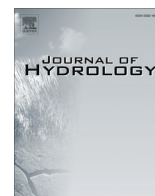


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Application of high-resolution spectral absorbance measurements to determine dissolved organic carbon concentration in remote areas



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SUMMARY

Accurate quantification of dissolved organic carbon (DOC) in surface and soil pore waters is crucial for understanding changes in water resources under the influence of climate and land use changes. Sampling and laboratory analysis of DOC content at a sufficient temporal frequency are especially difficult to achieve for natural DOC sources like the extensive boreal and arctic mire landscapes due to their remoteness. Therefore, the goals of this paper are (1) to investigate the performance of a portable, high-resolution ultraviolet–visible light spectroscopic method for determining the DOC content of surface and soil pore water samples from a boreal mire complex and (2) to compare the spectroscopic method with other DOC measurement techniques, e.g., the wet heated persulfate oxidation method and a laboratory, expulsion-based spectrophotometric method and (3) to assess different multivariate models that relate absorbance measurements with DOC contents. The study indicates that high-resolution spectroscopic measurements provide a simple, robust and non-destructive method for measuring DOC content. These measurements are of short duration (<1 min) and the sample analysis is portable, rendering this method particularly advantageous for in situ investigations at remote field locations. The study also demonstrates that if absorbances at specific wavelengths are used as proxies for DOC concentration, it is recommended to create site-specific calibration models that include more than one wavelength to achieve the optimal accuracy of the proxy-based DOC quantification.

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1. Introduction

Recent efforts have been made to qualitatively and quantitatively characterize dissolved organic matter (DOM) in (near-) pristine and anthropogenically perturbed waterbodies (Ågren et al., 2008; Cory et al., 2011; Grayson and Holden, 2012; Freeman et al., 2001; Langergraber et al., 2004; Lauerwald et al., 2012; Ulanowski and Branfireun, 2013; Worrall et al., 2002, 2008). Several methods are available to determine the main component of DOM, i.e., the dissolved organic carbon (DOC) concentrations. Typically, the DOC content is determined by measuring the oxidation of organic matter to CO₂, which is generally accomplished using high-temperature combustion or persulfate oxidation (Aiken et al., 2002; Cory et al., 2011). Currently, special cuvette tests can be used to determine DOC content using the so-called expulsion method (e.g., LCK 385 and 386 test kits, Hach Lange GmbH, Germany; a detailed description of the expulsion method is presented in the method section). However, because these methods require bulky instruments, laboratory space and additional

consumables, they cannot be directly used under field conditions. Such limitations make it particularly difficult to obtain frequent measurements in remote areas (e.g., Russian boreal mires), which often lack information concerning DOM changes in their surface and soil pore waters. Moreover, these methods only provide information about DOC concentrations; the characterization of DOM quality (e.g., molecular size), which is helpful to promote an improved understanding of DOM turnover processes and their environmental controls, cannot be accomplished without additional size fractionation (e.g., the use of tangential flow filtration, chromatographic analysis) (e.g., Guo and Santschi, 2007; Pereira et al., 2014; Pokrovsky et al., 2005, 2006, 2010; Shvartsev et al., 2012) or characterization with a light-based proxy (absorbance or fluorescent emission) (e.g., Ågren et al., 2008; Chen et al., 2003; Cory and McKnight, 2005; Thurman, 1985; Weishaar et al., 2003).

Because DOM has distinctive spectrophotometric properties and absorbs at a wide range of wavelengths in the UV and visible radiation spectra, a variety of spectroscopic techniques have been used to provide information about the bulk content and qualitative characterization of DOM (e.g., Baker and Spencer, 2004; Baker et al., 2008; Carter et al., 2012; Grayson and Holden, 2012; McKnight et al., 1997; Pastor et al., 2003; Tipping et al., 1988, 2009; Wallage and Holden, 2010; Weishaar et al., 2003; Worrall

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et al., 2002). However, the specific absorbance spectra of different DOM sources vary considerably. Thus, when using spectroscopic measurements as proxies for DOC concentrations, it is necessary to conduct a local calibration against one of the conventional DOC content determination methods (such as high-temperature combustion or persulfate oxidation). In the absence of site-specific calibration, the absorbance-based determination of DOC concentration can be inaccurate due to the varying absorbance strength of different types of DOM and the interference of other elements. For example, absorbance measurements can be influenced by dissolved nitrate and iron (Weishaar et al., 2003; Xiao et al., 2013). Advantageously, measurements made with spectrophotometers generally require a small sample size and are simple to perform. Due to recent technological developments, several advanced, “off-the-shelf” ultraviolet–visible light (UV–Vis) spectrometers are currently available to determine DOC levels under field conditions (e.g., ProPS-UV, Trios GmbH, Germany; CarboVis, WTW GmbH, Germany; and spectro::lyser, carbo::lyser and multi::lyser, s::can, Austria). The ability to operate an instrument in the field is particularly advantageous for environmental studies conducted in remote areas, which may lack laboratory space and equipment. Although these types of spectrometers are already widely used for effluent water-quality monitoring (e.g., Langergraber et al., 2004; Rieger et al., 2004), only a few studies within the geo-ecological sciences have used these probes (e.g., Waterloo et al., 2006; Koehler et al., 2009; Grayson and Holden, 2012; Jeong et al., 2012; Strohmeier et al., 2013).

Considering the importance of DOM in the carbon cycle, its influence on the cycling of other elements and its potential increase in concentrations under the influence of climate change and other anthropogenic factors, it is particularly important to obtain accurate and rapid estimates of DOC in remote areas. Hence, this study has the five following objectives:

- (1) To evaluate the performance of a portable UV–Vis spectrometer in measuring DOC concentration in surface and soil pore waters from a boreal mire complex under remote field conditions.
- (2) To compare the DOC content derived using high-resolution absorption spectrophotometry with the content derived from other available DOC measurement methods, such as the wet heated persulfate oxidation method and the expulsion method.
- (3) To assess the application of high-resolution absorbance measurements for determining DOC concentration using a set of different local calibration methods based on multiple stepwise regression (MSR), partial least-squares (PLS) regression, principal-component regression (PCR) and the two-component model of Carter et al. (2012).
- (4) To determine the wavelengths conferring an optimal explanatory power for different DOM size fractions that can be used as proxies to determine the DOC content.
- (5) To explore the specific spectrophotometric absorption properties of DOM in surface and soil pore waters in different land cover types within a boreal river valley mire complex that is typical for the extensive boreal lowland areas in Russia.

2. Materials and methods

2.1. Study site

The study was conducted on water samples collected from the Ust-Pojeg mire complex (61°56'N, 50°13'E, 119 m A.S.L.) in the Komi Republic (Fig. 1). This boreal river valley mire complex consisted of minerogenous (fen), ombrogenous (bog), and transitional forest-mire (marginal forest swamp) zones. The marginal

forest swamp zone was located at the interface between the forest and mire zones and received water from both the mire and the forest mineral soils in addition to precipitation, the bog zone received water only from precipitation, and the fen zone was supplied by water from both precipitation and, marginally, from minerogenic throughflow. In the fen portion, excess water from snowmelt generates net outflow and high water tables, which is gradually reduced during the months April through July (Runkle et al., 2014). The vegetation cover varied among sites. The main species in the marginal forest swamp zone were *Picea obovata*, *Betula pendula*, *Alnus incana*, *Menyanthes trifoliata*, and *Sphagnum magellanicum*. The fen was predominantly vegetated with *Betula nana*, *Scheuchzeria palustris*, *Sphagnum spp.*, while the bog vegetation cover included mainly *Chamaedaphne calyculata*, *S. palustris*, *S. magellanicum* and *Sphagnum spp.* (Schneider et al., 2012).

The site's climate is boreal, humid and continental. The mean annual temperature was 1.1 °C for the period 1960–2011, and the mean annual precipitation at Syktyvkar for the period of 1973–1998 was 585 mm. The coldest month is January, and the warmest is July. The mean monthly air temperature is above 0 °C from April through October. Permanent snow cover begins at the end of October and continues until the beginning of April. Soils in all three investigated ecohydrological mire units were classified as Fibric Histosols according to the World Reference Base for Soil Resources (IUSS Working Group WRB., 2006; Langer, 2012). pH values varied among sites (outflow: 5.3; swamp-forest: 5.6; fen: 5.3; bog: 4.3). The details on hydrochemistry are presented in Table 1.

