

# Phosphorus Enhances Uptake of Dissolved Organic Matter in Boreal Streams

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## ABSTRACT

Retention of carbon (C), either by physical mechanisms or microbial uptake, 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 rates of C retention, in situ, following introduction of leachates derived from alder, poplar, and spruce trees subject to long-term fertilization with nitrogen (N) or phosphorus (P). Leachate C:N varied approximately twofold, 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 uptake 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.

**Key words:** dissolved organic matter (DOM); excitation–emission matrices (EEMs); headwater streams; litter leachate; nitrogen; nutrient limitation; phosphorus; uptake velocity ( $V_f$ ).

## INTRODUCTION

Decomposition of organic matter recycles essential elements within ecosystems and regulates the global carbon (C) cycle (Schlesinger and Andrews 2000; Cole and others 2007). In both terrestrial and aquatic ecosystems, decomposition and transformation of organic matter is influenced by nutrient availability as well as the molecular composition of the organic matter (Berg and Staaf 1980; Elwood and others 1981; Qualls and Haines 1992; Mineau and others 2013). Nutrient availability is changing on a global scale due to processes such as per-

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**Author contributions:** AEM and TKH designed the study. AEM collected and analyzed data and drafted the paper with significant contribution from TKH. All authors contributed to development of study design and editing of the paper.

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mafrost thaw (Jones and others 2005; McClelland and others 2007), fertilizer application (Vitousek and others 1997), and nitrogen (N) deposition (Galloway and others 2008; Mineau and others 2013). Global change also influences the molecular composition of detritus, defined by functional groups that determine reactivity, through the effects of atmospheric CO<sub>2</sub> concentration on plant tissue chemistry (Peñuelas and others 1997; Lindroth and others 2001), and geographic shifts in plant species composition (Chapin and others 2000; Johnstone and others 2010; Fraser and others 2014). Understanding how nutrients and molecular composition regulate decomposition and retention of organic C is thus critical to projecting changes in the global C cycle, but the relative influences of molecular composition and nutrient content on retention of C in ecosystems remain unclear.

Nutrients influence retention of organic matter through both biotic and abiotic mechanisms, which include microbial uptake and respiration, photochemical reactions, and sorption. Nutrient content of organic matter influences biological uptake because microbial decomposers require essential nutrients for cellular function (Sinsabaugh and others 2013). When nutrients are limiting, decomposers shift resource allocation from acquisition of C to nutrients, slowing the rate of decomposition (Sinsabaugh and others 1997). N content of organic matter typically increases decomposition of leaf litter in N-limited terrestrial ecosystems (Berg and Staaf 1980; Taylor and others 1989), and phosphorus (P) availability constrains decomposition of litter and leachates in P-limited terrestrial ecosystems (Hobbie and Vitousek 2000; Wieder and others 2008). In many streams, DON is readily taken up by microorganisms (Bernhardt and McDowell 2008; Balcarczyk and others 2009; Fellman and others 2009). Experimental fertilization indicates co-limitation of C uptake or decomposition by N and P in temperate streams (Mineau and others 2013; Rosemond and others 2015) and strong P limitation of respiration in a high-latitude stream (Peterson and others 1993). Nutrients also influence physical retention of dissolved organic matter (DOM), with high sorption capacity of stream sediments for dissolved organic P (Meyer 1979; Reddy and others 1999) and preferential sorption of N-rich DOM to particulates in rivers (McKnight and others 1992; Aufdenkampe and others 2001).

The molecular composition of organic matter may also determine whether it is retained or exported from ecosystems. Abiotic sorption of DOM to soils and sediments preferentially retains

