

## Seasonal patterns in nutrient bioavailability in boreal headwater streams

Mayra P. D. Rulli <sup>1\*</sup>, Ann-Kristin Bergström <sup>2</sup>, Ryan A. Sponseller<sup>2</sup>, Martin Berggren <sup>1</sup>

<sup>1</sup>Department of Physical Geography and Ecosystem Science, Lund University, Lund, Sweden

<sup>2</sup>Department of Ecology and Environmental Sciences, Umeå University, Umeå, Sweden

### Abstract

Changes in nutrient bioavailability due to increased loading of dissolved organic matter (DOM) may impact boreal freshwaters. Yet, the relative bioavailability of carbon (C), nitrogen (N), and phosphorus (P) associated with terrestrial DOM remains poorly understood. We applied short-term bioassays with natural bacterial inocula to determine seasonal variation in bioavailable organic nutrient pools from four boreal headwater streams in northern Sweden. Experiments were designed to exhaust bioavailable nutrients associated with DOM by inducing limiting conditions when all required resources except for the targeted nutrient (C, N, or P) are provided in excess. We hypothesized that the supply of different bioavailable nutrients to streams would reflect seasonal variations in terrestrial demand, hydrology, and temperature. The delivery of bioavailable DOM-associated resources from the four streams were, on average, 2%, 11%, and 38% of the total dissolved organic C, N, and P, respectively, emphasizing the relatively low C bioavailability in these DOM-rich waters. Bioavailable N : P ratios peaked in autumn for all sites, with lower values in winter and spring. Both in terms of relative (% of total) and absolute bioavailable organic nutrient concentrations, the seasonal pattern was characterized by systematically high values for the autumn period. Furthermore, links between bioavailable resources and temperature and hydrology varied across sites, time periods, and the different elements. Thus, elevated concentrations of bioavailable organic resources in autumn suggest the potential for leaf fall, as well as late season storms that rewet dry soils, to serve as considerable sources of C, N, and P to boreal aquatic ecosystems.

The dissolved organic matter (DOM) pool in boreal freshwaters originates mostly from terrestrial ecosystems (Jones 1992; Algesten et al. 2005). This allochthonous DOM is a major driver of bacterial metabolism (Jansson et al. 2000; Karlsson et al. 2002; Figueroa et al. 2016), which not only contributes to significant production of greenhouse gases (Lapierre et al. 2013), but also subsidizes the pelagic food web through bacterivores such as flagellates (Bergström et al. 2003). In this regard, the use of DOM by benthic and planktonic bacteria represents an important link between terrestrial and aquatic ecosystems (Berggren et al. 2010). During the last decades, concentrations of DOM have increased in many aquatic ecosystems in the northern hemisphere, including both fresh and coastal marine waters (Hansson et al. 2013). Such increases may be due to anthropogenic-

related factors, including land use change (Kritzberg 2017), climate warming (Evans et al. 2005; Larsen et al. 2011), recovery from atmospheric acid deposition (Monteith et al. 2007), and increases in precipitation (Hongve et al. 2004). Regardless, these changes in surface water chemistry have the potential to alter energy and nutrient supply to aquatic ecosystems (Lebret et al. 2018).

To serve as a nutrition and carbon source for biota, molecules should be susceptible to enzymatic breakdown by microbes, that is, they must be bioavailable (Berggren et al. 2015). However, DOM bioavailability is highly variable, and poor knowledge about this variability hampers our understanding of the ecological consequences of increasing DOM loading (Berggren et al. 2015; Creed et al. 2018). Research on nutrient control of aquatic ecosystems often focuses on total fractions of carbon (C), nitrogen (N), and phosphorus (P) or inorganic fractions of N and P but does not explicitly consider what is readily bioavailable for microorganisms. The disregard of organic fractions of C, N, and P when investigating bioavailability is a shortcoming because most potentially bioavailable nutrients in brown boreal freshwater ecosystems are bound to DOM molecules (Jones et al. 1988; Jansson 1998; Berggren et al. 2015). Some of these DOM molecules can be

\*Correspondence: [rulli.mayra@gmail.com](mailto:rulli.mayra@gmail.com)

This is an open access article under the terms of the [Creative Commons Attribution](https://creativecommons.org/licenses/by/4.0/) License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

Additional Supporting Information may be found in the online version of this article.

assimilated directly by biota, whereas others are too big to pass cell membranes, and thus, must first be transformed into assimilable molecules via the activities of membrane-associated or extracellular enzymes (Likens 2010; Berggren et al. 2015). Therefore, nutrients that can be assimilated either directly or facilitated by enzymes, that is, also including larger DOM molecules, must be accounted for to accurately define bioavailable nutrients (Stepanauskas et al. 2002; Berggren et al. 2015).

Loadings of N and P from terrestrial landscapes are well studied due to their contributions to eutrophication in fresh and coastal waters (Stepanauskas et al. 1999, 2002; Wiegner and Seitzinger 2004). Such inputs compromise water quality, degrade habitats, and reduce biological diversity, with negative effects on public health, fisheries, and recreation (Conley et al. 2009; Howarth et al. 2011; Fulweiler et al. 2012; Stutter et al. 2018). Yet, there are critical gaps in our ability to predict ecological responses to nutrient loading and, consequently, to manage ecosystems. In particular, conventional concepts of nutrient availability usually neglect the actual bioavailable nutrient fraction for microorganisms. Indeed, if organic components are dominant and more bioavailable than previously known, as shown, for example, by Lønborg et al. (2018) and Stepanauskas et al. (2002) for N and P, then using inorganic and total fractions to determine nutrient bioavailability will not give accurate results.

One challenge to understanding the contribution of DOM to aquatic nutrient availability is that inputs may not be static in space and time. Seasonal changes in DOM transport, production, and consumption have considerable effects on the concentrations and characteristics of DOM (Wallin et al. 2015; Broder et al. 2017). Moreover, land cover, hydrology of the catchment, and climate can all greatly impact DOM. For example, Broder et al. (2017) identified changes in hydrological condition as the main factor influencing concentration and quality of DOM in headwater streams. Others have shown the influence of different land covers and seasonally variable soil processes on the DOM contribution to aquatic productivity (Wiegner and Seitzinger 2004). Seasonal changes in environmental conditions as well as nutrient bioavailability also shift bacterial community composition and function within aquatic environments (Kritzberg et al. 2006). Although many studies have investigated the seasonal controls over bulk nutrient concentrations (Sponseller et al. 2014), only a few have considered the same dynamics for the actual bioavailable pools, and almost none have done so for organic C, N, and P simultaneously.

We explored the patterns and controls over C, N, and P bioavailability in four boreal headwater streams with contrasting characteristics, draining catchments dominated by forests or wetlands (mires). We hypothesized that seasonal variation in bioavailable C, N, and P supply are shaped by changes in terrestrial resource demand, hydrological forcing, and temperature. More specifically, given the temperature-

dependent mobilization of bioavailable P from soils (Jansson et al. 2012), together with peaks terrestrial N demand during the growing season and declines in bioavailable C during low flow periods (Berggren et al. 2009), we predicted that bioavailable N : P and C : P in streams would decrease during summer. We further expected this pattern to be particularly strong for streams draining mires, where summer increases in bioavailable P are especially pronounced (Jansson et al. 2012). Moreover, we expected bioavailable dissolved organic carbon (BDOC) and bioavailable dissolved organic nitrogen (BDON) to show the highest concentrations during the wet seasons (spring and autumn), especially during the spring flood. In contrast, during the drier summer growing season, the combination of high biotic activity with water flowing slow through deep and relatively less permeable soil layers should result in low BDOC and BDON delivery to streams (Fellman et al. 2009). However, hydrological dependence of resource mobilization should be more relevant to forested sites compared with boreal peat mires (Stepanauskas et al. 2000b), because mire-covered sites have a relatively more stable water table and uniform soil profile (Berggren et al. 2009).

We estimated the bioavailability of dissolved organic C (DOC), N (DON), and P (DOP) by applying an established multielement bioassay method from Soares et al. (2017) in a new way. Instead of using  $^3\text{H}$ -leucine incorporation as the response variable, we used bacterial abundance measured through flow cytometry. Flow cytometry is one of the most accurate and fastest methods to count microbial cells, and in combination with nucleic acid stains, this method can measure any microbial cell, independent of their physiological condition (Wang et al. 2010). By using this approach, our study aimed to answer the following research questions: (1) how do the absolute and relative abundances of bioavailable C, N, and P delivered via runoff change seasonally? and (2) how do landscape types (forest vs. mire covered catchments) influence the bioavailable nutrient pools? We also explored temporal bioavailability relationships to environmental variables (e.g., temperature, discharge, and precipitation) and other elements (e.g., chlorophyll [Chl] *a*) known to influence DOM transport as well as DOM concentration in surface waters and how it relates with changes in landscape type.

## Methods

### Site selection

Our study was performed in four headwater streams in northern boreal Sweden. For comparative purposes, we selected two streams draining forest catchments and two draining catchments with at least 50% of mire cover. Three of the streams are located in the Krycklan Catchment Study (64°23'N, 19°78'E) and are part of the ongoing Krycklan Catchment monitoring program, which includes weekly-to-monthly water chemistry measurements that date back almost

four decades (Laudon et al. 2013). We selected catchments C1 (Västrabäcken, 0.48 km<sup>2</sup>, 100% forest), C4 (Kallkällsmyren, 0.18 km<sup>2</sup>, 51% mire and 49% forest), and C2 (Risbäcklan, 0.12 km<sup>2</sup>, 100% forest), all of which are intensely studied catchments in the Krycklan Catchment. The fourth site is a larger stream just outside the Krycklan Catchment, C18 (Degerö stormyr, 3.12 km<sup>2</sup>, 69% mire and 31% forest; Ågren et al. 2008). Degerö Stormyr has also been under a monitoring program for at least the last two decades. The Krycklan Catchment is covered by 9% of mires and 87% of forests, which are dominated by Scots pine (*Pinus sylvestris*, 63%) and Norway spruce (*Picea abies*, 26%) with an understory of ericaceous shrubs (Laudon et al. 2013).

Climate in this region is a cold temperate humid type, which is characterized by continuous snow cover during winter (Laudon et al. 2013). The total long-term average annual precipitation in the Krycklan Catchment is 623 mm (1981–2010), reaching a highest of 918 mm in 1982 and a lowest of 446 in 1994 (Laudon et al. 2020). Approximately 30% of the precipitation falls as snow (Laudon et al. 2020). Mean annual temperature is 1.7°C, with minimum averages in January ( $-9.5 \pm 4.1^\circ\text{C}$ ) and maximum in July ( $14.7 \pm 1.7^\circ\text{C}$ ) (Tiwari et al. 2018). Laudon et al. (2020) identified an overall warming pattern in the Krycklan Catchment since the beginning of the continuous measurements in 1980 until present, with increases in air temperature of approximately 2°C. Finally, summer 2018 registered severe droughts in large parts of Europe, especially affecting Scandinavia and the rest of northern Europe, with unusually high temperatures (Johansson et al. 2020). For the Krycklan Catchment, this was one of the most severe droughts recorded since the monitoring program began (Gómez-Gener et al. 2020).