2.2. Sampling and filtration

Water samples were collected along a 1.2 km long transect in the south-western part of the Ust-Pojeg mire complex from bog, fen and marginal swamp areas and from the surface of the adjacent Pojeg River during several field campaigns from July 2010 to October 2011. The DOM fraction was separated using pre-combusted glass-fiber filters (0.7 µm GF/F, Whatman, UK) with a glass filtration set (Sartorius, Germany). Glass fiber filters were preferred for filtration because they are binder-free and do not add any contamination to the sample (Cory et al., 2011). To precondition the filtration system and avoid contamination from the filter prior to collecting samples, 30 ml of sample water was processed through the filter and then discarded. For further size fractionation, a tangential-flow filtration system was used to divide the overall bulk DOM fraction into two fractions: ≤1 kDa and ≤10 kDa, according to a protocol of Schwalger and Spitzzy (2009). For tangential flow filtration, polyethersulfone cassette membranes (nominal molecular-size cut-offs of 1 kDa and 10 kDa, Omega, Centramate, PALL Corporation, USA) were used. The samples were then acidified with HCl to pH < 2 and stored at 4 °C until laboratory analysis. Samples with precipitated DOM particles were excluded from further analyses.

2.3. High-resolution spectroscopic measurements using a portable UV–Vis probe

A submersible, portable multi-parameter UV–Vis probe (spectro::lyser, s::can Messtechnik GmbH, Austria) was used for the high-resolution spectroscopic measurements. All of the controller's electronics, including the data logger, are placed in four tubular anodized aluminum housings. The length of the probe is approximately 0.6 m. The spectro::lyser measures absorbance across the UV–Vis range (200–750 nm, at 2.5 nm intervals) and saves these values in an internal datalogger. The measurement range of the probe depends on the optical path length, which can range from 2 to 100 mm. In this study, a probe with a path length of 5 mm was used, with the manufacturer's estimated DOC concentration measurement range of 0–84 mg L⁻¹. Duplicate DOC content



Fig. 1. Study site location in North-East Europe. Green areas indicate wetland land cover types determined in the ESA GlobCover 2009 (ESA, 2010).

Table 1

Variability of the mean concentrations of chemical species measured in the mire complex. All concentrations are presented in mg L^{-1} , whereas the unit of SUVA_{254} is $\text{L mg}^{-1} \text{m}^{-1}$. The water samples were analyzed for the cation contents with an atomic absorption spectrometer ('AAS'; 1100B, Perkin-Elmer, USA).^a

Element	Outflow	Swamp-forest	Fen	Bog	≤ 10 kDa	≤ 1 kDa
SUVA_{254}	4.1	3.9	3.8	3.7	3.3	3.2
C_{Ca}	4.8	7.0	3.1	0.3	1.30	0.65
C_{Mg}	1.3	1.5	0.9	0.1	0.40	0.20
C_{Fe}	1.06	0.5	0.5	0.06	0.06	0.01
C_{K}	0.3	0.1	0.6	0.4	0.92	0.51
C_{Mn}	0.03	0.04	0.08	n/d	n/d	n/d
C_{Na}	1.3	1.2	1.3	0.7	n/a	n/a

^a n/a denotes not available because samples were not analysed for the given element, whereas n/d denotes not detected because content of the element was under the analytical measurement range.

measurements of unfiltered samples ($n = 41$) demonstrated standard deviations within the range of $0\text{--}0.3 \text{ mg L}^{-1}$. In this study, all results are from measurements that were performed with cleaned mirrors in a field station. Only for a limited period the probe was used in a submerged condition. The standard deviation for the probe performance in a submerged condition at the site's outflow channel for 104 min ($n = 104$, at 1 min intervals) demonstrated a consistent reproducibility ($44 \pm 0.1 \text{ mg L}^{-1}$). However, the frequency of mirror cleaning should be adjusted on a site-specific basis depending on the POC load and staining intensity.

Using the manufacturer's default global calibration, it is possible to simultaneously estimate the concentrations of total organic carbon (TOC), DOC and nitrate and the turbidity of the sample. In the global calibration, the DOC concentrations are calculated using multi-wavelength algorithms of a turbidity-compensated absorbance fingerprint. The details of the multi-wavelength algorithms are not provided by the manufacturer. According to Langergraber et al. (2003) the algorithms were created based on the UV-Vis spectra and reference DOC values. The turbidity-compensated fingerprint is calculated based on the relationship between the wavelength and scattering intensity as a function of the particle diameter, as described by Huber and Frost (1998) (cited by Langergraber et al., 2003).

2.4. Assessment of a portable UV-Vis spectrophotometer's performance for DOC content estimation

The instrument's built-in local calibration method was applied for a portable UV-Vis spectrometer (spectro::lyser; s::can

Messtechnik GmbH, Austria) prior to initiating the main sampling campaign (Fig. 2a). First, 30 water samples were collected from different areas of the study site and analyzed with the spectro::lyser. Next, DOC concentration measurements for the same samples were conducted using the wet heated persulfate oxidation method ("wet oxidation") (Aurora Model 1030, O-I-Analytica, USA), and the results were integrated into the local calibration algorithm of the spectro::lyser. This factory method uses an iterative approach to exclude poor spectra and then uses a selection of multivariate approaches to determine the best calibration (Fleischmann et al., 2001). Then, to evaluate the performance of the local calibration, 204 samples were analyzed using both the wet oxidation and the spectrophotometric methods. This evaluation was conducted through an ordinary least-squares (OLS) regression which regressed the DOC concentration values obtained from the portable UV-Vis spectrophotometer against the values obtained from the wet oxidation technique.

In the wet oxidation method, potassium hydrogen phthalate was used as a standard ($5, 20, 40 \text{ mg L}^{-1}$), analyzed before and after each sample run to ensure that there was no analytical drift within and among sample runs (up to 88 samples per run). The standard deviations of triplicate potassium hydrogen phthalate measurements were always lower than 0.4 mg L^{-1} (5 mg L^{-1} standard: $<0.16 \text{ mg L}^{-1}$, $n = 11$; 20 mg L^{-1} standard: $<0.23 \text{ mg L}^{-1}$, $n = 11$; 40 mg L^{-1} standard: $<0.34 \text{ mg L}^{-1}$, $n = 11$).

To check if particulate organic carbon could bias the DOC content measurements of the investigated mire water samples, a subset of samples ($n = 51$) was analyzed for differences between the filtered (using $0.7 \mu\text{m}$ pore-size glass-fiber filters, GF/F, Whatman, UK) and the unfiltered samples. To evaluate the significance of differences between filtered and unfiltered samples, a paired samples t-test was applied (SPSS 18.0, IBM Corp., Chicago, USA).

2.5. Comparison of DOC-measurement methods

Three independent methods were evaluated regarding their efficiency in determining a sample's DOC content. First, high-resolution absorbance measurements were conducted immediately following sample collection without acidification or filtration (spectro::lyser, s::can Messtechnik GmbH, Austria). Second, filtered and acidified samples were analyzed with a conventional laboratory technique – wet oxidation (Aurora Model 1030, O-I-Analytica, USA). Third, measurements with cuvette tests (LCK385 and LCK386 test kits, Hach Lange, Germany) using the expulsion

