin, and M. C. Mack. 2010. Changes in fire regime break the legacy lock on successional trajectories in Alaskan boreal forest. *Global Change Biology* 16:1281–1295.
- Jones, J. B., K. C. Petrone, J. C. Finlay, L. D. Hinzman, and W. R. Bolton. 2005. Nitrogen loss from watersheds of interior Alaska underlain with discontinuous permafrost. *Geophysical Research Letters* 32:1–4.
- Jones, J. B., E. H. Stanley, and P. J. Mulholland. 2003. Long-term decline in carbon dioxide supersaturation in rivers across the contiguous United States. *Geophysical Research Letters* 30:1–4.
- Judd, K. E., B. C. Crump, and G. W. Kling. 2006. Variation in dissolved organic matter controls bacterial production and community composition. *Ecology* 87:2068–2079.
- Kaiser, K., G. Guggenberger, and W. Zech. 1996. Sorption of DOM and DOM fractions to forest soils. *Geoderma* 74:281–303.
- Kiikkilä, O., A. Smolander, and V. Kitunen. 2013. Degradability, molecular weight and adsorption properties of dissolved organic carbon and nitrogen leached from different types of decomposing litter. *Plant and Soil* 373:787–798.
- Kothawala, D. N., C. A. Stedmon, R. A. Muller, G. A. Weyhenmeyer, S. J. Kohler, and L. J. Tranvik. 2014. Controls of dissolved organic matter quality: evidence from a large-scale boreal lake survey. *Global Change Biology* 20:1101–1114.
- Lindroth, R. L., B. J. Kopper, W. F. J. Parsons, J. G. Bockheim, D. F. Karnosky, R. Hendrey, George, K. S. Pregitzer, J. G. Isebrands, and J. Sober. 2001. Consequences of elevated carbon dioxide and ozone for foliar chemical composition and dynamics in trembling aspen (*Populus tremuloides*) and paper birch (*Betula papyrifera*). *Environmental Pollution* 115:395–404.
- Logue, J. B., C. A. Stedmon, A. M. Kellerman, N. J. Nielsen, A. F. Andersson, H. Laudon, E. S. Lindström, and E. S. Kritzberg. 2016. Experimental insights into the importance of aquatic bacterial community composition to the degradation of dissolved organic matter. *International Society for Microbial Ecology* 10:533–545.
- Lutz, B. D., E. S. Bernhardt, B. J. Roberts, and P. J. Mulholland. 2011. Examining the coupling of carbon and nitrogen cycles in Appalachian streams: the role of dissolved organic nitrogen. *Ecology* 92:720–732.
- McClelland, J. W., M. Stieglitz, F. Pan, R. M. Holmes, and B. J. Peterson. 2007. Recent changes in nitrate and dissolved organic carbon export from the upper Kuparuk River, North Slope, Alaska. *Journal of Geophysical Research* 112:1–13.
- McDowell, W. H. 1985. Kinetics and mechanisms of dissolved organic carbon retention in a headwater stream. *Biogeochemistry* 1:329–352.

- McKnight, D. M., E. W. Boyer, P. K. Westerhoff, P. T. Doran, T. Kulbe, and D. T. Andersen. 2001. Spectrofluorometric characterization of dissolved organic matter for indication of precursor organic material and aromaticity. *Limnology and Oceanography* 46:38–48.
- Meals, D. W., S. N. Levine, D. Wang, J. P. Hoffmann, E. A. Cassell, J. C. Drake, D. K. Pelton, H. M. Galarneau, and A. B. Brown. 1999. Retention of spike additions of soluble phosphorus in a northern eutrophic stream. *Journal of the North American Benthological Society* 18:185–198.
- Meentemeyer, V. 1978. Macroclimate and lignin control of litter decomposition rates. *Ecological Monographs* 59:465–472.
- Melillo, J. M., J. D. Aber, and J. F. Muratore. 1982. Nitrogen and lignin control of hardwood leaf litter decomposition dynamics. *Ecology* 63:621–626.
- Mineau, M. M., C. M. Rigsby, D. T. Ely, I. J. Fernandez, S. A. Norton, T. Ohno, H. M. Valett, and K. S. Simon. 2013. Chronic catchment nitrogen enrichment and stoichiometric constraints on the bioavailability of dissolved organic matter from leaf leachate. *Freshwater Biology* 58:248–260.
- Mineau, M. M., W. M. Wollheim, I. Buffam, S. E. G. Findlay, R. O. Hall, E. R. Hotchkiss, L. E. Koenig, W. H. McDowell, and T. B. Parr. 2016. Dissolved organic carbon uptake in streams: a review and assessment of reach-scale measurements. *Journal of Geophysical Research: Biogeosciences*.
- Mohamed, M. N., J. R. Lawrence, and R. D. Robarts. 1998. Phosphorus limitation of heterotrophic biofilms from the Fraser River, British Columbia, and the effect of pulp mill effluent. *Microbial Ecology*. Sept. Oct. 36:121–130.
- Murphy, J., and J. P. Riley. 1962. A modified single solution method for the determination of phosphate in natural waters. *Analytica Chimica Acta* 27:31–36.
- Murphy, K. R., C. A. Stedmon, P. Wenig, and R. Bro. 2014. OpenFluor- an online spectral library of auto-fluorescence by organic compounds in the environment. *Analytical Methods* 6:658–661.
- Mutschlecner, A., J. J. Guerard, J. B. Jones, and T. K. Harms. (n.d.). Regional and intra-annual stability of dissolved organic matter composition and lability in high-latitude rivers. Manuscript in preparation.
- Neff, J. C., and G. P. Asner. 2001. Dissolved organic carbon in terrestrial ecosystems: synthesis and a model. *Ecosystems* 4:29–48.
- Oviedo-Vargas, D., T. V. Royer, and L. T. Johnson. 2013. Dissolved organic carbon manipulation reveals coupled cycling of carbon, nitrogen, and phosphorus in a nitrogen-rich stream. *Limnology and Oceanography* 58:1196–1206.
- Peñuelas, J., M. Estiarte, and J. Llusà. 1997. Carbon-based secondary compounds at elevated CO₂. *Photosynthetica* 33:313–316.

