



## RESEARCH ARTICLE

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## Key Points:

- Land cover has a dominating influence on the composition of stream DOM
- Hydrological controls on stream DOM composition are minor
- There was no evidence of in-stream transformations to DOM composition

## Supporting Information:

- Supporting Information S1

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## The relative influence of land cover, hydrology, and in-stream processing on the composition of dissolved organic matter in boreal streams

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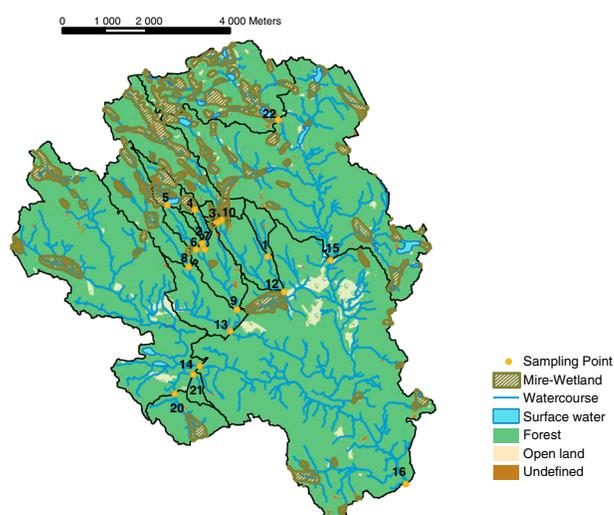
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**Abstract** Low-order boreal streams are particularly sensitive interfaces where dissolved organic matter (DOM) is transported from soils to inland waters. Disentangling the relative influence of key environmental factors suspected to influence stream water DOM composition is highly relevant to predicting the reactivity and fate of terrestrial DOM entering inland waters. Here we examined changes to DOM composition using absorbance and fluorescence, from 17 boreal streams ranging from first to fourth orders, over 14 months, including the rarely studied winter season, and two snowmelt periods ( $n = 836$ ). We also analyzed soil pore water samples from three forest soil lysimeters to a depth of 70 cm ( $n = 60$ ). Of five identified fluorescing parallel factor analysis components, two (C4 and C5) expressed a clear mire wetland or forest signature, providing distinct molecular markers of dominant land cover. In fact, land cover alone explained 49% of the variability in DOM composition. In contrast, seasonal fluctuations in hydrology only contributed to minor shifts (8%) in the composition of stream water DOM, while in-stream transformations to DOM composition were undetectable. These findings suggest that low-order boreal streams act as a passive pipe, since in-stream processing of DOM is restricted by short water residence times (6 h to 2 days). In addition, we demonstrated the sensitivity of optical approaches to distinguish between key terrestrial sources of DOM in the boreal landscape. By distinguishing the proportional leverage of key environmental controls on headwater stream DOM composition, we are better equipped to predict where and when key DOM transformations occur in the aquatic conduit.

### 1. Introduction

A sizable fraction of the global carbon pool is stored within boreal soils [Post *et al.*, 1982]. Consequently, the reactivity and ultimate fate of dissolved organic matter (DOM) released from boreal soils to inland waters is highly relevant to the global carbon cycle [Cole *et al.*, 2007; Raymond *et al.*, 2013; Tranvik *et al.*, 2009]. Low-order streams are particularly sensitive to changes in soil processes [Benstead and Leigh, 2012; Lowe and Likens, 2005], due to the immediate interface with catchment soils. For instance, changes to the timing and intensity of soil warming [Schelker *et al.*, 2013], soil frost [Haei *et al.*, 2010], and spring snowmelt [Ågren *et al.*, 2010] can strongly influence the export of DOM to headwater streams [Lepistö *et al.*, 2008]. While the terrestrial-aquatic interface is recognized as a location for hot spots and hot moments of biogeochemical cycling [McClain *et al.*, 2003], it remains unclear exactly where, and when, these losses and transformations occur to terrestrial DOM upon entering headwater streams.

Recently, CO<sub>2</sub> fluxes have been reported to be up to 3 times higher in small low-order streams, relative to high-order streams [Aufdenkampe *et al.*, 2011]. Even headwater streams of the cooler boreal region are significant sources of CO<sub>2</sub> [Koprivnjak *et al.*, 2010; Wallin *et al.*, 2013]. However, it remains a topic of ongoing debate to establish the relative importance of CO<sub>2</sub> evasion due to in-stream mineralization of DOM versus the lateral transport of CO<sub>2</sub> respired and weathered within catchment soils [Berggren *et al.*, 2007; Moody *et al.*, 2013]. In boreal inland waters, primary productivity and heterotrophic respiration rates are strongly regulated by a combination of cool temperatures, nutrient and light limitations, and the predominantly allochthonous DOM [Bergström *et al.*, 2005; Jansson *et al.*, 2000; Jonsson *et al.*, 2001; Karlsson *et al.*, 2009; Tranvik, 1988]. Since the flux of soil carbon to inland waters is highly sensitive to changing environmental



**Figure 1.** Map of the Krycklan study catchment (69 km<sup>2</sup>), with the location of all stream sites and land cover characteristics.

conditions [Gorham, 1991; Pimm *et al.*, 2009], it is critical to rank the relative influence of key environmental factors such as land cover, seasonally variable hydrological conditions and in-stream metabolism influencing the cycling of DOM released into aquatic ecosystems. Upon entering stream waters, some DOM can be lost or transformed via a set of processes including microbial mineralization and physico-chemical processes such as photooxidation and flocculation [Graneli *et al.*, 1996; Tranvik and Bertilsson, 2001; von Wachenfeldt and Tranvik, 2008]. Additionally, new sources of aquatic DOM can potentially be generated autochthonously from primary productivity [Hood *et al.*, 2005; Kraus *et al.*, 2011].

Land cover across the boreal region of Sweden is predominantly composed of forest (mean 63%) and wetlands including peat mires (mean 12%) [Kothawala *et al.*, 2014]. Differences in the molecular composition of DOM exported from wetlands versus forests can influence the biogeochemical functioning of DOM, such as bioavailability [Berggren *et al.*, 2007]. For instance, wetland-derived DOM tends to be highly colored, with high aromatic content, including a greater proportion of polyphenolic aromatic compounds [Ågren *et al.*, 2008b]. These structurally complex polyphenolic compounds may inhibit enzymatic processes regulating DOM degradation [Freeman *et al.*, 2001]. In fact, several studies have reported reduced bioavailability of stream DOM containing a higher proportion of wetland land cover [Ågren *et al.*, 2008a; Berggren *et al.*, 2007; Fellman *et al.*, 2008]. Thus, defining the influence of land cover on stream DOM composition is valuable.

Boreal streams undergo highly variable hydrological flow patterns, including a few weeks of intense spring runoff, along with periods of prolonged base flow during the summer and winter. Rapid fluctuations in stream runoff have a strong influence on the composition of DOM in ephemeral stream networks [Catalán *et al.*, 2013]. Variable hydrological conditions could mobilize DOM originating from differing soil horizons or influence the spatial connectivity of landscape elements, such as isolated wetlands [Creed *et al.*, 2003; Laudon *et al.*, 2011, 2012]. In light of projected increases to runoff in the studied region of the boreal ecozone [Intergovernmental Panel on Climate Change, 2013], understanding the influence of these strong hydrological dynamics on the chemical composition of stream DOM is highly relevant.