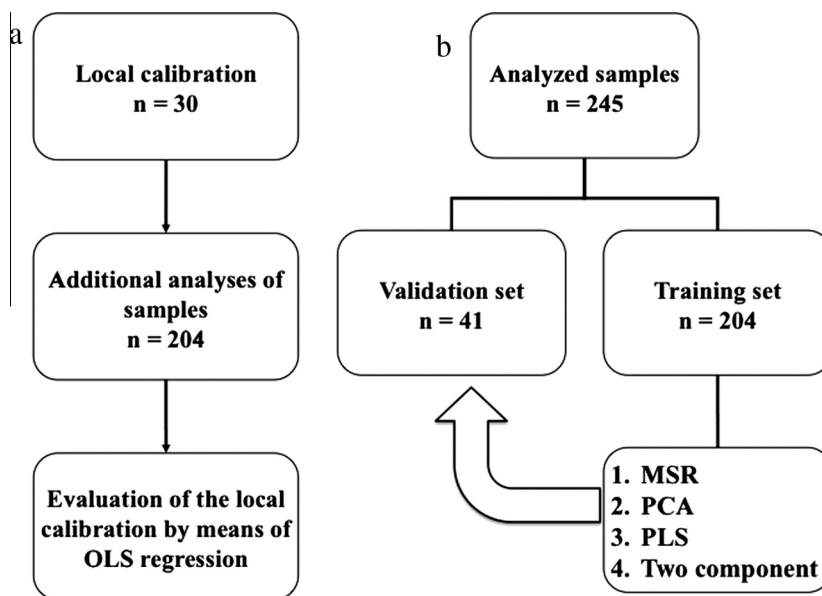


Fig. 2. a. The instrument's built-in local calibration procedure and b. Multilinear local calibration procedures to estimate DOC content. a. The samples were analyzed with the wet oxidation and spectroscopic methods ($n = 30$). Afterward, the values obtained from the wet oxidation method were included into the spectro::lyser's algorithm for DOC estimation using absorbance values as proxies. The local calibration was evaluated using simultaneous measurements of samples with the wet oxidation and spectrophotometric methods. b. The set of alternative multilinear local calibration procedures to determine DOC content. The DOC content of 245 samples was measured using the wet oxidation and spectrophotometric methods. To model the DOC values, 204 samples were used in the multiple stepwise (MSR), partial least-squares (PLS) principal-component regressions (PCR), and two-component model by Carter et al. (2012), while 41 samples were kept aside for validation.

method were conducted immediately following sample collection, filtration and acidification. The expulsion method analyses were completed in a two-stage process. First, the dissolved inorganic carbon (DIC) was released from the sample with the help of a shaker. Next, a cuvette was placed into a thermostat (100 °C) for two hours, where the organic matter underwent heat-oxidative digestion with sodium peroxydisulfate. The heat-oxidative digestion led to carbon dioxide production, which was transferred through a gas-permeable membrane into an indicator cuvette, where it reacted with lithium hydroxide. The indicator color changed based on the amount of carbon dioxide produced and was measured with a spectrophotometer before conversion into a DOC concentration.

In this study, the wet oxidation method is accepted as highly accurate and is considered the “gold standard” for comparison to the other methods. The differences between the methods were tested by a factorial ANOVA with the repeated-measures factorial design, followed by the Tukey HSD post hoc test (if the sphericity assumption was fulfilled) or the Bonferroni test (if the sphericity assumption was violated). The sphericity assumption was tested by Mauchly's test. The results were considered to be significant at $p < 0.05$. Additional statistical comparisons were based on the intercept and slope parameters of OLS regressions as well as their root mean square errors (RMSE) and coefficients of determination (r^2). All of the aforementioned statistical tests were conducted with the SPSS 18.0 software package (IBM Corp., Chicago, USA).

2.6. Testing different multilinear regression methods for local calibration

To test for an optimized estimate of DOC from the absorbance fingerprint, a set of calibrations based on different multilinear regression methods was performed with “real-life” samples ($n = 245$) (Fig. 2b). The following statistical methods were used for the multilinear calibration of the spectrophotometer measurements using the DOC values obtained from the wet-oxidation method as the predictand variable and the absorbance values at different wavelengths as predictor variables: multilinear OLS

regression with variable selection (i.e., multiple stepwise regression – MSR), partial least-squares regression (PLS), and principal-component regression (PCR). These methods were selected due to their applicability to datasets containing collinear variables and a larger number of variables than observations. The two-component model was selected as it produced good unbiased predictions of DOC content from absorbance data at 270 and 350 nm (Carter et al., 2012).

For all of the regressions, only the absorbance values from 250 nm to 740 nm at 2.5 nm intervals were used as input data. Wavelengths shorter than 250 nm were excluded from the analyses because inorganic substances can lead to interference at the lower end of the UV–Vis range (Tipping et al., 1988). Additionally, the following two ratios used for qualitative DOC characterization were added to the predictor variable list: Ratio 1: E4/E6 (absorbance at 465 nm divided by absorbance at 665 nm) and Ratio 2: E2/E3 (absorbance at 255 nm divided by absorbance at 365 nm). The E2/E3 ratio is inversely related to molecular size (Ågren et al., 2008; De Haan, 1993). The E4/E6 ratio is related to the molecular size of humic substances: the ratio for humic acids is usually < 5.0 , whereas the ratio for fulvic acids ranges from 6.0 to 8.5 (Thurman 1985; Peuravuori and Pihlaja, 1997).

Stepwise regression was conducted by combining forward selection and backward elimination. At each step, the best predictor variables were consecutively entered into the regression model only if the model performance was improved on the 5% significance criterion using an F-test. Variable addition halted when the inclusion of additional variables failed to lead to a further and significant increase of the coefficient of determination r^2 . Next, each variable selected for the model was tested and potentially removed if its significance after the inclusion of other variables generated a p-value greater than 0.1 (Dugard et al., 2010).

A different approach was used for the PCA and PCR regressions. These techniques reduce the number of dimensions in the data by computing latent linear variables (Varmuza and Filzmoser, 2009; Miller and Miller, 2010). However, the method by which these linear combinations are chosen differs. In PCR, the principal

components are generated to describe the maximum variation in the predictors without considering the strength of the relationship between the predictor and predictand variables (Miller and Miller, 2010). In PLS, the variables exhibiting a high correlation with the response variables are given extra weight (Miller and Miller, 2010).

The PCR and PCA analyses were conducted with the XLSTAT software package (Addinsoft, Paris, France), while the OLS and MSR analyses were performed with the SPSS 18.0 software package (IBM Corp., Chicago, USA). An independent subset ($n = 41$) of systematically selected samples from each group and covering the complete range of measured values was separated from the calibration dataset for validation. Values obtained applying the local calibration and the default global calibration were also compared with the results after application of the MSR, PCA and PLS regression calibrations.

Additionally, we tested the two-component model of Carter et al. (2012) for determining DOC content through two wavelengths of a spectral absorption fingerprint (i.e., 270 nm and 350 nm). The fixed parameters were the extinction coefficients from each component A and B at 350 nm, $Ea_{350} = 30 \text{ L g}^{-1} \text{ cm}^{-1}$ and $Eb_{350} = 0 \text{ L g}^{-1} \text{ cm}^{-1}$. These assumptions are based on findings indicating $35 \text{ L g}^{-1} \text{ cm}^{-1}$ as a possible upper limit on E340 and the consistency during fitting of a low absorption for the second (B) component at 350 nm. Thus, here component B is assumed to absorb light only in the UV range and to be completely transparent to light above 350 nm (Thacker et al., 2008). These assumptions were found necessary for a unique solution to be found by the modeling routine (Carter et al., 2012). Microsoft Excel Solver was used to find optimal parameter values by minimizing the sum of squared residuals. The adjusted parameters of the model were found to be $Ea_{270} = 47.81 \text{ L g}^{-1} \text{ cm}^{-1}$, $Eb_{270} = 1.8 \text{ L g}^{-1} \text{ cm}^{-1}$, $C_{\text{DOCc}} = 1.8 \text{ mg L}^{-1}$, where the E terms are extinction coefficients for each component at 270 nm and C_{DOCc} is the “invisible” DOC component that does not absorb light in any wavelength.

2.7. Evaluation of chemical species interference with DOC absorbance values

To evaluate the interference of other chemical species with the DOC absorbance values, a subset of samples was analyzed to determine the iron ($n = 66$) and nitrate contents ($n = 42$). The iron content was measured with an atomic absorption spectrometer (1100B, Perkin-Elmer, USA), while the nitrate content was measured with a cadmium-reduction photometric method (Photometer KFK-3, Zagorsky Optical-Mechanical Plant, Russia).

2.8. Identification of site and fraction specific wavelengths

To select the most site- and fraction-specific wavelengths, step-wise multiple regressions were performed for different size fractions ($\leq 1 \text{ kDa}$: $n = 22$; $\leq 10 \text{ kDa}$: $n = 22$), sites (marginal forest swamp: $n = 17$; bog: $n = 17$) and concentration ranges (low range: $15\text{--}25 \text{ mg L}^{-1}$, $n = 35$; intermediate range: $26\text{--}36 \text{ mg L}^{-1}$, $n = 35$; high range: $36\text{--}52 \text{ mg L}^{-1}$, $n = 35$). To illustrate the particular wavelength range at which the absorbance values significantly varied among sites, the DOC absorbance spectra were first normalized to the DOC content measured by wet oxidation and were then divided by the mean normalized (to DOC) absorbance spectra of the outflow site (during the summer).

