Potential terrestrial influence on transparent exopolymer particle concentrations in boreal freshwaters

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Abstract

Transparent exopolymer particles (TEP) are ubiquitous in aquatic ecosystems and contribute, for example, to sedimentation of organic matter in oceans and freshwaters. Earlier studies indicate that the formation of TEP is related to the in situ activity of phytoplankton or bacteria. However, terrestrial sources of TEP and TEP precursors are usually not considered. We investigated TEP concentration and its driving factors in boreal freshwaters, hypothesizing that TEP and TEP precursors can enter freshwaters via terrestrial inputs. In a field survey, we measured TEP concentrations and other environmental factors across 30 aquatic ecosystems in Sweden. In a mesocosm experiment, we further investigated TEP dynamics over time after manipulating terrestrial organic matter input and light conditions. The TEP concentrations in boreal freshwaters ranged from 83 to 4940 μg Gum Xanthan equivalent L⁻¹, which is comparable to other studies in freshwaters. The carbon fraction in TEP in the sampled boreal freshwaters is much higher than the phytoplanktonic carbon, in contrast to previous studies in northern temperate and Mediterranean regions. Boreal TEP concentrations were mostly related to particulate organic carbon, dissolved organic carbon, and optical indices of terrestrial influence but less influenced by bacterial abundance, bacterial production, and chlorophyll a. Hence, our results do not support a major role of the phytoplankton community or aquatic bacteria on TEP concentrations and dynamics. This suggests a strong external control of TEP concentrations in boreal freshwaters, which can in turn affect particle dynamics and sedimentation in the recipient aquatic ecosystem.

Extracellular polymeric substances (EPS) influence the formation of particles in the water column by acting as a glue (Droppo and Ongley 1994). The formed particles can contribute to sedimentation processes and transport of carbon to sediments, and thus represent an important component in the aquatic carbon cycle (Passow et al. 2001; de Vicente et al. 2009). Transparent exopolymer particles (TEP) represent one type of particles formed by EPS that has received increasing attention in the aquatic literature (Simon et al. 2002; Bar-Zeev et al. 2015). TEP are defined as transparent particles that are formed abiotically through the conjugation of exopolymers—mainly acid polysaccharides released by phytoplankton and bacteria (Passow 2002a) and are stainable with the dye Alcian Blue (Alldredge et al. 1993). The occurrence and formation of TEP depend on several environmental parameters (e.g., turbulence, multivalent cation concentrations, pH) and on the concentrations of precursors present (Kloareg and Quatrano 1988; Engel and Passow 2001). Higher TEP fluxes are frequently associated with peaks of particle fluxes to sediments in marine (Passow et al. 2001; Bar-Zeev et al. 2009) and inland (de Vicente et al. 2009) waters. For example, TEP contributed with up to 30% to the carbon export to the sediment in an oligotrophic reservoir in Spain (de Vicente et al. 2009). Thus, they constitute important components in the turnover, decomposition, and sedimentation of organic matter in aquatic ecosystems (Simon et al. 2002). In marine systems as well as in freshwaters, TEP are also known to support buoyancy of particulate organic carbon (POC) (Mari et al. 2017) and are important for particle formation (Grossart et al. 1998; Passow et al. 2001), biofilm development (Bar-Zeev et al. 2012), and they can also serve as a food source for higher trophic levels such as zooplankton or small fish (Grossart et al. 1998). In drinking water production, they are described as a fouling agent that clogs filters (Bar-Zeev et al. 2015). Hence, their involvement in various processes in aquatic ecosystems and
their role in sedimentation makes it important to better understand TEP formation and dynamics in inland waters.

The precursors of TEP can be produced by both phytoplankton and bacteria (Passow 2002b), and TEP concentrations are known to correlate well with phytoplankton abundance (Grossart et al. 1998; Berman and Viner-Mozzini 2001; Passow et al. 2001). However, most studies have been done in marine systems where phytoplankton production is a main source of organic matter (Mühlenbruch et al. 2018). The ubiquitous occurrence of TEP in lentic systems has also been confirmed (Grossart et al. 1997; Berman and Viner-Mozzini 2001; de Vicente et al. 2010) but its relationship with phytoplankton and bacteria is less conclusive. de Vicente et al. (2010) related TEP concentration to chlorophyll a (Chl a) both in Mediterranean lakes and in northern temperate lakes, whereas TEP and bacterial abundance were significantly and positively related only in Mediterranean lakes. However, the study by de Vicente et al. (2010) is the first and so far only study linking TEP concentrations with the ecosystem properties of lakes from different regions.