The source of stream water DOM, along with shifts in the molecular composition of DOM due to in-stream processing can be detected with a sensitive optical technique such as fluorescence spectroscopy [R. M. Cory *et al.*, 2007; Jaffe *et al.*, 2008; Stedmon *et al.*, 2003]. While optical approaches provide insight into bulk DOM composition, recent analytical advances have allowed the coupling of fluorescence with detailed molecular level information regarding the elemental and structural composition of DOM [Kellerman *et al.*, 2015; Stubbins *et al.*, 2014]. To this end, spectroscopic approaches, such as fluorescence and absorbance, are promising tools for detecting changes to the molecular composition of DOM, especially since low costs and ease of analysis enable the characterization of numerous samples across a wide range of temporal and spatial scales.

The overall objective of this study was to identify key factors influencing the variability in DOM molecular composition across a range of boreal low order streams. The study includes catchments spanning a wide forest-wetland land cover gradient and multiple seasons including high and base flow conditions. We included soil pore water DOM from different depths to examine the original composition of forest-derived DOM. To identify distinct molecular markers associated with wetland and forested derived stream DOM, we used excitation-emission fluorescence spectroscopy along with a multivariate approach called parallel factor analysis (PARAFAC). Here we use the term “molecular markers” exclusively to describe PARAFAC

**Table 1.** Landscape Characteristics, and the Mean  $\pm$  Standard Error of Dissolved Organic Carbon (DOC), and Number of Samples ( $n$ ), for All Subcatchments in the Krycklan Study Area

Site	Name	Area (km <sup>2</sup> )	Land cover category <sup>a</sup>	Forest (%)	Wetland (%)	Stream Order	DOC (mg L <sup>-1</sup> )	$n$
1	Risbäcken	0.48	Forest	98	2	1	21.1 $\pm$ 0.9	42
2	Västrabäcken	0.12	Forest	100	0	1	19.5 $\pm$ 1.0	38
3	Lillmyrbäcken	0.04	Wetland	24	76	1	35.8 $\pm$ 1.0	35
4	Kalkällsmyren	0.18	Wetland	58	42	1	29.0 $\pm$ 0.9	44
5	Stortjärnen Outlet	0.65	Wetland <sup>b</sup>	55	40	1	24.4 $\pm$ 0.9	42
6	Stortjärnsbäcken	1.28	Mixed	72	25	1	19.9 $\pm$ 0.9	44
7	Kalkällsbäcken	0.47	Mixed	84	16	2	23.5 $\pm$ 0.9	44
9	Nyängesbäcken	2.88	Mixed	85	13	3	17.7 $\pm$ 0.9	45
10	Stormyrbäcken	3.36	Mixed	75	25	2	19.5 $\pm$ 0.9	43
12	Nymrbäcken	5.44	Mixed	83	17	3	19.0 $\pm$ 0.9	45
13	Långbäcken	7.00	Forest	89	9	3	19.5 $\pm$ 0.9	45
14	Åhedbäcken	14.10	Forest	91	5	3	13.9 $\pm$ 0.8	45
15	Övre Krycklan	20.13	Forest	83	14	4	13.2 $\pm$ 0.9	43
16	Krycklan	68.91	Mixed	88	8	4	13.0 $\pm$ 0.9	41
20	Stream from Stormulkälsmyren	1.45	Mixed	88	10		11.6 $\pm$ 0.9	42
21	Stream from Mulkälen	0.26	Forest	99	1		19.3 $\pm$ 0.9	44
22	Bertjärn lake outlet	4.91	Wetland	68	29		17.5 $\pm$ 0.9	46

<sup>a</sup>Categories based on percent coverage by wetland areas: forest (0–5%), mixed (6–25%), and wetland (26–100%).

<sup>b</sup>Lake Outlet.

components that are closely correlated to land cover. Subsequently, we assessed the relative influence of land cover (% forest and % wetland), seasonal fluctuations in hydrology, and the extent of in-stream processing on the PARAFAC components as well as simple-to-use optical indexes.

## 2. Methods

### 2.1. Study Sites and Sampling

A set of 17 streams were sampled year round for 14 months between 17 April 2011 and 2 May 2012, as part of a regular monitoring program at the Krycklan experimental study catchment (Figure 1; 69 km<sup>2</sup>; 64°14' N, 19°46'E) in north-central Sweden [Laudon *et al.*, 2013]. Historically, levels of atmospheric acid deposition of both sulfate and reactive nitrogen have been low (0.21  $\pm$  0.07 g m<sup>-2</sup> y<sup>-1</sup> for sulfate-S and 0.32  $\pm$  0.1 g m<sup>-2</sup> y<sup>-1</sup> for inorganic N) [Oni *et al.*, 2013]. The study sites are representative of a relatively undisturbed boreal landscape, but ditching occurred in many streams during the early twentieth century to improve forest productivity, and some selective forest cutting and clear-cuts have occurred. Sampling of Krycklan streams generally occurred every other week, with greater sampling frequency during spring melt (2 to 3 days), and monthly sampling during the stable winter base flow conditions following the protocol described in Laudon *et al.* [2013]. This resulted in each stream being sampled between 36 and 47 times, depending on the availability of water during dry periods. Streams drained catchments ranging in size from 0.03 to 69 km<sup>2</sup>, and stream order from first to fourth orders (Figure 1 and Table 1). Forest vegetation was primarily Scots Pine (*Pinus sylvestris* L.) in dryer upslope regions and Norway spruce (*Picea abies* L.) in lower wetter regions. Vegetation in wetland areas was pre dominantly peat composed of *Sphagnum* [Yurova *et al.*, 2008]. The land cover in catchments varied from 24 to 100% forests, 0 to 76% wetlands, which are classified as mires, and <5% covered by lakes. Sites were divided into three land cover categories based on the area of mire-wetland cover and were defined accordingly as wetland (>26% mire area), mixed (6 to 25% mire area) and forest (<6% mire area) sites.

Soil water in the forest (site 2) was sampled along a transect consisting of three nested sites with suction lysimeters at soil depths ranging from 5 to 90 cm, located 4 m (S4), 12 m (S12), and 22 m (S22) from the stream [N. Cory *et al.*, 2007]. Riparian soils were covered with *Sphagnum* with high organic carbon content up to a depth of 60 cm. Most of the well-drained upland forest soils were podzols [Laudon *et al.*, 2004b; Petrone *et al.*, 2007]. Soil waters were only analyzed for a limited time period of 5 weeks prior to spring melt.

## 2.2. Climate and Hydrology

The climate was typical of the boreal ecozone with a 30 year mean annual temperature of 1.7°C, and precipitation of 600 mm, of which 35% falls as snow [Laudon *et al.*, 2013]. Total runoff in the 2011 and 2012 calendar years were 222 mm and 414 mm, respectively. The majority of snowmelt occurred within a 2 month period between April and early June 2011, accounting for 37% and 30% of the total annual runoff for 2011 and 2012, respectively. There were several months of base flow conditions in the summer (May–July 2011) and winter (November 2011 to March 2012). Discharge ( $Q$ ) was measured using a 90° V notched weir housed in a heated shed (located at a headwater catchment, site 7 [see Laudon *et al.*, 2013]), and mean daily specific discharge ( $L s^{-1}$ ) was calculated from continuous readings of stream water height in the weir house measured with pressure transducers and established stage-discharge rating curves built up from salt dilutions or time-volume methods [Laudon *et al.*, 2013]. Mean daily discharge was converted to specific daily runoff ( $mm d^{-1}$ ) by scaling discharge to catchment area.