- Peterson, B. J., L. Deegan, J. Helfrich, J. E. Hobbie, B. Moller, T. E. Ford, A. Hershey, A. Hiltner, G. Kipphut, M. A. Lock, D. M. Fiebig, V. Mckinley, M. C. Miller, J. R. Vestal, B. J. Peterson, L. Deegan, J. Helfrich, J. E. Hobbie, M. Hullar, B. Moller, T. E. Ford, M. C. Miller, and J. R. Vestal. 1993. Biological responses of a tundra river to fertilization. *Ecology* 74:653–672.
- Prescott, C. E., L. Vesterdal, C. M. Preston, and S. W. Simard. 2004. Influence of initial chemistry on decomposition of foliar litter in contrasting forest types in British Columbia. *Canadian Journal of Forest Research* 34:1714–1729.
- Qualls, R. G., and B. L. Haines. 1992. Biodegradability of dissolved organic matter in forest throughfall, soil solution, and stream water. *Soil Science Society of America Journal* 56:578–586.
- R Core Team. 2016. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. www.r-project.org.
- Raymond, P. A., J. Hartmann, R. Lauerwald, S. Sobek, C. McDonald, M. Hoover, D. Butman, R. Striegl, E. Mayorga, C. Humborg, P. Kortelainen, H. Dürr, M. Meybeck, P. Ciais, and P. Guth. 2013. Global carbon dioxide emissions from inland waters. *Nature* 503:355–9.
- Reddy, K. R., R. H. Kadlec, E. Flaig, and P. M. Gale. 1999. Phosphorus retention in streams and wetlands: a review. *Critical Reviews in Environmental Science and Technology* 29:83–146.
- Redfield, A. C. 1958. The biological control of chemical factors in the environment. *American Scientist* 46:205–221.
- Rosemond, A. D., J. P. Benstead, P. M. Bumpers, V. Gulis, J. S. Kominoski, D. W. P. Manning, K. Suberkropp, and J. B. Wallace. 2015. Experimental nutrient additions accelerate terrestrial carbon loss from stream ecosystems. *Science* 347:318–321.
- Ruess, R. W., M. D. Anderson, J. M. Mcfarland, K. Kielland, K. Olson, and D. L. Taylor. 2013. Ecosystem-level consequences of symbiont partnerships in an N-fixing shrub from interior Alaskan floodplains. *Ecological Monographs* 83:177–194.
- Schade, J. D., E. C. Seybold, T. Drake, S. Spawn, W. V. Sobczak, K. E. Frey, R. M. Holmes, and N. Zimov. 2016. Variation in summer nitrogen and phosphorus uptake among Siberian headwater streams. *Polar Research* 35:1–28.
- Schlesinger, W., and J. Andrews. 2000. Soil respiration and the global carbon cycle. *Biogeochemistry* 48:7–20.
- Schreeg, L. A., M. C. Mack, and B. L. Turner. 2013. Nutrient-specific patterns in leaf litter solubility across 41 lowland tropical woody species. *Ecology* 94:94–105.
- Shanin, V., A. Komarov, Y. Khoraskina, S. Bykhovets, T. Linkosalo, and R. Mäkipää. 2013. Carbon turnover in mixed stands: modelling possible shifts under climate change. *Ecological Modelling* 251:232–245.