### Sampling and filtration

We sampled streams for bioassay experiments on seven occasions from September 2018 to October 2019 (Supporting Information Fig. S1). Samples collected in September and October were considered autumn samples, those collected during snow cover periods (December and March) were considered winter samples, while samples collected in April were defined as spring samples, and those collected in July and August summer samples. We collected water from each stream using acid-washed low-density polyethylene containers (4 liter). Before sampling, we rinsed the containers with stream water three times. Collected samples were kept in the dark and cool at approximately 4°C during transit to the laboratory where we processed them within 24 h of sampling. Here, we filtered water samples in a two-step process, first through a glass microfiber filter (ca. 0.7 µm pore size, grade GF/F, Whatman) used for chlorophyll analysis, and thereafter through a membrane nylon filter (0.2 µm pore size, Whatman) to eliminate most bacterial cells. We stored filtered samples at 4°C in the dark until bioassay preparation, which occurred immediately after or at latest within a week after filtration. In

the latter case, we filtered the samples again through a 0.2 µm filter before the bioassay to ensure minimum presence of bacterial cells. For the purpose of creating a bacterial inoculum free from bacterivores and other larger particles, we also collected additional water from the four streams, as well as from other two nearby streams on each sampling occasion and then filtered this through a 1.2 µm membrane filter (Milipore). To obtain a diverse natural standard bacteria community that could be used as inoculum for all bioassays, we mixed the same proportion of water from each stream, ensuring that difference in species composition of the bacterial community did not influence bioavailability measurements (Martinez et al. 1996; Soares et al. 2017).

### Bioavailability assays

We prepared the bioassays (150 mL final volume) using 0.2-µm filtered stream water in 250 mL acid-washed glass bottles with polypropylene caps (Duran). To each incubation bottle, we added 5% (v/v) of modified (excluding C, N, and P) L16 medium (Lindström 1991) containing micronutrients, vitamins, and trace metals necessary for bacterial growth. We then inoculated the samples with 2% (v/v) of the standard inoculum (described above). We induced strong C, N, or P limitation by adding combinations of C (as C<sub>6</sub>H<sub>12</sub>O<sub>6</sub>, final concentration 20 mg C L<sup>-1</sup>), N (as NH<sub>4</sub>NO<sub>3</sub>, 2000 µg N L<sup>-1</sup>), and P (as Na<sub>2</sub>HPO<sub>4</sub>, 200 µg P L<sup>-1</sup>) in different sets of replicate incubation bottles. For C limitation bioassay samples, N and P were added; for N limitation, C and P were added; and for P limitation conditions, C and N were added. We prepared either two (September 2018) or four (all other time points) replicates of each nutrient condition for each stream. A spike of C (1000 µg C L<sup>-1</sup>), N (100 µg N L<sup>-1</sup>), or P (10 µg P L<sup>-1</sup>) was added to half of the C, N, or P-limited bioassay bottles, respectively, that is, to either one (September 2018) or two (all others) of the four replicates.

The added spikes worked as internal standards, permitting calculations of C, N, or P assimilation (content) per cell that was produced in response to the spike (see calculations below). Thus, we used the spiked samples to verify that limitation was achieved (Stepanuskas et al. 2000a) and to be able to correctly calculate bioavailable nutrient concentrations in the nonspiked samples, based on cell yields multiplied by the known nutrient contents per cell (Stepanuskas et al. 1999; Soares et al. 2017). We incubated the bottles for a period of 7 days in a dark constant chamber at 20°C, which is a standard temperature for bioavailability experiments (del Giorgio and Davis 2003; Soares et al. 2017). Samples for bacterial abundance were taken (in triplicates for September 2018 and in duplicates for all remaining time points) from each incubation bottle at six time points during the incubation period (after 0, 1, 2, 3, 4, and 7 d) and fixed with filtered (0.2 µm) glutaraldehyde (3% final concentration). We measured bacterial abundance through flow cytometry immediately after the end of the incubation period or at latest within a month.

To be able to control for potential C, N, and P contamination from the inoculum, the L16 growth medium, the nutrient solutions, and general sample handling, we prepared control samples in the same way as described above (including filtration) but using Milli-Q water instead of stream water.

### Flow cytometry

Flow cytometry is an efficient and sensitive method used to measure single cells passing through a detector (Jahan-Tigh et al. 2012). This method is advantageous since it enables rapid analysis (thousands of cells per second), high accuracy (< 5% machine error) and high sample throughput and automation (Vives-Rego et al. 2000; Wang et al. 2010). Through this method, we used nutrient-dependent bacterial growth (as bacterial abundance) as a proxy to estimate bioavailable resources (Stepanauskas et al. 1999, 2000b).

Bacterial abundance was measured based on Stepanauskas et al. (2000a) flow cytometric method. Five microliters of SYTO 13 nucleic acid stain (5 mM, Molecular Probes) was diluted in 995  $\mu\text{L}$  of Milli-Q water (25  $\mu\text{M}$  conc.). We pipetted 190  $\mu\text{L}$  of each sample collected during the incubation period into a well of a 96-well plate. We then stained each sample with 10  $\mu\text{L}$  of the diluted SYTO 13 solution (1.25  $\mu\text{M}$  final conc.) and left in the dark for 10 min. The samples were then analyzed in a BD Accuri C6<sup>®</sup> flow cytometer (BD Biosciences) with a BD CSampler (BD Biosciences) for automated 96-well plate analysis. The flow cytometer was controlled with the BD CSampler<sup>™</sup> software, calibrated to count the number of particles in 50  $\mu\text{L}$  of a 200  $\mu\text{L}$  sample (ca. 50 s).

Control bioassays had significantly lower cell counts compared to stream bioassays (*t* test,  $p \leq 0.0001$ ) (Supporting Information Fig. S2a), and averaged values from all replicates were used for DOC, DON, and DOP bioavailability calculations.

For most samples, the first bacterial abundance peak was reached at day two (Supporting Information Fig. S2b). Overall, secondary peaks were common during the incubation period, that is, cell abundances increasing again during the second half of the incubation period. Such secondary peaks were observed in 48% of samples with no spike added and in 33% of samples with spike added. We sought to identify a single time point for which the cell abundances generally showed a primary (first) peak. We then used a combination of the approaches applied by Stepanauskas et al. (2000a) and Soares et al. (2017) for bioavailability estimates. We used a spike-and-response approach (Stepanauskas et al. 2000a) with a fixed short-term time frame to get only the readily bioavailable fractions (Soares et al. 2017). Therefore, for each incubation sample, we only used abundance from day 2 to estimate nutrient bioavailability.

For a few N and P incubation samples, the measured bacterial abundances were higher in nonspiked than in spiked cells, and thus the resulting bioavailability estimates were slightly negative. These samples were removed from the analysis and considered as below the detection range.

We based our DOM bioavailability measurements in three main assumptions:

1. The added spike was 100% bioavailable and fully used during the incubations, as shown by Stepanauskas et al. (1999). Incubations with spike had substantially higher bacterial abundance than the ones without a spike and showed a clear decrease in abundance after the peak was reached. Soares et al. (2017) showed a linear relationship between the resource (spike) added and biomass yield.
2. Distinct bioavailable C, N, and P compounds produce an equal increase in bacterial biomass per mol of C, N, or P. In a similar approach to determine BDOM, reported elsewhere, nitrate and ammonium additions yielded equal increases in bacterial abundance per unit of added N (Stepanauskas et al. 1999).
3. All bioavailable C, N, or P were exhausted when the bacterial growth reached a primary peak since we are estimating only readily bioavailable nutrients. However, DOM utilization could possibly have continued after the primary bacterial growth peak, causing underestimation of long-term bioavailability (Stepanauskas et al. 1999). Hence, our estimates of bioavailable resources should be viewed as short-term DOM bioavailability.

### Chemical analyses and calculations

Stream water for DOC analysis was filtered through Whatman GF/F and membrane filters (described above) placed into pre-acid-washed 40 mL amber borosilicate glass vials to the brim, and securely closed with silicone septum caps. Samples were kept cold (ca. 4°C) until analysis at the Ján Veizer Stable Isotope Laboratory, University of Ottawa. Filtered stream samples for analyses of total dissolved nitrogen (TDN),  $\text{NH}_4\text{-N}$  (ammonium),  $\text{NO}_3\text{-N}$  (nitrate), total dissolved phosphorus (TDP), and  $\text{PO}_4\text{-P}$  (phosphate, represented by soluble reactive P [SRP]) were kept frozen until analysis, which was conducted at the Department of Ecology and Genetics, Uppsala University. Here, SRP was used as an approximate measure of  $\text{PO}_4\text{-P}$  as it has been used as a standard method in several studies (Worsfold et al. 2016) and due to a lack of a more accurate method to measure orthophosphate concentrations in natural water samples. Analysis of TDP and  $\text{PO}_4\text{-P}$  was done through colorimetry using the molybdenum blue method (Murphy and Riley 1962; Menzel and Corwin 1965). For TDP oxidation, potassium persulfate and autoclavation was used. Analysis of  $\text{NH}_4\text{-N}$  and  $\text{NO}_3\text{-N}$  was conducted in a Metrohm IC system (883 Basic IC Plus and 919 Autosampler Plus). Lower detection limits for TDP,  $\text{PO}_4\text{-P}$ , and  $\text{NO}_3\text{-N}$  was 1  $\mu\text{g L}^{-1}$  while  $\text{NH}_4\text{-N}$  was 2  $\mu\text{g L}^{-1}$ . Values lower than the detection limit were reported as < 1 and < 2  $\mu\text{g L}^{-1}$ , respectively. Concentrations of TDN were analyzed on a Shimadzu TNM-L instrument with lower detection limit of 50  $\mu\text{g L}^{-1}$ . We calculated DON and DOP by subtracting the inorganic fractions

(NO<sub>3</sub>-N and NH<sub>4</sub>-N for N; PO<sub>4</sub>-P for P) from TDN and TDP, respectively.