As described before, most studies on TEP in inland waters focused on the role of phytoplankton and aquatic bacteria for TEP formation and its dynamics (de Vicente et al. 2010; Thuy et al. 2015; Callieri et al. 2017). An external source to the aquatic systems has rarely been considered although terrestrial biofilms in soils can also be embedded in a matrix of hydrated EPS (Flemming and Wingender 2010) that might be flushed into aquatic systems. Chateauneuf et al. (2012) and de Vicente et al. (2009) were the first ones to conclude that TEP precursors can be imported to aquatic systems directly from the terrestrial surroundings. Chateauneuf et al. (2012) found a high share of TEP contributing to particles in Arctic floodplain lakes, in particular, after a flood and where riverine influence was strong. Although they conclude that TEP precursors can originate in terrestrial systems, their study focused on the role of autotrophic production to TEP formation. As of now, the role of terrestrial input and its influence on TEP concentrations in aquatic ecosystems have not explicitly been investigated. This could be especially relevant in boreal inland waters, which are highly influenced by terrestrial inputs of organic matter (Kothawala et al. 2014).

Our study aims to investigate the concentration of TEP and its driving factors in boreal freshwaters. Here, we present the first cross-system study (including peats, streams, rivers, and lakes) of TEP combined with results from an experimental mesocosm study. We hypothesized that TEP can directly flow from the terrestrial environment into freshwater systems with a strong aquatic-terrestrial linkage. To test the role of terrestrial organic matter inputs, phytoplankton, and bacteria on TEP concentrations, we conducted a field survey measuring TEP concentrations and several abiotic and biotic parameters in 30 boreal freshwaters in Sweden that we compared with a data set from an earlier study (de Vicente et al. 2010). In addition, we set up a mesocosm study where we manipulated light conditions and input of terrestrial dissolved organic matter (DOM) and measured TEP concentrations over time. This knowledge will contribute to a better understanding of the effect of terrestrial inputs, phytoplankton, and bacteria on TEP concentrations in freshwaters and the contribution of TEP to sedimentation along gradients of natural inland waters.

Materials and methods

Field survey—sampling

We sampled a total of 30 freshwater systems in four regions in Sweden in 10 d (28 August to 6 September 2016): Småland, Jämtland, Bergslagen, and Uppland, including three wetland peats, seven streams and rivers (hereafter referred to as running waters), and 20 lakes (Supporting Information Table S1). The different boreal freshwaters represent a gradient of aquatic-terrestrial linkages with peats representing water before entering freshwaters, while most running waters form a second group with a high connection to the terrestrial surrounding and finally lakes that in general have the lowest terrestrial influence. At each site, we took a water sample (~25 L) and measured temperature, oxygen, conductivity (HQ40d, Hach, Loveland, Colorado, U.S.A.), and pH (Hanna HI991300; Woonsocket, Rhode Island, U.S.A.) directly on site. Peat water samples were taken from standing surface waters in the wetland peat, running water samples from the surface, and the lakes were sampled at 0.5 m depth. Besides TEP, several physical and chemical parameters were measured to assess the influence of environmental variables on TEP concentrations, including dissolved organic carbon (DOC), total phosphorus (TP), total nitrogen (TN), suspended particulate matter (SPM), POC, particulate nitrogen (PN), carbon and nitrogen ratio (C:N) of particles, anions (F−, Cl−, and SO42−), cations (Na+, K+, Ca2+), organic acids (acetate and glycolate), and absorbance and fluorescence of DOM. Additionally, proxies for primary production and bacterial influence were determined (Chl.a, bacterial abundance, and bacterial protein production).

Mesocosm experiment—setup and sampling

The mesocosm experiment was part of a larger study that aimed to investigate the influence of light and addition of terrestrial DOM on biogeochemical processes and the food web (refer to Nydahl et al. 2019 for more details). In August/September 2016 for a period of 4 weeks, 20 mesocosms were set up in Lake Erken in Sweden (meso-eutrophic, dimictic; 59°50'09.7"N 18°37'52.7"E) with four different treatments in a 2 x 2 factorial design and with five replicates each. The mesocosms were made of polyethylene cylinders that were open at the top with diameters between 92 and 101 cm and a height of 2 m. The mesocosms were attached at the end of a floating wooden jetty. The treatments included (1) “Control”—the control without any addition or manipulation; (2) “Reverse Osmosis” (RO)—the addition of terrestrial DOM (see below for
description), increasing background DOC concentrations by 4 mg L$^{-1}$; (3) “Shade”—the coverage of the mesocosm with a black gauze (nylon chiffon) on top and black polyethylene film outside of the mesocosms to decrease light (shade); and (4) “Shade_RO”—the combination of the latter two. The terrestrial DOM was obtained through a RO concentration made from a humic stream draining a forested wetland (59°92'N, 17°34'E; stream DOC concentration 37.7 mg L$^{-1}$) in May/June 2016 and stored in the dark at 4°C. After a prefiltration step (0.2 μm) through 10-in. filter cartridges, DOC was concentrated by RO using a Real Soft PROS/2S unit as described by Serkiz and Perdue (1990), to a final concentration of approximately 800 mg L$^{-1}$.

