Large differences between carbon and nutrient loss rates along the land to ocean aquatic continuum—implications for energy:nutrient ratios at downstream sites

Gesa A. Weyhenmeyer,1* Daniel J. Conley2
1Department of Ecology and Genetics/Limnology, Uppsala University, Uppsala, Sweden
2Department of Geology, Lund University, Lund, Sweden

Abstract

The balance between the availability of energy and nutrients is decisive for the growth and survival of organisms. Here, we evaluated how energy, in this study expressed as total carbon (TC), is lost along the land to ocean aquatic continuum (LOAC) in relation to nutrients, i.e., total phosphorus (TP), total nitrogen (TN), total iron (TFe), and dissolved silica (DSi). For the evaluation, we used data from 4774 lakes, 149 streams, and 52 river mouths from the boreal region. We found that the loss of all chemical variables followed a first order decay function along the LOAC with shortest half-lives for TFe and DSi (410 d and 568 d, respectively). The half-life of TC was more than twice as long as for TFe and DSi, resulting in rapidly increasing TC:TFe and TC:DSi ratios along the LOAC. In contrast, TC:TP and TC:TN ratios decreased along the LOAC. The TC and TFe concentration declines along the LOAC were quantitatively similar to the TC and TFe concentration declines from winter to summer, indicating that similar drivers are responsible for spatial and seasonal TC and TFe losses in inland waters. We conclude that the energy:nutrient ratio rapidly changes along the LOAC with an increasing surplus of energy in relation to TFe and DSi the longer water stays in the landscape. These findings have implications for the growth of aquatic organisms along the LOAC, where organisms are likely to become increasingly iron and silica limited with increasing water retention in the landscape.

All aquatic organisms require energy in form of carbon and nutrients, in particular phosphorus and nitrogen but also iron, silicon, and other micronutrients, for critical cellular processes (Sterner and Elser 2002). For prediction of growth and survival of aquatic organisms, it is therefore essential to understand the quantity as well as the stoichiometry of carbon and nutrients in aquatic ecosystems. This field of research is well established, and numerous studies are available where energy:nutrient ratios in aquatic ecosystems have been analyzed in detail (e.g., Sterner et al. 1992; Hecky et al. 1993; Stelzer and Lamberti 2001; Hillebrand et al. 2014). Identified major drivers for variations in energy:nutrient ratios in aquatic ecosystems are land use (e.g., Arbuckle and Downing 2001), atmospheric deposition (e.g., Elser et al. 2009), and hydrology (e.g., Frost et al. 2009). There are, however, still some unknowns, one of them being knowledge on how stable the energy:nutrient ratio is when water travels through a lake-rich landscape, i.e., along the land to ocean aquatic continuum (LOAC) of the boreal region. This knowledge gap makes predictions of the survival of aquatic organisms along the LOAC of the boreal region uncertain. There is, for example, a possibility that limitations for the growth of aquatic organisms along the LOAC, where organisms are likely to become increasingly iron and silica limited with increasing water retention in the landscape.

*Correspondence: gesa.weyhenmeyer@ebc.uu.se

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

Special Issue: Headwaters to Oceans: Ecological and Biogeochemical Contrasts Across the Aquatic Continuum
Edited by: Marguerite Xenopoulos, John A. Downing, M. Dileep Kumar, Susanne Menden-Deuer, and Maren Voss

S183
The carbon loss is known to increase with increasing water retention in the landscape (Algesten et al. 2004; Catalan et al. 2016). Based on this knowledge, we suggest that a substantial amount of carbon and probably also of nitrogen which in the boreal region shows a close linkage to carbon (Khalili et al. 2010) is lost along the LOAC when water passes through lakes. In addition, we expect that the loss of carbon and nitrogen along the LOAC exceeds the loss of phosphorus and micronutrients since carbon and nitrogen can be lost in form of gases at the water-atmosphere interface while phosphorus, silicon, and iron cannot. These expectations are, however, only correct when all elements have the same primary upstream source with no major additional inputs along the LOAC. Such a requirement might be fulfilled when the energy and nutrient change along the LOAC follows a first order decay function which has earlier been reported for organic carbon in the boreal region (Algesten et al. 2004; Weyhenmeyer et al. 2012a; Müller et al. 2013).

We tested here whether concentrations of total carbon (TC; with separate analyses of the organic and inorganic fraction of TC), total phosphorus (TP), total nitrogen (TN), total iron (TFe), and dissolved silica (DSi) in Swedish boreal waters follow a first order decay function along the LOAC during autumn water column mixing. Since TC, TP, TN, TFe, and DSi in Swedish boreal waters most probably have their main source in the surrounding forest floor in the relatively sparsely populated Swedish boreal region, we hypothesized that TC, TP, TN, TFe, and DSi autumn concentrations in Swedish boreal waters decline according to a first order decay function along the LOAC. We further hypothesized that the carbon to nutrient ratios, i.e., TC:TP, TC:TN, TC:TFe, and TC:DSi ratios, significantly decrease along the LOAC of the boreal region, indicating a faster TC than TP, TN, TFe, and DSi loss as a consequence of CO₂ and CH₄ emissions. As a final step, to better understand drivers for changes in the energy:nutrient ratio along the LOAC, we compared changes in TC, TP, TN, TFe, and DSi autumn concentrations along the LOAC with seasonal changes in TC, TP, TN, TFe, and DSi concentrations. We hypothesized that concentration declines along the LOAC during autumn are slower than concentration declines from winter to summer due to strong TC, TP, TN, TFe, and DSi concentration declines by seasonally driven mineralization, benthic primary production as well as pelagic primary production induced sedimentation. We tested the three hypotheses with thousands of lake, stream, and river mouth data from the Swedish boreal and hemiboreal region.