2.9. Evaluation of DOC stability after a storage period

To test whether storage affected the samples' spectroscopic features and changed their DOC content, repeated absorbance and DOC concentration measurements were made before and after four

months of storage at $4 \text{ }^\circ\text{C}$ at several wavelengths across the UV–Vis spectrum (250 nm, 280 nm, 330 nm, 632.5 nm, and 737.5 nm) with the portable multi-parameter UV–Vis probe. A paired samples t-test analyses was used to evaluate the significance of the differences between the treatments (SPSS 18.0 software package, IBM Corp., Chicago, USA).

3. Results

3.1. Performance of a portable UV–Vis spectrophotometer

The locally calibrated high-resolution absorbance measurements were consistent with the measurements by wet oxidation, as seen in the results of the OLS regression for an independent validation dataset ($r^2 = 0.99$, $\text{RMSE} = 1.61 \text{ mg L}^{-1}$, $p < 0.001$, $n = 204$) (Fig. 3). Over the full range of measured concentrations, the intercept and slope parameters of the linear OLS regression ($b = 1.36 \pm 0.21 \text{ mg L}^{-1}$, $m = 0.97 \pm 0.01$) appeared acceptable; however, logarithmic fitting demonstrates that the spectrophotometric method overestimates the DOC concentrations when smaller than 10 mg L^{-1} (Fig. 4).

For the investigated mire waters, the spectrophotometer satisfactorily discriminated the DOC content from the particulate organic carbon (POC) in the water samples without filtration. No significant difference in DOC content estimated using the spectro::lyser was detected between the unfiltered and filtered samples. The DOC content comprised $86 \pm 9\%$ of TOC as measured by the portable spectrophotometer (TOC: $16\text{--}54 \text{ mg L}^{-1}$; POC: $1\text{--}18 \text{ mg L}^{-1}$, $\text{POC}_{\text{average}} = 3.6 \pm 2.9 \text{ mg L}^{-1}$), though other laboratory-based estimates of POC were not performed on these samples. The DOC concentrations measured in the unfiltered samples were on average $0.3 \pm 1.2 \text{ mg L}^{-1}$ higher than the DOC concentrations measured in the filtered samples. In contrast to the DOC values, the absorbance values (200–740 nm, at 2.5 nm intervals) demonstrated significantly different values for unfiltered and filtered samples. This result indicates that the absorbance values produced solely in the spectro::lyser absorbance fingerprint without implementing the manufacturer's turbidity compensation algorithm may not be used to characterize DOC unless the samples are filtered.

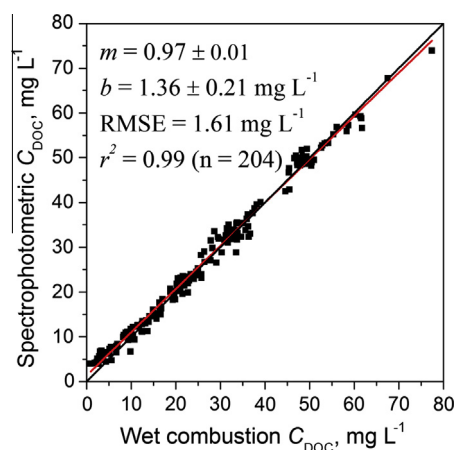


Fig. 3. Evaluation of the instrument's built-in local calibration method. Linear regression ($y = mx + b$) of the DOC values measured by wet oxidation (Aurora 1030) and spectrophotometric (spectro::lyser) techniques. The global calibration is compared in Table 2. The root mean square error (RMSE) indicates non-explained variance. Regression offset parameters b significantly deviating from zero and slope parameters m significantly deviating from one indicate biases between the wet oxidation and spectrophotometric methods.

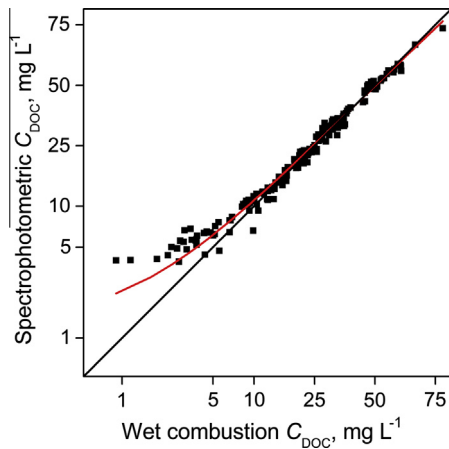


Fig. 4. Logarithmic fitting (log–log plot) of the DOC values measured by wet oxidation (Aurora 1030) and spectrophotometric (spectro::lyser) techniques. The graph indicates that spectrophotometric method overestimates DOC concentrations $< 10 \text{ mg L}^{-1}$.

No influence of nitrate and iron on the absorbance values was detected. A preliminary screening revealed low concentrations of iron in the samples ($0.4 \pm 0.5 \text{ mg L}^{-1}$, max 2.2 mg L^{-1} , $n = 66$). The nitrate concentration was always less than 0.06 mg L^{-1} ($n = 42$).

3.2. Comparison of different DOC measurement methods

The cross-method test of 27 systematically selected samples showed generally close agreement between the different methods (Fig. 5). Statistical test parameters of the regressions comparing the DOC values measured with wet oxidation against other methods reveal good agreement for all of the techniques (cuvette test: $r^2 = 0.96$, $\text{RMSE} = 2.04 \text{ mg L}^{-1}$; spectrophotometric: $r^2 = 0.97$, $\text{RMSE} = 1.71 \text{ mg L}^{-1}$). The uncalibrated cuvette method overestimated the DOC concentration over the full range of measured concentration ($b = 1.01 \pm 0.75$, $m = 1.05 \pm 0.04$), whereas the spectrophotometric method slightly overestimated DOC values only at low concentrations (high intercept $b = 1.41 \pm 0.63 \text{ mg L}^{-1}$, $m = 0.94 \pm 0.03$) (Fig. 5).

While the cuvette tests were acceptable for the comparison set of samples ($n = 27$), their results were not encouraging when used for an extended set of samples. During the measurements of 117 samples, 25% of the cuvette-test analyses were inaccurate and

displayed negative values for the organic-rich samples, perhaps due to high ambient laboratory temperature conditions. After excluding the negative results, however, the remaining values consistently underestimated DOC concentrations compared to the wet-oxidation method ($b = 1.01 \pm 0.03$, $m = 0.72 \pm 0.76$, $r^2 = 0.93$, $\text{RMSE} = 3.97 \text{ mg L}^{-1}$, $p < 0.001$, $n = 117$). This subset of the extended data exhibited lower r^2 and m values as well as higher RMSE values compared to the 27-sample comparison data set.

The factorial ANOVA with a repeated-measures factorial design demonstrated a significant difference among the methods ($p < 0.001$). The Bonferroni pairwise comparison demonstrated that no significant difference was detected between the wet oxidation and spectrophotometric methods, whereas the cuvette test results differed significantly from the wet oxidation and spectrophotometric methods ($p < 0.05$).

3.3. Comparison of multilinear local calibration models

The comparison of spectrophotometric measurements based on the different multilinear calibration methods is presented in Table 2. Compared to the global calibration, the instrument's built-in local calibration method showed better accuracy when compared with the wet oxidation DOC measurements (lower intercept parameter values and slope parameter values close to one for both training and validation sets) and showed higher r^2 values and lower RMSE values of the OLS regression equations used for calibration comparison (Table 2). Measurements applying the global calibration overestimated the DOC content compared to measurements applying the built-in local calibration for the subset of independent samples. The DOC values from the PLS, PCR, MSR and two-component calibrations produced accurate DOC estimates for the training set as can be seen by regression slopes m near 1 and models with high explanatory power r^2 values also near to 1. The PCR calibration method produced the model with the highest explanatory power (r^2) and lowest RMSE values for the training set. However, for the validation set, the measurements applying the PCR calibration method demonstrated the lowest r^2 and highest RMSE values, demonstrating the effect of over-parameterization of the PCR calibration model. Furthermore, for the validation set the PCR model produced the model with the highest offset ($b = 3.78 \text{ mg L}^{-1}$). The two-component model demonstrated the lowest offset of the model ($b = -0.16 \pm 0.7 \text{ mg L}^{-1}$), whereas its RMSE value was higher than the values of PLS, MSR and local calibrations for the validation set (Table 2). The PLS, PCR, MSR and