Calculations of nutrient bioavailability for bioavailable DOC (BDOC), DON (BDON), and DOP (BDOP) were based on Stepanauskas et al. (2000a) and Soares et al. (2017). First, nutrient content per bacterial cell in C, N, or P-limited incubations (Nutrient<sub>cell</sub>) was calculated as in Eq. 1,

$$\text{Nutrient}_{\text{cell}} = \frac{s}{A_{\text{spike}} - A_{\text{stream}}} \quad (1)$$

where  $s$  is the concentration of glucose ( $\mu\text{g C L}^{-1}$ ), nitrate ( $\mu\text{g N L}^{-1}$ ), or phosphate ( $\mu\text{g P L}^{-1}$ ) spike added to C, N, or P-limited incubations, respectively,  $A_{\text{spike}}$  is the average bacterial cell abundance (cells mL<sup>-1</sup>) in incubations with stream C, N, or P and their respective spikes,  $A_{\text{stream}}$  is the average bacterial cell abundance in incubations with only stream C, N, or P (cells mL<sup>-1</sup>). Concentrations of BDOC, BDON, and BDOP ( $\mu\text{g C/N/P L}^{-1}$ ) were calculated as in Eq. 2,

$$B = (\text{Nutrient}_{\text{cell}} \times A_{\text{stream}}) - I \quad (2)$$

where  $I$  is the concentration of inorganic nutrient fraction in the stream water ( $\mu\text{g L}^{-1}$ ). For carbon,  $I$  was ignored.

The dissolved nonbioavailable organic fraction (Dn) of C, N, or P ( $\mu\text{g L}^{-1}$ ) was calculated as in Eq. 3,

$$\text{Dn} = T - B - I \quad (3)$$

where  $T$  is the total resource/nutrient pool for each nutrient. For carbon,  $I$  was again ignored.

### Statistical analysis

We tested the differences in bioavailable nutrient concentrations among streams as well as for each stream between time points using one-way ANOVA with Tukey's post hoc tests. We tested differences in bioavailable and total resource ratios between the streams with paired  $t$  tests, while differences between stream type (forest or mire) as well as differences between cell counts from the control and the other treatments were tested with two-sample  $t$  tests. We used Pearson correlations to test whether averaged nutrient bioavailability was correlated with average stream water DOC, TDN, TDP, dissolved inorganic N and P, chlorophyll, air temperature, discharge, and precipitation and for the forest and mire streams. In this region, discharge patterns are strongly driven by the snowmelt flood, and thus by accumulation of snowpack throughout the winter. Precipitation is normally represented as snowfall from November to April and as rainfall from May to October, and these rainfall events can also generate floods that influence stream chemistry. For our correlation analysis, we were interested in the short-term implications of precipitation inputs and calculated this as the accumulation of rainfall and/or snowfall during the 2 weeks prior to sampling. All

variables, except precipitation and air temperature, were log<sub>10</sub>-transformed. Bioavailability concentration was also log<sub>10</sub>-transformed to obtain a normal distribution for parametric testing. Nutrient ratios and bioavailable resource ratios were all presented in molar log-transformed ratios (Isles 2020). All statistical test results were considered significant when  $p < 0.05$ . To statistically compare differences between dates in single streams, sample size ( $n$ ) in the analyses included values from replicate incubations and bacterial abundance counts for each sampled stream, giving in total four bioavailability estimates per site and date. We conducted all statistical analyses using R (R Core Team 2020).

## Results

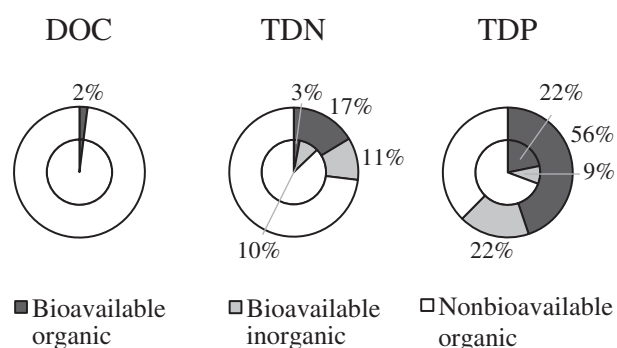
### Nutrient and DOM composition in the streams

On average, higher PO<sub>4</sub>-P and NH<sub>4</sub>-N concentrations were observed in the two mire streams combined, compared with the two forest streams combined ( $t$ -test,  $p = 0.01$  and  $< 0.0001$ , respectively). Across sites and during the study period, NO<sub>3</sub>-N, NH<sub>4</sub>-N, and TDN concentrations ranged from 5.5 to 150  $\mu\text{g L}^{-1}$ ,  $< 2$  to 127.9  $\mu\text{g L}^{-1}$ , and 285 to 741.6  $\mu\text{g L}^{-1}$ , respectively (Supporting Information Fig. S3). Phosphate phosphorus (PO<sub>4</sub>-P) and TDP ranged from  $< 1$  to 7.3  $\mu\text{g L}^{-1}$  and 6.3 to 20.2  $\mu\text{g L}^{-1}$ , respectively (Supporting Information Fig. S3). Finally, across all sites, DOC ranged from 16.4 to 43.7  $\text{mg L}^{-1}$ ; DON and DOP ranged from 130.7 to 665.2  $\mu\text{g L}^{-1}$  and 5.7 to 16  $\mu\text{g L}^{-1}$ , respectively.

### Overall DOM bioavailability estimates

Our estimates of bioavailable resource concentrations from the four streams, averaged for all analytical replicates, ranged from 118 to 2370  $\mu\text{g C L}^{-1}$  for BDOC, 0.7 to 304  $\mu\text{g N L}^{-1}$  for BDON, and 1 to 21  $\mu\text{g P L}^{-1}$  for BDOP. Concentrations of BDON and BDOP, averaged for all replicate observations ( $n = 108$ ), were significantly different between stream types, that is, streams C1 and C2 (forest sites) had distinct concentrations when compared to C4 and C18 (mire sites) ( $t$ -test,  $p = 0.0003$  for N and  $p < 0.0001$  for P). There were no significant differences between the two forested sites nor between the two mire sites. For BDOC concentrations, only C4 showed significant difference from the other sites (ANOVA,  $p < 0.0001$ ), with concentrations twice as high as C18 (Tukey's post hoc test,  $p = 0.0002$ ) and about three times higher than C1 and C2 (Tukey's post hoc test,  $p < 0.0001$ ).

Bioavailable organic resource fractions, averaged for all replicate observations ( $n = 108$ ), relative to the total pool were highest for P, intermediate for N, and lowest for C, with 56%, 17%, and 2% of the total pool for mire sites, and 22%, 3%, and 2% for forest sites, respectively (Fig. 1). The organic forms represented most of the bioavailable resources for N and P in mire sites, corresponding to 69% of the bioavailable dissolved P and 58% (mire) of the bioavailable dissolved N. For the forest sites, organic P was the dominant bioavailable form (71%),



**Fig. 1.** Proportion of bioavailable organic, bioavailable inorganic, and nonbioavailable organic nutrient fractions of DOC, TDN, and TDP. Chart shows averaged data for the two forest (C1 and C2; inner pie) and the two mire (C4 and C18; outer pie) streams for all seven sampling dates (from September 2018 to October 2019).

whereas organic N only accounted for 30% of the bioavailable pool. In general, the relative organic contribution to bioavailability was greater for P than N.

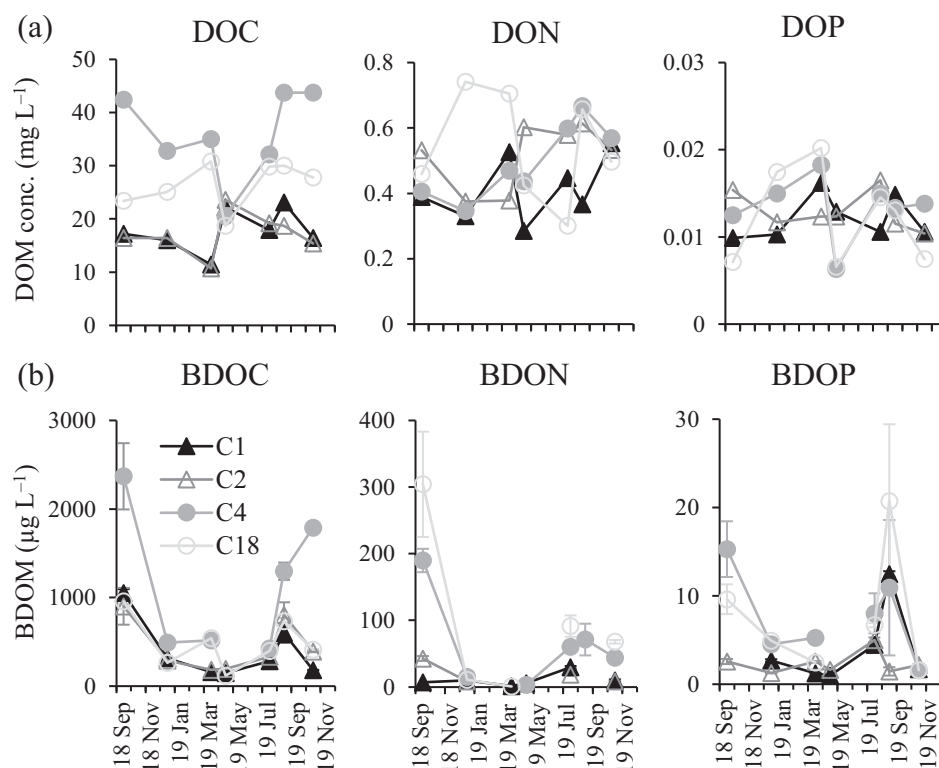
### Seasonal patterns of bioavailable DOM

Temporal variation in BDOC, BDON, and BDOP concentrations during the studied period (September 2018 to October 2019) was similar for mire and forest streams for all three resources. Nevertheless, averaged bioavailable resources concentrations in each stream, for all replicate observations, were significantly different between the time points (ANOVA,  $p < 0.0001$  for C and P, and  $p = 0.003$  for N,  $n = 15$ –27). When considering all streams, the lowest bioavailability values were observed in April 2019 for DOC ( $153 \mu\text{g C L}^{-1} \pm 11.1$  SE) and DOP ( $1.3 \mu\text{g P L}^{-1} \pm 0.09$  SE) and March 2019 for DON ( $1.02 \mu\text{g N L}^{-1} \pm 0.23$  SE), while the highest values were observed in September 2018 for DOC ( $1319 \mu\text{g C L}^{-1} \pm 206$  SE) and DON ( $162 \mu\text{g N L}^{-1} \pm 39.6$  SE) and August 2019 for DOP ( $10.5 \mu\text{g P L}^{-1} \pm 3.2$  SE) (Table 1). However, both BDON and BDOP had samples with values below detection in a few of the sampling dates (Table 1), particularly for N during spring and late summer. Estimated BDOM concentrations for each sampling occasion, stream, and replicates from the bioassay incubations are shown in Supplementary Table S1. Moreover, total DOC and DOP concentrations in the mire streams decreased drastically from the March to the late April sampling (spring flood; Fig. 2a).

Bioavailable resource concentrations per time point showed particularly high variability in the case of C. With exceptionally high values, the BDOC in September 2018 differed significantly from nearly all other time points in all four streams (Table 1). With the exception of C1, BDON in September 2018 also differed significantly from all other time points (Table 1). Conversely, BDOP in September 2018 was significantly different only from October 2019 for C4, while July 2019 differed significantly from most time points for the other three streams (Table 1).