**Methods**

**Lake, stream, and river mouth data**

To study changes in the energy:nutrient ratio along the LOAC within the boreal region, we used water chemical data from 4774 lakes (including Sweden’s two largest lakes Vänern and Vättern), 149 streams (including three very small headwater streams), and 52 river mouths distributed across the entire boreal and hemiboreal areas of Sweden (Fig. 1). All data have been collected as part of the Swedish national freshwater inventory program (http://webstar.vatten.slu.se/db.html). The inventory database includes thousands of lake, stream, and river mouth data. We chose here all waters for which data were available on total organic carbon (TOC) concentrations (in mg L⁻¹), TP concentrations (in μg L⁻¹), TN concentrations (in μg L⁻¹), TFe concentrations (in mg L⁻¹), and molybdate-reactive silicon which represents the amount of the dissolved fraction of total silicon.

![Fig. 1. Map of Sweden, showing 4975 sampling sites distributed across Sweden.](image-url)
(DSi; in mg L\(^{-1}\)). All measurements of the chemical variables were performed on unfiltered water and analyzed by the Swedish Board for Accreditation and Conformity (SWEDAC) accredited laboratory at the Swedish University of Agricultural Sciences following standard limnological procedures. The laboratory has been responsible for the Swedish National Freshwater Inventory Program and data quality check since the beginning in the 1960s so that data quality can be considered as being comparable between sites and years. TOC was measured with a Shimadzu (Swedish Standard SS-EN 1484 1st ed.), TP was measured with a Technicon Autoanalyser (Swedish Standard SS-EN ISO 6878:2005; modified), TN was measured with a Shimadzu (since 2007; Swedish Standard SS-EN 12260:2004) or with Technicon Traacs 800 (before 2007; Swedish Standard SS-EN ISO 11905 modified for Traacs), TFe was measured with a Varian Vista AX (since 2008) or PerkinElmer (Swedish Standard SS-EN ISO 11885:2009), and DSI was measured with an Autoanalyser (since 2007) or Technicon Traacs 800 (Bran and Leubbe Method). A detailed method description including analytical precision and range is available at http://www.slu.se/en/departments/aquatic-sciences-assessment/laboratories/geochemical-laboratory/water-chemical-analyses/.

In addition to measured TOC, TP, TN, TFe, and DSI, we calculated dissolved inorganic carbon (DIC; in mgC L\(^{-1}\)) from water temperature, alkalinity (only positive values were selected), pH (only values > 5.4 were used to minimize incorrect estimates of CO\(_2\) concentrations), and altitude according to Weyhenmeyer et al. (2012). This approach has limitations but gives reasonable results for Swedish boreal waters as described in Weyhenmeyer et al. (2015). Concentrations of DIC together with TOC were then used to calculate TC concentrations. This calculation neglects particulate inorganic carbon, which we assume being negligible in the humic boreal waters. Of minor importance is also the fraction of particulate organic carbon (POC), i.e., studies have shown that commonly <3% of TOC is POC in boreal waters (Kortelainen et al. 2006; von Wachenfeldt and Tranvik 2008; Laudon et al. 2011). Thus, TOC in our study is clearly dominated by DOC. Likewise, TN in Swedish boreal waters is usually clearly dominated by dissolved organic nitrogen (Khaliili et al. 2010).

All data from Swedish the National Freshwater Inventory Program can freely be downloaded at http://webstar.vatten.slu.se/db.html. Most study lakes were small (excluding Sweden’s two largest lakes Vänern and Vättern) with a median lake area of 0.16 km\(^2\) (97.5% quartile: 11.25 km\(^2\)), and a median mean lake depth of 3.2 m (97.5% quartile: 11 m). The lakes, streams, and river mouths examined were generally nutrient-poor (median TP: 10 μg L\(^{-1}\), 97.5% quartile: 65 μg L\(^{-1}\); median TN: 0.41 mg L\(^{-1}\), 97.5% quartile: 1.42 mg L\(^{-1}\)) and rather humic (median TOC: 14.7 mg L\(^{-1}\), 97.5% quartile: 46.4 mg L\(^{-1}\); median pH: 6.7, 97.5% quartile: 7.7). All samples were collected at a water depth of 0.5 m (except of the very small headwater streams where surface-water samples were taken).

For the assessment of spatial variation in TC, TOC, DIC, TP, TN, TFe, and DSI concentrations along the LOAC, we used site-specific long-term median values during autumn. The early autumn period is the time when the annual large-scale national inventory takes place in about 1000 lakes. In addition to the large-scale sampling, there is a monthly or at least four times a year sampling program for several streams and lakes and the river mouths. To achieve consistency in the data material, we restricted our spatial analysis to data from the early autumn period when water temperatures are around 4°C and the water column is well mixed. When more than one autumn value during a year was available, we chose the autumn value when water temperatures were closest to 4°C. Since our sites were re-sampled a few times since 1990, we used the median of available autumn values. The number of available autumn values for each site varied but since year-to-year variation in autumn water chemistry at around 4°C water temperature usually remains small compared to the spatial variation (Weyhenmeyer et al. 2012b) we consider the median being suitable for analyzes of spatial variation.

Apart from spatial variation, we also considered seasonal variation. From the 4975 inland waters, we selected waters that were sampled during different months for more than 15 yr. This selection gave 395 inland waters, comprising 131 streams, 210 lakes, Sweden’s two largest lakes Vänern and Vättern, and 52 river mouths.