## 2.3. Water Chemistry, Absorbance, and Fluorescence Spectroscopy

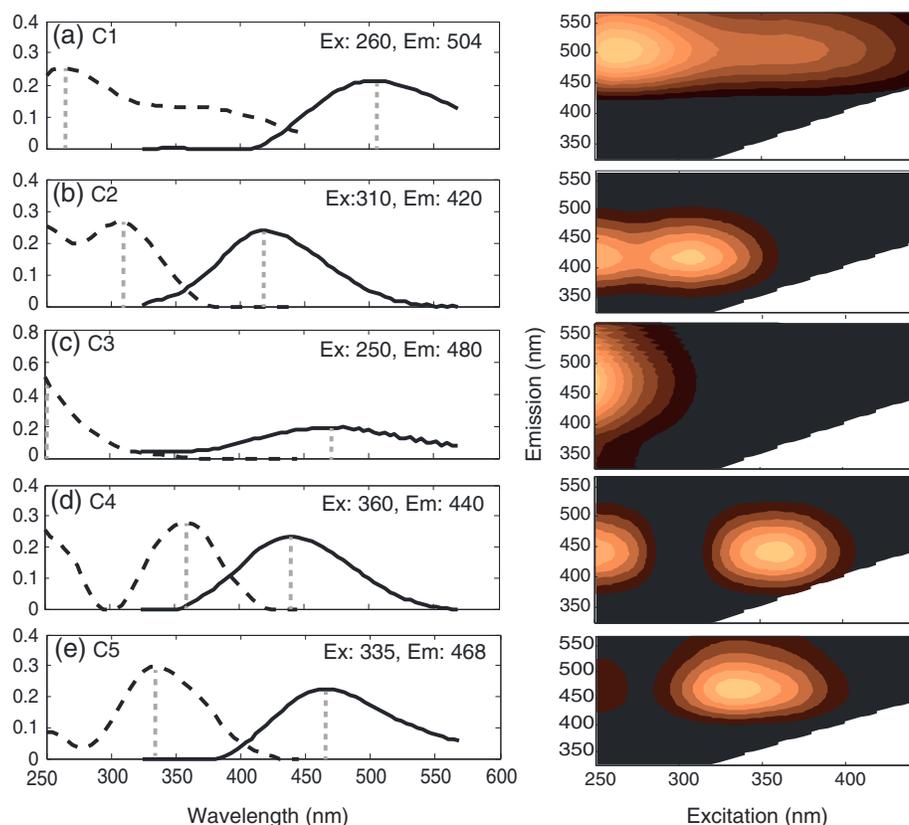
Routine chemical analyses were conducted to quantify total organic carbon and pH [Laudon *et al.*, 2013]. All samples were filtered prior to spectral analysis using pre rinsed regenerated cellulose 0.45  $\mu m$  syringe filters (Satorius Stedim, Göttingen, Germany). The absorbance spectra of filtered DOM was measured across a wavelength range from 200 to 600 nm, at 1 nm intervals at a scan speed of 240  $nm min^{-1}$  and slit width of 2 nm using a Lambda 40 UV-visible spectrophotometer (Perkin Elmer, Waltham, MA, USA). Samples were measured in a 1 cm quartz cuvette using Milli-Q water as the blank. Two absorbance metrics were assessed: (i) the specific UV absorbance (SUVA;  $L \cdot mg^{-1} C \cdot m^{-1}$ ) was calculated as the dissolved organic carbon (DOC) normalized absorbance at 254 nm, which is positively related to aromatic content [Weishaar *et al.*, 2003], with values spanning from <1 to a maximum of 6.0, and (ii) the absorbance ratio, measured at 254 and 365 nm (A254:A365).

Fluorescence scans of synchronous excitation-emission matrices (EEM) were measured using a fluorescence spectrophotometer (SPEX FluoroMax-2, Horiba Jobin Yvon, New Jersey, USA) and a 1 cm quartz cuvette. Samples were measured in ratio mode ( $S_c/R_c$ ), whereby sample signal ( $S$ ) and lamp reference signal ( $R$ ) were corrected ( $c$ ) for instrument specific biases. Excitation wavelengths ( $\lambda_{ex}$ ) spanned from 250 to 445 nm, with an increment of 5 nm, while emission wavelengths ( $\lambda_{em}$ ) ranged from 300 to 600 nm at increments of 4 nm. Excitation and emission slit widths were set to 5 nm, and the integration time was 0.1 s. All EEMs were blank-subtracted using the EEM of Milli-Q water. All fluorescence spectra were corrected for inner filter effects using the absorbance-based approach previously tested on the concentration range of this data set [Kothawala *et al.*, 2013; Lakowicz, 2006]. Fluorescence intensity was normalized to Raman units (RU) by dividing the fluorescence intensity by the Raman area of pure water integrated at an excitation of 350 nm and over an emission range of 380 to 420 nm [Lawaetz and Stedmon, 2009]. All corrections were performed using the DOMcorr toolbox [Murphy *et al.*, 2010].

Commonly used fluorescence indexes were explored to evaluate if differences in DOM quality could be detected. The fluorescence index (FI) was calculated as the ratio between emission at wavelengths of 470 and 520 nm, at an excitation of 370 nm [Cory *et al.*, 2010; McKnight *et al.*, 2001]. The FI typically ranges between 1.2 and 1.8, with lower FI values (<1.4) related to higher aromatic content typical of terrestrially-derived DOM, while higher values (>1.4) suggest lower aromaticity and a greater extent of microbially processed DOM [McKnight *et al.*, 2001]. The freshness index ( $\beta: \alpha$ ) was calculated as the ratio of emission at 380 nm ( $\beta$  peak), divided by the emission maxima between 420 and 435 nm ( $\alpha$  peak), at an excitation of 310 nm [Parlanti *et al.*, 2000]. The freshness index measures the extent of blue-shifting along this fluorescence band, and the blue-shifted  $\beta$  peak has been found to be the result of biological activity [Parlanti *et al.*, 2000].