**Table 1.** Concentrations of BDOC, BDON, and BDOP on seven sampling occasions (columns) for each stream. Values show means of three (September 2018) or four (all the others) analytical replicates and standard deviations are provided within parentheses. Distinct index letters within rows identify dates significantly different from each other ( $p < 0.05$ ), which were determined by the one-way ANOVA test and Tukey's post hoc test. The one-way ANOVA tests were performed based on  $\log_{10}$ -transformed data, and thus index letters were also based on  $\log_{10}$ -transformation.

| Stream                       | Sep 2018                | Dec 2018                 | Mar 2019               | Apr 2019                | Jul 2019                | Aug 2019                | Oct 2019                |
|------------------------------|-------------------------|--------------------------|------------------------|-------------------------|-------------------------|-------------------------|-------------------------|
| BDOC, $\mu\text{g C L}^{-1}$ |                         |                          |                        |                         |                         |                         |                         |
| C1                           | 1050 <sup>d</sup> (75)  | 314 <sup>b</sup> (21)    | 158 <sup>a</sup> (5)   | 148 <sup>a</sup> (16)   | 281 <sup>b</sup> (9)    | 599 <sup>c</sup> (218)  | 178 <sup>a</sup> (12)   |
| C2                           | 900 <sup>d</sup> (361)  | 286 <sup>b,c</sup> (13)  | 182 <sup>a</sup> (6)   | 195 <sup>ab</sup> (17)  | 331 <sup>c</sup> (24)   | 804 <sup>d</sup> (287)  | 387 <sup>c</sup> (14)   |
| C4                           | 2370 <sup>c</sup> (649) | 489 <sup>a</sup> (30)    | 516 <sup>a</sup> (21)  | 118 <sup>a</sup> (51)   | 424 <sup>a</sup> (19)   | 1296 <sup>b</sup> (195) | 1788 <sup>c</sup> (118) |
| C18                          | 956 <sup>d</sup> (54)   | 262 <sup>b</sup> (8)     | 542 <sup>cd</sup> (20) | 5 <sup>ab</sup>         | 397 <sup>b,c</sup> (25) | 731 <sup>d</sup> (73)   | 411 <sup>bc</sup> (10)  |
| BDON, $\mu\text{g N L}^{-1}$ |                         |                          |                        |                         |                         |                         |                         |
| C1                           | 7 <sup>ab</sup>         | 10 <sup>b</sup> (5)      | 1.2 <sup>a</sup> (1.2) | 5 <sup>ab</sup>         | 30 <sup>b</sup> (4)     | 10 <sup>b</sup> (3)     | 10 <sup>b</sup> (3)     |
| C2                           | 43 <sup>c</sup> (6)     | 8 <sup>a</sup> (0.3)     | 1.2 <sup>a</sup> (1.2) | 3 <sup>a</sup> (0.5)    | 19 <sup>b</sup> (2)     | 71 <sup>c</sup> (48)    | 9 <sup>a</sup> (1.6)    |
| C4                           | 190 <sup>d</sup> (30)   | 15 <sup>b</sup> (1)      | 0.7 <sup>a</sup>       | 3 <sup>a</sup> (0.5)    | 60 <sup>c</sup> (15)    | 71 <sup>c</sup> (48)    | 44 <sup>c</sup> (1.4)   |
| C18                          | 304 <sup>d</sup> (137)  | 11 <sup>b</sup> (3)      | 0.7 <sup>a</sup>       | 1 <sup>a</sup> (0.1)    | 91 <sup>c</sup> (32)    | 68 <sup>c</sup> (5)     | 68 <sup>c</sup> (5)     |
| BDOP, $\mu\text{g P L}^{-1}$ |                         |                          |                        |                         |                         |                         |                         |
| C1                           | 3 <sup>a,c</sup> (0.5)  | 3 <sup>d</sup> (0.3)     | 1.2 <sup>b</sup> (0.1) | 1 <sup>a</sup> (0.1)    | 4 <sup>e</sup> (0.5)    | 12 <sup>f</sup> (0.6)   | 1.7 <sup>c</sup> (0.1)  |
| C2                           | 15 <sup>b</sup> (5)     | 1.3 <sup>ab</sup> (0.07) | 3 <sup>b,c</sup> (0.1) | 1.6 <sup>ab</sup> (0.1) | 5a <sup>c</sup> (0.5)   | 1.5 <sup>a</sup> (0.9)  | 2 <sup>ab</sup> (0.2)   |
| C4                           | 10 <sup>b</sup> (3)     | 5 <sup>ab</sup> (1)      | 5 <sup>ab</sup> (0.5)  | 8 <sup>ab</sup> (4.5)   | 8 <sup>ab</sup> (4.5)   | 11 <sup>ab</sup> (15)   | 1.6 <sup>a</sup> (0.4)  |
| C18                          | 10 <sup>b</sup> (3)     | 5 <sup>a</sup> (0.1)     | 2 <sup>a</sup> (0.1)   | 7 <sup>a</sup> (0.5)    | 21 <sup>a</sup> (17)    | 21 <sup>a</sup> (17)    | 1.6 <sup>a</sup> (0.1)  |



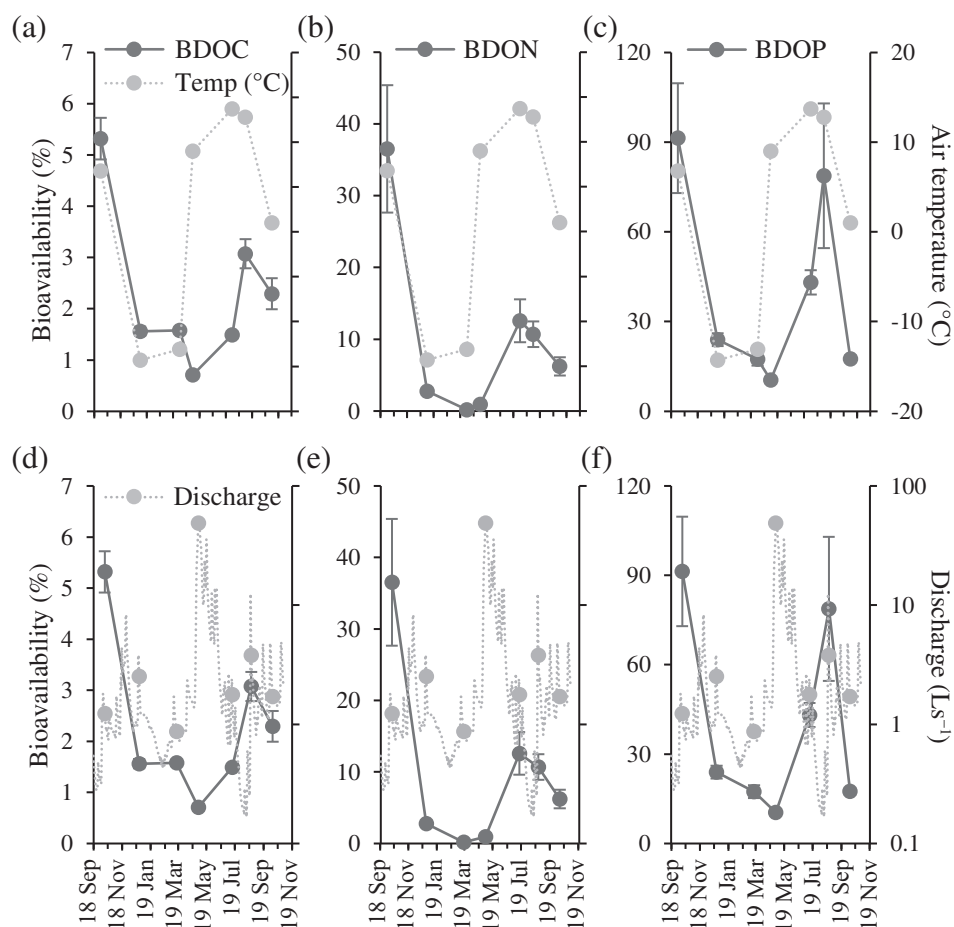
**Fig. 2.** Symbols show measured dissolved organic nutrients expressed in the form of (a) total DOM concentrations as DOC, DON, and DOP and (b) bioavailable DOM concentrations as BDOC, BDON, and BDOP. Interpolation lines are drawn between measurement points from September 2018 to October 2019. The open and closed triangles represent the two forested catchments, and the different circle symbols show the two mire-dominated catchments. The charts are structured in three columns (across rows a and b) that show the different element fractions of DOM: carbon (left), nitrogen (middle), and phosphorus (right). Error bars denote  $\pm$  SE. Note the different scaling on the y-axes.

Contrary to our expectations, our estimates showed that BDOC and BDON were low during the spring flood and increased during the summer (not considering samples below the detection range). Bioavailable resource concentrations were, in general, higher in autumn 2018 than the other seasons covered in this study, especially for mire streams (C4 and C18) (Fig. 2). DOC bioavailability was, generally, higher in autumn 2018 (September 2018) for all streams. Stream four (C4) had up to six times higher BDOC in autumn ( $2370 \mu\text{g C L}^{-1}$ ,  $\pm 375$  SE) than in winter, spring, and summer, with the high values in both 2018 and 2019 autumn samples (September 2018, October 2019) (Fig. 2b). For the other three streams (C1, C2, and C18), BDOC reached higher values during autumn 2018, then peaked again in late summer 2019 (August 2019) but decreased by autumn 2019 bioassays (Fig. 2). BDOC reached 6% of the total DOC pool during autumn 2018 (4–6%,  $\pm 0.1$ –0.3 SE). Mire streams (C4 and C18) had, on average, seven times higher BDON in autumn 2018 than other time points. These two streams reached 56% ( $\pm 9$  SE) BDON relative to the total nitrogen pools in the same season. BDON for forested streams (C1 and C2) did not have significant changes for most time points, but it was higher in autumn 2018 for C2 and July 2019 for C1 compared to the

other seasons. Concentrations of DOP bioavailable resource varied between streams over time. Mire streams had, on average, higher DOP bioavailability during autumn 2018, reaching 128% ( $\pm 16$  SE) of the total phosphorus pool. Both forested streams showed higher phosphorus bioavailability during summer 2019, August for C1 and July for C2 (Fig. 2).

#### DOM bioavailability and environmental drivers

DOM bioavailability estimates were correlated to some of the measured environmental factors, especially air temperature, as well as stream nutrient composition. Average bioavailability for each site, in general, followed temperature patterns across seasons (Fig. 3a–c). Changes in mean daily air temperature were positively correlated with BDOC, BDON, and BDOP for forest and mire streams (Pearson correlation, for forest  $p = 0.004$  for C, 0.0002 for N, and 0.019 for P; for mire  $p = 0.004$  for N and 0.03 for P; Table 2), except for BDOC for mire streams which showed no significant correlation with air temperature (Pearson correlation,  $p = 0.3$ ; Table 2). BDON showed the most similar pattern in relation to temperature, and consequently, the strongest correlation (Pearson correlation,  $p = 0.0002$ ; Table 2), with bioavailability increasing slightly during spring and decreasing again in autumn when



**Fig. 3.** Symbols show average percentage of (a, d) BDOC, (b, e) BDN, and (c, f) BDOP shown over time together with (a–c) air temperature or (d–f) daily discharge (from the outlet stream C7). Interpolation lines are drawn between measurement points from September 2018 to October 2019. Error bars denote  $\pm$  SE.