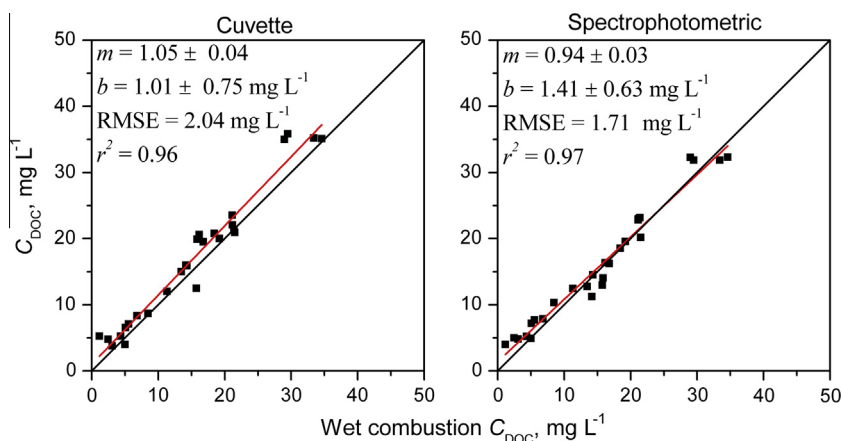


Fig. 5. Performance of different DOC concentration measurement methods compared to the wet oxidation method. DOC values ($n = 27$) obtained with the cuvette tests (LCK385 and LCK386 test kits, Hach Lange) and by the spectrophotometric method (spectro::lyser) were linearly regressed ($y = mx + b$, OLS) against values analyzed with the wet oxidation method (Aurora 1030). The root mean square error (RMSE) indicates non-explained variance. Regression offset parameters b significantly deviating from zero and slope parameters m significantly deviating from unity indicate biases between the wet oxidation and spectrophotometric methods.

Table 2

Statistical parameters of linear OLS regressions ($y = mx + b$) of the spectrophotometric measurements obtained from the calibration based on partial least-squares (PLS), principal-component (PCR) and multiple stepwise (MSR) regressions, the local and global calibrations as well as from the two-component model of Carter et al. (2012). Spectrophotometric measurements were regressed against DOC values measured by the wet oxidation method (Aurora 1030).

	Statistical parameters	PLS ^a	PCR ^a	MSR ^b	Local calibration	Global calibration	Two-component model
Training set, $n = 204$	r^2	0.991	1.0	0.992	0.991	0.989	0.99
	RMSE (mg L ⁻¹)	1.65	0.36	1.55	1.61	2.53	1.66
	m	0.99 ± 0.01	1.0 ± 0.002	0.99 ± 0.01	0.97 ± 0.01	1.39 ± 0.01	0.99 ± 0.01
	b (mg L ⁻¹)	0.25 ± 0.21	0.01 ± 0.05	0.22 ± 0.2	1.36 ± 0.21	-1.5 ± 0.33	0.25 ± 0.21
Validation set, $n = 41$	r^2	0.981	0.706	0.986	0.983	0.982	0.985
	RMSE (mg L ⁻¹)	2.80	13.9	2.43	2.55	3.79	3.39
	m	0.95 ± 0.02	1.0 ± 0.1	0.95 ± 0.02	0.91 ± 0.02	1.29 ± 0.3	0.94 ± 0.02
	b (mg L ⁻¹)	-0.44 ± 0.7	3.78 ± 3.6	-0.29 ± 0.6	0.85 ± 0.6	-2.05 ± 0.9	-0.16 ± 0.7

^a The number of parameters used for PLS and PCR were 197 (absorbance values of the 250–740 nm wavelengths at every 2.5 nm intervals).

^b The MSR used absorbance values at selected wavelengths with the highest explanatory power (257.5, 380, 730 and 292.5 nm).

two-component model each had a regression slope close to 1 for the validation set. Measurements applying the MSR calibration resulted in the highest explanatory power and the lowest RMSE value for the validation set (RMSE = 2.43 mg L⁻¹, $r^2 = 0.98$). The MSR analyses included only a limited number of wavelengths and resulted in the following best-fit equation:

$$C_{\text{DOC}} = (4.95 + 0.3 m \times A_{257.5} - 1.1 m \times A_{380} + 1.4 m \times A_{730} + 0.2 m \times A_{292.5}) \text{mg L}^{-1} \quad (1)$$

Detailed results of the MSR models for the different size fractions, sites and concentrations are presented in Table 3. The MSR indicated that, in most cases, the highest r^2 and lowest RMSE of

the prediction were achieved when more than one wavelength was included in the model. The inclusion of wavelengths in the range 600–740 nm also increased the accuracy.

The number of possible models decreased when the samples were separated by site type and within limited concentration ranges. A relatively high number of suitable models were obtained for the groups with a low concentration range. For all of the subgroups, the highest contribution to r^2 was attributed to the absorbance values in the UV range. As indicated in Table 3, several of the models included an additional molecular size index (Ratio 2: A_{255}/A_{365}). The absorbance characteristics differed among the sites (Fig. 6). Specifically, the absorbance values demonstrated stronger differences among the sites for values within the 360–420 nm

Table 3

Statistical test parameters of the multiple stepwise regression (MSR) applied to the spectrophotometric absorbance values as predictor variables against DOC values measured with the wet oxidation (Aurora 1030) technique as predictand variable.^a

Sample subset	N	Wavelength used in the model, nm	r^2	RMSE, mg L ⁻¹	F_{change}
All samples, $n = 204$	1	257.5	0.99	1.85	16943.77
	2	257.5, 380	0.99	1.63	60.36
	3	257.5, 380, 730	0.99	1.60	9.06
	4	257.5, 380, 730, 292.5	0.99	1.56	7.70
	5	257.5, 380, 730, 292.5, Ratio 2	0.99	1.55	5.38
DOC ($\leq 0.7 \mu\text{m}$), $n = 158$	1	295	0.98	1.89	9787.354
	2	295, 322.5	0.99	1.73	32.23
	3	295, 322.5, 250	0.99	1.67	11.27
	4	295, 322.5, 250, 275	0.99	1.56	24.69
	5	295, 322.5, 250, 275, 720	0.99	1.47	18.56
	6	295, 322.5, 250, 275, 720, 737.5	0.99	1.44	7.44
	7	295, 322.5, 250, 275, 720, 737.5, 632.5	0.99	1.43	4.71
$\leq 1 \text{ kDa}$, $n = 22$	1	Ratio 2	0.60	0.92	30.41
$\leq 10 \text{ kDa}$, $n = 22$	1	250	0.93	1.31	282.84
	2	250, 632.5	0.97	0.89	24.05
	3	250, 632.5, 642.5	0.98	0.75	8.99
	4	250, 632.5, 642.5, 672.5	0.99	0.58	12.63
	5	250, 632.5, 642.5, 672.5, 677.5	0.99	0.52	5.74
	6	250, 632.5, 642.5, 672.5, 677.5, 565	0.99	0.42	9.06
	7	250, 632.5, 642.5, 672.5, 677.5, 565, 655	0.99	0.36	6.98
	8	250, 632.5, 642.5, 672.5, 677.5, 565, 655, 695	0.99	0.30	6.30
Marginal forest swamp, $n = 17$	1	250	0.62	1.36	24.32
	2	250, 645	0.77	1.10	8.89
Bog, $n = 17$	1	305	0.73	1.38	40.58
Low range: 15–25 mg L ⁻¹ , $n = 35$	1	275	0.82	1.27	151.41
	2	275, 357.5	0.85	1.19	5.53
	3	275, 357.5, 495	0.87	1.13	4.77
	4	275, 357.5, 495, 497.5	0.89	1.07	4.49
Middle range: 26–36 mg L ⁻¹ , $n = 35$	1	295	0.44	2.13	26.01
	2	295, Ratio 2	0.54	1.97	6.71
High range: 36–52 mg L ⁻¹ , $n = 35$	1	250	0.80	1.72	133.95
	2	250, Ratio 2	0.88	1.34	22.19

^a The absorbance values from 250 nm to 740 nm at 2.5 nm intervals, Ratio 1 (A_{465}/A_{665}), and Ratio 2 (A_{255}/A_{365}) were used as input data. MSR was implemented separately for the different fractions, sites and concentrations. All F statistics are significant, stepwise regression stopped when further addition of absorbance values at any wavelength did not add a significant contribution to the model. F_{change} indicates the difference made by including new predictors to the model.