- Shutova, Y., A. Baker, J. Bridgeman, and R. K. Henderson. 2014. Spectroscopic characterisation of dissolved organic matter changes in drinking water treatment: from PARAFAC analysis to online monitoring wavelengths. *Water Research* 54:159–169.
- Sinsabaugh, R. L., S. Findlay, P. Franchini, and D. Fischer. 1997. Enzymatic analysis of riverine bacterioplankton production. *Limnology and Oceanography* 42:29–38.
- Sinsabaugh, R. L., S. Manzoni, D. L. Moorhead, and A. Richter. 2013. Carbon use efficiency of microbial communities: stoichiometry, methodology and modelling. *Ecology Letters* 16:930–939.
- Solórzano, L. 1969. Determination of ammonia in natural waters by the phenolhypochlorite method. *Limnology and Oceanography* 14:799–801.
- Stedmon, C. A., and R. Bro. 2008. Characterizing dissolved organic matter fluorescence with parallel factor analysis: a tutorial. *Limnology and Oceanography: Methods* 6:572–579.
- Stedmon, C. A., S. Markager, and R. Bro. 2003. Tracing dissolved organic matter in aquatic environments using a new approach to fluorescence spectroscopy 82:239–254.
- Stream Solute Workshop. 1990. Concepts and methods for assessing solute dynamics in stream ecosystems. *Journal of the North American Benthological Society* 9:95–119.
- Taylor, B. R., D. Parkinson, and W. F. J. Parsons. 1989. Nitrogen and lignin content as predictors of litter decay rates: a microcosm test. *Ecology* 70:97–104.
- Toohey, R. C., N. M. Herman-Mercer, P. F. Schuster, E. Mutter, and J. C. Koch. 2016. Multi-decadal increases in the Yukon River Basin of chemical fluxes as indicators of changing flowpaths, groundwater, and permafrost. *Geophysical Research Letters* 43:12,120–12,130.
- Ussiri, D. A. N., and C. E. Johnson. 2004. Sorption of organic carbon fractions by spodosol mineral horizons. *Soil Science Society of America Journal* 68:253.
- Van Cleve, K., J. Yarie, R. Erickson, and C. T. Dyrness. 1993. Nitrogen mineralization and nitrification in successional ecosystems on the Tanana River floodplain, interior Alaska. *Canadian Journal of Forest Research* 23:970–978.
- Weishaar, J., G. Aiken, B. Bergamaschi, M. Fram, R. Fujii, and K. Mopper. 2003. Evaluation of specific ultra-violet absorbance as an indicator of the chemical content of dissolved organic carbon. *Environmental Science and Technology* 37:4702–4708.
- Wickland, K. P., J. C. Neff, and G. R. Aiken. 2007. Dissolved organic carbon in Alaskan boreal forest: sources, chemical characteristics, and biodegradability. *Ecosystems* 10:1323–1340.
- Wieder, W. R., C. C. Cleveland, and A. R. Townsend. 2008. Tropical tree species composition affects the oxidation of dissolved organic matter from litter. *Biogeochemistry* 88:127–138.
- Wollheim, W. M., C. J. Vörösmarty, B. J. Peterson, S. P. Seitzinger, and C. S. Hopkinson. 2006. Relationship between river size and nutrient removal. *Geophysical Research Letters* 33:1–4.

Wymore, A. S., Z. G. Compson, W. H. McDowell, J. D. Potter, B. A. Hungate, T. G. Whitham, and J. C. Marks. 2015. Leaf-litter leachate is distinct in optical properties and bioavailability to stream heterotrophs. *Freshwater Science* 34:857–866.

2.7 Tables

Table 2.1. Physico-chemical conditions of headwater streams. Concentrations represent the means of $n = 6$ replicate water samples collected prior to uptake experiments ($n = 3$ for C1). Discharge represents the mean of $n = 8$ measurements taken at the top and bottom of each experimental reach, measured twice daily on each of two days ($n = 4$ for C1). BDL = below detection limit. Stoichiometric ratios are given in molar units.