hydrophobic molecules (Kaiser and others 1996; Ussiri and Johnson 2004), including those of higher aromatic content (McKnight and others 1992), and compounds of high molecular weight (Meier and others 2004; Pérez and others 2011). Opposite patterns in export of organic matter may occur as a result of active biological decomposition, because when molecules are large 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 and others 2013). Indeed, among bacteria, the genetic traits linked to the use of high molecular weight molecules are less common than those linked to use of low molecular weight molecules (Logue and others 2016), indicating potential constraints of molecular composition on biotic decomposition. For example, in terrestrial studies, lignin, a molecule of high aromatic content that is derived from terrestrial vegetation, tends to resist decomposition (Meentemeyer 1978), whereas monomeric carbohydrates are reactive (Bernhardt and McDowell 2008). Due in part to variation in molecular composition, species-based variation in leaf litter chemistry explains more variation in decomposition than temperature across biomes globally (Cornwell and others 2008) and species-specific patterns in chemistry are correlated with biodegradability of leaf leachates (Cleveland and others 2004; Wickland and others 2007). The search for general patterns linking organic molecules to ecologically relevant patterns in C fluxes is complicated by the diverse chemical constituents of organic matter and the potential priming effect of labile substrates on subsequent decomposition of recalcitrant molecules (Guenet and others 2010).

The relative effects of nutrients and molecular composition of C on retention of C in ecosystems remain unresolved, in part because nutrient content and molecular composition 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 and others 2005; Lutz and others 2011). Separating the effects of molecular composition and nutrient content on retention is critical to predicting whether C is stored in ecosystems or respired as CO<sub>2</sub>, 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 retention of dissolved organic C (DOC), using boreal streams as a model system for examining the relative importance of these two factors. The boreal

forest biome stores a large fraction of Earth's C, but warming climate and an intensifying fire regime contribute to uncertainty regarding storage and flux of C (McGuire and others 2009; Chapin and others 2010), particularly in aquatic ecosystems (Stackpoole and others 2017). Terrestrially derived organic matter 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 C (Cole and others 2007), potentially offsetting the terrestrial storage term in C budgets (Butman and others 2015) and contributing to hot spots of CO<sub>2</sub> evasion within the landscape (Jones and others 2003; Raymond and others 2013). However, the composition of DOM and its reactivity vary depending on contributing terrestrial sources (McKnight and others 2001; Guerard and others 2009). The limitation status of the boreal streams included in the present study is unknown, but heterotrophic respiration in boreal streams elsewhere may be limited by C or nutrients, depending on seasonality (Burrows and others 2017) and strong P limitation of primary production and heterotrophic activity has been demonstrated in an Arctic stream (Peterson and others 1993). Therefore, 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 or due to preferential sorption of N- or P-containing organic molecules. To test these hypotheses, we contrasted in situ uptake of DOM derived from leaf leachates of three plant species that were known to vary in molecular composition (Van Cleve and others 1993) and in nutrient content due to growth under long-term fertilization with N or P (Ruess and others 2013). Thus, our approach includes factorial contrasts of the effects of C quality, N, and P availability on C uptake in stream ecosystems.

## METHODS

### 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 km<sup>2</sup>, respectively (Table 1). Stream temperature during the study ranged 2–5 °C.

### 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 (20 × 20 m) of alder (*Alnus incana* ssp. *tenuifolia*), poplar (*Populus balsamifera* L.), and white spruce (*Picea glauca*) fertilized with N (100 kg N ha<sup>-1</sup> year<sup>-1</sup> as NH<sub>4</sub>NO<sub>3</sub>) or P (80 kg P ha<sup>-1</sup> year<sup>-1</sup> as P<sub>2</sub>O<sub>5</sub>) as well as unfertilized control plots (30 × 30 m). Fertilization has occurred annually since 1997 for N and since 2004 for P (Ruess and others 2013). Leaves were harvested in September 2013 and 2014 from nine plots: alder control, alder N, alder P, poplar control, poplar N, poplar P, spruce control, spruce N, and spruce P (Supplementary Table 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) that allowed for infiltration of water while containing leaf fragments, 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 h. Resulting leachates were filtered through 0.7-µm glass fiber filters (Whatman GF/F) and frozen until use (≤ 7 days). Logistics prevented immediate analysis of leachate samples, and although we recognize freezing alters

**Table 1.** Physicochemical Conditions of Headwater Streams

Stream	Discharge (L/s)	Cl <sup>-</sup> recovery (%)	DOC (mg C/L)	TDP (µg P/L)	DON (µg N/L)	NH <sub>4</sub> <sup>+</sup> (µg N/L)	NO <sub>3</sub> <sup>-</sup> (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

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.

fluorescence of DOM in sample-specific ways that are difficult to predict (Spencer and others 2007), the high concentration of DOM in leachate samples should have prevented the loss of detectable fluorescence signals.