## 2.4. Statistical Analysis

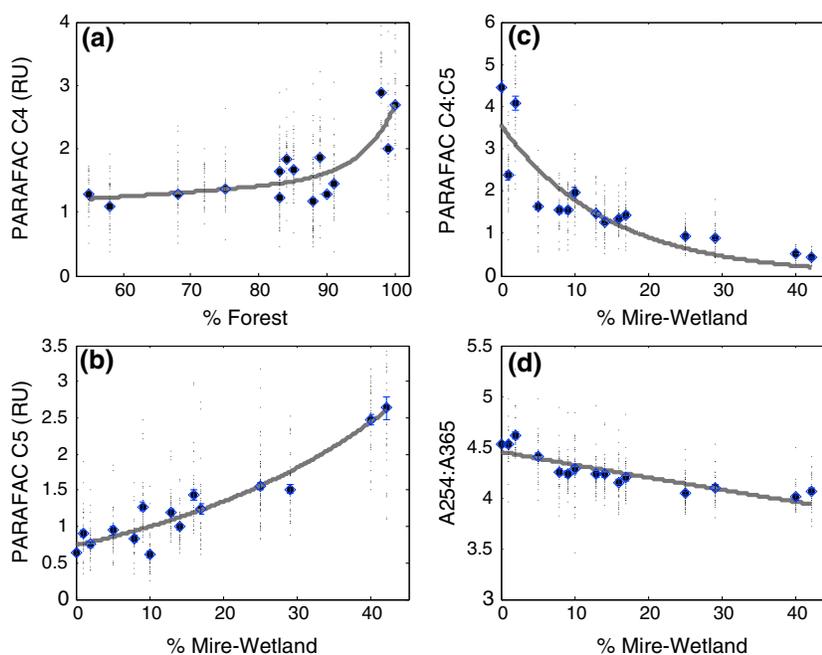
A multi way statistical approach called parallel factor analysis (PARAFAC) was applied to decompose the fluorescence signal into underlying fluorescent components [Bro, 1997]. PARAFAC statistically identifies the intensity of underlying components across the data set, which is useful for tracing the source and fate of individual fluorescent components in the environment [Stedmon and Bro, 2008]. Individual PARAFAC components are considered to represent groups of compounds with similar molecular composition and



**Figure 2.** Excitation (dashed lines) and emission (solid lines) loadings for five PARAFAC components (C1 to C5), with peak maxima identified with grey dashed lines. Associated contour plots of three-dimensional spectra to the right.

biogeochemical functioning [Ishii and Boyer, 2012]. Here we used PARAFAC to characterize DOM fluorescence across a data set of EEMs including 836 stream samples and 55 soil water samples. The fluorescence of water, called Rayleigh scatter, is largely removed from sample EEMs during blank subtraction. However, prior to PARAFAC analysis, data in the region of first- and second-order Rayleigh scatter were removed by inserting a band of zeros to ensure that any residual scatter was not inadvertently modeled. Samples with strong leverage were evaluated and potentially removed as outliers. After initial analysis, four, five, or six components appeared to adequately describe the data set. After detailed examination of the sum of squared residuals, and performing a set of 10 random initializations followed by split-half analysis, it became apparent that five independent components best described the variability in fluorescence across the data set of EEMs, while minimizing residuals. A commonly used toolbox, DOMFluor [Stedmon and Bro, 2008] was used to perform all steps involved in PARAFAC analysis. In addition to assessing the intensity of PARAFAC components ( $C_i$ ), changes to the relative composition of fluorescence components was evaluated and expressed as a percentage (% $C_i$ ) of the sum of all five component intensities ( $\sum C_i$ ) using,  $\%C_i = C_i / \sum C_i \times 100\%$ .

We conducted a redundancy analysis (RDA) to identify the relationship between all five PARAFAC components, land cover predictors (%wetland and %forest), runoff, catchment area, and season. RDA is a multivariate regression technique [Legendre and Legendre, 2012], whereby a matrix of response variables (the PARAFAC component scores) are related to a matrix of predictors (land cover, runoff, catchment area, and season), allowing us to disentangle their relative influence on the composition of DOM. PARAFAC component scores were Hellinger transformed [Legendre and Legendre, 2012] prior to analysis. The Hellinger transformation was performed so as to facilitate comparisons between proportions of PARAFAC components, as opposed to their absolute magnitude. Seasons were treated as categorical variables representing spring snowmelt, summer base flow, autumn high flow, and winter base flow. RDA analysis and variance partitioning was performed in Canoco v.5.



**Figure 3.** Relationships between (a) the percentage of forest cover and the fluorescence intensity of PARAFAC component C4, (b) the percentage of mire-wetland cover and component C5, (c) the percentage of mire-wetland area and C4:C5, and (d) the percent mire-wetland area with the absorbance ratio (A254:A365). Regressions are based on median values for each stream (large diamonds), with error bars representing the standard error and fine dots showing the temporal variability (April 2011 to July 2012).

To identify if there were any shifts to bulk DOM composition with runoff, we considered if there were statistically significant differences in the slope of relationships between (i) the relative intensity of components (% $C_i$ ) and (ii) the DOC normalized fluorescence intensities ( $C_i$ /[DOC]) with runoff. Prior to analysis, we grouped sites based on land cover (wetland or forest) and season (spring melt or other seasons).

Since the %wetland cover and [DOC] were strongly correlated, we used variance partitioning to determine what fraction of the variance in PARAFAC components could be explained by just [DOC] alone, the % wetland cover alone, the shared influence of [DOC] and %wetland, and the unexplained fraction.

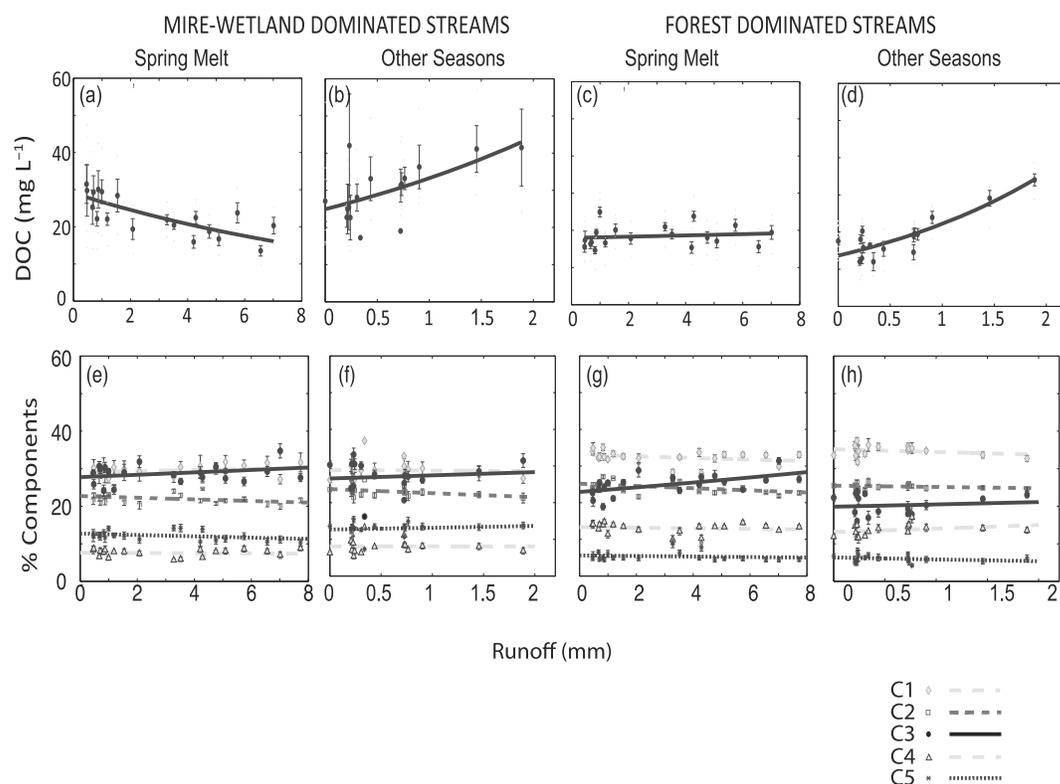
Analysis of variance (ANOVA) was used to test for differences in group means (seasons, land cover types, and stream order), and two-way ANOVA was used to identify interactions between drivers.