**Catchment and climate data**

For each of the 4975 inland waters, we had data on nine catchment variables, derived from the Geographic Information System (GIS; resolution: 100 m × 100 m): size of catchment area of the inland water (in case a lake is considered the size of the catchment area excludes the area of the study lake; in km\(^2\) and abbreviated as CA), altitude at the sampling site (in m.a.s.l.), percentage of agricultural land in the catchment (in % of CA), percentage of pasture in the catchment (in % of CA), percentage of urban and exploited land in the catchment (in % of CA), percentage of coniferous forest in the catchment (in % of CA), percentage of other forest in the catchment (i.e., all forest but excluding coniferous forest; in % of CA), percentage of open wetland (in % of CA), and percentage of open water (i.e., the sum of lake surface area in the CA; in % of CA). For lakes, we also had data on mean lake depth and size of surface area. Using GIS, we overlapped the lake and stream database with the database on climate variables from the Swedish Meteorological and Hydrological Institute at http://www.smhi.se and downloaded site-specific (i.e., interpolated data at the sampling point) long-term means (i.e., 1961–1990) of annual precipitation (in mm), annual mean air temperature (here defined as temperature region; °C), and the number of growing degree days (in days).
Energy and nutrient concentration changes along the LOAC

Energy and nutrient concentration changes along the LOAC are expected to show a dependency on the time water travels through the landscape, according to what has been found for organic carbon quantity and quality (e.g., Algesten et al. 2004; Kellerman et al. 2014; Kothawala et al. 2014). The long-term mean water retention time through the landscape upstream of a lake (WRT\textsubscript{landscape}) has been determined for a variety of Swedish lakes (Müller et al. 2013) but was not available for the majority of our study lakes and streams. However, the percentage of open water in the catchment of a site can be used as a reasonable proxy for WRT\textsubscript{landscape} (Weyhenmeyer et al. 2014). We therefore chose in this study to classify lakes and streams according to their percentage of open water in the catchment. We used eight categories: (1) very small headwater streams with zero percentage open water in CA (three in total), (2) streams and lakes with <5% open water in CA (98 streams and 1390 lakes, i.e., 1488 inland waters in total), (3) streams and lakes with ≥5% and <10% open water in CA (31 streams and 1472 lakes, i.e., 1503 inland waters in total), (4) streams and lakes with ≥10% and <20% open water in CA (17 streams and 1443 lakes, i.e., 1460 inland waters in total), (5) streams and lakes with ≥20% and <30% open water in CA (0 streams and 361 lakes), (6) lakes with ≥30% and <40% open water in CA (0 streams and 83 lakes), (7) Sweden’s largest lakes Vänern and Vättern with a lake water retention of 9 yr and 58 yr, respectively, and (8) Sweden’s river mouth (52 in total). Twenty-three lakes were not allocated to a category since the % open water in CA ranged between 40% and 94% making the number of lakes in a category too small.

We transferred each % open water category to a WRT\textsubscript{landscape} value where <5% open water in CA corresponded to 46 d, ≥5% and <10% open water in CA to 235 d, ≥10% and <20% open water in CA to 319 d, ≥20% and <30% open water to 897 d, and ≥30% and <40% open water in CA to 1275 d. The transfer was based on a significant linear relationship between % open water and WRT\textsubscript{landscape} in the % open water range less than 40% using data from Müller et al. (2013) ((WRT\textsubscript{landscape} = 37.8%) open water in CA – 48.5; \(R^2 = 0.30, \ p < 0.0001, \ n = 1659\)). Thus, changes along the LOAC were in this study expressed as changes along a WRT\textsubscript{landscape} gradient.

To test the first of our three hypotheses, we applied a first order decay function for TC, TOC, DIC, TP, TN, TFe, and DSi concentration changes along the WRT\textsubscript{landscape} gradient according to:

\[
N(t) = N_0 \cdot e^{-kt}
\]

where \(N(t)\) is the concentration at time \(t\) along the WRT\textsubscript{landscape} gradient, \(N_0 = N(0)\) is the concentration at WRT\textsubscript{landscape} = 0 which corresponds to the concentration in headwaters, and \(k\) is the decay coefficient (in days). We also determined the half-life of TC, TOC, DIC, TP, TN, TFe, and DSi concentration decay along WRT\textsubscript{landscape} gradient according to:

\[
t_{1/2} = \frac{\ln(2)}{k}
\]

Finally, we compared the energy and nutrient concentration decline along the LOAC with the energy and nutrient concentration decline from winter to summer, i.e., from January to August. For this comparison, we used monthly median values of the concentrations in the 395 inland waters with time series data and applied the same kind of first order decay function and half-life as we did for the energy and nutrient concentration decline along the LOAC (i.e., we applied Eqs. 1, 2).

Statistics

All statistical tests were run in JMP, version 12.0. Due to the non-normal distribution of the data, tested by a Shapiro-Wilk test, we used median values for averages and applied partial least squares (PLS) regression models when we predicted concentrations of chemical variables in inland waters by 12 catchment and climate variables. We chose PLS because of the method’s insensitivity to X-variable’s interdependency and the insensitivity to deviations from normality (Wold et al. 2001). PLS is commonly used to find fundamental relations between two matrices (\(X\) and \(Y\)) where the variance in \(X\) is taken to explain the variance in \(Y\). In PLS, \(X\)-variables are ranked according to their relevance in explaining \(Y\), commonly and also in this study expressed as Variable Importance in Projection (VIP value; Wold et al. 2001). The higher the VIP values are the higher is the contribution of an X-variable to the model performance. We consider VIP-values exceeding 1.0 as important X-variables. Apart from PLS, we also used the nonparametric Wilcoxon test to compare concentrations of chemical variables between lakes and streams.

Results

Catchment and climate drivers of carbon and nutrient concentrations in boreal waters

Using data from the 4975 boreal inland waters and applying PLS regression models, we found that long-term site-specific median concentrations of all chemical variables, i.e., TC, TOC, DIC, TP, TN, TFe, and DSi showed a dependency on temperature region, site-specific number of growing degree days, and altitude (Table 1). We further found that the percentage of coniferous forest and open water in the CA was important for TOC, TFe, and DSi concentrations in boreal inland waters but not for DIC, TP, and TN concentrations. DIC, TP, and TN concentrations were instead influenced by the percentage of agricultural land and pasture in the catchment (Table 1), although the percentage of
agricultural in the catchment was <10% for 90% of the inland waters and exceeded 5% only in the catchments of 815 boreal waters, mainly in the catchments of the river mouths. Removing the 815 boreal waters from the PLS analyses, DIC and TN concentrations were no longer influenced by the percentage of agricultural and pasture in the catchment (VIP values <1.0 for % agricultural and % pasture in CA) but instead became influenced by the percentage of coniferous forest (VIP values >1.0 for % coniferous in CA). Only TP concentrations still showed a dependency on the percentage of agricultural land and pasture. TP became also dependent on the percentage of urban and exploited land in the catchment (VIP values >1.0).