Stream	Discharge L/s	Cl ⁻ recovery %	DOC mg C/L	TDP μg P/L	DON μg N/L	NH ₄ ⁺ μg N/L	NO ₃ ⁻ μg N/L	C:N	C:P
C1	39.0	72.8	3.50	2.6	134.9	12.2	271.3	9.8	3475.0
C2	13.0	88.5	1.72	1.3	BDL	25.4	483.0	4.2	3437.9
C3	6.0	50.7	1.80	1.6	51.7	20.6	399.2	4.5	2975.4
C4	20.9	73.4	1.06	2.2	BDL	12.2	649.1	1.9	1224.5
P6	21.5	77.4	2.34	3.2	9.5	14.6	330.2	7.7	1863.3

Table 2.2. Chemistry of leaves grown in long-term fertilization plots and their leachates. Values represent means of n = 5 analytical replicates. Significant differences are indicated by upper case letter for differences between the controls of each species (species effect) and lower case letters for differences among nutrient treatments within a species (nutrient effect). Letters are omitted where there is no significant difference. Statistics were not performed for calculations of solubility, which lack replication. Stoichiometric ratios are given in molar units.

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Species	Nutrient Treatment	DOC g C/L	TDP mg/L	DON mg N/L	NH ₄ ⁺ mg N/L	NO ₃ ⁻ mg N/L
Alder	Control	6.16	28.5 ^a	100.95	1.88 ^A	4.62
	N	6.62	33.7 ^a	92.06	1.07	5.54
	P	7.07	199.2 ^b	92.87	1.12	5.60
Poplar	Control	7.31	17.1 ^a	84.54 ^a	2.75 ^{A,a}	3.80
	N	7.20	28.0 ^a	179.35 ^b	9.05 ^b	2.90
	P	7.76	322.2 ^b	84.30 ^a	4.44 ^c	2.26
Spruce	Control	6.12	12.6	73.04 ^a	0.50 ^B	2.56
	N	6.11	28.0	150.46 ^b	0.54	3.16
	P	5.22	34.1	92.95 ^a	0.56	2.20

Table 2.2 continued

Species	Nutrient Treatment	Leachate C:N	Dry mass C:N	Leachate C:P	Dry mass C:P	Soluble C %	Soluble N %	Soluble P %
Alder	Control	72.1	23.4 ^A	559.0 ^{A,a}	1545.8 ^{A,a}	12.3	4.0	36.9
	N	78.8	27.3	506.9 ^{a,b}	1850.9 ^a	13.2	4.7	46.4
	P	86.5	30.3	91.6 ^b	467.4 ^b	14.4	5.3	65.7
Poplar	Control	93.6 ^a	56.5 ^{B,a}	1117.3 ^{B,a}	3630.2 ^{B,a}	15.4	8.5	47.3
	N	43.9 ^b	27.0 ^b	688.8 ^a	3391.6 ^a	15.2	8.9	76.0
	P	100.2 ^a	101.4 ^c	62.1 ^b	246.3 ^b	17.2	17.8	60.9
Spruce	Control	97.8 ^a	61.3 ^{B,a}	1269.3 ^{B,a}	2445.8 ^{C,a}	12.1	8.0	21.7
	N	49.7 ^b	41.3 ^b	891.7 ^{a,b}	2484.8 ^a	12.1	10.9	52.7
	P	72.0 ^{a,b}	62.0 ^a	553.4 ^b	635.0 ^b	10.4	10.2	16.3

Table 2.3. Breakthrough curve-integrated metrics of DOC uptake estimated from addition of leaf leachate. S_w , uptake length; V_f , uptake velocity; U , areal uptake. Values represent means across five streams with 95% confidence intervals.

Species	Nutrient Treatment	S_w (m)	V_f (mm/min)	U (mg m ⁻² day ⁻¹)
Alder	Control	548 ± 198	2.72 ± 1.17	798 ± 253
	N	554 ± 280	3.18 ± 1.91	732 ± 231
	P	440 ± 151	3.66 ± 2.38	1118 ± 279
Poplar	Control	586 ± 213	2.92 ± 2.33	854 ± 361
	N	538 ± 190	2.84 ± 1.78	851 ± 367
	P	369 ± 144	4.46 ± 3.80	1097 ± 471
Spruce	Control	488 ± 158	2.94 ± 1.70	955 ± 443
	N	477 ± 167	3.13 ± 1.86	842 ± 222
	P	577 ± 241	3.17 ± 2.65	735 ± 250

2.8 Figure legends

Figure 2.1. Molecular composition of leachates derived from control and fertilized leaves of alder, poplar, and spruce. (A) Percent of fluorescence intensity at the maximum (F_{max}) for components identified in leachates by PARAFAC analysis. Error bars indicate one standard error. (B) Loadings of principal component 1, which summarizes optical characteristics of the leachate. The principal component was negatively correlated with the humic-like component C2, and positively correlated with the protein-like component C5 and the slope ratio, for which larger values correspond to smaller average molecular weight of DOM. Center line indicates the median, boxes represent 25th and 75th percentiles, and whiskers 1.5 times the inter-quartile range. Letters indicate significant differences between leachates based on a Tukey's honest significance difference test.