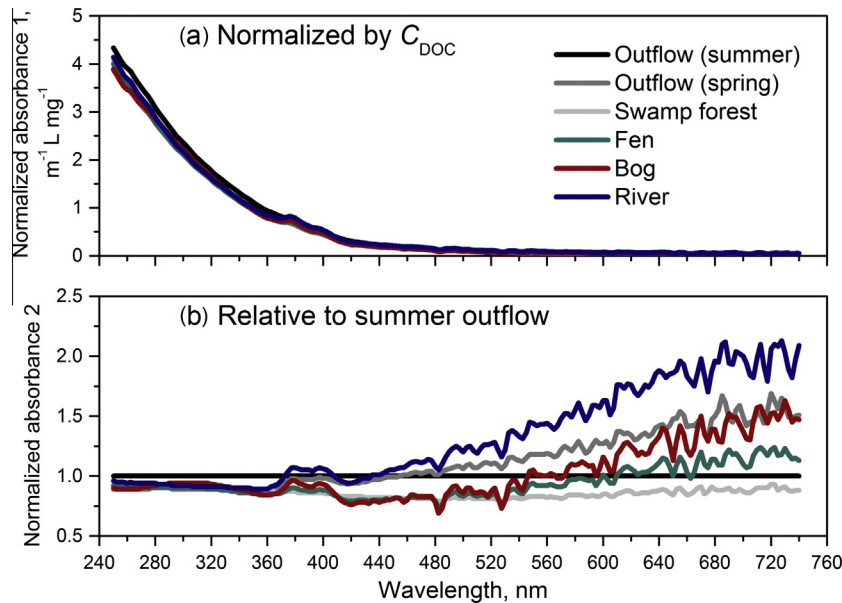


Fig. 6. Normalized absorbance spectra of DOC-containing water samples. All calculations are conducted for the sites' mean absorbance spectra (marginal forest swamp, $n = 16$; fen, $n = 16$; bog, $n = 2$; outflow summer period, $n = 2$; outflow spring, $n = 3$; and river, $n = 4$). From the marginal forest swamp, fen and bog samples were collected during the summer-fall (June–September) baseflow period, while the river samples were collected during the snowmelt (April–May). a. Absorbance spectra values normalized by the DOC-content for all sites, respectively. b. Absorbance values normalized by the DOC content divided by the corresponding normalized absorbance values of the DOC of the mean outflow (summer values) for better comparability of relative differences across the spectra.

range. At wavelengths above 420 nm, the variations between sites in terms of the absorbance intensities increased.

3.4. Influence of storage on qualitative and quantitative DOC characteristics

The spectrophotometric measurements that were performed to assess sample stability after storage (4 months at $< 4^{\circ}C$, $n = 12$) revealed small variations in both DOC content and light absorption, generally indicating a reduction in DOC concentration ($\Delta DOC = -0.97 \pm 1 \text{ mg L}^{-1}$, $\Delta A_{250} = -0.02 \pm 3.4 \text{ m}^{-1}$; $\Delta A_{280} = 0.6 \pm 2.6 \text{ m}^{-1}$; $\Delta A_{330} = 0.6 \pm 1.7 \text{ m}^{-1}$; $\Delta A_{632.5} = 0.6 \pm 1.2 \text{ m}^{-1}$; $\Delta A_{737.5} = 1.1 \pm 0.004 \text{ m}^{-1}$). The paired samples t-test analyses demonstrated that the differences were not significant at the 95% confidence interval for the DOC and individual absorbance values with the exception of absorbance at 737.5 nm.

4. Discussion

4.1. Local calibration and DOC discrimination capability of the spectro::lyser

The spectro::lyser probe has been used in a limited number of geo-ecological studies. However, not all of these studies used a built-in local calibration algorithm of the probe to improve the estimation of DOC content. For example, Waterloo et al. (2006) only used single wavelengths as proxies when estimating DOC (during their first study period, the absorbance was 350 nm; a second period utilized absorbance at 255 nm), and the absorbance values at these wavelengths were calibrated based on a regression against the DOC concentrations measured using a TOC analyzer (Shimadzu, Japan). However, the use of a single wavelength can lead to low-accuracy results, and Wallage and Holden (2010) have also demonstrated that some samples may demonstrate identical absorbance values at the 400 nm wavelength but differ in their DOC concentrations by 50%. Several studies suggested that methods relying on absorbance at more than one wavelength offer a much-improved DOC concentration estimate compared with the

use of only a single wavelength (e.g., Downing et al., 2009; Fichot and Benner, 2011; Sandford et al., 2010; Tipping et al., 2009). Similarly, in the current study, the accuracy of the models was improved by including an increased number of wavelengths. The improvement in relative accuracy is particularly important for low concentrations ($1\text{--}5 \text{ mg L}^{-1}$) where small improvements in absolute accuracy carry proportionately larger improvements in relative accuracy.

Koehler et al. (2009) measured DOC concentrations continuously in a stream draining an Atlantic bog using the spectro::lyser probe, and samples collected using a 24-bottle auto-sampler were further analyzed using the heat-combustion method (TOC-V cpH, Shimadzu Scientific Instruments, USA). The results from the heat combustion method were then used to correct the spectro::lyser measurements for a linear offset (Koehler et al., 2009). The DOC concentrations in the latter study ranged from 2.7 to 11.5 mg L^{-1} , and the r^2 value of a regression between DOC concentration measurements of the two different methods was 0.6 during a storm period and 0.3 during a dry period. Koehler et al. (2009) did not use a built-in local calibration, and the DOC concentration range that they covered was narrower than that presented here. Grayson and Holden (2012) did not estimate DOC values with the global calibration of the spectro::lyser because the relationship between the absorbance at specific wavelengths and DOC had been found to considerably vary among sites. Instead, this study focused on changes of different absorbance values over time as a proxy for indicating temporal changes in the absorbance characteristics of DOM. Recently, Strohmeier et al. (2013) conducted a local, customized calibration with regular cross-checks against the heat combustion method (TOC-V_{CPN} Analyzer, Shimadzu, Japan); the DOC values used in their calibration ranged from 2.6 to 33.8 mg L^{-1} . The present study describes the performance of the built-in local calibration and a wider range of DOC values ($1\text{--}78 \text{ mg L}^{-1}$) and includes the separation of the study site into portions, such as marginal forest swamp, fen, bog, river, and runoff points. Moreover, in addition to the bulk DOC fraction ($\leq 0.7 \mu\text{m}$), the following two low-molecular size DOC fractions were tested: $\leq 1 \text{ kDa}$ ($1\text{--}7 \text{ mg L}^{-1}$) and $\leq 10 \text{ kDa}$ ($3\text{--}21 \text{ mg L}^{-1}$).

In the current study, it was possible to obtain accurate DOC estimates with the portable UV–Vis spectrophotometer by pre-calibrating the system with 30 samples. It may be possible to achieve even better results by increasing the number of samples used during the pre-calibration phase. However, in many cases, it may not be possible to obtain enough samples prior to the main sampling campaign. Therefore, the local calibration step can be performed for a sub-set of representative samples during the sampling campaign, and previously collected absorbance fingerprint results can be uploaded into a new calibration algorithm. The local calibration between spectrophotometric measurements and DOC concentration analyses with any conventional laboratory method (e.g., heat combustion or wet oxidation) should be performed during the same day. If these guidelines are not followed, the spectrophotometric features of DOM may change slightly during storage, and thus a newly obtained local calibration algorithm could produce biased results. The recalculation of calibrated DOC estimates is automatically completed by the spectro::lyser.

This study identified no significant differences in the DOC content of filtered and unfiltered samples of mire surface and soil pore waters, indicating that the spectro::lyser is a suitable tool to determine DOC content in a nondestructive and time-efficient manner in the presence of low contents of particulate organic matter at the field station. However, it should be noted that all of the natural samples collected contained a DOC content of approximately 90% of the total organic carbon. Therefore, if the spectro::lyser is used at sites with high POC loads, accurate detection of the DOC fraction may be difficult. Jeong et al. (2012) demonstrated that DOC concentrations under high-flow conditions were overestimated by a portable spectrophotometer (carbo::lyser, s::can, Austria) due to inaccurate turbidity compensation. Therefore, these authors suggested that additional correction algorithms should be established to accommodate the wide range of flow conditions that may occur at a specific study.