**Carbon and nutrient concentration changes along the LOAC**

We examined TC, TOC, DIC, TP, TN, TFe, and DSI concentration changes in lakes and streams along the WRT\textsubscript{landscape} gradient and found that all concentrations generally decreased with increasing WRT\textsubscript{landscape} (Fig. 2). The median concentration decrease in inland waters along the WRT\textsubscript{landscape} gradient in the catchment followed a first order decay function with the shortest half-life for TFe and DSI, with 410 d and 568 d, respectively (Table 2). Longest half-lives were observed for TP, DIC, and TN with 1777 d, 2236 d, and 2567 d, respectively. TC and TOC had half-lives along the LOAC of 1260 d and 1195 d, respectively, corresponding to a bit more than 3 yr (Table 2). The half-lives remained very similar when we restricted our data analysis to the 4160 boreal waters with less than 5% agriculture in the CA (i.e., half-lives for TFe and DSI were 408 d and 559 d, respectively, and for TP and TN, 2310 d and 3466 d, respectively). Thus, loss rates along the LOAC were in the order (from fastest to slowest decline): TFe > DSI > TOC > TP > DIC > TN (Table 2). The fast decline in TFe and DSI concentrations along the LOAC resulted in a general significant linear increase in TC:TFe and TC:DSI ratios along the WRT\textsubscript{landscape} gradient (Fig. 2; $R^2 > 0.96$, $p < 0.01$, $n = 5$). In contrast, TN:TP ratios remained rather constant along the WRT\textsubscript{landscape} gradient (Fig. 2), and TC:TP, TOC:TP, TC:TN, and TOC:TN ratios showed a general significant linear decrease ($R^2 > 0.93$, $p < 0.01$, $n = 5$). In the river mouths, TOC:TP and TOC:TN reached a median of 578 and 16, respectively (range for TOC:TP based on concentrations in mg L$^{-1}$:42–1358; range for TOC:TN based on concentrations in mg L$^{-1}$:63–39). These ratios, in particular the TOC:TP ratio, were low compared to global averages which were estimated being 1467 for TOC:TP and 21 for TOC:TN (Chen et al. 2015).

Apart from TC, TOC, DIC, TP, TN, TFe, and DSI concentration declines along the LOAC, we also found a significant general decline in the percentage of coniferous forest along the WRT\textsubscript{landscape} gradient when we used median values for each WRT\textsubscript{landscape} category ($R^2 = 0.96$, $p < 0.01$, $n = 5$). The percentage of coniferous forest in the catchment decreased from, on average, 58% at WRT\textsubscript{landscape} equal to 46 d to, on average, 41% at WRT\textsubscript{landscape} equal to 1275 d. The percentage of agriculture in the catchment did not show a dependency on WRT\textsubscript{landscape} within the WRT\textsubscript{landscape} range of 46–1275 d.

To better understand concentration changes along the LOAC comprising both lentic and lotic ecosystems, we compared the chemical concentrations, i.e., site specific long-term median autumn values, between the 4772 boreal lakes (Vänern and Vättern were excluded) and the 149 boreal streams. We found that TFe and DSI concentrations were significantly lower in the boreal lakes compared to the boreal streams during autumn (Wilcoxon group comparison test: $p < 0.0001$). Also TN concentrations were slightly lower in lakes than in streams in autumn (Wilcoxon group comparison test: $p < 0.01$) while

### Table 1. Importance of 12 catchment and climate variables for the prediction of TC, TOC, DIC, TP, TN, TFe, and DSI concentrations (for abbreviations see methods) in 4975 boreal inland waters during autumn using PLS regression. The importance is expressed as a VIP value. The higher the VIP values is the more important is the input variable for the model performance. VIP values >1.0 (in bold) are considered as important for the model performance.

| X-variable                        | VIP TC | VIP TOC | VIP DIC | VIP TP | VIP TN | VIP TFe | VIP DSI |
|-----------------------------------|--------|---------|---------|--------|--------|---------|---------|
| Temperature region (°C)            | 1.53   | 1.57    | 1.12    | 1.16   | 1.54   | 1.39    | 1.25    |
| Growing degree days (d)           | 1.50   | 1.55    | 1.11    | 1.16   | 1.51   | 1.42    | 1.27    |
| Altitude (m.a.s.l.)                | 1.47   | 1.48    | 1.09    | 1.22   | 1.41   | 1.33    | 1.33    |
| Open water (%)                     | 1.09   | 1.20    | 0.43    | 0.35   | 0.60   | 1.47    | 1.93    |
| Agricultural land (%)              | 1.00   | 0.56    | 1.87    | 1.98   | 1.46   | 0.64    | 0.48    |
| Pasture (%)                        | 0.97   | 0.66    | **1.27** | **1.29** | **1.19** | 0.81    | 0.62    |
| Coniferous forest (%)              | 0.82   | **1.28** | 0.85    | 0.71   | 0.69   | **1.24** | **1.30** |
| Precipitation (mm yr$^{-1}$)       | 0.78   | 0.56    | 0.72    | 0.70   | 0.48   | 0.24    | 0.77    |
| Open wetland (%)                   | 0.66   | 0.70    | 0.64    | 0.60   | 0.69   | **1.11** | 0.55    |
| Urban and exploited land (%)       | 0.46   | 0.31    | **1.10** | 0.79   | 0.41   | 0.31    | 0.28    |
| Size of CA (km$^{2}$)              | 0.35   | 0.36    | 0.15    | 0.19   | 0.18   | 0.29    | 0.21    |
| Other forest (%)                   | 0.18   | 0.33    | 0.35    | 0.30   | 0.28   | 0.26    | 0.19    |
TC, TOC, DIC, and TP concentrations were not significantly different between lakes and streams in autumn (Wilcoxon group comparison test: \( p > 0.05 \)).