Figure 2.2. Retention of DOC following addition to streams as leaf leachate. Nutrient treatment refers to the fertilization regime for the plots from which leaves were collected. Letters indicate significant differences among nutrient treatments within a species. There were no significant differences between species.

2.9 Figures

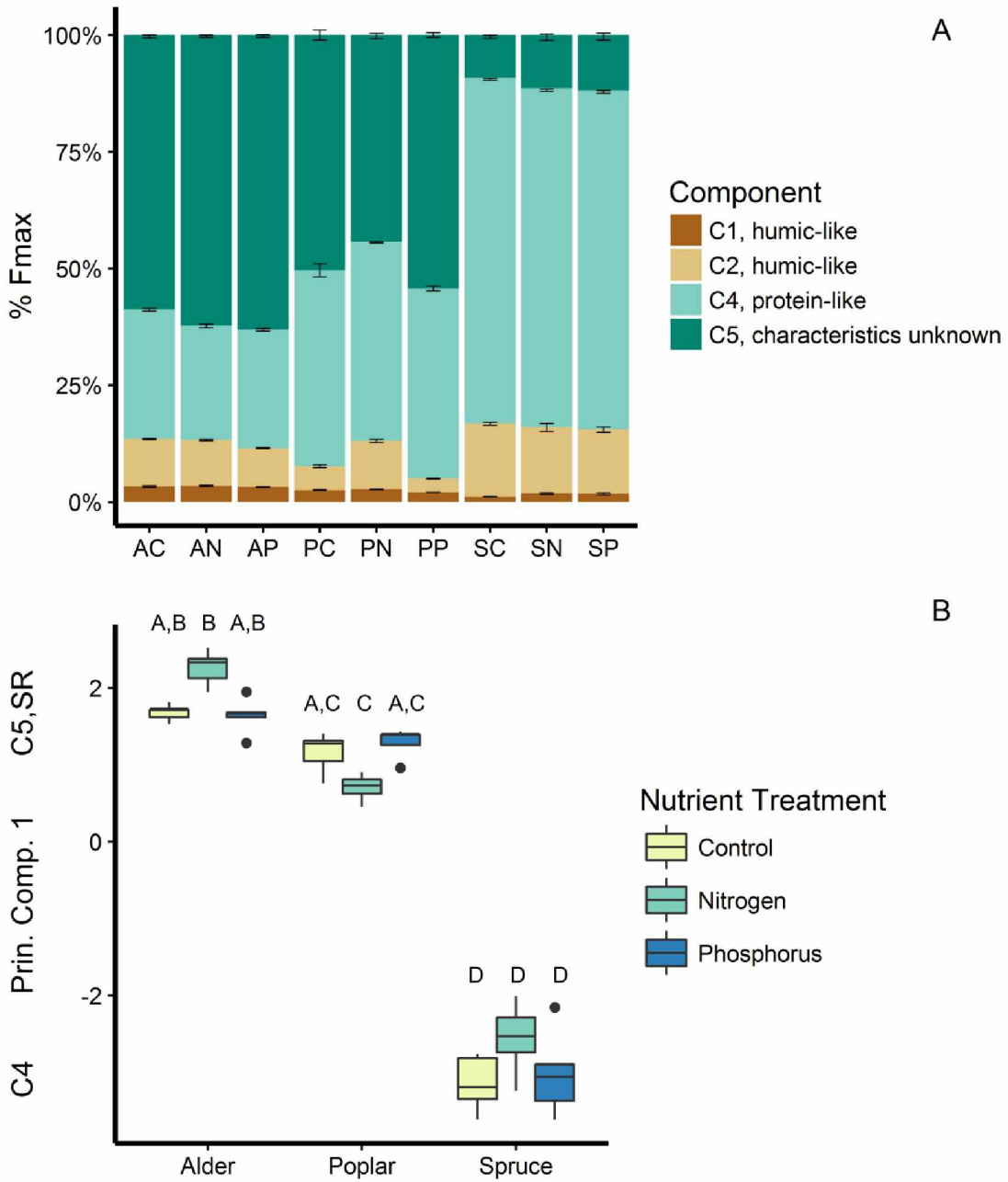


Figure 2.1.