We measured uptake of DOC following slug additions 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 (250–350 m) was chosen such that travel time was approximately 30 min. 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<sup>-</sup>/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 ad-

ded DOC could be reliably detected above background without significantly altering reaction kinetics from ambient conditions. Total mass of DOC added ranged from 14.8 to 29.3 g depending on discharge and background DOC concentration. 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. Although we did observe loss of Cl<sup>-</sup> during each experiment, conductivity returned to the ambient value after each experiment, and therefore release of stored Cl<sup>-</sup> likely occurred on a longer timescale than our experiments or resulted in an increase in concentration below the limit of detection and therefore did not influence subsequent experiments.

## Chemical Analysis and Fluorescence

Samples from the uptake experiments were analyzed for concentration of Cl<sup>-</sup> 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<sub>4</sub><sup>+</sup>), nitrate (NO<sub>3</sub><sup>-</sup>), and total dissolved P (TDP). Chloride and NO<sub>3</sub><sup>-</sup> 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<sup>-</sup>/L and 0.5 µg NO<sub>3</sub><sup>-</sup>-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<sub>4</sub><sup>+</sup> was measured by automated colorimetry (Smartchem 170, West-



co 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 the 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  $\mu\text{m}$ , Pall GN-6) using a fluorometer (Jobin-Yvon Horiba Aqualog-800-C, Horiba Instruments) with a 1-cm quartz cuvette (Firefly Scientific). Molecular composition is therefore described in this study by distribution of chromophoric functional groups. Excitation-emission matrices (EEMs) were collected over an excitation range of 240–600 nm every 3 nm and an emission range of 247–604 nm every 2.33 nm with an integration time of 0.1 s 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 leachate samples as well as 184 stream samples from boreal and arctic Alaska (Mutschlecner 2017) that were added to improve the statistical power of the model. 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 and others (2003) and included split-half analysis, inspection of residuals, and random initialization from 10 iterations 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 data published in the OpenFluor database (Supplementary Table 2). PARAFAC components 1-4 were matched to previously described compo-

nents in the OpenFluor database using a 0.95 Tucker convergence coefficient (Murphy and others 2014). Component 5 appeared to consist of two components, but the model was unable to resolve them separately, and this component did not match those present in OpenFluor, but visually matched previously reported excitation and emission curves (Supplementary Table 2; Cory and McKnight 2005). In addition to analysis of fluorescence spectra, we calculated specific ultraviolet absorbance at 254 nm normalized to DOC concentration (SUVA), which is associated with greater aromatic content (Weishaar and others 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 400 nm with log-linear fits (Helms and others 2008).

## DOC Uptake

Biogeochemical retention of DOC in the streams was calculated by mass balance using the method described by Covino and others (2010) to estimate breakthrough curve-integrated measures of uptake. Kinetics of leachate uptake could not be assessed because the reactive fraction of the DOC added was unknown. The mass of tracer recovered ( $T_{\text{MR}}$ ) was calculated for both the conservative ( $\text{Cl}^-$ ) and reactive (DOC) tracers by multiplying the time-integrated tracer concentration ( $T_{\text{C}}$ ) by discharge ( $Q$ ):

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

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

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

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 ( $T_{\text{MR}}(\text{Cl}\%)$ ) was applied to the mass of the added reactive tracer (DOC) to account for hydrologic storage of C (HS):

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

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

$$R = \text{TR} - \text{HS}$$

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<sup>-</sup> 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 sorptive or biological demand, was calculated as stream discharge divided by the product of uptake length and average stream width. Finally, the areal uptake rate ( $U$ , mg m<sup>-2</sup> day<sup>-1</sup>) was calculated by multiplying  $V_f$  by the geometric mean of conservative (based on Cl<sup>-</sup> recovery) and observed background-corrected DOC concentrations throughout the breakthrough curve.