**Table 2.** Nutrient bioavailability (BDOC [ $\mu\text{g C L}^{-1}$ ], BDN [ $\mu\text{g N L}^{-1}$ ], and BDOP [ $\mu\text{g P L}^{-1}$ ]) correlated with stream water DOC, TDN, TDP, discharge, air temperature, chlorophyll, precipitation and dissolved inorganic N and P for the forest and mire streams, and all sampling occasions. Pearson correlation coefficients (*cor*) are shown. Numbers marked in bold are significant. Numbers in parentheses indicate a different number of samples than shown in row *n*. Exact *p*-values are shown in Supplementary Table S2.

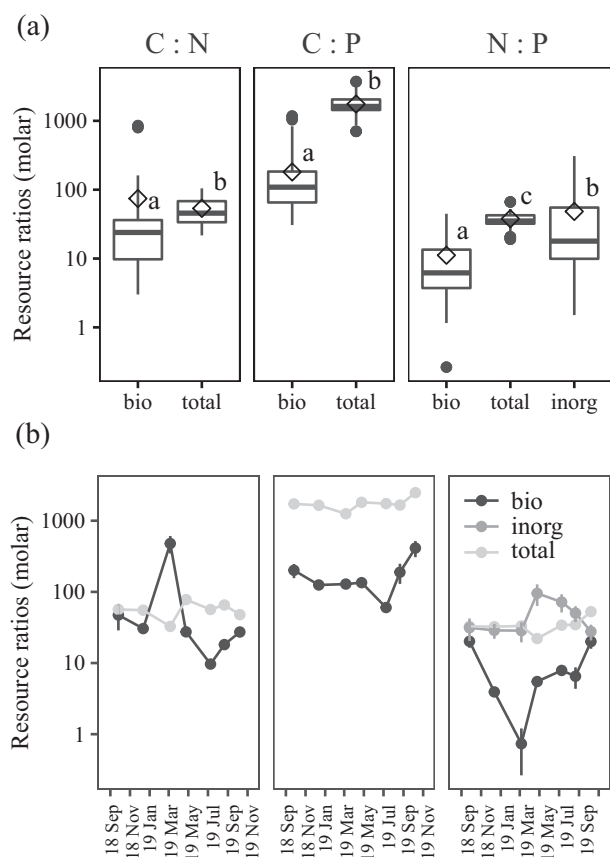
|                    | Forest         |                |               | Mire            |                 |              |
|--------------------|----------------|----------------|---------------|-----------------|-----------------|--------------|
|                    | BDOC           | BDN            | BDOP          | BDOC            | BDN             | BDOP         |
| Log10 DOC          | 0.16           | <b>0.58***</b> | 0.16          | <b>0.82***</b>  | <b>0.37*</b>    | −0.04        |
| Log10 TDN          | 0.13 (31)      | 0.2            | 0.01          | 0.1 (35)        | −0.2            | −0.15 (35)   |
| Log10 TDP          | −0.06 (49)     | 0.08 (30)      | 0.19          | 0.13 (45)       | −0.06 (35)      | −0.01        |
| Log10 discharge    | −0.27          | −0.1           | −0.28         | <b>−0.55***</b> | <b>−0.62***</b> | 0.12         |
| Air temperature    | <b>0.38**</b>  | <b>0.62***</b> | <b>0.33*</b>  | 0.14            | <b>0.45**</b>   | <b>0.32*</b> |
| Log10 Chl <i>a</i> | 0.16           | <b>0.4*</b>    | <b>0.6***</b> | 0.13            | <b>0.38*</b>    | 0.29         |
| Precipitation      | <b>0.65***</b> | 0.23           | <b>0.31*</b>  | 0.27            | 0.18            | <b>0.31*</b> |
| Log10 DIN          | <b>−0.38**</b> | <b>−0.45*</b>  | −0.23         | −0.17           | 0.27            | <b>0.35*</b> |
| Log10 DIP          | 0.21           | 0.27           | −0.14         | <b>0.42**</b>   | 0               | −0.13        |
| <i>n</i>           | 54             | 31             | 49            | 50              | 39              | 45           |

\**p* < 0.05.

\*\**p* < 0.01.

\*\*\**p* < 0.001.





**Fig. 4.** (a) Average resource ratios of carbon to nitrogen (C : N,  $n = 76$ ), carbon to phosphorus (C : P,  $n = 97$ ) and nitrogen to phosphorus (N : P,  $n = 65$ ) for bioavailable (bio), total and inorganic (inorg, for N : P,  $n = 108$ ) fractions. Data in (a) shown as boxplots. Different letters represent statistical significance obtained with  $t$  test ( $p < 0.05$ ). Means are shown as diamond. (b) Temporal patterns in resource ratios for all streams. Ratios of N : P are shown for total, bioavailable organic (bio), and inorganic (inorg) fractions. Error bars denote  $\pm$  SE.

temperatures decreased (Fig. 3). All three resources (C, N, and P) had higher bioavailability in September when temperature was high, and then decreased in December and March when temperatures were the lowest registered during the study. However, resource bioavailability was still decreasing in April for both C and P when temperatures were increasing and discharge was the highest (spring flood; Fig. 3).

Discharge during the sampling period, measured for the stream outlet (C7), was the highest when averaged relative bioavailability for all resources was the lowest (second lowest for BDN; Fig. 3d–f). However, discharge was only negatively correlated with BDOC and BDN (Pearson correlation,  $p < 0.0001$ ) in mire streams (Table 2). Furthermore, suspended Chl *a* and precipitation were also significantly correlated with nutrient bioavailability. Chl *a* was positively correlated with BDN (Pearson correlation,  $p = 0.02$ ) and BDOP (Pearson correlation,  $p < 0.0001$ ) in forest streams, but only with BDN in

mire streams (Pearson correlation,  $p = 0.016$ ; Table 2). Precipitation was positively correlated with BDOC and BDOP in forest streams, but only with BDOP (Pearson correlation,  $p = 0.036$ ) in mire streams (Table 2; Supplementary Table S2).

### Overall and temporal patterns of resource ratios

Mean nutrient ratios (averaged for all replicate observations) calculated for the total pools of nutrients (i.e., bioavailable organic and inorganic + nonbioavailable organic nutrient fractions) were, in general, significantly higher than those calculated for bioavailable fractions (Fig. 4a). Average total C : N ratio (median 45,  $\pm$  2 SE) was two times higher than bioavailable C : N ratio (median 24  $\pm$  21 SE, Fig. 4a; Supplementary Table S3). In contrast, averaged C : P total ratio was over 10 times higher than bioavailable C : P ratio (median 109  $\pm$  23 SE). Similarly, averaged bioavailable N : P ratio (median 6.2  $\pm$  1.1 SE) was a fifth lower than averaged N : P total ratio (35  $\pm$  1.5 SE) and about a third lower than N : P inorganic ratios (18  $\pm$  6.6 SE).

Total ratios of C : P and N : P remained higher than bioavailable ratios throughout the seasons (Fig. 4b). For C : N, bioavailable ratios ( $\log_{10}$  mean 2.51,  $\pm$  0.1 SE) were higher than total C : N ratios ( $\log_{10}$  mean 1.49,  $\pm$  0.04 SE; Fig. 4b) in March 2019. Moreover, highest values for bioavailable N : P ratios were observed during the autumn while the lowest were in winter (Fig. 4b). We also observed a considerable increase of bioavailable N : P from winter (March) to spring flood (end of April), when also inorganic N : P ratios were at their highest. However, although C : P ratios decreased from the spring to the first summer measurement (July), there was no similar decrease in N : P ratios during the summer, contradicting our hypothesis.

### Discussion

Increased loading of DOM to aquatic ecosystems affects potential nutrient supplies to aquatic primary and secondary producers and consequently impacts the functioning of freshwater ecosystems (Peura et al. 2014; Trochine et al. 2014). Our results show that BDOM derived from catchment soils and peatlands can be an important nutrient source, especially of N and P, for heterotrophic microbes in DOM-rich boreal surface waters. Yet, the bioavailability of dissolved organic inputs also varied seasonally for all three nutrients (C, N, and P). Thus, our study highlights the dynamic nature of organic nutrient fractions that should be considered when predicting bioavailability for heterotrophic microbes in boreal freshwater ecosystems. Moreover, as boreal ecosystems are particularly vulnerable to ongoing climate changes (Laudon et al. 2013), a better understanding of bioavailability and stoichiometry of the bioavailable resources in these ecosystems may be fundamental to predict and manage potential responses to climate pressures in northern regions, for example, changes in nutrient limitation due to shifts in DOM export as a consequence of altered precipitation and stream flow.

### The key importance of organic nutrient fractions

Our observed concentrations of bioavailable organic nutrients agree with previously published results and reinforce the importance of organic bioavailable resources for heterotrophic microbes in DOM-rich waters. For example, absolute concentrations of BDOC ( $153\text{--}1319\ \mu\text{g C L}^{-1}$ , mean:  $569\ \mu\text{g C L}^{-1}$ ) and BDON ( $0.7\text{--}304\ \mu\text{g N L}^{-1}$ , mean:  $49\ \mu\text{g N L}^{-1}$ ) were similar to those reported for boreal freshwater lakes (Soares et al. 2017), as well as cedar bog wetlands (Wiegner and Seitzinger 2004) and a temperate estuary (Knudsen-Leerbeck et al. 2017) and boreal streams (Stepanauskas et al. 2000b). Bioavailable DOP concentrations ( $1\text{--}21\ \mu\text{g P L}^{-1}$ , mean:  $4.8\ \mu\text{g P L}^{-1}$ ) were also similar to those reported by Soares et al. (2017) and Jansson et al. (2012) for two boreal streams (streams 1 and 4 of the present study). Furthermore, organic forms of N and P dominated the total pools of these nutrients (91% and 84%, respectively) in the four streams, from which 11% and 40% were bioavailable, respectively. These values are in the range previously recorded for BDON in freshwater mires in southern Sweden (Stepanauskas et al. 1999) and a few rivers in the Bothnian Bay drainage area (Stepanauskas et al. 2002). Bioavailable DOP fractions relative to the total DOP pool were intermediate to values reported for boreal lakes (Soares et al. 2017) and Baltic Sea rivers (Stepanauskas et al. 2002). Our findings also support the observation from Soares et al. (2017) that relative nutrient bioavailability increase from BDOC to BDON and from BDON to BDOP. Furthermore, DOC bioavailability was considerably lower compared to N and P. Hence, our study emphasizes the argument that C may be highly important in controlling heterotrophic metabolism in boreal aquatic ecosystems with high absolute DOC concentrations (Burrows et al. 2015; Soares et al. 2017). Therefore, future changes in loadings of C due to climate change and anthropogenic actions could have a great impact on ecological processes of boreal aquatic ecosystems.