For the experiment, the mesocosms were filled with prefiltered (200 μm) water from Lake Erken to remove bigger zooplankton and phytoplankton to a volume of approximately 1000 L. After this, zooplankton was added, from a sample collected on the same day in the center of Lake Erken by net tows, to similar concentrations as before prefiltration (34 individuals L$^{-1}$ of mainly cyclopoids and cladocerans) in order to have similar zooplankton communities in all mesocosms at the start of the experiment. The manipulations were started 1 d before the first sampling. A second manipulation was conducted with the addition of three young-of-the-year perch (Perca fluviatilis L.) of similar biomass (1.5 ± 0.4 g; mean ± standard deviation) to all mesocosms after the second sampling (1 week after the first). All mesocosms were manually mixed in the morning and afternoon with a disk attached to a pole.

The mesocosms were sampled every week for the following 4 weeks ending up with a total of five sampling points. A water sample of 15–18 L was taken with a tube sampler (1.5 m long, ~3 L) and divided for the following analyses: TEP, DOC and absorbance and fluorescence of DOM, SPM and its carbon and nitrogen content, Chl $a$, and bacterial abundance and production. Values of pH, oxygen concentration, conductivity, and water temperature were measured directly in the mesocosms at 1 m depth at the day of sampling with a YSI (EXO2 Multiparameter Sonde, YSI, Yellow Springs, Ohio, U.S.A.).

**Abiotic parameters**

**Transparent exopolymer particles**

TEP concentrations were determined colorimetrically (Passow and Alldredge 1995). Depending on the particle concentration, between 2 and 60 mL of sampled water was filtered onto 0.4 μm polycarbonate filters (25 mm diameter) (Whatman, Dassel, Germany). The filters were stained with Alcian Blue solution at a pH of 2.5. Subsequently, the filters were soaked in 80% sulfuric acid (6 mL) within 24 h and measured spectrophotometrically (UV/Vis Spectrometer Lambda 40; Perkin Elmer; Waltham, Massachusetts, U.S.A.) at 787 nm, using empty, stained filters as blanks. Alcian Blue absorption was calibrated using a solution of the polysaccharide Gum Xanthan (GX) and TEP concentrations are expressed in μg of GX equivalents per liter (μg GX eq L$^{-1}$). The calculated TEP concentrations were converted to carbon units using the conversion factor of 0.75 μg C per μg GX L$^{-1}$ proposed by Engel and Passow (2001). Thereby, we were able to compare carbon in TEP and phytoplankton directly by using the same unit. Unfortunately, the TEP concentrations from two samples (one peat and one stream) could not be determined due to very high particle concentrations that clogged the filters immediately. Therefore, the data and analysis reported here for the field samples were only done with 28 samples.

**DOC, TN, and TP**

DOC concentrations were analyzed on filtered water (precombusted GF/F, Whatman) and measured on a Total Carbon Analyzer (Sievers M9 Laboratory Analyzer, GE Analytical Instruments, Boulder, Colorado, U.S.A.). The concentrations of the field survey samples of TN in the water were measured by a TN analyzer (Shimadzu TOC-L/TNM-L, Kyoto, Japan) and TP concentrations were measured photometrically (UV/Vis Spectrometer Lambda 40; Perkin Elmer; Waltham, Massachusetts, U.S.A.) using the molybdenum-blue method (Menzel and Corwin 1969).

**Absorbance and fluorescence of DOM**

The absorbance and fluorescence were measured on filtered water (precombusted GF/F, Whatman). Absorbance spectra were measured at 1 nm intervals from 200 to 600 nm with a Lambda 40 UV–visible spectrophotometer (Perkin Elmer, Waltham, U.S.A.). From this data, absorbance at 420 nm (A420) and 440 nm (A440) and the DOC-specific UV absorbance (SUVA; L mg C$^{-1}$ m$^{-1}$) at 254 nm, an indicator of the aromaticity of DOM, were calculated based on the absorbance at 254 nm divided by DOC concentration (Weishaar et al. 2003). Excitation-emission matrices (EEMs) from lake water samples were analyzed with a fluorescence spectrophotometer (SPEX FluoroMax-4, Horiba Jobin Yvon, Japan) in a 1 cm quartz cuvette (or 0.5 cm if absorbance at 200 nm > 2 cm$^{-1}$). More detailed descriptions of the absorbance and fluorescence and following parameters can be found in the Supporting Information. The EEMs were used to calculate the humification index (HIX = ratio of areas under the emission curve at 435–480 nm and 300–345 nm plus 435–480 nm at an excitation wavelength 254 nm) (Ohno 2002), fluorescence index (Fl = emission intensity at 470 nm divided with that of 520 nm at 370 nm excitation) (Cory and McKnight 2005), and freshness index (βα = ratio of emission intensity at 380 nm and maximum intensity between 420 and 435 nm at an excitation wavelength of 310 nm) (Parlanti et al. 2000).