**Seasonal variations in carbon and nutrient concentrations**

Using data from the 395 boreal inland waters, we found a clear seasonal decline for DSI, DIC, TC, and TFe concentrations from January to August but not for TOC, TP, and TN concentrations (Table 2). The seasonal decline followed a first order decay function when we used monthly median values of the 395 inland waters during the period January to August (Table 2). When we only examined the seasonal concentration development in the three small headwater streams, we did not detect a significant first order seasonal decline from January to August for any examined chemical variable, although there was a general concentration decline from March to June.

![Box plots showing seasonal variations in carbon and nutrient concentrations](image-url)
**Table 2.** Decay coefficient $\lambda$ and half-lives for TC, TOC, DIC, TP, TN, TFe, and DSI concentrations (for abbreviations see methods) along a landscape water retention time gradient (WRT$_{landscape}$) using Eqs. 1, 2 with median data of 4895 boreal inland waters for each WRT$_{landscape}$ category shown in Fig. 2. Additionally, median values for each month from January to August with data from 395 inland waters were used to determine $\lambda$ and half-lives for TC, TOC, DIC, TP, TN, TFe, and DSI concentrations. n.s. is the abbreviation for not significant.

| Variable | First order decay along the LOAC | First order decay from Jan to Aug |
|----------|---------------------------------|----------------------------------|
| TC       | $R^2 = 0.96, p < 0.01, n = 5$   | $R^2 = 0.62, p < 0.05, n = 8$    |
| TOC      | $R^2 = 0.95, p < 0.01, n = 5$   | n.s.                             |
| DIC      | $R^2 = 0.98, p < 0.01, n = 5$   | $R^2 = 0.75, p < 0.01, n = 8$    |
| TP       | $R^2 = 0.97, p < 0.01, n = 5$   | n.s.                             |
| TN       | $R^2 = 0.98, p < 0.01, n = 5$   | n.s.                             |
| TFe      | $R^2 = 0.97, p < 0.01, n = 5$   | n.s.                             |
| DSI      | $R^2 = 0.97, p < 0.01, n = 5$   | $R^2 = 0.51, p < 0.05, n = 8$    |

**Discussion**

Aquatic chemical concentration declines along the LOAC, often described as retention, are well known and have been attributed to the efficient filtering capacity of inland waters (e.g., Billen et al. 1991; Conley et al. 2000; Cook et al. 2010; Bouwman et al. 2013). In this study, we could confirm chemical concentration declines along the LOAC in the following order (from fastest to slowest decline): TFe > DSI > TOC > TC > DP > DIC > TN (Table 2). We also showed that TFe, DSI, TOC, TC, TP, DIC, and TN concentrations in Swedish boreal waters during autumn decline according to a first order decay function along the LOAC (Table 2), supporting one of our three hypotheses.

When we formulated the hypothesis of a first order decay along the LOAC, we assumed that all variables have their main source in the surrounding forest floor of the Swedish boreal region. This assumption was fulfilled for TC, TOC, TFe, and DSI concentrations as they were mainly driven by the percentage of coniferous forest in the catchment (Table 1). DIC, TP, and TN concentrations, however, were mainly driven by the percentage of agriculture and pasture in the catchment (Table 1) albeit the percentage of agriculture and pasture in the catchment was generally less than 10%. Despite the differences in the catchment drivers of chemical variables, we found a very similar significance in the first order decay along the WRT$_{landscape}$ gradient for all variables (Table 2). A first order decay along the WRT$_{landscape}$ gradient suggests that WRT$_{landscape}$ is an important driver for a concentration decline along the LOAC of the boreal region, which supports previous studies (e.g., Melli 1992; Dillon and Molot 2005; Humborg et al. 2008; Weyhenmeyer et al. 2012; Müller et al. 2013). WRT$_{landscape}$ is particularly long for lakes. Lakes can act as buffers, slowing down the movement of water and thereby providing critical time for physical and biochemical processes to take place (Essington and Carpenter 2000; Goodman et al. 2011; Hanson et al. 2011; Lottig et al. 2013). The importance of in-lake physical and biochemical processes for a concentration decline along the WRT$_{landscape}$ gradient becomes, however, only apparent when catchment characteristics do not substantially change along the
gradient. It has, for example, been shown that nutrient concentrations can also substantially increase toward downstream sites when catchment characteristics rapidly change along lake chains of high altitude regions (Sadro et al. 2012). Physical and biochemical processes that can result in a concentration decline along the LOAC of the lake-rich boreal region are sedimentation, flocculation, microbial and photochemical mineralization, emission, biological uptake, and dilution (Fig. 4). Sedimentation and flocculation directly affect all chemical variables except DIC and DSi, which only after biological uptake might be subjected to sedimentation and flocculation. In Swedish boreal waters, flocculation has been identified as an important loss process for TFe and TOC since both TFe and TOC primarily occur in the colloidal and dissolved phase (von Wachenfeldt et al. 2009; Köhler et al. 2013). Flocculation has also been suggested being the main reason why TFe concentrations decline faster than TOC concentrations along a WRT landscape gradient (Köhler et al. 2013; Weyhenmeyer et al. 2014). Thus, our observed fast TFe loss rates along the LOAC of the lake-rich boreal region which exceeded the TC and TOC loss rates are probably a result of flocculation.

Apart from flocculation, a loss of TOC, in particular of DOC, along the LOAC has been attributed to microbial and photochemical mineralization, resulting in a gain of CO₂ and CH₄ with consequent substantial CO₂ and CH₄ emissions from inland waters (Algesten et al. 2004; Cole et al. 2007; Raymond et al. 2013). Also Catalan et al. (2016) attributed major DOC losses along the LOAC to mineralization and found DOC half-lives of 2.5 ± 4.7 yr. These DOC half-lives are comparable to the TOC half-life of this study where we found half-lives being a bit more than 3 yr (Table 2). Thus, microbial and photochemical mineralization might be an important process to explain organic carbon losses along the LOAC. Mineralization with a consequent CO₂ and CH₄ production would also explain why we found substantially longer DIC than TOC half-lives along the LOAC. However, despite a possible DIC production along the LOAC, DIC concentrations still declined along the LOAC (Table 2). We attribute this decline to CO₂ and CH₄ emissions as well as to biological uptake.