### Seasonal patterns of bioavailable DOC, DON, and DOP

Our results contrast with some previous observations for BDOC in cedar bog wetland streams (Wiegner and Seitzinger 2004) and BDON in other boreal streams in the same region as the present study (Stepanauskas et al. 2000b) that highlighted the importance of flooding for bioavailability. Instead, our seasonal patterns are more similar to those documented elsewhere, including observed increases in forest DON utilization in summer (Seitzinger et al. 2002) and decreases of BDOC with discharge in a black water river (Leff and Meyer 1991). Leff and Meyer (1991) suggest that declining bioavailability with discharge may be due to bacterial composition and differences in resource use, since many of the new bacterial cells washed in with the spring flood may be inactive and thus less capable of growth. Despite the differences in hydrology and biogeochemistry of the Leff and Meyer (1991) study sites as compared to ours, this effect of composition could possibly have affected our experiments,

which were based on a fresh inoculum obtained during the spring flood. Therefore, further studies on DOM bioavailability considering the native bacterial community composition would be interesting to confirm and understand better these seasonal patterns. Alternatively, low DOC and DON bioavailability during spring flood may be caused by snowmelt dilution of DOM (Ågren et al. 2007; Broder et al. 2017), which is especially important for mire sites (Buffam et al. 2007). Moreover, the correlations of BDOC and BDON with total DOC and discharge in mire streams support the snowmelt dilution effect on DOM bioavailability in this study.

Unusual climate conditions during this study could be another reason why our observations deviated from past studies in boreal streams. For example, the high concentrations of bioavailable DOM observed in this study for autumn 2018 may be associated with the severe drought the previous summer (Gómez-Gener et al. 2020; Johansson et al. 2020). Dry periods induce changes in DOM quality mobilized in soils (Smith et al. 2017) and supplied to streams (Broder et al. 2017). Long drought periods cause reduction of microbial biomass in soils, increasing losses of nutrients to soils due to various factors, such as build-up of dead cells, release of intracellular organic solutes, and exposure of reactive organic matter on formerly protected soil surfaces (Borken and Matzner 2009; Khan et al. 2019). Additionally, accumulation of nonmicrobial biomass substrates on land, such as fresh litter, during drought periods represents an important fraction of available resources to microorganisms (Borken and Matzner 2009; Hensgens et al. 2020) during the rewetting process. With autumn storms and rewetting, released labile DOM from these various sources is carried out via runoff to surface waters and can be used by heterotrophic microorganisms enhancing bacterial growth in these systems. Furthermore, DOM molecular size and aromaticity usually decrease under drought conditions, and thus when the ground water table starts to increase again, more labile DOM will be delivered to surface waters (Broder et al. 2017). Rain events that terminate dry periods in both autumn and spring are more likely to deliver more labile DOM to streams (Broder et al. 2017). Together, our results highlight the potential key importance of DOM accumulation during drought and autumn storms for export of bioavailable nutrients to aquatic systems. This seasonal time window has traditionally received much less attention than spring snow melt as a driver of resource supply to northern streams. With potential increases in the frequency of summer drought in this region (Spinoni et al. 2018), flushing of dissolved resources during autumn could emerge as an increasingly important process.

The BDOP seasonal patterns supported our expectations that concentrations would be highest during summer, although concentrations were also high in autumn 2018 for mire streams. The increase in P may be associated with high mineralization of P and export from soils due to warmer soil temperatures in the summer (Jansson et al. 2012). Seasonal

variations in BDOP as well as BDOC and BDON followed patterns similar to air temperature, which was reflected in the positive correlation between air temperature and bioavailable DOM (except for mire BDOC) (Table 2). Therefore, not only BDOP but also BDOC (only forest) and BDON were temperature dependent. This temperature dependency, together with the correlation between BDON and BDOC with discharge, may explain spring decreases and summer increases of these resources. Regardless, the similarities in temporal variation of BDOM between streams types suggest that the export of BDOC, BDON, and BDOP from catchments dominated by mire or forest cover was regulated by similar mechanisms. Yet, the absolute concentrations were much higher in mire compared to forest streams during autumn 2018 for all three resources, and during summer for BDOP only (Fig. 2). Jansson et al. (2012) identified similar temporal variations for BDOP in boreal headwater streams (two of the streams investigated in the present study, C2 and C4). They argued that both temporal variation and the quality of BDOP were likely to follow the same pattern in a range of boreal headwater streams. Our study brings this view one step further, and we thus suggest that not only BDOP but also temporal variations in absolute BDOC and BDON concentrations may follow the same patterns regardless of catchment characteristics.

### BDOM stoichiometry

Stoichiometry studies enable predictions of multiple nutrients as they assess the elemental balance between relevant resources and the organisms that use them (Sterner and Elser 2002). Nevertheless, we lack knowledge of the actual supply of bioavailable nutrients to aquatic organisms and this supply is central to understanding the elemental nutrient imbalances that organisms experience. Berggren et al. (2015) argued that simultaneous measurements of nutrient bioavailability is a way to better understand these imbalances that bacterial communities face in the natural environment. Here, we observed that BDOC : BDOP and BDON : BDOP were consistently lower than total fractions of C : P and N : P, similar to Thompson and Cotner (2018) for C : P. Therefore, bioavailability estimates based on bulk or total fractions of nutrients appear to overestimate the actual bioavailable DOC and DON pool, or underestimate the bioavailable DOP pool, for heterotrophic microbes.

The relatively higher inorganic N : P ratios during the spring flood could be associated with a general high demand of N by terrestrial plants during the summer (Bergström et al. 2008), whereas P may be in excess in the terrestrial ecosystem, being mineralized efficiently in soils at high temperatures (Jansson et al. 2012). Similarly, we hypothesized that BDON : BDOP ratios would be high in spring and autumn but low in summer. However, our findings did not support this hypothesis, but instead showed that BDON : BDOP ratios were substantially higher during autumn (for both studied years) than spring. These results are interesting in relation to a recent

study in the same study area, suggesting the importance of fresh litter (on land), with high emphasis on understory, for DOM bioavailability in surface water (Hensgens et al. 2020). They argued that DOC and DON leaching from litter are highly correlated and that fresh litter produces highly labile DON on the forest floor during autumn. Additionally, more recalcitrant DOM is leached as time passes (Hensgens et al. 2020). Labile DOM from fresh litter, therefore, could be an important energy source for microorganism in boreal forest soils as well as for microbial heterotrophs in surface water during autumn. In addition, this study highlights the distinct views on resource limitation depending on which resource fraction is focused on, for example, looking at inorganic or total N : P ratios would suggest P limitation during spring, while the bioavailable N : P ratios reported here indicate N limitation. Hence, we stress the importance of considering the real bioavailability of nutrients to microorganisms in studies addressing resource limitation.

### Nutrient bioavailability estimates using flow cytometry

The strength of the bioassay approach using flow cytometry is in accounting for both metabolic diversity and natural chemistry (Stepanauskas et al. 1999). Our control incubation samples showed that only a negligible number of bacterial cells grew when there was a lack of the limiting resource, which confirmed the dependency between the limiting resource and bacterial growth. However, samples with measurements considered below the detection range, that is, samples with calculated negative bioavailability values and which were removed from the analyses, could have a potential impact on the results. For instance, a fraction of the N-limited incubation samples in April and August had below detection values. Nonetheless, measurements from site C4 from both April and August samples had consistent values across all replicates and supported the patterns described earlier in the Discussion section. Additionally, bacterial abundance for this site was comparable to previously described in the literature using a similar approach (Stepanauskas et al. 1999). On the other hand, if the samples considered below the detection threshold were not removed from the analysis and instead were considered as zeros, that is, no bioavailable resource, seasonal patterns would have been affected. For example, averaged BDON estimates during the spring flood would be even lower than reported here, but summer estimates would also be lower and closer to our expectations.

The BDOP estimates using the bioassay and flow cytometry approach were mostly consistent and resulted in concentrations comparable to other approaches (Jansson et al. 2012). However, mire samples from September 2018 and August 2019 had concentrations higher than that of the measured stream TDP, which resulted in relative BDOP values above 100%. A reason for this could be luxury P uptake by the microbial cells. Luxury P uptake is the ability of both auto and heterotrophic microbes to assimilate more P, usually as

polyphosphate, than necessary for immediate growth when in conditions of high P concentrations (Khoshmanesh et al. 2002; Solovchenko et al. 2019). Therefore, if luxury uptake happened during the mentioned occasions of the experiment, P content per cell as well as BDOP estimates could have been overestimated resulting in BDOP values higher than stream TDP measurements.

Our bioavailability estimates were performed over a 7-d period and most resources were consumed within the first 2 d. Bioavailability assessments are generally done in a much longer time frame (e.g., 100 d); however, short incubation times for assessing bioavailability estimates are indeed relevant to streams because of their short water residence times (Berggren et al. 2015). Furthermore, the use of shorter time frame incubations for bioavailability measurements prevents recycling of resources by bacteria and reflects the readily bioavailable resource pool to heterotrophic microbes at any point in time (Soares et al. 2017). Despite the short time scale, there may have been recycling of resource and this may be reflected in the secondary peaks registered for bacterial abundance measured in some incubations (especially for C; Supporting Information Fig. S2b). We accounted for this by using only day 2 in our estimates for all incubations, and thus considering just the readily bioavailable resource pool. In addition, the use of glucose as C spike in C incubations with added resource could potentially lead to overestimation of bacterial growth and consequent conservative estimates of BDOC, that is, glucose may support the fastest growing part of the community which may differ from the community supported by natural C sources (Soares et al. 2017). Nonetheless, our BDOC estimates were similar to bioavailability measurements using distinct methods. Thus, our short-term estimates of bioavailable DOM are most likely accurate, making this method an interesting and more accessible approach to be used in future bioavailability estimate studies.

## Conclusions

Boreal freshwater ecosystems will respond to changes in environmental conditions as climate change progresses (Laudon et al. 2013). The effect of these changes to primary and secondary production as well as food web dynamics in boreal freshwaters is still hard to predict (Creed et al. 2018). Our study shows that for a better understanding of aquatic production, more focus should be given to the real bioavailability and stoichiometry of the bioavailable resource pools, that is, considering both the inorganic and organic resources. We identified similar temporal variation of bioavailable DOC, DON, and DOP in streams from forest and mire catchments suggesting that both types are regulated by similar mechanisms. Nevertheless, absolute concentrations of these resources were generally higher for mire streams during autumn 2018. We showed that organic C, N, and P bioavailability changed seasonally and we suggest that autumn

processes, including rewetting of soils that were dry during summer as well as production of resources from litterfall, could have a greater importance for resource stoichiometry and heterotrophic microbes production than previously thought. Moreover, we propose that bioavailable N : P ratios could be affected by drought events, with amplified N : P ratios during the autumn and reduced increase from winter to spring. The idea that the autumn season could play such an important role in bacterial production calls for further research on seasonal bioavailability of organic resources to both auto- and heterotrophic microbes to increase our understanding of the main mechanisms driving change in stoichiometry with season.