**Ion chromatography (anions, cations, and organic acids)**

The ion chromatography was only done for the field survey. First, the samples for measurements of anions, cations, and organic acids were filtered over 0.2 μm Acrodisc Supor hydrophilic polysulfone membranes (Pall Laboratory, Port Washington, New York, U.S.A.) and stored frozen. Then,
the analysis was conducted on a Metrohm IC system (883 Basic IC Plus and 919 Autosampler Plus).

**SPM, POC, and C:N**

Samples for SPM/POC/C:N from the field survey were collected on glass microfiber filters (precombusted GF/F, Whatman). The filters were freeze-dried prior to analysis and subsequently acidified with 3% HCl in order to eliminate any particulate inorganic C (see Nieuwenhuize et al. 1994), and dried in a desiccator. SPM was calculated from the weight of the filter after filtering and drying minus the empty weight. Subsequently, the organic C (POC) and N (PN) contents were analyzed on an Elemental Combustion System (Costech Instruments, Cernusco s/Nav., Italy) and the C:N ratios we report here refer to the ratio POC:PN.

**Biotic parameters**

**Chlorophyll a**

The suspended materials for the Chl a analyses from the field survey were collected in duplicates on glass microfiber filters (precombusted GF/F, Whatman) and the Chl a was extracted from the filter by leaching with ethanol for 5 min at 75°C and Chl a contents were determined with a spectrophotometer (UV/Vis Spectrometer Lambda 40; Perkin Elmer; Waltham, Massachusetts, U.S.A.), following standard guidelines (Jespersen and Christoffersen 1987). A conversion factor of 40 μg C per μg Chl a−1 (Banse 1977) was applied to roughly estimate the phytoplankton C content. The samples from the mesocosm experiment were filtered onto GF/C filters (precombusted, Whatman) and processed as described above.

**Bacterial abundance**

Bacterial abundance from the field survey samples was counted under the epifluorescence microscope at 1000X magnification (Nikon Eclipse E600, Tokyo, Japan) after staining with SYBR Gold (Invitrogen, Darmstadt, Germany) (Shibata et al. 2006). Samples from the mesocosms were counted using a flow cytometer (CyFlow space, Partec, Münster, Germany) according to Székely et al. (2013).

**Bacterial production**

Bacterial protein production was determined via incorporation of 3H-leucine into the protein fraction using the protocol of Smith and Azam (1992).

**Statistics**

First, a principal component analysis (PCA) of the field survey and the environmental data from de Vicente et al. (2010) was performed to compare the studied regions and visualize the differences. The data were standardized through centered log-ratio, followed by z-scale transformations and PCA was done in R using the “prcomp” function and an ellipse with a 95% probability was built around each region. For the field data, multivariate linear regressions by means of partial least squares projections (PLS regression; Eriksson et al. 2001) were run to determine the environmental factors explaining the variances in TEP concentrations in boreal freshwaters with TEP concentrations as dependent (Y) variables and a set of environmental factors (Table 1) as independent (X) variables. Log-transformations were done for data with skewness < 2 and minimum/maximum ratio > 0.1 and subsequently data were mean-centered and scaled to unit variance prior to analysis. The relevance of independent variables is given as the variable influence on projections (VIP) and values > 1 are considered as highly influential, between 1 and 0.7 as moderately influential, and < 0.7 as less influential. Model evaluation was done...

### Table 1. Environmental variables and VIP values for PLS regressions.

| Parameter                        | Abbreviation | Transformed? | VIP values |
|----------------------------------|--------------|--------------|------------|
| Transparent exopolymer particle concentration | TEP          | Log10        | —          |
| Dissolved organic carbon concentration | DOC         | Log10        | 1.68       |
| Absorbance at 420 nm             | A420         | Log10        | 1.63       |
| Total phosphorus concentration   | TP           | Log10        | 1.59       |
| Particulate organic carbon concentration | POC        | Log10        | 1.38       |
| Total nitrogen concentration     | TN           | Log10        | 1.28       |
| Specific UV absorbance at 254 nm | SUVA         | No           | 1.17       |
| Particulate nitrogen concentration | PN          | Log10        | 1.17       |
| Humification index               | HIX          | Log10        | 1.17       |
| pH                               | pH           | No           | 1.08       |
| Bacterial abundance              | BA           | Log10        | 0.98       |
| Fluoride concentration           | F            | Log10        | 0.97       |
| Oxygen concentration             | O₂           | Log10        | 0.96       |
| Chl a concentration              | Chl a        | Log10        | 0.93       |
| Freshness index                  | FRESH        | No           | 0.93       |
| Sodium concentration             | Na           | Log10        | 0.91       |
| Chloride concentration           | Cl           | Log10        | 0.69       |
| Carbon to nitrogen ratio of particles | CN       | No           | 0.69       |
| Conductivity                     | Cond         | Log10        | 0.66       |
| Fluorescence index               | FI           | No           | 0.59       |
| Acetate and glycolate concentration | Acetate/glycolate | Log10        | 0.59       |
| Water temperature                | T            | No           | 0.55       |
| Bacterial carbon production      | BCP          | Log10        | 0.43       |
| Calcium concentration            | Ca           | Log10        | 0.32       |
| Potassium concentration          | K            | Log10        | 0.29       |
| Sulfate concentration            | SO₄          | Log10        | 0.18       |
on the basis of the $R^2Y$ and $Q^2$ values where $R^2Y$ gives the explained variance of $X$ by $Y$ (goodness of fit) and $Q^2$ the predicted variation of the model. In addition, permutation tests (100 times permutations) were run to assess the explained variance that can be attributed to random chance. PLS regressions were performed in SIMCA 14 (Umetrics, Umeå, Sweden).