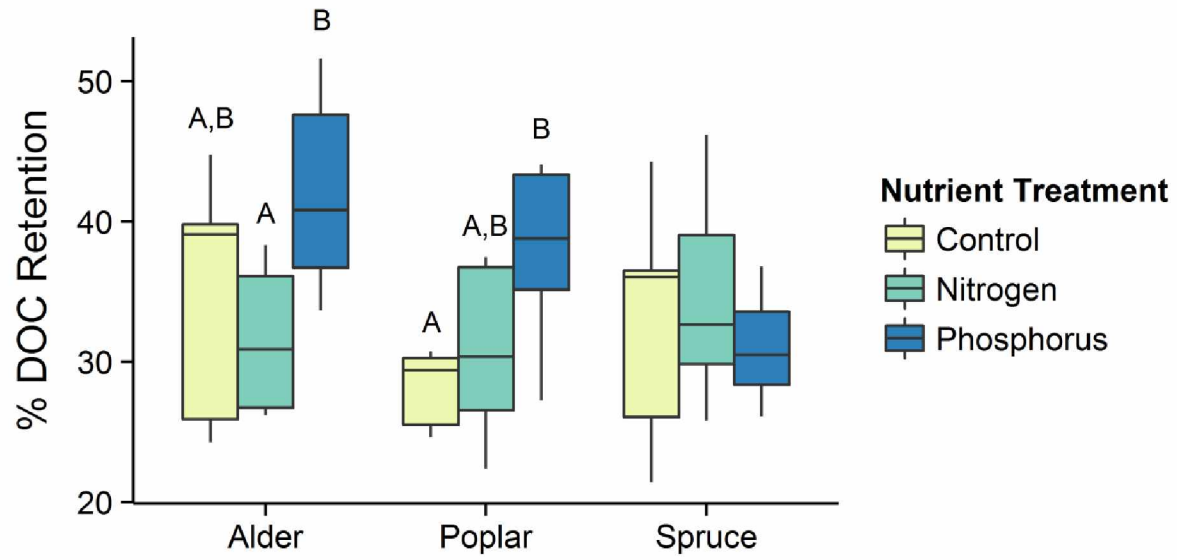


Figure 2.2.

2.10 Supplementary Information

Supplementary Table 2.1. Chemistry of leaves grown in long-term fertilization plots collected in September 2013 and 2014. Values represent means of n = 3 analytical replicates.

Species	Nutrient Treatment	% C		% N		% P	
		2013	2014	2013	2014	2013	2014
Alder	Control	50.85	49.24	2.63	2.79	0.07	0.09
	N	50.70	49.47	2.10	2.12	0.08	0.07
	P	49.63	48.43	1.74	1.99	0.36	0.25
Poplar	Control	47.96	46.86	1.33	0.82	0.04	0.03
	N	48.60	46.38	2.55	1.78	0.04	0.03
	P	47.15	42.86	0.53	0.49	0.30	0.76
Spruce	Control	49.62	51.64	0.94	0.97	0.07	0.05
	N	50.83	50.46	1.13	1.70	0.06	0.05
	P	48.94	51.06	0.74	1.14	0.23	0.19

Supplementary Table 2.2. Fluorescent components found in leachates as identified by PARAFAC analysis. C3 was present in stream water samples included in the model, but not in leachates.

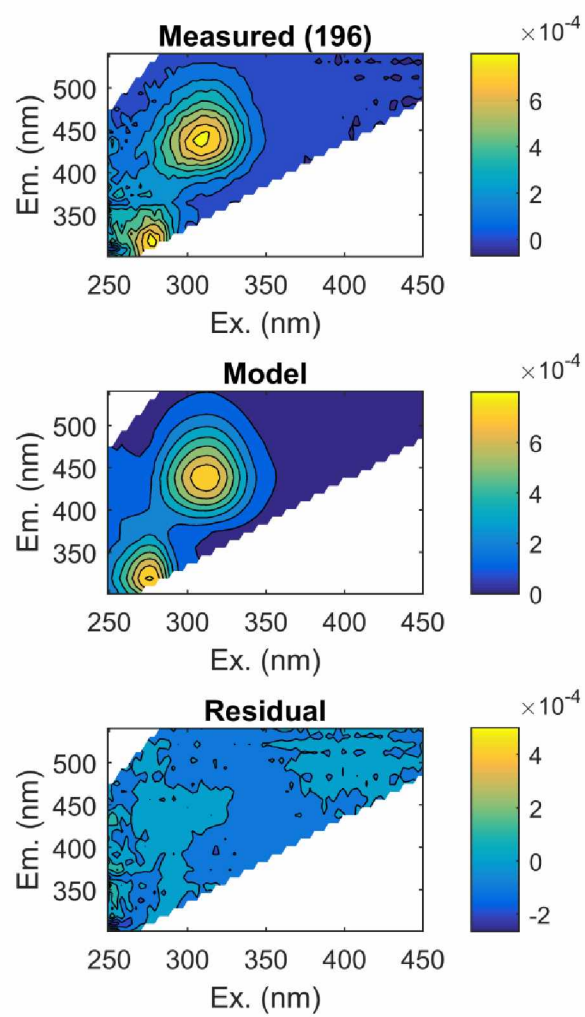
Component	Excitation Max nm	Emission Max nm	Description	Reference
C1	340	460	Humic-like	C1, Kothawala et al. 2014
C2	300	390	Humic-like	C2, Shutova et al. 2014
C3	290/400	500	High molecular weight fraction of terrestrially derived humic-like material	C3, Kothawala et al. 2014
C4	275	320	Protein-like	C4, Dainard et al. 2015
C5	315	450	Characteristics unknown	C10, Cory and McKnight 2005