## 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 and others 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) 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 versus 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 and others 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 (PC1) explained 75% of variance and was used in models as described above to test for species and nutrient fertilization effects on leachate chemistry. To test the potential relationship of DOM composition with C uptake, we performed regression analyses of PC1 on uptake metrics ( $V_f$  and  $U$ ). Models incorporated fixed effects of PC1 and nutrient treatment and a random effect of stream, with model selection conducted following the approach described previously.

## RESULTS

### 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). 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). 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). The nine leachates varied in nutrient concentrations but not in concentration of DOC (Table 2). DON concentration for N-fertilized poplar and spruce 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). 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). Soluble C, the percent of total leaf C that was recovered as DOC in leachates, varied little across species and fertilization treatments (Table 2). Soluble N was lowest for alder compared to the other species, and soluble P was lowest for spruce (Table 2).

The leachates from different species varied in molecular composition based on optical measures (Figure 1). These measures only pertain to chromophoric and fluorophoric fractions of the DOM pool, but they revealed strong species-based dif-

**Table 2.** Chemistry of Leaves Grown in Long-Term Fertilization Plots and Their Leachates

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

Species	Leaf 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 <sup>A</sup>	559.0 <sup>A,a</sup>	1545.8 <sup>A,a</sup>	12.3	4.0	36.9
	N	78.8	27.3	506.9 <sup>a,b</sup>	1850.9 <sup>a</sup>	13.2	4.7	46.4
	P	86.5	30.3	91.6 <sup>b</sup>	467.4 <sup>b</sup>	14.4	5.3	65.7
Poplar	Control	93.6 <sup>a</sup>	56.5 <sup>B,a</sup>	1117.3 <sup>B,a</sup>	3630.2 <sup>B,a</sup>	15.4	8.5	47.3
	N	43.9 <sup>b</sup>	27.0 <sup>b</sup>	688.8 <sup>a</sup>	3391.6 <sup>a</sup>	15.2	8.9	76.0
	P	100.2 <sup>a</sup>	101.4 <sup>c</sup>	62.1 <sup>b</sup>	246.3 <sup>b</sup>	17.2	17.8	60.9
Spruce	Control	97.8 <sup>a</sup>	61.3 <sup>B,a</sup>	1269.3 <sup>B,a</sup>	2445.8 <sup>C,a</sup>	12.1	8.0	21.7
	N	49.7 <sup>b</sup>	41.3 <sup>b</sup>	891.7 <sup>a,b</sup>	2484.8 <sup>a</sup>	12.1	10.9	52.7
	P	72.0 <sup>a,b</sup>	62.0 <sup>a</sup>	553.4 <sup>b</sup>	635.0 <sup>b</sup>	10.4	10.2	16.3

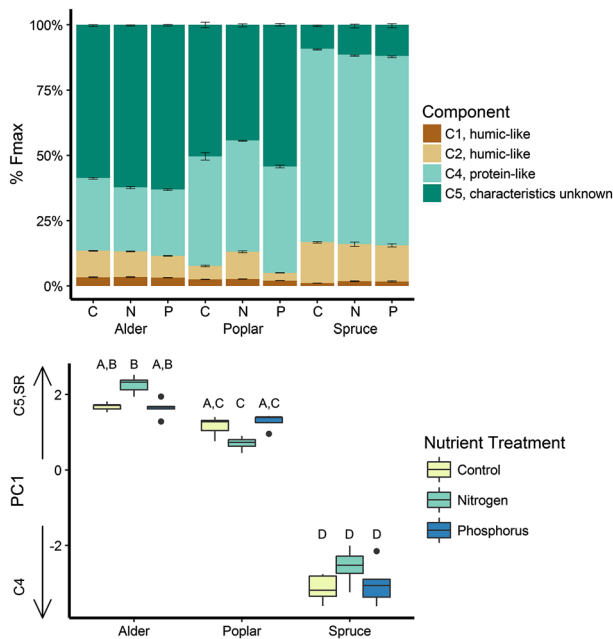
Values represent means of  $n = 5$  analytical replicates. Significant differences were determined for leachate chemistry and leaf dry mass chemistry and are indicated by upper case letter for differences between the controls of each species (species effect) and lower case letters for differences among nutrient treatments within a species (nutrient effect). Letters are omitted where there is no significant difference. Stoichiometric ratios are given in molar units.