To evaluate differences between the treatments from the mesocosm experiment over the whole experiment, we performed nonparametric Friedman’s tests (Friedman 1937) as the data from the mesocosms did not meet the requirements for parametric repeated measures tests. When the null hypothesis was rejected, post hoc tests using the Fisher’s least significant difference criterion were applied with a Bonferroni correction (Dunn 1961) to compare the treatments. These analyses were done in R 3.4.3 with the “friedman” function of the agricolae package (de Mendiburu 2015). The significance level was set at $p < 0.05$ unless stated otherwise. All analyses and graphs were built in R 3.4.3 (R Core Team 2018).

**Results**

**Field survey**

TEP concentrations in the field survey ranged from 83 to 4940 μg GX eq L$^{-1}$ (1110 ± 1420 μg GX eq L$^{-1}$; mean ± standard deviation). Mean values for the different types of waters were 3220 ± 1920 μg GX eq L$^{-1}$ ($n = 2$) for peats, 1550 ± 1680 μg GX eq L$^{-1}$ ($n = 6$) for running waters, and 768 ± 1120 μg GX eq L$^{-1}$ ($n = 20$) for lakes (Fig. 1a). The corresponding carbon content in the TEP fraction (TEP-C) amounted to 833 ± 1062 μg C L$^{-1}$ (range from 62 to 3703 μg C L$^{-1}$) and the mean values for the different types of waters were 2410 ± 1440 μg C L$^{-1}$ ($n = 2$) for peats, 1160 ± 1260 μg C L$^{-1}$ ($n = 6$) for running waters, and 576 ± 843 μg C L$^{-1}$ ($n = 20$) for lakes. To evaluate the importance of TEP-C and phytoplankton-C and compare the different aquatic ecosystems from our study with de Vicente et al. (2010), we calculated the TEP-C to phytoplankton-C ratios. These ratios ranged between 0.8 and 129 (20.4 ± 28.0; mean ± standard deviation) in boreal freshwaters (Fig. 1b). The study in Mediterranean and north temperate lakes by de Vicente et al. (2010) revealed lower ratios of TEP-C compared to phytoplankton-C with the same groups (b). Boxplots indicate median, first, and the third quartile (box), and the whiskers from the ends of the boxes to the outermost data point falls within 1.5 times the interquartile range. The ratios are displayed on a logarithmic scale. With only two data points, BOR Peat samples are not shown as violin or boxplots. The sample size ($n$) is given below the graph.

The first two components of the PCA using the environmental data set from this study and de Vicente et al. (2010) clearly separated the three regions (Boreal, Mediterranean, and North-temperate) (Fig. 2). The first two components explained 79% of the variance between the sites with the first one explaining 43% and the second one 36%. The separation of the boreal data from this study (BOR) and the north temperate region (NT) in de Vicente et al. (2010) is mainly driven by A440 and bacterial abundance, which are both higher in NT (Fig. 2). Hence, the first component can be interpreted as a terrestrial gradient. The pH, conductivity, DOC concentrations, and Chl a separated the Mediterranean data (MED) from BOR and NT along the second component and represents a trophic gradient. The pH, conductivity, DOC concentrations, and Chl a were all the highest in MED (Fig. 2).

The PLS model built for the measured boreal TEP concentrations extracted one significant component, explaining 66.7% of the variance in TEP concentrations (Fig. 3). Model predictability was high ($Q^2 = 0.576$) and the background correlation low ($R^2Y = 0.111$). The best explaining variables revealed by the loadings plot and VIP values were DOC concentration and A420 as well as POC, PN, and the nutrients TP and TN (Fig. 3).
In addition, other optical indicators, such as SUVA$_{254}$ and HIX, were highly influential, but also pH. Both bacterial abundance and Chl$_a$ were only moderately influential.

Mesocosms
The TEP concentrations, Chl$_a$, and the TEP-C to phytoplankton-C ratio differed significantly between the treatments in the mesocosm experiment whereas POC concentrations and bacterial abundances were not significantly different between the treatments (Friedman’s test; Table 2; Fig. 4a–d). The TEP concentrations were higher in the treatments with RO concentrate added (RO and Shade_RO) at the beginning of the experiment (Fig. 4a). After 2 weeks, from the third sampling and onward, these differences in TEP concentrations between the treatments decreased until the end of the experiment when they were all on a similar level as the control. For Chl$_a$ concentrations, the control was significantly different from all treatments and showed a decrease in Chl$_a$ over the course of the experiment (Fig. 4b). The treatments with the shading cloth showed a slight increase in Chl$_a$ but also high standard deviations whereas the Shade_RO treatment stayed at a higher level until the end of the experiment.