### 3. Results

#### 3.1. Component Intensities and Land Cover

All five PARAFAC components describing stream DOM fluorescence were characteristic of fluorescence derived from terrestrial origin and are generalized as having humic-like fluorescence [Fellman *et al.*, 2010] (Figure 2). A distinct protein-like peak was not detected nor was there any systematic evidence of residual fluorescence in the protein-like region. Of the five components, C1, C2, and C3 are among the most commonly observed components found across PARAFAC studies [Ishii and Boyer, 2012], with C2 and C3 being located where Peak M and Peak A are located, respectively [Fellman *et al.*, 2010]. C4 and C5 are unique, yet their excitation and emission spectra fit the criteria for being viable components [Bro, 1997], and they predominantly cover the region associated with Peak C [Fellman *et al.*, 2010].

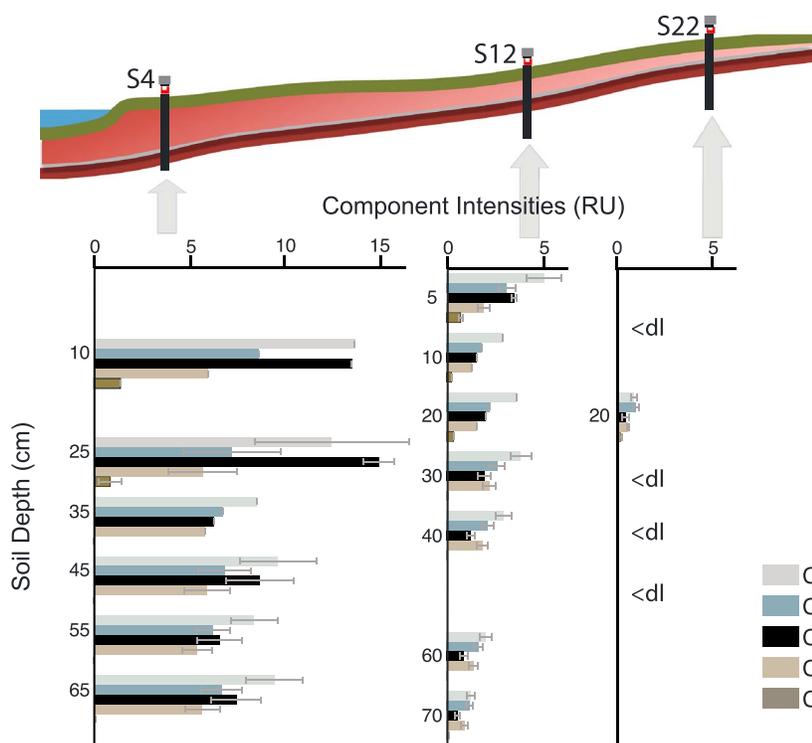
It was apparent that two PARAFAC components, C4 and C5, were most strongly correlated to land cover, specifically the %forest and %wetland area, respectively (Figures 3a and 3b). The intensity of C4 was found to increase substantially at sites when forest cover was in the range from 80 to 100% ( $R^2 = 0.74$ ;  $P < 0.0001$ ; Figure 3a). In contrast, there was a continual positive increase in the intensity of C5 with %wetland cover



**Figure 4.** (a–d) Relationships between runoff and dissolved organic carbon (DOC) concentrations or (e–h) the percentage of PARAFAC components (components C1 to C5 identified in legend) for streams draining catchments dominated by forest or mire-wetland, and depending on the season, either during spring snowmelt or all other seasons. Note that the scale of the x axis is variable between seasons. Equations for the curve fits and significance of the slope are presented in Table S1 in the supporting information.

( $R^2=0.91$ ;  $P<0.0001$ ; Figure 3b). In fact, when streams were categorized into land cover classes (forest, mixed, and wetland), it was clear that streams draining wetlands contained significantly greater amounts of C5 (mean  $\pm$  standard error;  $2.58 \pm 0.05$  Raman Units (RU),  $n=216$ ) than mixed ( $1.22 \pm 0.04$  RU,  $n=315$ ), or forest streams ( $0.92 \pm 0.05$  RU,  $n=219$ ), with only trace amounts of C5 found in soil water from upland forests ( $0.13 \pm 0.09$  RU,  $n=55$ ) (supporting information Figure S1a). In contrast, C4 was most abundant in upland forest soil water ( $3.26 \pm 0.11$  RU), with significantly lower intensities in streams draining forests ( $2.16 \pm 0.06$  RU), mixed vegetation ( $1.47 \pm 0.05$  RU), and wetlands ( $1.22 \pm 0.06$  RU) (Figure S1a). The relative intensity of forest to wetland components (C4:C5) resulted in a particularly good relationship with % wetland (Figure 3c;  $R^2=0.53$ ,  $P<0.0001$ ). Thus, from here on, we refer to C4 as the forest component and C5 as the wetland component, with each representing distinct molecular markers of contrasting terrestrial DOM sources. We found the simple absorbance ratio (A254:A365) had a strong negative linear relationship with %wetland area (Figure 3d;  $R^2=0.61$ ,  $P<0.0001$ ). Components C1, C2, and C3 were poorly differentiated by land cover and were abundant in all streams, with one notable exception whereby C3, rather than C1, was most abundant at wetland streams (Figure S1b).

While PARAFAC components, C4 and C5, can be extrapolated from future data sets of stream DOM fluorescence spectra, we found it useful to examine if a direct ratio of fluorescence intensities at wavelengths corresponding to the peak maxima of forest component, C4 ( $\lambda_{\text{ex}}=360$ ,  $\lambda_{\text{em}}=440$ ), and wetland component, C5 ( $\lambda_{\text{ex}}=335$ ,  $\lambda_{\text{em}}=468$ ), could be used as a simple and direct predictor of DOM source. This simple peak picking approach resulted in a strong positive relationship with %forest ( $R^2=0.60$ ,  $P<0.0001$ ), and negative relationship with %wetland ( $R^2=0.58$ ,  $P<0.0001$ ). From here on we refer to this index as the forest-wetland index, which is solely provided as an alternative to PARAFAC components for distinguishing between land cover types.



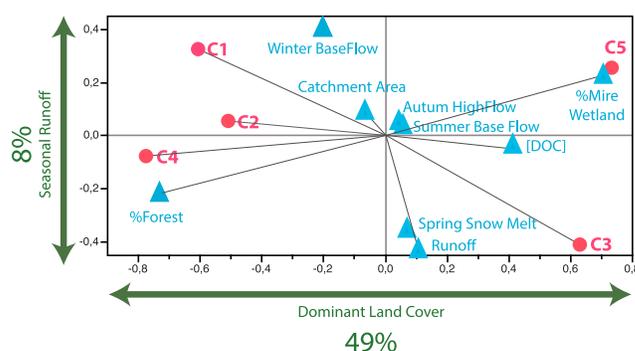
**Figure 5.** PARAFAC component intensities in Raman units (RU) within the soil profiles of three soil lysimeter pits, S4, S12, and S22, situated 4, 12, and 22 m from the stream, respectively, at a forest dominated site. In some cases, samples at S22 were available; however, fluorescence intensities were too low for detection (<dl).