ferences. Spruce leachates had the greatest average molecular weight, as indicated by SR, and the greatest aromaticity, as indicated by SUVA (Supplementary Table 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 1). Through comparisons with OpenFluor, Component 1 (Kothawala and others 2014) and Component 2 (Shutova and others 2014) were identified as humic-like (Table 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 and others 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, as different compounds may exhibit similar fluorescence (Murphy and others 2014). Component 4 and

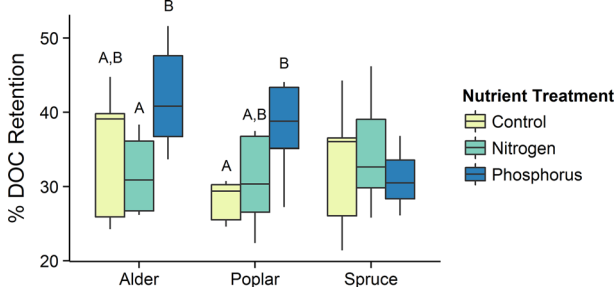
Component 5 differed most strongly between species. For example, spruce leachates had the greatest relative loading of Component 4 and the lowest relative loading of Component 5 (Figure 1). The first component of the principal components analysis conducted on the four PARAFAC components as well as SUVA and SR was positively related to SR and Component 5, and negatively related to Component 4 (protein-like) from the PARAFAC analysis (Supplementary Table 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 1).

### Whole-Stream C Retention

The five headwater streams had similar chemical and hydrologic characteristics. Ambient DOC concentration ranged from 1.06 to 3.5 mg/L and nutrient concentrations varied little among streams (Table 1). Stream discharge varied between streams, ranging 6.4–39 L/s. Chloride was used as a conservative tracer to account for physical retention of water and solutes during C addition exper-



**Figure 1.** Molecular composition of leachates derived from control and fertilized leaves of alder, poplar, and spruce. *Top panel:* percent of fluorescence intensity at the maximum (Fmax) for components identified in leachates by PARAFAC analysis. Error bars indicate one standard error. *Bottom panel:* loadings of principal component 1 (PC1), which summarizes chromophoric and fluorophoric characteristics of the leachate. PC1 was negatively correlated with the protein-like component C4, and positively correlated with the uncharacterized 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 are 1.5 times the interquartile range. *Letters* indicate significant differences between leachates based on a Tukey's honestly significant difference test.



**Figure 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 of control or nutrient treatments among species.

iments. Recovery of  $\text{Cl}^-$  ranged from 40 to 92% of the mass added across all sites, with a median recovery of 75% across all experiments. Stream C3 demonstrated the lowest average  $\text{Cl}^-$  recovery (51%).

Retention of DOC ranged from 20 to 52% of the DOC added as leachate (Figure 2). Peak DOC concentration during the leachate addition was 1.1 mg DOC/L above background, on average (range 0.4–2.3 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 of 300 for the interaction model, 305 and 302 for models including only species or nutrient effects, respectively, indicated a significant effect of nutrients. The model including the interaction term was selected for further analysis, and fixed effects of species and nutrients explained 25% of the variance in uptake rate, with fixed plus random effects, which accounted for differences among streams, explaining a total of 64% of the variance in DOC uptake. Post hoc tests indicated that retention of DOC was significantly greater for leachates from P-fertilized alder relative to N-fertilized alder, and for P-fertilized poplar relative to unfertilized poplar, but there was no difference among nutrient treatments for spruce (Figure 2). Further, post hoc tests revealed that retention of DOC did not differ among tree species grown in control plots (Figure 2).