4.2. Method comparison

Generally, different analytical techniques are expected to give slightly differing results for the same sample due to measurement systematic under- or overestimation. Because commonly used DOC measurement methods estimate the DOC content relative to standards that are comparatively easy to oxidize (e.g., potassium hydrogen phthalate or glucose), the DOC content in natural waters can be underestimated; its complex mixture includes many refractory organic compounds that are more difficult to oxidize and consequently may have unequal oxidation potentials (Aiken et al., 2002). For example, in the case of caffeine, high-temperature systems have overestimated the concentration, while the concentration was underestimated using Pt–persulfate systems (Aiken et al., 2002). Therefore, unless they are inter-calibrated, measurements taken with different instruments should be avoided in long-term studies because variations in instrument performance could be misinterpreted as a change in environmental conditions.

Overall, the cuvette tests performed at a relatively satisfactory level. However, as previously mentioned, some of the carbon-rich samples generated negative values, which can be explained by the unusually high room temperatures during the laboratory analyses in Syktyvkar, Russia (up to 35 °C). According to the manufacturer, different types of test kits must be stored at either room-temperature (+15 to +25 °C) or in the refrigerator (+2 to +8 °C). Furthermore, consideration should be given to the differences in detection limits before selecting the proper cuvette tests for the desired measurements. For example, the LCK 385 kit measures within a range of 3–30 mg L⁻¹, while the LCK 386 kit's range is 30–300 mg L⁻¹. Therefore, it is desirable to have in advance an estimate of the expected DOC concentration. Moreover, the cuvette test requires a special disposal procedure after use.

Table 4 details a further comparison of these different methods for operation in remote field conditions. The high-resolution absorption measurements demonstrated a number of advantages of using this robust, small-sized, on-line spectrophotometer over the conventional single- or dual-wavelength spectrophotometers. The usage of many wavelengths makes it possible for the probe to automatically estimate and compensate for turbidity in order to estimate the DOC content, which in turn reduces the necessity for an additional filtration step.

The UV–Vis probe can be used for both in situ real-time measurements at a field site and in the offline mode in the laboratory, and the method does not require filtration to determine the DOC content for samples with low POC content. The high-frequency absorbance measurements made under field conditions enable the use of this method for detecting rapid temporal fluctuations in DOC content and quality at a study site, which can be used to quickly detect changes in response to environmental or meteorological conditions (e.g., storms). This probe can be used also for an adaptive sampling approach with higher sampling during high-flow events such as the snowmelt period and a reduced observation frequency during the base flow period. This approach allows a more efficient use of resources to deliver higher quality flux estimates than for traditional fixed-frequency approaches.

Moreover, in situ measurements reduce requirements for sample transportation, storage and dilution. Furthermore, this method requires low level technical maintenance and may be used in a challenging environment (e.g., industrial wastewater systems or high-flow events, such as snowmelt or floods). However, care should be taken at sites with high sediment loads. Thus, it is advisable to frequently monitor the condition of the sensors and to complete, additionally to auto-cleaning with pressurized air, a manual cleaning if the sensors are disturbed by fouling. The increased power consumption due to the auto-cleaning cleaning system should be also considered.

The spectrophotometer's ability to operate using a battery or solar power supply render it possible to work in remote locations, although frequent charging is required for high-frequency measurement campaigns (e.g., every two days for measurements with 30 min frequency) using the standard battery of the instrument

Table 4

Comparison of the methods^a: heat combustion (Shimadzu TOC-L), wet oxidation (Aurora 1030), expulsion cuvette test (Hach Lange) and spectrophotometry (spectro::lyser).

Parameters	Wet oxidation, heat combustion	Cuvette tests	Spectrophotometric measurements
Premeasurement calibration	✓	✓	–
Local site specific calibration	–	–	✓
Operation in the field	–	–	✓
Filtration for DOC separation	✓	✓	–
Additional consumables (e.g., acids)	✓	✓	–
Special disposal requirements	–	✓	–
Time per 1 sample measurement	3–5 min	125 min ^b	<1 min

^a “✓” indicates the parameter is required by the method, while “–” indicates the parameter is not required by the method.

^b 125 min., including 120 min. for thermostat incubation prior to the measurements, cooling and measurement with the spectrophotometer (Hach Lange, Germany).

(battery capacity 20 Ah, probe power consumption 20 W). It is possible to increase battery life by using the probe in sleep/power saving mode, though this setting was not tested in the current study. The data logger on the spectrophotometer can store one month of data taken at 30-min time intervals (Langergraber et al., 2003). Moreover, when determining the DOC content, several absorbance coefficients can provide a qualitative description of the DOC's aromatic property, molecular size and humification degree for filtered samples, which can be advantageous when tracing biochemical changes (Peuravuori and Pihlaja, 1997; Worrall et al., 2002; Berggren et al., 2007; Baker et al., 2008).

4.3. Different multilinear calibration methods

For a multilinear calibration, the MSR, PCR and PLC regressions were chosen because they can be used with datasets that have collinear variables and a larger number of predictor variables than observations (Varmuza and Filzmoser, 2009; Miller and Miller, 2010). The current study shows that although the MSR analyses included a limited number of variables into the model, it produced a model with the highest explanatory power and the lowest RMSE value when applied to an independent validation dataset. The MSR model also produced lower RMSE values compared to the two-component model for the training and validation sets. MSR was also preferable over PLS and PCR because the regression models produced by PCR and PCA included all of the variables and were thus difficult to interpret. Generally, interpretation is feasible if no more than approximately a dozen variables are used in a model (Varmuza and Filzmoser, 2009). Furthermore, due to its possible overparameterization, the PCR model failed to produce accurate estimates for the validation dataset (RMSE: 13.9 mg L⁻¹) in contrast to the training set (RMSE: 0.36 mg L⁻¹).

4.4. Multiple stepwise regression results for different sub-sets

As previously mentioned, the spectro::lyser produces a fingerprint report with a wide range of wavelengths; this detailed output enables researchers to select the most suitable wavelength for the qualitative and quantitative characterization of DOC. As indicated in Table 3, the MSR models included wavelengths that were also commonly used previously as proxies for DOC concentration. Wavelengths that have previously been used as proxies include 254 nm (Baker et al., 2008; Tipping et al., 2009), 272 nm (Baker et al., 2008), 320 nm (Pastor et al., 2003), 340 nm (Tipping et al., 1988; Baker and Spencer, 2004; Baker et al., 2008; Tipping et al., 2009; Grayson and Holden, 2012), 365 nm (Baker et al., 2008), 400 nm (Worrall et al., 2002; Wallage and Holden, 2010; Grayson and Holden, 2012) and 410 nm (Baker et al., 2008).

As indicated in Fig. 6, the highest absorbance values normalized to DOC content were recorded at wavelengths below 400 nm. According to the review by Korshin et al. (1997), most of the chromophores that absorb light at wavelengths below 400 nm are aromatic groups with various degrees and types of substitution, including mono- and polysubstituted phenols and diverse aromatic acids. These chromophores are primarily associated with the humic fraction of the DOM (Korshin et al., 1997). The increased variation of absorbance intensities at wavelengths greater than 450 nm may be caused by turbidity differences among samples (i.e., due to differences in the sizes, shapes, colors and reflectivities of particles) because the wavelengths at which the absorbance is affected by turbidity are located within the visible range between 450 and 650 nm (Jeong et al., 2012).

For all of the models apart from the ≤1 kDa fraction, the absorption in the UV-range wavelengths best explained the sample variance. The absorption of UV light is caused by π-electron changes in energy levels and reflects aromatic, carbonylic and carboxylic

electron systems and their conjugates (Abbt-Braun and Frimmel, 1999). The natural waters of terrestrial reserves, such as peatlands, have high aromatic carbon levels; therefore, DOC concentrations strongly correlate with absorbance values in the UV range. The DOC concentration of the ≤1 kDa fraction most probably did not correlate with the UV-range wavelengths because this fraction included a low molecular organic carbon fraction without contribution of the aromatic carbon that generally occurs in the high molecular fraction.

The MSR analyses for the different sites, fractions and concentrations demonstrated that calibration model performances can be improved when more than one wavelength-specific absorbance (2–4) is included in the model (Table 3). In the current study, a preliminary screening for iron and nitrate contents in the samples revealed low concentrations, which are not assumed to significantly interfere with DOM absorption. In other water samples with higher iron and nitrate contents, possible interference from these species should be considered.