### 3.2. Component Intensities and Hydrological Fluxes Across Seasons

Both positive and negative DOC-runoff relationships were found across this data set. The direction of the DOC-runoff relationship depended on the predominant land cover and seasonality (Figures 4a–4d and Table S1). For instance, at wetland sites a negative DOC-runoff relationship was observed during spring melt (Figure 4a;  $P = 0.0002$ ), while a positive DOC-runoff was observed during all other seasons (Figure 4b;  $P = 0.013$ ). Forest sites also had a positive DOC-runoff relationship during all other seasons (Figure 4d;  $P < 0.0001$ ), but during spring melt the DOC-runoff relationship was directionless (Figure 4c;  $P > 0.5$ ). The relationship between all five PARAFAC component intensities and runoff mimicked the patterns observed with DOC concentration (not shown). Thus, we attempted to identify shifts to PARAFAC components with runoff by comparing the slope of their relative intensities. Accordingly, if the slope of the relationship between %C<sub>i</sub>, or C<sub>i</sub>/[DOC] and runoff did not differ significantly from zero, the composition of DOM would be considered unaffected by runoff. Even with this approach, we could only detect one significant shift in the percentage of components relative to each other with runoff (Figures 4e–4h and Table S1). The %C<sub>3</sub> became more abundant at the highest runoff levels found during spring melt at forest sites (Figure 4g;  $P = 0.008$ ). The positive influence of runoff on %C<sub>3</sub> suggests that it was the only component to be actively mobilized from forest sites at elevated runoff and higher water table.

### 3.3. Component Intensities in Upland Soil Pore Waters

To further understand the spatial distribution and origin of PARAFAC components leached from forest soils, we examined the abundance of components in soil water from three soil lysimeters located along a transect spanning from the riparian area (S4), to increasingly well-drained upland soils (S12 and S22; Figure 5). Only the upper 20 cm of the upland site S22 was assessed since fluorescence intensities were too close to detection limits for the other soil depths. At the riparian (S4) and upland (S12) lysimeters the composition of DOM was similar between deeper horizons (45 to 65 cm); however, the upper horizons (10 and 25 cm) contained greater amounts of C<sub>3</sub>. In addition, the amount of forest component (C<sub>4</sub>) remained constant with



**Figure 6.** Redundancy analysis (RDA) with PARAFAC components as response variables (red circles) and environmental variables as explanatory variables (blue triangles). RDA analysis includes all stream water samples ( $n = 747$ ). Continuous variables have lines running to the origin.

depth (10 to 65 cm) at the riparian site (Figure 5), but comprised a greater proportion of total fluorescence (%C4) with increasing depth (not shown). Only trace amounts of the wetland component (C5) could be detected within the uppermost horizons (5 to 25 cm) of the riparian or upland sites (S4 or S12) (Figure 5). The fluorescence intensity of C1 and C2 remained constant with depth, with C1 remaining greater than C2. The intensity and proportion of C3 were particularly large in the upper horizons (10 and 25 cm) of the riparian site (Figure 5).

### 3.4. Influence of Stream Order and RDA Analysis

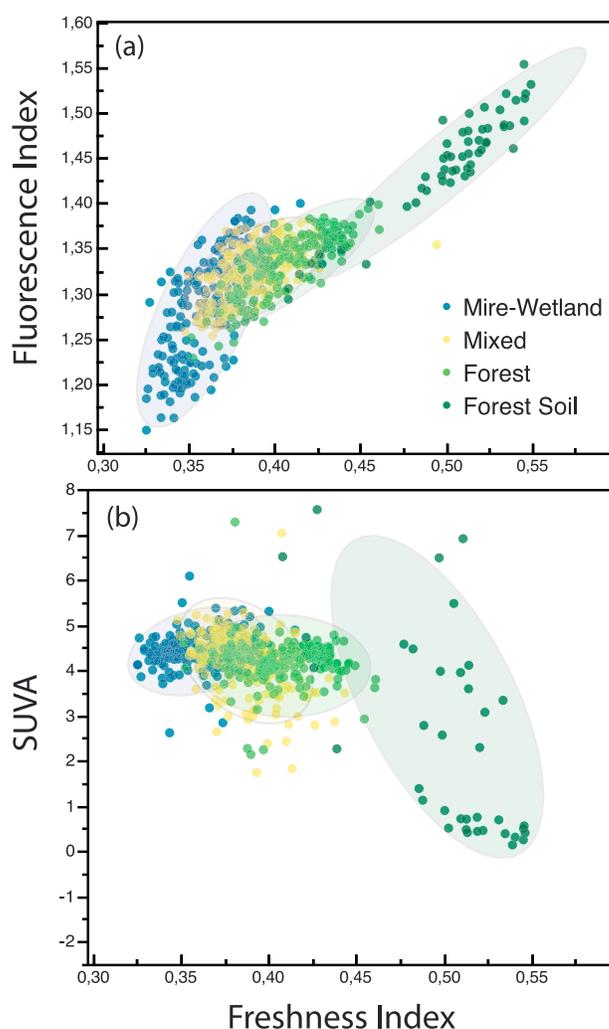
Since a multitude of variables were correlated with stream order (including land cover), we assessed the influence of stream order relative to other key explanatory variables using RDA analysis with the full spatial and temporal data set (Figure 6). Rather than classifying streams as being one of four stream order classes, we used  $\log(\text{catchment area})$ , as a more effective continuous variable, similar to stream order representing proximity to the headwaters. Catchment area would provide an indication of in-stream processing. From RDA analysis, it was clear that catchment area had very little influence on DOM fluorescence composition, as it plotted near the origin of the first and second axes, indicating that catchment area (and stream order) had very little leverage in explaining the variability of PARAFAC components (Figure 6). The RDA analysis confirmed that land cover, particularly the %wetland and %forest were dominant variables driving the composition of DOM, explaining 49% of the total variability in PARAFAC components (axis 1). It was also clear that the seasonally variable level of runoff was of secondary importance (axis 2), only explaining a further 8% of the variability in PARAFAC components (Figure 6). The contribution of individual variables toward the total explained variability (58.8%) is provided in Table S2 in the supporting information, with catchment area explaining only 1% of the variability.

### 3.5. %Wetland or [DOC]

While land cover was a particularly important predictor of the composition of DOM, [DOC] and % wetland were strongly correlated; thus, we attempted to distinguish their relative influence on DOM composition using variance partitioning. Variance partitioning revealed that 26% of the variability in PARAFAC components could be explained by %wetland cover alone, with only 0.9% explained by [DOC] alone, while 12% of the variability was shared and 60% was unexplained by either %wetland or [DOC].