Metrics of C spiraling were calculated for each slug, producing breakthrough curve-integrated estimates of uptake under experimentally elevated DOC concentration. Across all experiments, mean  $S_w$  was 509 m, mean  $V_f$  was 3.2 mm/min, and mean  $U$  was 887 mg m<sup>-2</sup> day<sup>-1</sup> (Table 3). Mixed effects models indicated no significant relationship between PC1, a metric of molecular composition of DOC, and  $V_f$  (marginal  $R^2 = 0.03$ ) or  $U$  (marginal  $R^2 = 0.13$ ), indicating that the variation in C composition observed among species did not influence C uptake.

## DISCUSSION

Contrasting uptake of C derived from plant species that differ in molecular composition of C and in nutrient content due to long-term fertilization revealed that nutrient content, particularly P, was the primary influence on retention of DOC in boreal headwater streams. Leachates from alder, poplar, and spruce differed in molecular composition of organic C, as demonstrated by differences in optical measures, but similar rates of DOC retention occurred for leachates derived from the three species



**Table 3.** Breakthrough Curve-Integrated Metrics of DOC Uptake Estimated from Addition of Leaf Leachate

Species	Nutrient treatment	$S_w$ (m)	$V_f$ (mm/min)	$U$ (mg m <sup>-2</sup> day <sup>-1</sup> )
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

Values represent means across five streams with 95% confidence intervals.  
 $S_w$ , uptake length;  $V_f$ , uptake velocity;  $U$ , areal uptake.

grown under unfertilized conditions, suggesting that molecular composition of leachates did not influence DOC retention.

Enhanced DOC retention for leachates with a higher P content supports the hypothesis that nutrients constrain processing of organic C in aquatic ecosystems. Our study, like most studies of whole-stream uptake of DOC, cannot distinguish between biotic and abiotic mechanisms of retention, which include sorption, photooxidation, temporary storage in transient storage zones, and biological uptake, all of which occur on timescales compatible with the reaction rates measured here and may be coupled to result in ecosystem-level retention of C (McDowell 1985; Findlay and Sobczak 1996; McKnight and others 2002; Kaplan and others 2008). Photooxidation is likely insignificant in the headwater streams studied here, which are closed-canopy or heavily shaded. Transient storage cannot account for all of the observed DOC retention, as total retention exceeded physical retention determined from the conservative tracer in all experiments. P-enriched DOM stimulated uptake of DOC for the leachates that contained the greatest P content relative to unfertilized controls, whereas no effect of P was observed for P-fertilized spruce, which contained only double the P content of unfertilized spruce. Strong sorption capacity for P by the streambed (Meyer 1979; Reddy and others 1999) may have contributed to preferential uptake of P-rich DOM, and sorption of organic P can also facilitate concentration of the enzymes that decompose DOC (Olsson and others 2012). Alternatively, strong heterotrophic demand for P-containing molecules might have stimulated uptake due to P limitation of microbes. Average ambient N:P in the study streams

was 546:1, over 30 times the Redfield ratio of 16:1 (Redfield 1958), evidence for P limitation. P limitation of DOM processing has been documented in other streams, as evidenced by heterotrophic responses to P fertilization (Elwood and others 1981; Peterson and others 1993; Mohamed and others 1998). Increased phosphatase activity also occurs in response to enrichment with labile C (Oviedo-Vargas and others 2013), indicating the close coupling of C and P cycling in some streams.

In contrast to the effect of P on C uptake, we did not observe an effect of N on DOC uptake. One explanation for this result is that the leachates were relatively more P-enriched than N-enriched (for example, P-fertilized poplar contained 19 times more P than unfertilized poplar, but N-fertilized poplar contained only 2 times more N than unfertilized leaves) and thus were more likely to provide P in excess of a limitation threshold than were N-fertilized leachates, or reduced our ability to detect preferential sorption of N (McKnight and others 1992). Higher solubility of P than N from the leaves caused the contrast in leachate chemistry, with 47% solubility for P compared to 8% percent for N, which is similar to solubilities observed in tropical leaves (Schreeg and others 2013). Lack of an effect of N on DOC retention might also support the notion that P, rather than N, is the primary limiting nutrient in these streams. The effects of organic P addition in P-limited ecosystems may be stronger than the effect of N addition in N-limited ecosystems because of larger relative differences in P availability of substrates compared to microbial demand than occur for N (Hobbie and Vitousek 2000). For example, sorption of inorganic P by sediments may reduce the availability of P in the water column, contributing to stronger limitation

of decomposers by P than N, and resulting in high demand for organic P from DOM. Finally, we found no support for a hypothesis that an association between N content and simple molecular composition of DOM enhances the lability of DOC (Lutz and others 2011), as leachates enriched in DON were retained at a similar rate to unenriched leachates.