Supplementary Table 2.3. Leachate optical properties. Values represent means of $n = 5$ replicates with 95% confidence intervals. UV-vis scans were used to calculate specific ultraviolet absorbance (SUVA), which is associated with greater aromatic content (Weishaar et al. 2003), and spectral slope ratio (SR), which is negatively correlated with DOM molecular weight (Helms et al. 2008).

Species	Nutrient Treatment	SUVA $\text{L mg}^{-1} \text{m}^{-1}$	SR
Alder	Control	0.6 ± 0.0	0.6 ± 0.0
	N	0.6 ± 0.1	0.7 ± 0.0
	P	0.6 ± 0.1	0.5 ± 0.0
Poplar	Control	0.6 ± 0.2	0.6 ± 0.0
	N	0.7 ± 0.2	0.6 ± 0.0
	P	1.0 ± 0.1	0.5 ± 0.0
Spruce	Control	1.8 ± 0.1	0.3 ± 0.0
	N	1.8 ± 0.3	0.2 ± 0.1
	P	1.9 ± 0.4	0.2 ± 0.0

Supplementary Table 2.4. Loadings of optical measures included in the principal component analysis. C1–C5 were determined by PARAFAC analysis of EEMS and specific ultraviolet absorbance (SUVA) and slope ratio (SR) were determined from leachate absorbance.

	Component 1 loading
C1	0.402
C2	-0.359
C4	-0.458
C5	0.465
SUVA	0.305
SR	0.437



Supplementary Figure 2.1. Measured fluorescence, fitted model, and residuals of a representative leachate EEM included in PARAFAC analysis.

General Conclusion

This study furthers our understanding of spatial and temporal patterns in the molecular composition and biolability of DOM, as well as the mechanisms that drive DOM retention in arctic and boreal streams. DOM composition follows seasonal patterns in hydrology with distinct characteristics during spring freshet. I showed that ambient riverine DOM is generally recalcitrant, but DOM from leaf leachates is rapidly taken up by streams. Together, these results suggest that export is the dominant fate of ambient DOM in high-latitude streams, but that C processing is highly sensitive to inputs of bioavailable DOM.

Correlations between DOM composition and biolability are tenuous and highly nuanced. I found that measures of molecular composition of DOM in streamwater were only weakly correlated to biolability, and that differences in the molecular composition of DOM from leaf leachates did not correspond to differences in uptake. In this study I used optical properties to characterize molecular composition, but the compounds that drive biolability may not be optically detectable. Other studies have found DOM biolability to be correlated with protein-like fractions of the DOM pools (Balcarczyk et al. 2009, Fellman et al. 2009), but I did not observe this pattern. Contradictions within the literature over what constitutes biolabile DOM highlight that caution should be applied when using composition to infer biolability.

Laboratory (Chapter 1) and field (Chapter 2) experiments revealed evidence of coupling between C and P cycles. In Chapter 1, I found that the addition of P to laboratory incubations of streamwater increased the rate of decomposition. In Chapter 2, I found that DOC uptake was greater for leaf leachates with a higher concentration of P. Together, these results emphasize that accurate predictions of C processing must also account for P availability. If P concentrations are low, rates of DOM decomposition will be constrained, and therefore rates of CO₂ release may be

lower than expected. Many studies of high-latitude DOM dynamics do not measure P, and there have been even fewer tests of P-limitation. I observed a strong influence of both ambient P concentration and experimental fertilization with P on DOM uptake and decomposition in streams. Increased P availability has the potential to increase rates of C processing and therefore shift the balance of C pools within high-latitude ecosystems