## References

- Ågren, A., I. Buffam, M. Berggren, K. Bishop, M. Jansson, and H. Laudon. 2008. Dissolved organic carbon characteristics in boreal streams in a forest-wetland gradient during the transition between winter and summer. *Eur. J. Vasc. Endovasc. Surg.* **113**: G03031. doi:10.1029/2007JG000674
- Ågren, A., I. Buffam, M. Jansson, and H. Laudon. 2007. Importance of seasonality and small streams for the landscape regulation of dissolved organic carbon export. *Eur. J. Vasc. Endovasc. Surg.* **112**: G03003. doi:10.1029/2006JG000381
- Algesten, G., S. Sobek, A. K. Bergström, A. Jonsson, L. J. Tranvik, and M. Jansson. 2005. Contribution of sediment respiration to summer CO<sub>2</sub> emission from low productive boreal and subarctic lakes. *Microb. Ecol.* **50**: 529–535. doi:10.1007/s00248-005-5007-x
- Berggren, M., H. Laudon, and M. Jansson. 2009. Hydrological control of organic carbon support for bacterial growth in boreal headwater streams. *Microb. Ecol.* **57**: 170–178. doi:10.1007/s00248-008-9423-6
- Berggren, M., R. A. Sponseller, A. R. Alves Soares, and A. K. Bergström. 2015. Toward an ecologically meaningful view of resource stoichiometry in DOM-dominated aquatic systems. *J. Plankton Res.* **37**: 489–499. doi:10.1093/plankt/fbv018
- Berggren, M., L. Ström, H. Laudon, J. Karlsson, R. Giesler, A. K. Bergström, and M. Jansson. 2010. Lake secondary production fueled by rapid transfer of low molecular weight organic carbon from terrestrial sources to aquatic consumers. *Ecol. Lett.* **13**: 870–880. doi:10.1111/j.1461-0248.2010.01483.x
- Bergström, A. K., M. Jansson, S. Drakare, and P. Blomqvist. 2003. Occurrence of mixotrophic flagellates in relation to bacterioplankton production, light regime and availability of inorganic nutrients in unproductive lakes with differing humic contents. *Freshw. Biol.* **48**: 868–877. doi:10.1046/j.1365-2427.2003.01061.x
- Bergström, A. K., A. Jonsson, and M. Jansson. 2008. Phytoplankton responses to nitrogen and phosphorus enrichment in unproductive Swedish lakes along a gradient of

- atmospheric nitrogen deposition. *Aquat. Biol.* **4**: 55–64. doi:10.3354/ab00099
- Borken, W., and E. Matzner. 2009. Reappraisal of drying and wetting effects on C and N mineralization and fluxes in soils. *Glob. Chang. Biol.* **15**: 808–824. doi:10.1111/j.1365-2486.2008.01681.x
- Broder, T., K. H. Knorr, and H. Biester. 2017. Changes in dissolved organic matter quality in a peatland and forest headwater stream as a function of seasonality and hydrologic conditions. *Hydrol. Earth Syst. Sci.* **21**: 2035–2051. doi:10.5194/hess-21-2035-2017
- Buffam, I., H. Laudon, J. Temnerud, C. M. Mörth, and K. Bishop. 2007. Landscape-scale variability of acidity and dissolved organic carbon during spring flood in a boreal stream network. *Eur. J. Vasc. Endovasc. Surg.* **112**: G01022. doi:10.1029/2006JG000218
- Burrows, R. M., E. R. Hotchkiss, M. Jonsson, H. Laudon, B. G. McKie, and R. A. Sponseller. 2015. Nitrogen limitation of heterotrophic biofilms in boreal streams. *Freshw. Biol.* **60**: 1237–1251. doi:10.1111/Fwb.12549
- Conley, D. J., H. W. Paerl, R. W. Howarth, D. F. Boesch, S. P. Seitzinger, K. E. Havens, C. Lancelot, and G. E. Likens. 2009. Eutrophication: Time to adjust expectations response. *Science* **324**: 724–725. doi:10.1126/science.324\_724
- Creed, I. F., and others. 2018. Global change-driven effects on dissolved organic matter composition: Implications for food webs of northern lakes. *Glob. Chang. Biol.* **24**: 3692–3714. doi:10.1111/gcb.14129
- Evans, C. D., D. T. Monteith, and D. M. Cooper. 2005. Long-term increases in surface water dissolved organic carbon: Observations, possible causes and environmental impacts. *Environ. Pollut.* **137**: 55–71. doi:10.1016/j.envpol.2004.12.031
- Fellman, J. B., E. Hood, D. V. D'Amore, R. T. Edwards, and D. White. 2009. Seasonal changes in the chemical quality and biodegradability of dissolved organic matter exported from soils to streams in coastal temperate rainforest watersheds. *Biogeochemistry* **95**: 277–293. doi:10.1007/s10533-009-9336-6
- Figueroa, D., O. F. Rowe, J. Paczkowska, C. Legrand, and A. Andersson. 2016. Allochthonous carbon—a major driver of bacterioplankton production in the subarctic northern Baltic Sea. *Microb. Ecol.* **71**: 789–801. doi:10.1007/s00248-015-0714-4
- Fulweiler, R. W., N. N. Rabalais, and A. S. Heiskanen. 2012. The eutrophication commandments. *Mar. Pollut. Bull.* **64**: 1997–1999. doi:10.1016/j.marpolbul.2012.07.025
- del Giorgio, P. A., and J. Davis. 2003. Patterns in dissolved organic matter lability and consumption across aquatic ecosystems, p. 399–424. *In* S. E. G. Findlay and R. L. Sinsabaugh [eds.], *Aquatic ecosystems: Interactivity of dissolved organic matter*. Academic Press.
- Gómez-Gener, L., A. Lupon, H. Laudon, and R. A. Sponseller. 2020. Drought alters the biogeochemistry of boreal stream networks. *Nat. Commun.* **11**: 1–11. doi:10.1038/s41467-020-15496-2
- Hansson, L. A., and others. 2013. Food-chain length alters community responses to global change in aquatic systems. *Nat. Clim. Change* **3**: 228–233. doi:10.1038/Nclimate1689
- Hensgens, G., H. Laudon, M. Peichl, I. A. Gil, Q. Zhou, and M. Berggren. 2020. The role of the understory in litter DOC and nutrient leaching in boreal forests. *Biogeochemistry* **149**: 87–103. doi:10.1007/s10533-020-00668-5
- Hongve, D., G. Riise, and J. F. Kristiansen. 2004. Increased colour and organic acid concentrations in Norwegian forest lakes and drinking water - a result of increased precipitation? *Aquat. Sci.* **66**: 231–238. doi:10.1007/s00027-004-0708-7
- Howarth, R., F. Chan, D. J. Conley, J. Garnier, S. C. Doney, R. Marino, and G. Billen. 2011. Coupled biogeochemical cycles: Eutrophication and hypoxia in temperate estuaries and coastal marine ecosystems. *Front. Ecol. Environ.* **9**: 18–26. doi:10.1890/100008
- Isles, P. D. F. 2020. The misuse of ratios in ecological stoichiometry. *Ecology* **101**: e03153. doi:10.1002/ecy.3153
- Jahan-Tigh, R. R., C. Ryan, G. Obermoser, and K. Schwarzenberger. 2012. Flow cytometry. *J. Investig. Dermatol.* **132**: 1–6. doi:10.1038/jid.2012.282
- Jansson, M. 1998. Nutrient limitation and bacteria-phytoplankton interactions in humic lakes, p. 177–196. *In* L. J. Tranvik and D. O. Hessen [eds.], *Aquatic humic substances: Ecology and biogeochemistry*. Springer-Verlag.
- Jansson, M., M. Berggren, H. Laudon, and A. Jonsson. 2012. Bioavailable phosphorus in humic headwater streams in boreal Sweden. *Limnol. Oceanogr.* **57**: 1161–1170. doi:10.4319/lo.2012.57.4.1161
- Jansson, M., A. K. Bergstrom, P. Blomqvist, and S. Drakare. 2000. Allochthonous organic carbon and phytoplankton/bacterioplankton production relationships in lakes. *Ecology* **81**: 3250–3255. doi:10.2307/177416
- Johansson, J. M., Å. K. Watne, P. E. Karlsson, G. P. Karlsson, H. Danielsson, C. Andersson, and H. Pleijel. 2020. The European heat wave of 2018 and its promotion of the ozone climate penalty in southwest Sweden. *Boreal Environ. Res.* **25**: 39–50.
- Jones, R. I. 1992. The influence of humic substances on lacustrine planktonic food-chains. *Hydrobiologia* **229**: 73–91. doi:10.1007/BF00006992
- Jones, R. I., K. Salonen, and H. Dehaan. 1988. Phosphorus transformations in the epilimnion of humic lakes - abiotic interactions between dissolved humic materials and phosphate. *Freshw. Biol.* **19**: 357–369. doi:10.1111/j.1365-2427.1988.tb00357.x
- Karlsson, J., M. Jansson, and A. Jonsson. 2002. Similar relationships between pelagic primary and bacterial production in clearwater and humic lakes. *Ecology* **83**: 2902–2910. doi:10.2307/3072025