Previous studies in both terrestrial and aquatic ecosystems have conflicted on whether molecular composition (Coq and others 2010; Hättenschwiler and Jørgensen 2010; Kiikkilä and others 2013) or nutrient content (Cleveland and others 2006; Wieder and others 2008; Mineau and others 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 influences retention of DOM. 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 and others 2007; Bernhardt and McDowell 2008; Burrows and others 2013; Wymer and others 2015) and also contrasts with in situ estimates of DOC uptake that positively correlated with the protein content of soil-derived leachates (Fellman and others 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 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 and others 2016), indicating that leachate-derived DOC is highly reactive. Ambiguity in the effects of particular components of DOM on the rate of retention might also explain difficulty in assigning capacity for ecosystem retention of DOM to particular molecular attributes. For example, both humic and non-humic proportions of leached DOM are readily biodegraded (Cleveland and others 2004). Such patterns might result if microorganisms are adapted to the compounds found in leachates (Judd and others 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.

Net retention of a significant fraction of the added leachate in all experiments indicated the high reactivity of leachate-derived DOM and estimates of spiraling metrics provide context for this reactivity at the network scale. Values of uptake length ( $S_w$ ) that were longer than the experimental

reaches indicates that although leachate was reactive, rates of biotic and/or physical mechanisms of C retention in these boreal headwater streams are lower than those typically observed for nutrients in headwater streams (for example, Ensign and Doyle 2006). However, uptake lengths were on average shorter than the total length of each of the studied headwater streams (1.9–4.3 km), indicating that cumulative uptake of C along each headwater stream may contribute to significant retention of reactive DOC. Further, uptake metrics were estimated under experimentally elevated DOC concentration, likely yielding overestimates of  $S_w$  under first-order or efficiency loss kinetics. Finally, the high reaction rates of leachate-derived DOC can lead to overestimates of watershed-scale retention of the ambient DOM pool (Mineau and others 2016). Following the approach of Mineau and others (2016), we adjusted  $V_f$  determined from leachate releases using the rate of DOC loss for laboratory incubations of ambient DOC in stream water collected at the same time as the present study from Poker Creek, which includes all five study streams as tributaries (Mutschlecner 2017). We used this adjusted  $V_f$  to calculate relative retention of DOC ( $R$ ) for the entire headwater stream network as  $R = 1.0 - e^{(-V_f/HL)}$ , where hydraulic load (HL) is equal to discharge/width \* length (Wollheim and others 2006) and estimated that approximately 2% of DOC is retained within the headwater stream network. This low removal rate is attributed to recalcitrance of ambient DOM in these streams during summer, with approximately 1–3% DOC loss over 21-day incubations (Mutschlecner 2017), but the extent to which fresh DOM such as that derived from leaf litter, compared to degraded sources contribute to ecosystem retention of C remains unclear. Further, input of labile or nutrient-rich DOM from leaves may have a priming effect on transformation and uptake of ambient DOM in streams (Thouin and others 2009; Hotchkiss and others 2014). Sources of DOM change with the stream discharge regime (Mutschlecner 2017), with leaf litter contributing most strongly to high-latitude streams during spring freshet (Spencer and others 2008), suggesting strong potential for retention of DOC in streams as discharge declines and litter leachate is prevalent following snowmelt.

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 and others 2010; Shanin and others 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 and others 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 and others 2007; Butman and others 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 and others 2015). We have demonstrated coupling between C and nutrient cycles due to P-limitation of DOC retention in boreal streams. Accounting for the coupling of C and nutrient cycles will improve estimates of C storage (Finzi and others 2011), and identifying the nutrients limiting C processing in heterotrophic streams, including stream networks of high latitudes, may provide a key refinement to C budgets.

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