### 3.6. Spectral Indexes and Land Cover

Upon exploring the usefulness of well-used optical indexes at detecting differences in DOM quality between land covers, we found the freshness and fluorescence indexes to be far more effective than SUVA (Figure 7a). We note that quenching of fluorescence due to iron (Fe) interference was ruled out since Fe:DOC was low (mean 0.011 and maximum 0.04) [Poulin *et al.*, 2014]. Consequently, indexes based on fluorescence were robust to Fe interference. The freshness index indicated significantly different values between land cover types with a mean  $\pm$  standard error of  $0.36 \pm 0.02$  for wetland sites ( $n = 216$ ),  $0.39 \pm 0.01$  for mixed sites ( $n = 315$ ), and  $0.41 \pm 0.02$  for forest sites ( $n = 219$ ) ( $P < 0.0001$ ) (Figure 7a). Likewise, there were significant differences in the fluorescence index for wetland  $1.28 \pm 0.06$  ( $n = 216$ ), mixed  $1.32 \pm 0.03$  ( $n = 315$ ), and forest  $1.33 \pm 0.03$  ( $n = 219$ ) sites ( $P < 0.0001$ ) (Figure 7a). The freshness and fluorescence indexes for upland forest soils ( $0.51 \pm 0.01$  and  $1.46 \pm 0.03$ , respectively) were significantly higher than all stream samples ( $0.38 \pm 0.03$  and  $1.32 \pm 0.05$ , respectively; Figure 7a). In contrast, SUVA was far poorer at differentiating DOM quality from different land cover types (Figure 7b), with approximately 10% of samples containing  $> 0.5 \text{ mg L}^{-1}$  Fe, and potentially giving rise to some Fe interferences [Weishaar *et al.*, 2003]. There was no significant difference in the SUVA of streams draining forest ( $4.10 \pm 0.05$ ) and mixed ( $4.21 \pm 0.04$ ) sites;



**Figure 7.** Grouping of boreal stream dissolved organic matter based on common optical indexes: fluorescence index, specific absorbance (SUVA), and freshness index, by land cover.

export more labile low molecular weight substances including amino acids and organic acids [Berggren *et al.*, 2009], which may be recently excreted photosynthates from mycorrhizal plant roots [Berggren *et al.*, 2010]. The distinct land cover molecular markers identified here (e.g., C4, C5, and wetland-forest ratio) provide useful tools to predict the fraction of DOM originating from forest versus wetland sources.

Despite the overlapping fluorescence spectra of wetland (C5) and forest (C4) components, and excitation-emission maxima in close proximity to each other (Figure 2), RDA analysis confirmed the independent nature of C4 and C5, as driven by land cover. The broad distribution of land cover (up to 100% forest, and up to 76% wetland) and large sample size ( $n = 836$ ), included in this data set, were likely important factors contributing to the resolution of these two spectral regions (C4 and C5). These distinct spectroscopic molecular markers associated with wetland- and forest-derived DOM clearly demonstrate that the source of terrestrially derived DOM can be clearly resolved into land cover sub categories.

The variable direction of DOC-runoff relationships depending on both land cover and seasonality (Figure 4) provided an ideal foundation for evaluating hydrological controls on DOM quality. Statistically significant positive DOC-runoff relationships during seasons other than spring melt indicate an unlimited abundance of DOC in the soils and that transport is only limited by water flux. In contrast, negative DOC-runoff relationships at wetland sites during spring melt are attributed to a dilution effect resulting from the formation of an impermeable frozen layer of peat, restricting the flow of water to shallow surface and

however, wetland streams had significantly higher SUVA ( $4.41 \pm 0.05$ ) than the forest and mixed streams ( $P < 0.0001$ ) (Figure 7b). In contrast, a wide range of variability was observed in the SUVA of forest soil samples spanning from  $<1$  to  $>6$  (Figure 7b).

#### 4. Discussion

This detailed study of more than 800 samples across 17 low-order boreal streams spanning over multiple seasons reveals that land cover, specifically the relative coverage of wetland to forest area, was the dominating factor controlling DOM composition (49%), while hydrological controls were minor (8%), and finally in-stream processing of DOM did not contribute to changes in DOM composition.

We identified unique molecular markers associated with forest- and wetland-derived DOM, which provide critical insight into both bioavailability and the ultimate fate of DOM passing through inland waters (Figure 3). Although the proportion of wetland cover is known to strongly influence the areal export rates of DOC [Dillon and Molot, 1997; Kortelainen, 1993], previous investigations have found that forest-derived DOM is up to 2 times more bioavailable than wetland-derived DOM [Ågren *et al.*, 2008a; Berggren *et al.*, 2007]. In fact, forest-derived DOM has been found to

overland flow, hence constraining transport of DOC [Laudon *et al.*, 2011]. At forest sites, DOC-runoff relationships remained stable during spring melt, suggesting a balance between subsurface flow paths passing through more organic rich riparian soils and a dilution effect. Both positive and negative DOC-runoff relationships have been previously reported at Krycklan sites [Laudon *et al.*, 2004a, 2011, 2012; Oni *et al.*, 2013], and other study sites [Eckhardt and Moore, 1990; Eimers *et al.*, 2008], yet it has remained unclear until now how highly variable runoff levels influence the molecular composition of DOM. We found the percentage of PARAFAC components, and therefore DOM composition, to remain surprisingly stable as a function of runoff. One notable exception was found at forest sites, where C3 increased relative to other components (%C3) during peak spring melt (Figure 3g). This was likely explained by the higher abundance of C3 in uppermost soil horizons (10 and 25 cm). In contrast, the deeper riparian soil horizons (45 to 65 cm) maintained a highly homogeneous molecular composition (Figure 5). Consequently, the composition of stream water DOM reflected deeper riparian soil DOC during low flow seasons, until the water table rose to saturate the upper 25 cm soils during spring melt, potentially mobilizing more C3 from riparian soils to stream waters. While the analysis of soil waters was limited to 5 weeks prior to spring melt, the spatial distribution of components in the soil horizons provided an indication as to why C3 was most abundant during spring melt. Previous studies in Swedish boreal headwaters have also demonstrated that in-stream chemistry is largely regulated by riparian soils (e.g., lysimeter S4) [Seibert *et al.*, 2009], and stream runoff has been directly related to groundwater depth [Grabs *et al.*, 2012; Laudon *et al.*, 2004b]. The stable fluorescence characteristics of stream water DOM is likely explained by the selective soil-solution partitioning of organic matter associated with aluminum and iron in the riparian soil water of the forest and wetland sites, respectively [Köhler *et al.*, 2014]. We note that C5 is highly comparable to the region of fluorescence found to be selectively removed by flocculation during drinking water treatment [Lavonen, 2015], and consequently C5 may be in part regulated by adsorption processes. Significant changes to DOM composition in soil water have been previously reported, whereby hydrological fluxes were linked to soil degradation and adsorption-desorption processes [Shen *et al.*, 2015]. Since our analysis of soil water was temporally limited, we were unable to confirm these controls.

No significant changes to DOM composition could be detected spatially from first to fourth order streams, suggesting trace levels of in-stream degradation or production. Substantial DOM losses (almost 45%) have been previously attributed to respiration in a boreal stream study performed a much larger spatial scale (3025 km<sup>2</sup>), with a catchment area 45 times larger than Krycklan [Jonsson *et al.*, 2007]. In fact, significant DOM losses and changes to DOM composition are commonly reported in higher order streams and river outlets, including a large arctic fluvial basin [R. M. Cory *et al.*, 2007; Larson *et al.*, 2014; Williams *et al.*, 2010; Wilson and Xenopoulos, 2008]. A particularly noteworthy finding arising from PARAFAC analysis of Krycklan streams, however, was the absence of any distinguishable protein-like fluorescence. The intensity of protein-like fluorescence remained undetectable even during summer months at the outflow of a small headwater lake (Site 5, Stortjärnen Outlet), where we expect primary productivity and respiration rates to be highest. Previous studies have linked protein-like fluorescence to by-products of microbial consumption and primary production of algal-derived DOM [Guillemette and del Giorgio, 2012; Lønborg *et al.*, 2010], and the amount of bioavailable DOM has been positively related to protein-like fluorescence (Peak T) [Fellman *et al.*, 2008, 2009; Guillemette and del Giorgio, 2011]. In fact, a recent boreal lake study including over 500 sites spanning across the full geographical range of Sweden found protein-like fluorescence to be a common feature of lake spectra, which was positively related to water residence time [Kothawala *et al.*, 2014]. The presence or absence of protein-like fluorescence can be interpreted as a result of both the supply and demand of labile substrates. Here we suggest the lack of protein-like fluorescence as being the result of low bacterial activity, or low supply, along with a high demand for labile substrates.