- Khan S. U., P. S. Hooda, M. S. A. Blackwell, and R. Busquets. 2019. Microbial Biomass Responses to Soil Drying-Rewetting and Phosphorus Leaching. *Front. Environ. Sci.* **7**: 133. doi:10.3389/fenvs.2019.00133
- Khoshmanesh, A., B. T. Hart, A. Duncan, and R. Beckett. 2002. Luxury uptake of phosphorus by sediment bacteria. *Water Res.* **36**: 774–778. doi:10.1016/S0043-1354(01)00272-X
- Knudsen-Leerbeck, H., M. Mantikci, M. Bentzon-Tilia, S. J. Traving, L. Riemann, J. L. S. Hansen, and S. Markager. 2017. Seasonal dynamics and bioavailability of dissolved organic matter in two contrasting temperate estuaries. *Biogeochemistry* **134**: 217–236. doi:10.1007/s10533-017-0357-2
- Kritzberg, E. S. 2017. Centennial-long trends of lake browning show major effect of afforestation. *Limnol. Oceanogr.: Letters* **2**: 105–112. doi:10.1002/lo.10041
- Kritzberg, E. S., S. Langenheder, and E. S. Lindström. 2006. Influence of dissolved organic matter source on lake bacterioplankton structure and function - implications for seasonal dynamics of community composition. *FEMS Microbiol. Ecol.* **56**: 406–417. doi:10.1111/j.1574-6941.2006.00084.x
- Lapierre, J. F., F. Guillemette, M. Berggren, and P. A. del Giorgio. 2013. Increases in terrestrially derived carbon stimulate organic carbon processing and CO<sub>2</sub> emissions in boreal aquatic ecosystems. *Nat. Commun.* **4**: 2972. doi:10.1038/ncomms3972
- Larsen, S., T. Andersen, and D. O. Hessen. 2011. Climate change predicted to cause severe increase of organic carbon in lakes. *Glob. Chang. Biol.* **17**: 1186–1192. doi:10.1111/j.1365-2486.2010.02257.x
- Laudon, H., I. Taberman, A. Agren, M. Futter, M. Ottosson-Lofvenius, and K. Bishop. 2013. The Krycklan Catchment Study—a flagship infrastructure for hydrology, biogeochemistry, and climate research in the boreal landscape. *Water Resour. Res.* **49**: 7154–7158. doi:10.1002/Wrcr.20520
- Laudon, H., and others. 2021. Northern landscapes in transition: Evidence, approach and ways forward using the Krycklan Catchment Study. *Hydrological Processes* **35**: e14170. doi:10.1002/hyp.14170
- Lebret, K., S. Langenheder, N. Colinas, O. Ostman, and E. S. Lindstrom. 2018. Increased water colour affects freshwater plankton communities in a mesocosm study. *Aquat. Microb. Ecol.* **81**: 1–17. doi:10.3354/ame01858
- Leff, L. G., and J. L. Meyer. 1991. Biological availability of dissolved organic carbon along the Ogeechee river. *Limnol. Oceanogr.* **36**: 315–323. doi:10.4319/lo.1991.36.2.0315
- Likens, G. E. 2010. *Biogeochemistry of inland waters*. Elsevier Science.
- Lindström, K. 1991. Nutrient requirements of the dinoflagellate *Peridinium gatunense*. *J. Phycol.* **27**: 207–219. doi:10.1111/j.0022-3646.1991.00207.x
- Lønborg, C., X. A. Alvarez-Salgado, S. Duggan, and C. Carreira. 2018. Organic matter bioavailability in tropical coastal waters: The Great Barrier Reef. *Limnol. Oceanogr.* **63**: 1015–1035. doi:10.1002/lno.10717
- Martinez, J., D. C. Smith, G. F. Steward, and F. Azam. 1996. Variability in ectohydrolytic enzyme activities of pelagic marine bacteria and its significance for substrate processing in the sea. *Aquat. Microb. Ecol.* **10**: 223–230. doi:10.3354/ame010223
- Menzel, D. H., and N. Corwin. 1965. The measurement of total phosphorus in seawater based on the liberation of organically bound fractions by persulphate oxidation. *Limnol. Oceanogr.* **10**: 280–282. doi:10.4319/lo.1965.10.2.0280
- Monteith, D. T., and others. 2007. Dissolved organic carbon trends resulting from changes in atmospheric deposition chemistry. *Nature* **450**: 537–540. doi:10.1038/nature06316
- Murphy, J., and J. P. Riley. 1962. A modified single solution method for the determination of phosphate in natural waters. *Anal. Chim. Acta* **27**: 31–36. doi:10.1016/S0003-2670(00)88444-5
- Peura, S., H. Nykanen, P. Kankaala, A. Eiler, M. Tirola, and R. I. Jones. 2014. Enhanced greenhouse gas emissions and changes in plankton communities following an experimental increase in organic carbon loading to a humic lake. *Biogeochemistry* **118**: 177–194. doi:10.1007/s10533-013-9917-2
- R Core Team. 2020. R: A language and environment for statistical computing.
- Seitzinger, S. P., R. W. Sanders, and R. Styles. 2002. Bioavailability of DON from natural and anthropogenic sources to estuarine plankton. *Limnol. Oceanogr.* **47**: 353–366. doi:10.4319/lo.2002.47.2.0353
- Smith, A. P., B. Bond-Lamberty, B. W. Benschoter, M. M. Tfaily, C. R. Hinkle, C. Liu, and V. L. Bailey. 2017. Shifts in pore connectivity from precipitation versus groundwater rewetting increases soil carbon loss after drought. *Nat. Commun.* **8**: 1335. doi:10.1038/s41467-017-01320-x
- Soares, A. R. A., A. K. Bergström, R. A. Sponseller, J. M. Moberg, R. Giesler, E. S. Kritzberg, M. Jansson, and M. Berggren. 2017. New insights on resource stoichiometry: Assessing availability of carbon, nitrogen, and phosphorus to bacterioplankton. *Biogeosciences* **14**: 1527–1539. doi:10.5194/bg-14-1527-2017
- Solovchenko, A. E., T. T. Ismagulova, A. A. Lukyanov, S. G. Vasilieva, I. V. Konyukhov, S. I. Pogosyan, E. S. Lobakova, and O. A. Gorelova. 2019. Luxury phosphorus uptake in microalgae. *J. Appl. Phycol.* **31**: 2755–2770. doi:10.1007/s10811-019-01831-8
- Spinoni, J., J. V. Vogt, G. Naumann, P. Barbosa, and A. Dosio. 2018. Will drought events become more frequent and severe in Europe? *Int. J. Climatol.* **38**: 1718–1736. doi:10.1002/joc.5291
- Sponseller, R. A., J. Temnerud, K. Bishop, and H. Laudon. 2014. Patterns and drivers of riverine nitrogen (N) across alpine, subarctic, and boreal Sweden. *Biogeochemistry* **120**: 105–120. doi:10.1007/s10533-014-9984-z

- Stepanauskas, R., V. F. Farjalla, L. J. Tranvik, J. M. Svensson, F. A. Esteves, and W. Granéli. 2000a. Bioavailability and sources of DOC and DON in macrophyte stands of a tropical coastal lake. *Hydrobiologia* **436**: 241–248. doi:[10.1023/a:1026537905947](https://doi.org/10.1023/a:1026537905947)
- Stepanauskas, R., N. O. G. Jørgensen, O. R. Eigaard, A. Zvikas, L. J. Tranvik, and L. Leonardson. 2002. Summer inputs of riverine nutrients to the Baltic Sea: Bioavailability and eutrophication relevance. *Ecol. Monogr.* **72**: 579–597. doi:[10.2307/3100058](https://doi.org/10.2307/3100058)
- Stepanauskas, R., H. Laudon, and N. O. G. Jørgensen. 2000b. High DON bioavailability in boreal streams during a spring flood. *Limnol. Oceanogr.* **45**: 1298–1307. doi:[10.4319/lo.2000.45.6.1298](https://doi.org/10.4319/lo.2000.45.6.1298)
- Stepanauskas, R., L. Leonardson, and L. J. Tranvik. 1999. Bioavailability of wetland-derived DON to freshwater and marine bacterioplankton. *Limnol. Oceanogr.* **44**: 1477–1485. doi:[10.4319/lo.1999.44.6.1477](https://doi.org/10.4319/lo.1999.44.6.1477)
- Sterner, R. W., and J. J. Elser. 2002. *Ecological stoichiometry: The biology of elements from molecules to the biosphere.* Princeton Univ. Press.
- Stutter, M. I., D. Graeber, C. D. Evans, A. J. Wade, and P. J. A. Withers. 2018. Balancing macronutrient stoichiometry to alleviate eutrophication. *Sci. Total Environ.* **634**: 439–447. doi:[10.1016/j.scitotenv.2018.03.298](https://doi.org/10.1016/j.scitotenv.2018.03.298)
- Thompson, S. K., and J. B. Cotner. 2018. Bioavailability of dissolved organic phosphorus in temperate lakes. *Front. Environ. Sci.* **6**: 62. doi:[10.3389/fenvs.2018.00062](https://doi.org/10.3389/fenvs.2018.00062)
- Tiwari, T., R. A. Sponseller, and H. Laudon. 2018. Extreme climate effects on dissolved organic carbon concentrations during snowmelt. *Eur. J. Vasc. Endovasc. Surg.* **123**: 1277–1288. doi:[10.1002/2017jg004272](https://doi.org/10.1002/2017jg004272)
- Trochine, C., M. E. Guerrieri, L. Liboriussen, T. L. Lauridsen, and E. Jeppesen. 2014. Effects of nutrient loading, temperature regime and grazing pressure on nutrient limitation of periphyton in experimental ponds. *Freshw. Biol.* **59**: 905–917. doi:[10.1111/Fwb.12314](https://doi.org/10.1111/Fwb.12314)
- Vives-Rego, J., P. Lebaron, and G. Nebe-von Caron. 2000. Current and future applications of flow cytometry in aquatic microbiology. *FEMS Microbiol. Rev.* **24**: 429–448. doi:[10.1111/j.1574-6976.2000.tb00549.x](https://doi.org/10.1111/j.1574-6976.2000.tb00549.x)
- Wallin, M. B., G. A. Weyhenmeyer, D. Bastviken, H. E. Chmiel, S. Peter, S. Sobek, and L. Klemetsson. 2015. Temporal control on concentration, character, and export of dissolved organic carbon in two hemiboreal headwater streams draining contrasting catchments. *Eur. J. Vasc. Endovasc. Surg.* **120**: 832–846. doi:[10.1002/2014jg002814](https://doi.org/10.1002/2014jg002814)
- Wang, Y., F. Hammes, K. De Roy, W. Verstraete, and N. Boon. 2010. Past, present and future applications of flow cytometry in aquatic microbiology. *Trends Biotechnol.* **28**: 416–424. doi:[10.1016/j.tibtech.2010.04.006](https://doi.org/10.1016/j.tibtech.2010.04.006)
- Wiegner, T. N., and S. P. Seitzinger. 2004. Seasonal bioavailability of dissolved organic carbon and nitrogen from pristine and polluted freshwater wetlands. *Limnol. Oceanogr.* **49**: 1703–1712. doi:[10.4319/lo.2004.49.5.1703](https://doi.org/10.4319/lo.2004.49.5.1703)
- Worsfold, P., I. McKelvie, and P. Monbet. 2016. Determination of phosphorus in natural waters: A historical review. *Anal. Chim. Acta* **918**: 8–20. doi:[10.1016/j.aca.2016.02.047](https://doi.org/10.1016/j.aca.2016.02.047)

#### Acknowledgments

We would like to thank the technical staff at the Krycklan Catchment Study for sample collection and shipping whenever needed. This research was funded by FORMAS - A Swedish Research Council for Sustainable Development (grant 2017-00772). The study was further supported by the Royal Physiographic Society of Lund (Fysiografen).

#### Conflict of Interest

None declared.

Submitted 07 April 2021

Revised 06 October 2021

Accepted 01 March 2022

Associate editor: Robert O. Hall, Jr.