CO₂ and CH₄ emissions from boreal inland waters are usually substantial (Tranvik et al. 2009; Raymond et al. 2013), and they are probably the reason why we found a faster TC than TN and TP loss along the LOAC (Fig. 2), which supports our second hypothesis of declining TC:TN and TC:TP ratios along the LOAC of the boreal region. Declining TC:TN and TC:TP ratios indicate a surplus of TN and TP in relation to TC toward downstream sites. In the Swedish boreal region, lakes are typical downstream sites and WRT landscape can be rather long. In contrast, WRT landscape is usually comparably short for the Swedish river mouth since they receive most of their water from a large main stream and not from lakes (Müller et al. 2013). Consequently, chemical concentrations remained rather high at the river mouths (Fig. 2). Particularly high were TP and TN concentrations at the river mouths which we attribute to an additional TP and TN input by increasing agricultural activity downstream. The high TP and TN concentrations at the river mouths resulted in rather low TC:TP, TOC:TP, TC:TN, and TOC:TN ratios. The TOC:TP as well as the TN:TP ratios in the Swedish river mouths correspond to ratios which are typical for mesotrophic to eutrophic lakes (Chen et al. 2015). Since waters of the Swedish boreal region are
generally considered being nutrient-poor (Bergström et al. 2005), such low TOC:TP and TN:TP ratios at the river mouths might be rather unexpected but they clearly show that little agricultural activity in the catchment as well as point sources from cities which are located close to the coast can substantially influence energy:nutrient ratios, in particular energy:macronutrient ratios.

Additional TP and TN input from agriculture and point sources along the LOAC might explain our observed comparably long half-lives for these nutrients. Originally, we assumed that TN is lost faster from the water column than TP due to an additional loss at the water-atmosphere interface in form of gas. This was not the case, resulting in rather stable TN:TP ratios along the LOAC (Fig. 2). Several reasons are possible for stable TN:TP ratios along the LOAC. A possible nitrogen loss through denitrification might be countered by nitrogen fixation (Cook et al. 2010) or the nitrogen loss at the water-atmosphere interface might be countered by an additional leakage from agricultural and boreal soils which usually is larger for nitrogen than for carbon and phosphorus (Vanni et al. 2001; Khalili et al. 2010).

In contrast to TN and TP, TFe and DSI were lost much faster along the LOAC than TC and TOC (Table 2), resulting in rapidly increasing TC:TFe and TC:DSI ratios with increasing WRTlandscape. Generally, we found that the forest driven variables TFe, DSI, TC, and TOC showed up to six times shorter half-lives than the agricultural driven variables DIC, TN, and TP (Table 2). Shorter half-lives for TFe, DSI, TC, and TOC might partly be caused by an additional input of TN and TP along the LOAC and partly by a dilution effect due to a decline in the percentage of coniferous forest, which is the main driver for TFe, DSI, TC, and TOC. However, since the half-lives of TFe and TC along the WRTlandscape gradient were quantitatively similar to the half-lives determined from seasonal patterns (Table 2), a strong dilution effect for TFe, DSI, TC, and TOC along the LOAC is rather unlikely. It is more likely that flocculation and DOC mineralization are mainly responsible for fast TFe, TC, and TOC declines along the LOAC of the lake-rich boreal region.

Dissolved Si is the only chemical variable examined with a rapid loss along the LOAC which has no direct coupling to flocculation and DOC mineralization. DSI is known to rapidly decline in lakes and reservoirs as a response of biological uptake, in particular by diatoms (Schelske 1985; Conley et al. 2000). If diatom growth is a main driver determining concentrations in boreal waters, we might see a congruent DSI and DIC concentration pattern which is well known from the oceans (Li et al. 2011). Along the LOAC a congruent pattern was not evident since DSI declined much faster than DIC, partly due to CO2 production by DOC mineralization. In addition, DSI fluxes from agricultural landscapes are significantly lower than for forested watersheds due to intense biological cycling in croplands and managed grasslands (Vandevenne et al. 2015). However, on a seasonal scale, we observed a 1:1 relationship between a DSI and DIC concentration decline from winter to summer (Fig. 3). Thus, we suggest that diatoms play an important role for seasonal biogeochemical cycles in the Swedish boreal waters although most of our study inland waters were nutrient-poor. The seasonal loss was much faster than the loss along the LOAC that supports our third hypothesis, but only for the biological driven variables DSI and DIC. All other variables showed either no seasonal loss at all, i.e., TOC, TN, and TP or a similar loss between seasons and along the LOAC, i.e., TC and TFe. We suggest that TC, TOC, TN, TP, and TFe are less influenced by seasonally strongly varying benthic and pelagic primary production compared to DSI and DIC. The fast seasonal DSI loss toward summer might result in DSI limitation for phytoplankton growth, particularly at downstream sites. Such DSI limitation is well known from studies in estuaries (Conley and Malone 1992; O’Boyle et al. 2015).

We conclude that lakes are particularly efficient sinks for TFe and DSI, resulting in a rapid decline of TC:TFe and TC:DSI ratios along the LOAC of the lake-rich boreal region. The observed increasing imbalance of the energy and micronutrient supply during downstream flow path might be critical for the survival of some aquatic organisms, like, e.g., diatoms at downstream sites. We suggest that micronutrient limitation might become relevant in waters with WRTlandscape > 3 yr since in these boreal waters both TFe and DSI were almost depleted (Fig. 2). Since WRTlandscape is highly influenced by variations in runoff, micronutrient limitation in boreal waters might become more or less widespread, depending on whether the climate becomes drier or wetter. Iron and silica limitation needs to be considered in order to understand biodiversity and ecosystem functioning.