Opposite to the TEP concentrations, the changes in Chl$_a$ mostly occurred at the second sampling point and later. The POC concentrations did not show any corresponding changes (Fig. 4c) and also the bacterial abundance showed no differences between the treatments but decreased slightly in all treatments on the third sampling point (Fig. 4d).

Discussion
Our study is the first cross-system analysis of TEP concentrations spanning across the inland water continuum, and is based on samples ranging from peat surface waters to streams, rivers, and lakes. We found high TEP concentrations in all sampled systems, comparable to other studies in freshwaters (Fig. 1a). However, the fraction of carbon in TEP in the sampled boreal freshwaters is much higher than the phytoplanktonic carbon, in contrast to previous studies in other regions (de Vicente et al. 2010). Boreal TEP concentrations were mostly related to POC, DOC, and optical indices of terrestrial influence such as A$_{420}$, HIX, and SUVA$_{254}$ but less influenced by bacterial abundance, bacterial production, and Chl$_a$. In the mesocosm experiment, TEP concentrations increased already 1 d after the addition of terrestrial DOM in the corresponding treatments, whereas Chl$_a$ and bacterial abundances showed no differences to the control, suggesting that abiotic factors also play a role in TEP dynamics. These results suggest that terrestrial sources may contribute to the high concentrations of TEP we found throughout the boreal aquatic continuum. Thus, a strong aquatic-terrestrial coupling might introduce TEP and its precursors from the terrestrial...
surrounding and increase TEP concentrations in the adjacent aquatic system. This may facilitate flocculation, including the coprecipitation of TEP with colored organic matter of terrestrial origin (von Wachenfeldt et al. 2009).

**High contributions of TEP to particles in boreal freshwaters**

TEP concentrations ranged from 83 to 4940 μg G X e L⁻¹ across a wide range of boreal waters, which is at the same level as the data from de Vicente et al. (2010), which ranged from 36 to 1460 μg GX e L⁻¹ in north temperate lakes of the Wisconsin and Michigan’s Upper Peninsula and from 66 to 9040 μg GX e L⁻¹ in Mediterranean lakes in southern Spain (Fig. 1a). The TEP concentrations reported from marine ecosystems are substantially lower, ranging from 23 to 791 μg GX L⁻¹ (compiled by Bar-Zeev et al. 2011). We found that the ratios of TEP-C to phytoplankton-C are considerably higher in boreal freshwaters than in Mediterranean or north-temperate lakes (Fig. 1b). The TEP-C in boreal freshwaters also makes up a considerable fraction of the total organic carbon pool, ranging from 1.3% to 22% with an average of 5.9%. The data set from de Vicente et al. (2010) only allows to compare the TEP-C to the DOC pool which is, on average, less than half with 2.4% for each region (MED and NT have the same average) and these numbers would be even lower when considering the total organic C pool. Thus, our results point toward an important role of TEP in boreal freshwaters, potentially regulating flocculation, sedimentation, and C turnover in boreal freshwaters as it is known to further stimulate the aggregation of solids and other particles (Passow 2002a).

However, it has to be noted that the conversion factor for TEP-C has been determined experimentally from phytoplankton cultures and ranges from 0.51 to 0.88 μg TEP-C L⁻¹ per μg GX L⁻¹ (Engel and Passow 2001). Therefore, the TEP-C as well as the TEP-C-to-phytoplankton-C ratios has to be considered as semi-quantitative (Filella 2014). Nonetheless, these values are still indicative of gradients and allow to a certain extent the comparison of TEP and phytoplankton across systems and regions such as the comparison with de Vicente et al. (2010) that use the same conversion factors. The current literature lacks studies about the carbon content of TEP from phytoplankton or macrophytes especially in freshwater ecosystems. We hence call for successive studies, aiming at better assessing TEP-C variabilities from autochthonous but also terrestrial derived sources in marine and freshwater ecosystems.

**Sources of TEP in aquatic ecosystems**

In earlier studies, sources of TEP were mostly attributed to the in situ production of phytoplankton and bacteria (de Vicente et al. 2010; Chateauvert et al. 2012). Here, we

### Table 2. Statistical results for the Friedman’s test of the mesocosm data.

| Parameter                        | TEP (μg GX eq L⁻¹) | POC (mg L⁻¹) | Chl a (μg L⁻¹) | Bacterial abundance (× 10⁶ cells L⁻¹) | TEP-C : Phyto-C |
|----------------------------------|-------------------|-------------|--------------|-------------------------------------|----------------|
| Friedman’s test                  | χ² | df | ANOVA p   | χ² | df | ANOVA p   | χ² | df | ANOVA p   | χ² | df | ANOVA p   |
| Treatment                        | Con  | RO | Shade_RO  | Con  | RO | Shade_RO  | Con  | RO | Shade_RO  | Con  | RO | Shade_RO  |
| Treatment                        | 14.04 | 3  | 0.0029    | 6.36 | 3  | 0.0953    | 12.12 | 3  | 0.0070    | 5.4  | 3  | 0.1447    |

Superscript values for Friedman’s test are bold if < 0.05.