References

- Balcarczyk, K. L., J. B. Jones, R. Jaffé, and N. Maie. 2009. Stream dissolved organic matter bioavailability and composition in watersheds underlain with discontinuous permafrost. *Biogeochemistry* 94:255–270.
- Chapin, F. S., E. S. Zavaleta, V. T. Eviner, R. L. Naylor, P. M. Vitousek, H. L. Reynolds, D. U. Hooper, S. Lavorel, O. E. Sala, S. E. Hobbie, M. C. Mack, and S. Díaz. 2000. Consequences of changing biodiversity. *Nature* 405:234–42.
- Cory, R. M., C. P. Ward, B. C. Crump, and G. W. Kling. 2014. Sunlight controls water column processing of carbon in arctic fresh waters. *Science* 345:925–928.
- Fellman, J. B., E. Hood, R. T. Edwards, and J. B. Jones. 2009. Uptake of allochthonous dissolved organic matter from soil and salmon in coastal temperate rainforest streams. *Ecosystems* 12:747–759.
- Fraser, R. H., T. C. Lantz, I. Olthof, S. V. Kokelj, and R. A. Sims. 2014. Warming-induced shrub expansion and lichen decline in the western Canadian Arctic. *Ecosystems*:1151–1168.
- Guerard, J. J., P. L. Miller, T. D. Trouts, and Y. Chin. 2009. The role of fulvic acid composition in the photosensitized degradation of aquatic contaminants. *Aquatic Sciences* 71:160–169.
- Harms, T. K., J. W. Edmonds, H. Genet, I. F. Creed, D. Aldred, A. Balser, and J. B. Jones. 2016. Catchment influence on nitrate and dissolved organic matter in Alaskan streams across a latitudinal gradient. *Journal of Geophysical Research: Biogeosciences* 121:350–369.
- Johnstone, J. F., T. N. Hollingsworth, F. S. Chapin, and M. C. Mack. 2010. Changes in fire regime break the legacy lock on successional trajectories in Alaskan boreal forest. *Global Change Biology* 16:1281–1295.
- Jones, J. B., E. H. Stanley, and P. J. Mulholland. 2003. Long-term decline in carbon dioxide supersaturation in rivers across the contiguous United States. *Geophysical Research Letters* 30:1–4.
- Kling, G. W., G. W. Kipphut, and M. C. Miller. 1991. Arctic lakes and streams as gas conduits to the atmosphere: implications for tundra carbon budgets. *Science* 251:298–301.
- McKnight, D. M., E. W. Boyer, P. K. Westerhoff, P. T. Doran, T. Kulbe, and D. T. Andersen. 2001. Spectrofluorometric characterization of dissolved organic matter for indication of precursor organic material and aromaticity. *Limnology and Oceanography* 46:38–48.
- O'Donnell, J. A., G. R. Aiken, D. K. Swanson, S. Panda, K. D. Butler, and A. P. Baltensperger. 2016. Dissolved organic matter composition of Arctic rivers: linking permafrost and parent material to riverine carbon. *Global Biogeochemical Cycles*.
- Peterson, B. J., R. M. Holmes, J. W. McClelland, C. J. Vorosmarty, R. B. Lammers, A. I. Shiklomanov, I. A. Shiklomanov, and S. Rahmstorf. 2002. Increasing river discharge to the arctic ocean. *Science* 298:2171–2173.

- Raymond, P. A., J. Hartmann, R. Lauerwald, S. Sobek, C. McDonald, M. Hoover, D. Butman, R. Striegl, E. Mayorga, C. Humborg, P. Kortelainen, H. Dürr, M. Meybeck, P. Ciais, and P. Guth. 2013. Global carbon dioxide emissions from inland waters. *Nature* 503:355–9.
- Romanovsky, V. E., D. S. Drozdov, N. G. Oberman, G. V. Malkova, A. L. Kholodov, S. S. Marchenko, N. G. Moskalenko, D. O. Sergeev, N. G. Ukraintseva, A. A. Abramov, D. A. Gilichinsky, and A. A. Vasiliev. 2010. Thermal state of permafrost in Russia. *Permafrost and Periglacial Processes* 21:136–155.
- Schuur, E. A. G., A. D. McGuire, G. Grosse, J. W. Harden, D. J. Hayes, G. Hugelius, C. D. Koven, and P. Kuhry. 2015. Climate change and the permafrost carbon feedback. *Nature* 520:171–179.
- Sleighter, R. L., and P. G. Hatcher. 2007. The application of electro-spray ionization coupled to ultrahigh resolution mass spectrometry for the molecular characterization of natural organic matter. *Journal of Mass Spectrometry* 42:559–574.
- Tarnocai, C., J. G. Canadell, E. A. G. Schuur, P. Kuhry, G. Mazhitova, and S. Zimov. 2009. Soil organic carbon pools in the northern circumpolar permafrost region. *Global Biogeochemical Cycles* 23:1–11.
- Walvoord, M. A., and R. G. Striegl. 2007. Increased groundwater to stream discharge from permafrost thawing in the Yukon River basin: potential impacts on lateral export of carbon and nitrogen. *Geophysical Research Letters* 34:1–6.