References
Algesten, G., S. Sobek, A. K. Bergström, A. Ågren, L. J. Tranvik, and M. Jansson. 2004. Role of lakes for organic carbon cycling in the boreal zone. Glob. Chang. Biol. 10: 141–147. doi:10.1111/j.1365-2486.2003.00721.x
Arbuckle, K. E., and J. A. Downing. 2001. The influence of watershed land use on lake N: P in a predominantly agricultural landscape. Limnol. Oceanogr. 46: 970–975. doi:10.4319/lo.2001.46.4.0970
Aufdenkampe, A. K., E. Mayorga, P. A. Raymond, J. M. Melack, S. C. Doney, S. R. Alin, R. E. Aalto, and K. Yoo. 2011. Riverine coupling of biogeochemical cycles between land, oceans, and atmosphere. Front. Ecol. Environ. 9: 53–60. doi:10.1890/100014
Battin, T. J., S. Luysaart, L. A. Kaplan, A. K. Aufdenkampe, A. Richter, and L. J. Tranvik. 2009. The boundless carbon cycle. Nat. Geosci. 2: 598–600. doi:10.1038/ngeo618
Bergström, A. K., P. Blomqvist, and M. Jansson. 2005. Effects of atmospheric nitrogen deposition on nutrient limitation and
phytoplankton biomass in unproductive Swedish lakes. Limnol. Oceanogr. 50: 987–994. doi:10.4319/lo.2005.50.3.0987

Billen, G., C. Lancelot, and M. Meybeck. 1991. N, P, and Si retention along the aquatic continuum from land to ocean. John Wiley & Sons.

Bouwman, A. F., M. F. P. Bierkens, J. Griffioen, M. M. Hefting, J. J. Middelburg, H. Middelkoop, and C. P. Slomp. 2013. Nutrient dynamics, transfer and retention along the aquatic continuum from land to ocean: Towards integration of ecological and biogeochemical models. Biogeosciences 10: 1–22. doi: 10.5194/bg-10-1-2013

Catalan, N., R. Marce, D. N. Kothawala, and L. J. Tranvik. 2016. Organic carbon decomposition rates controlled by water retention time across inland waters. Nat. Geosci. 9: 501–505. doi:10.1038/ngeo2720

Chen, M., G. M. Zeng, J. C. Zhang, P. Xu, A. W. Chen, and L. H. Lu. 2015. Global landscape of total organic carbon, nitrogen and phosphorus in lake water. Sci. Rep. 5: 15043. doi: 10.1038/srep15043

Cole, J. J., and others. 2007. Plumbing the global carbon cycle: Integrating inland waters into the terrestrial carbon budget. Ecosystems 10: 171–184. doi:10.1007/s10021-006-9013-8

Conley, D. J., and T. C. Malone. 1992. Annual cycle of dissolved silicate in Chesapeake Bay: Implications for the production and fate of phytoplankton biomass. Mar. Ecol. Prog. Ser. 81: 121–128. doi:10.3354/meps081121

Conley, D. J., P. Stalnacke, H. Pitkanen, and A. Wilander. 2000. The transport and retention of dissolved silicate by rivers in Sweden and Finland. Limnol. Oceanogr. 45: 1850–1853. doi:10.4319/lo.2000.45.8.1850

Cook, P. L. M., K. T. Aldridge, S. Lamontagne, and J. D. Brookes. 2010. Retention of nitrogen, phosphorus and silicon in a large semi-ard riverine lake system. Biogeochemistry 99: 49–63. doi:10.1007/s10533-009-9389-6

Dean, W. E., and E. Gorham. 1998. Magnitude and significance of carbon burial in lakes, reservoirs, and peatlands. Geology 26: 535–538. doi:10.1130/0091-7613(1998)026<0535:MASOCB>2.3.CO;2

Dillon, P. J., and L. A. Molot. 2005. Long-term trends in catchment export and lake retention of dissolved organic carbon, dissolved organic nitrogen, total iron, and total phosphorus: The Dorset, Ontario, study, 1978-1998. J. Geophys. Res. Biogeosci. 110: doi:10.1029/2004gb002003

Elser, J. J., and others. 2009. Shifts in lake N:P stoichiometry and nutrient limitation driven by atmospheric nitrogen deposition. Science 326: 835–837. doi:10.1126/science.1176199

Essington, T. E., and S. R. Carpenter. 2000. Nutrient cycling in lakes and streams: Insights from a comparative analysis. Ecosystems 3: 131–143. doi:10.1007/s100210000015

Frost, P. C., J. P. Benstead, W. F. Cross, H. Hillebrand, J. H. Larson, M. A. Xenopoulos, and T. Yoshida. 2006. Threshold elemental ratios of carbon and phosphorus in aquatic consumers. Ecol. Lett. 9: 774–779. doi:10.1111/j.1461-0248.2006.00919.x

Frost, P. C., L. E. Kinsman, C. A. Johnston, and J. H. Larson. 2009. Watershed discharge modulates relationships between landscape components and nutrient ratios in stream seston. Ecology 90: 1631–1640. doi:10.1890/08-1534.1

Goodman, K. J., M. A. Baker, and W. A. Wurtsbaugh. 2011. Lakes as buffers of stream dissolved organic matter (DOM) variability: Temporal patterns of DOM characteristics in mountain stream-lake systems. J. Geophys. Res. Biogeosci. 116. doi:10.1029/2011jg001709

Hanson, P. C., D. P. Hamilton, E. H. Stanley, N. Preston, O. C. Langman, and E. L. Kara. 2011. Fate of allochthonous dissolved organic carbon in lakes: A quantitative approach. PLoS One 6. doi:10.1371/journal.pone.0021884