*Treatments with the same letter are not significantly different.

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tested the hypothesis that TEP can also be introduced from the terrestrial surrounding in systems with a high aquatic-terrestrial linkage. A potential source of terrestrial TEP precursors is soil biofilms that are embedded in a matrix of hydrated EPS produced by soil microorganisms (Flemming and Wingender 2010). Several results in our study point toward a larger contribution to TEP from terrestrial sources than from algal and bacterial in situ activity in boreal freshwaters. We found high TEP concentrations in boreal freshwaters with a tendency toward higher values for peat surface waters and running waters (Fig. 1a) and the carbon bound in TEP was much higher than carbon in phytoplankton, which is opposite to the results of de Vicente et al. (2010) (Fig. 1b). These Mediterranean sites with high DOC, Chl $\alpha$, and conductivity correspond to brackish shallow lagoons within the Doñana system, classified as hypereutrophic and markedly productive (López-Archilla et al. 2012). Thus, we can interpret the second axis of the PCA as a trophic gradient, implying that, for the same terrestrial influence, eutrophic systems with high phytoplanktonic biomass will have higher TEP concentrations (Fig. 2). The oligotrophic conditions and low concentrations of Chl $\alpha$ in boreal aquatic ecosystems might preclude them of internally producing as high amounts of TEP as shown in reservoirs (Thuy et al. 2015) and eutrophic lakes (de Vicente et al. 2010). The average percent of extracellular release of DOM by phytoplankton is estimated to be 13% of total carbon fixation (Baines and Pace 1991). Ranges in marine ecosystems are given from < 5% to 50% of primary production (Mari et al. 2017 and references therein). Most studies range between 10% and 20%, which corresponds to our literature value of 13% by Baines and Pace (1991) that includes marine and freshwater ecosystems. The DOM pool excreted by phytoplankton mainly consists of carbohydrates and polysaccharides contribute with 80% to the extracellular release (Myklestad et al. 1989). A rough estimate of primary production rates calculated from the measured Chl $\alpha$ concentrations based on Baines et al. (1994) revealed a mean value of 20.9 $\mu$g CL $^{-1}$ h $^{-1}$ (range from 0.1 to 266.9 $\mu$g CL $^{-1}$ h $^{-1}$) for our data. Assuming an extracellular release of 13% with 80% polysaccharides accounts for a release of polysaccharides-C of 2.2 $\mu$g CL $^{-1}$ h $^{-1}$ (range from 0.01 to 27.8 $\mu$g CL $^{-1}$ h $^{-1}$), which represents a mean of 0.8% (range from 0.1% to 3.5%) of the measured TEP-C concentrations. This approximation of the possible extracellular carbon release by phytoplankton suggests that primary production in boreal waters might not be high enough to sustain the measured concentrations of TEP. In addition to phytoplankton, macrophytes and phytobenthos can also release TEP precursors that can flocculate and contribute to the TEP pool.
(Chateauvert et al. 2012). We did not explicitly assess the contribution of these groups as possible TEP sources in this study although their role on TEP formation could be relevant in small and shallow boreal lakes (Ask et al. 2009). However, the macrophyte presence in the lakes, based on the observations and pictures from the field, was minimal. Furthermore, the smaller and shallower lakes in this study are not expected to have a substantial macrophyte influence due to relatively short water retention times and highly colored DOM, which is linked to terrestrial inputs and limited light availability (Attermeyer et al. 2018; Supporting Information Table S1).

Neither Chl a nor bacterial abundances and production rates were highly influential factors for TEP concentrations in boreal freshwaters as revealed by the PLS model (Fig. 3). In contrast, high correlations were found for concentrations of POC, DOC, and terrestrial DOM optical indicators. Macrophytes (Zhang et al. 2013) and sediments (Yang et al. 2014) can also release humic-like DOM that could potentially influence these patterns. However, the main contributors to DOM increases related to macrophytes seem to be either non-chromophoric (Catalán et al. 2014) or compounds occurring in the protein-like region (Zhang et al. 2013). Moreover, the humic-like peak from macrophytes generally appears at lower emission wavelengths than terrestrial-like influence, not leading to increases in HIX (Catalán et al. 2014) as we found here. In addition, the water columns were mixed and oxic at the time of samplings in late summer, which is limiting the sedimentary DOM release (Yang et al. 2014). Hence, we can conclude that TEP concentrations observed in the field survey might be mainly driven by terrestrial inputs, explaining the high TEP concentrations measured for boreal freshwaters in this study.