Incubation studies may provide some insight into the undetectable changes we observed to in-stream DOM composition. One previous study has reported unusually high DOM loss rates (73% over 10 days) [Moody *et al.*, 2013]; however, stream water was incubated under intense solar radiation, which would be highly uncommon for low-order boreal streams that tend to be well shaded by the dense tree canopy. Based on most dark bioassay studies, <10% of the DOM pool is utilized within 7 to 11 days [Köhler *et al.*, 2002; Moran and Hodson, 1990; Tranvik, 1988], and likewise for Krycklan streams [Berggren *et al.*, 2007]. In fact, shorter bioassays (3 days) better reflect the time scale of water passing through low-order streams (0 to 2 days) and tend to result in less than 3% loss of the total DOC pool [Guillemette and del Giorgio, 2011]. The undetectable

protein-like fluorescence observed here provides further evidence of low microbial activity and trace levels of labile substrates which may be tightly cycled by the microbial community, localized within benthic biofilms [Battin *et al.*, 2003]. The lack of protein-like fluorescence was, however, surprising, as a comparable study on several hundred Swedish boreal lakes, including a high number of low-order lakes, detected a clear and reoccurring protein-like peak [Kothawala *et al.*, 2014]. In addition, the protein-like fluorescence detected from these Swedish lakes was confirmed to be well correlated to elemental composition (H:C and O:C) of proteins using detailed Fourier transform ion cyclotron resonance mass spectrometry (FTICR-MS) [Kellerman *et al.*, 2015]. A key limitation to fluorescence is that detection is focused on functional groups containing fluorophores, which are assumed to be homogeneous across all DOM compounds. At present, there remains a need for future studies to clearly link the protein-like fluorescence to stream metabolism, particularly in low-order boreal streams. Currently, measurements of stream ecosystem metabolism including benthic measurements and the water column in boreal systems remain rare [Attermeyer *et al.*, 2013; Burrows *et al.*, 2014; Franke *et al.*, 2013]. While there is likely some heterotrophic uptake and autotrophic production of DOM within headwater streambeds, rates of mineralization, production, and exchange between biofilms and the overlying water column may not be high enough to significantly alter bulk molecular characteristics of DOM. In addition, with movement downstream, we can expect continual lateral inputs of allochthonous DOM from the surrounding catchment [Müller *et al.*, 2013], which may offset the detection of internally produced DOM. Both light and nutrients are likely limiting factors for bacterial and algal activity in these low-order boreal streams. Although soil-derived DOM can be particularly sensitive to photodegradation [Cory *et al.*, 2014; Olefeldt *et al.*, 2013], in the case of low order boreal streams, light penetration through the forest canopy is strongly limited and light-induced degradation only becomes relevant when DOM enters lakes or wider river systems, where water residence times also increase by an order of magnitude, to months or years [Köhler *et al.*, 2002; Larson *et al.*, 2014].

Fluorescence and absorbance are highly sensitive easy-to-use approaches that can provide valuable insight into the molecular composition of stream DOM. However, optical measures are limited to bulk DOM characteristics, and there is a need for more studies coupling fluorescence characteristics with detailed molecular analysis, such as FTICR-MS [Kellerman *et al.*, 2015; Stubbins *et al.*, 2014] to expand our knowledge base regarding the growing library of PARAFAC components [Murphy *et al.*, 2014]. The potential for iron interference resulting in fluorescence quenching was ruled out due very low levels of Fe:DOC (mean  $\text{mg}_{\text{Fe}}:\text{mg}_{\text{DOC}}$ : 0.011), which would result in <1% loss of total fluorescence intensity [Poulin *et al.*, 2014].

The lack of significant changes to DOM composition found here support those of a previous modeling study at the Krycklan sites, which estimated that maximum levels of in-stream DOC losses are not likely greater than  $0.09 \text{ mg L}^{-1}$  during base flow (i.e., 0.5% of mean base flow [DOC]), when maximum stream water residence times would be 2 days [Tiwari *et al.*, 2014]. Across the full Krycklan catchment ( $68 \text{ km}^2$ ), stream water residence times vary from a mere 6 h during peak flow, to a maximum of 2 days at base flow [Tiwari *et al.*, 2014]. At this short time scale, and given the small loss of total DOC relative to substantial background concentrations (mean  $20.7 \pm 7.3 \text{ mg L}^{-1}$ ), it is not surprising that we found negligible in-stream changes to the composition of DOM. Water residence times have consistently been identified among the most relevant factors influencing the ambient concentration and composition of DOM in aquatic environments [Algesten *et al.*, 2004; Hanson *et al.*, 2011; Kellerman *et al.*, 2014; Kohler *et al.*, 2013; Kothawala *et al.*, 2014; Weyhenmeyer *et al.*, 2012]. In fact, the half-life of DOM for oligotrophic boreal lakes and rivers has been estimated to be 8 years across a broad geographical scale [Weyhenmeyer *et al.*, 2012], while terrestrial and autochthonous components of DOM have been estimated to have highly variable half-lives of 8.0 and 2.5 years, respectively, across a large eutrophic boreal lake [Kohler *et al.*, 2013]. In comparison, the residence time for DOM passing through headwater streams is extremely brief (hours to days) and highly restricts the opportunity for significant in stream DOM degradation. In summary, the combination of light and nutrient limitation along with the lack of labile DOM substrates (no protein-like fluorescence) and extremely short residence times (6 h to 2 days) all likely contributed to the undetectable changes to the composition of DOM.

## 5. Conclusions

1. This study shows negligible changes to DOM fluorescence characteristics during its residence in a first- to fourth-order stream network and collectively suggests that in-stream DOM degradation in low-order

boreal streams is negligible. Hence, although there are substantial transformations and loss of DOM in the inland water continuum, making it an “active pipe” (in the sense of *Cole et al.* [2007]), low-order streams act more like a “passive pipe.” The expectation of detecting changes to DOM composition within low-order streams may be unrealistic due to nutrient and light limitations along with the short residence time of a few hours to two days.

2. The dominant influence of wetland sources in regulating stream water DOM composition was determined, and unique molecular markers for DOM (PARAFAC components C4 and C5) derived from wetland versus forest sources were identified. In fact, 49% of the variability in stream water DOM composition was explained by land cover alone (%forest versus %wetland).
3. Seasonal fluctuations in hydrology only had a minor influence (8%) on stream DOM composition, which was largely a direct reflection of DOM found within forest riparian soil waters. One exception was found for a fluorescence component (C3), which was mobilized from upper forest soil horizons (20 cm) during peak discharge at spring melt.
4. The results of this study suggest that changes to the molecular composition of DOM is of greater relevance to the overall aquatic conduit where water residence times extend to time scales longer than that typical of low order streams, particularly during passage through lakes, higher-order streams, and ultimately to coastal waters.

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