Hecky, R. E., P. Campbell, and L. L. Hendzel. 1993. The stoichiometry of carbon, nitrogen, and phosphorus in particulate matter of lakes and oceans. Limnol. Oceanogr. 38: 709–724. doi:10.4319/lo.1993.38.4.0709

Hillebrand, H., J. M. Cowles, A. Lewandowska, D. B. Van, D. Waal, and C. Plum. 2014. Think ratio! A stoichiometric view on biodiversity-ecosystem functioning research. Basic Appl. Ecol. 15: 465–474. doi:10.1016/j.baae.2014.06.003

Humberg, C., E. Smedberg, M. R. Medina, and C. M. Morth. 2008. Changes in dissolved silicate loads to the Baltic Sea - the effects of lakes and reservoirs. J. Mar. Syst. 73: 223–235. doi:10.1016/j.jmarsys.2007.10.014

Kellerman, A. M., T. Dittmar, D. N. Kothawala, and L. J. Tranvik. 2014. Chemodiversity of dissolved organic matter in lakes driven by climate and hydrology. Nat. Commun. 5. doi:10.1038/ncomms4804

Khallil, M. I., J. Temmerud, M. Froberg, E. Kari, and G. A. Weyhenmeyer. 2010. Nitrogen and carbon interactions between boreal soils and lakes. Global Biogeochem. Cycles 24. doi:10.1029/2009gb003668

Köhler, S. J., D. Kothawala, M. N. Futter, O. Liungman, and L. Tranvik. 2013. In-lake processes offset increased terrestrial inputs of dissolved organic carbon and color to lakes. PLoS One 8. doi:10.1371/journal.pone.0070598

Kortelainen, P., T. Mattsson, L. Finer, M. Ahltaian, S. Saukkonen, and T. Sallantaus. 2006. Controls on the export of C, N, P and Fe from undisturbed boreal catchments, Finland. Aquat. Sci. 68: 453–468. doi:10.1007/s00203-006-0833-6

Kothawala, D. N., C. A. Stedmon, R. A. Muller, G. A. Weyhenmeyer, S. J. Kohler, and L. J. Tranvik. 2014. Controls of dissolved organic matter quality: Evidence from a large-scale boreal lake survey. Glob. Chang. Biol. 20: 1101–1114. doi:10.1111/gcb.12488

Laudon, H., M. Berggren, A. Ägren, I. Buffam, K. Bishop, T. Grabs, M. Jansson, and S. Köhler. 2011. Patterns and dynamics of dissolved organic carbon (DOC) in boreal streams: The role of processes, connectivity, and scaling. Ecosystems 14: 880–893. doi:10.1007/s10021-011-9452-8

Li, D. D., A. Lerman, and F. T. Mackenzie. 2011. Human perturbations on the global biogeochemical cycles of coupled
Vandevenne, F. I., and others. 2015. Landscape cultivation

Tranvik, L. J., and others. 2009. Lakes and reservoirs as regu-

Sterner, R. W., and J. J. Elser. 2002. Ecological stoichiometry:

Stelzer, R. S., and G. A. Lamberti. 2001. Effects of N: P ratio

Schelske, C. L. 1985. Biogeochemical silica mass balances in

Meili, M. 1992. Sources, concentrations and characteristics

Weyhenmeyer and Conley Carbon and nutrient loss rates

Vanni, M. J., W. H. Renwick, J. L. Headworth, J. D. Auch, and
M. H. Schaus. 2001. Dissolved and particulate nutrient

Von Wachenfeldt, E., and L. J. Tranvik. 2008. Sedimentation in
boreal lakes - the role of flocculation of allochthonous dissolved

Von Wachenfeldt, E., D. Bastviken, and L. J. Tranvik. 2009. Microbiologically induced flocculation of allochthonous dissolved organic carbon in lakes. Limnol. Oceanogr. 54: 1811–1818. doi:10.4319/lo.2009.54.5.1811

Weyhenmeyer, G. A., M. Fröberg, E. Karlton, M. Khalili, D. Kothawala, J. Temnerud, and L. J. Tranvik. 2012a. Selective decay of terrestrial organic carbon during transport from land to sea. Glob. Chang. Biol. 18: 349–355. doi:10.1038/srep07732

Weyhenmeyer, G. A., P. Kortelainen, S. Sobek, R. Müller, and M. Rantakari. 2012b. Carbon dioxide in boreal surface waters: A comparison of lakes and streams. Ecosystems 15: 1295–1307. doi:10.1007/s10021-012-9585-4

Weyhenmeyer, G. A., Y. T. Prairie, and L. J. Tranvik. 2014. Browning of boreal freshwaters coupled to carbon-iron interactions along the aquatic continuum. PLoS One 9. doi:10.1371/journal.pone.008104

Weyhenmeyer, G. A., S. Kosten, M. B. Wallin, L. J. Tranvik, E. Jeppesen, and F. Roland. 2015. Significant fraction of CO₂ emissions from boreal lakes derived from hydrologic inorganic carbon inputs. Nat. Geosci. 8: 933–936. doi:10.1038/ngeo2582

Wold, S., M. Sjöström, and L. Eriksson. 2001. PLS-regression: A basic tool of chemometrics. Chemometr. Intell. Lab. Syst. 58: 109–130. doi:10.1016/S0169-7439(01)00155-1

Acknowledgments

Many thanks go to the Swedish Environmental Protection Agency and the staff of the laboratory of the Dept. of Aquatic Sciences and Assessment for financing, sampling, and analyzing thousands of water samples. Financial support was received from the European Union’s Horizon 2020 research and innovation program under the Marie Skłodowska-Curie grant agreement 643052 (C-CASCADES project), the Swedish Research Council (Grant 2014-5910 and Grant 2016-04153), and from the Knut and Alice Wallenberg Foundation (KAW project and Wallenberg Scholar). This work profited from the international networks abbreviated as GLEON, NETLAKE, and DOMQUA.

Conflict of Interest

None declared.

Submitted 15 October 2016
Revised 27 February 2017
Accepted 11 April 2017

Associate editor: Marguerite Xenopoulos