A strong aquatic-terrestrial coupling of TEP dynamics corresponds also to results from Chateauvert et al. (2012). The authors show that among Arctic floodplain lakes, the highest mean TEP-C concentrations occurred in the lake with the strongest riverine influence and lowest levels of autochthonous autotrophic production. Furthermore, the highest TEP concentrations were found immediately after a flood (Chateauvert et al. 2012), when autochthonous autotrophic production was at a seasonal low and colored DOM at a seasonal high, suggesting an uncoupling of primary production and occurrence of TEP and further supporting a terrestrial source of TEP to aquatic ecosystems also in other regions beyond the boreal zone.

Abiotic processes influencing TEP dynamics in aquatic ecosystems

In aquatic ecosystems, TEP can be formed from dissolved and colloidal precursors, a process that is mostly controlled by abiotic factors. The results from the mesocosm experiment give first hints on abiotic controls of TEP dynamics linked to terrestrial inputs. Here, we manipulated the terrestrial DOM input by addition at the start of the experiment. Thus, any changes in TEP during the course of the experiment were due to dynamics in the mesocosms. Interestingly, after 1 d, TEP concentrations were already higher in the treatments with RO added, whereas Chl a levels were similar across treatments at the first sampling point (Fig. 4).

It is likely that TEP precursors were introduced via the addition of the RO concentrate, which can aggregate and form TEP when abiotic conditions change. Abiotic processes that cause TEP precursors to aggregate and form TEP include chemical crosslinking by divalent cations (Ca$^{2+}$ and Mg$^{2+}$) (Kloareg and Quatrano 1988) and hydrogen bonds (Chin et al. 1998) but also light-induced flocculation. About 10% of DOM may spontaneously assemble into particles such as TEP (Chin et al. 1998) and photo-flocculation has been shown to cause an increase in TEP concentrations within hours after sun exposure (Shammi et al. 2017). Both processes could explain the high TEP concentrations already in the beginning in the mesocosms with RO added. The RO concentrate was stored in the dark before addition and the sudden exposure to sunlight could have caused a rapid increase in TEP. Finally, also changes in pH and conductivity due to the addition of the RO concentrate to the lake water in the mesocosms can influence TEP dynamics (Fig. 3) and favor their formation (de Vicente et al. 2010). Hence, as discussed in the preceding paragraph, terrestrial organic matter entering freshwaters can contain TEP but also TEP precursors that can flocculate spontaneously or induced by light due to changing abiotic conditions.

Over the course of the experiment, TEP concentrations declined in the treatments with RO added (Fig. 4a). This decrease could have been induced by bacterial degradation and dissolution (Grossart and Simon 1998) or grazing (Passow 2002a), but sedimentation or adhesion to the walls of the mesocosms (de Vicente et al. 2009; Bar-Zeev et al. 2012) are also possible explanations. Furthermore, light can also reverse the flocculation process and dissolve flocs (Ortega-Retuerta et al. 2009). After the 4 weeks of the experiment, TEP concentrations were back to the starting levels. In this mesocosm experiment, it is difficult to assess the relative importance of the factors that contributed to the declining TEP concentrations. Future studies should therefore focus on the mechanisms for the formation and breakdown of TEP and TEP precursors introduced from terrestrial surroundings to better understand their role for biogeochemical cycles in freshwaters. Furthermore, the comparison of the regions where TEP data are currently available (this study and de Vicente et al. 2010) shows different environmental characteristics (Fig. 2), which might act differently on TEP concentrations and their dynamics, warranting further investigations.

Conclusion

The high TEP concentrations in a broad range of boreal freshwaters could not be explained by phytoplankton and bacterial influences, but correlated to several indicators of
terrestrially derived organic matter. Thus, we suggest that there is a strong control from the terrestrial surrounding by direct inputs in the form of TEP and its precursors, although other sources such as macrophytes, phyto- benthos, and sediments also have to be accounted for. As the terrestrial organic matter enters aquatic ecosystems, it is exposed to changes in abiotic conditions, such as pH or exposure to light, which can trigger abiotic TEP formation from dissolved and colloidal precursors. The formation of TEP constitutes a link between the dissolved and POC pools, and exerts a strong influence on flocculation, sedimentation, and carbon turnover (Passow 2002a). Particles with a high TEP content have also been shown to persist in surface waters for longer periods (Mari et al. 2017). Hence, the relative amount of TEP to solid particles determines if POC is exported downstream or sediments locally. Considering sources of TEP external to aquatic ecosystems helps to explain the wide range of TEP concentrations found across freshwaters. We encourage future studies to consider terrestrial inputs of TEP and its precursors. This is especially relevant for aquatic ecosystems that are experiencing increasing concentrations of allochthonous organic matter due to climatic extreme events, such as storms, where huge amounts of dissolved and particulate organic matter can be introduced (Dhillon and Inamdar 2013).

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Conflict of Interest
None declared.

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