The relationship between DOC concentration and absorbance varied at different sites. A decreased number of predictor absorbances in the acceptable models was observed after separating the samples according to the sites or different concentration intervals. The relatively high number of useful predictor absorbance values in the acceptable models for the “low range group” can be explained because this group included samples collected from different sites during the snowmelt period, thus incorporating samples that presented a mixture of DOC from different sources that became hydrologically connected during the snowmelt period. Similar findings were obtained by Baker et al. (2008), who observed that their entire mixed catchment exhibited the strongest relationship between the absorbance coefficients at 340 nm and DOC concentration; however, DOC from the peat subcatchment had the most significant relationship with absorbance at 272 nm. Thus, even within a single catchment, it may be necessary to distinguish sub-sites to create specific regression calibration functions that generate optimal results. It may also be sub-optimal to use the same wavelengths to determine the organic carbon content during different seasons because the specific absorbance values of different DOC sources may considerably vary over time. Therefore, it is necessary to create seasonal site-specific calibration curves. For long-term spectrophotometric estimates of DOC concentrations, it can be important to periodically calibrate the changes in water color against the measured DOC concentrations using a wet oxidation or heat combustion method. However, the current study lacks samples from different seasons, so the assumption of seasonal variations in DOM quality was not tested.

5. Conclusions

The UV–Vis submersible spectrophotometric probe facilitates the rapid, robust measurement of DOC content for samples with low POC content under remote field conditions. A recommendation for further studies is to test this probe's performance for samples with high POC content. Additionally, the fingerprint results of filtered samples enable researchers to trace biogeochemical changes through the ratios of absorbance values at specific wavelengths, which provide information about the DOM composition. This study also demonstrates that studies that use absorbance values as a proxy for DOC content determination should include more than one wavelength (2–5) in their absorbance–concentration models. Moreover, in addition to the widely used wavelengths in the range of 254–400 nm, the inclusion of absorbance values at the wavelengths of 600 nm and 740 nm may significantly increase the accuracy of DOC estimates. This improvement is especially noticeable in the ≤10 kDa DOM size fraction, where including a higher

wavelength in the stepwise regression model improved modeled concentrations by more than 30% on an RMSE basis. Our findings also encourage the development of site- and season-specific multiple regression models for accurate DOC content estimation. Application of the high-resolution absorbance method complementary to conventional methods can be beneficial for many water research-related disciplines, potentially providing the biogeochemical research community with a new opportunity to expand its understanding of DOM fluctuations at different sites and over different seasons.

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References

- Abbt-Braun, G., Frimmel, F.H., 1999. Basic characterization of Norwegian NOM samples – similarities and differences. *Environ. Int.* 25, 161–180. [http://dx.doi.org/10.1016/S0160-4120\(98\)00118-4](http://dx.doi.org/10.1016/S0160-4120(98)00118-4).
- Ågren, A., Buffam, I., Berggren, M., Bishop, K., Jansson, M., Laudon, H., 2008. Dissolved organic carbon characteristics in boreal streams in a forest-wetland gradient during the transition between winter and summer. *J. Geophys. Res.* 113, 1–11. <http://dx.doi.org/10.1029/2007JG006674>.
- Aiken, G., Kaplan, L.A., Weishaar, J., 2002. Assessment of relative accuracy in the determination of organic matter concentrations in aquatic systems. *J. Environ. Monit.* 4, 70–74. <http://dx.doi.org/10.1039/b107322m>.
- Baker, A., Spencer, R.G.M., 2004. Characterization of dissolved organic matter from source to sea using fluorescence and absorbance spectroscopy. *Sci. Total Environ.* 333, 217–232. <http://dx.doi.org/10.1016/j.scitotenv.2004.04.013>.
- Baker, A., Bolton, L., Newson, M., Spencer, R.G.M., 2008. Spectrophotometric properties of surface water dissolved organic matter in an afforested upland peat catchment. *Hydrol. Process.* 22, 2325–2336. <http://dx.doi.org/10.1002/hyp.6827>.
- Berggren, M., Laudon, H., Jansson, M., 2007. Landscape regulation of bacterial growth efficiency in boreal freshwaters. *Glob. Biogeochem. Cycles* 21, 1–9. <http://dx.doi.org/10.1029/2006GB002844>.
- Carter, H.T., Tipping, E., Koprivnjak, J.-F., Miller, M.P., Cookson, B., Hamilton-Taylor, J., 2012. Freshwater DOM quantity and quality from a two-component model of UV absorbance. *Water Res.* 46, 4532–4542. <http://dx.doi.org/10.1016/j.watres.2012.05.021>.
- Chen, W., Westerhoff, P., Leenheer, J.A., Booksh, K., 2003. Fluorescence excitation-emission matrix regional integration to quantify spectra for dissolved organic matter. *Environ. Sci. Technol.* 37, 5701–5710. <http://dx.doi.org/10.1021/es034354c>.
- Cory, R.M., McKnight, D.M., 2005. Fluorescence spectroscopy reveals ubiquitous presence of oxidized and reduced quinones in dissolved organic matter. *Environ. Sci. Technol.* 39, 8142–8149. <http://dx.doi.org/10.1021/es0506962>.
- Cory, R.M., Boyer, E.W., McKnight, D.M., 2011. Spectral methods to advance understanding of dissolved organic carbon dynamics in forested catchments. In: Levina, D.F., Carlyle-Moses, D., Tanaka, T. (Eds.), *Forest Hydrology and Biogeochemistry, Ecological Studies*. Springer, Netherlands, pp. 117–135.
- De Haan, H., 1993. Solar UV-light penetration and photodegradation of humic substances in peaty lake water. *Limnol. Ocean.* 38, 1072–1076. <http://dx.doi.org/10.4319/lo.1993.38.5.1072>.
- Downing, B.D., Boss, E., Bergamaschi, B.A., Fleck, J.A., Lionberger, M.A., Ganju, N.K., Schoellhamer, D.H., Fujii, R., 2009. Quantifying fluxes and characterizing compositional changes of dissolved organic matter in aquatic systems in situ using combined acoustic and optical measurements. *Limnol. Ocean.: Method* 7, 119–131. <http://dx.doi.org/10.4319/lom.2009.7.119>.
- Dugard, P., Todman, J., Staines, H., 2010. *Approaching Multivariate Analysis, 2nd Edition: A Practical Introduction*, second ed. Routledge.
- ESA, 2010. GlobCover 2009 (Global Land Cover Map) Released on 21 December, 30. <<http://due.esrin.esa.int/globcover/>>.
- Fichot, C.G., Benner, R., 2011. A novel method to estimate DOC concentrations from CDOM absorption coefficients in coastal waters. *Geophys. Res. Lett.* 38, L03610. <http://dx.doi.org/10.1029/2010GL046152>.
- Fleischmann, N., Langergraber G., Weingartner A., Hofstaedter, F., Nusch S., Maurer P., 2001. On-line and in-situ measurement of turbidity and COD in wastewater using UV/VIS spectrometry. SCAN Media Library. <http://www.s-can.at/medialibrary/publications/p_2001_06.pdf> (accessed 17.10.2013).
- Freeman, C., Evans, C., Monteith, D., Reynolds, B., Fenner, N., 2001. Export of organic carbon from peat soils. *Nature* 412, 785. <http://dx.doi.org/10.1038/35090628>.
- Grayson, R., Holden, J., 2012. Continuous measurement of spectrophotometric absorbance in peatland streamwater in northern England: implications for understanding fluvial carbon fluxes. *Hydrol. Process.* 26, 27–39. <http://dx.doi.org/10.1002/hyp.8106>.
- Guo, L., Santschi, P.H., 2007. Ultrafiltration and its applications to sampling and characterisation of aquatic colloids. In: Lead, J.R., Wilkinson, K.J. (Eds.), *Environmental Colloids and Particles*. John Wiley & Sons, Ltd., pp. 159–221. Doi: 10.1002/9780470024539.ch4.
- Huber, E., Frost, M., 1998. Light scattering by small particles. *J. Water SRT – Aqua* 47, 87–94. <http://dx.doi.org/10.1046/j.1365-2087.1998.00086.x>.
- IUSS Working Group WRB, 2006. World reference base for soil resources 2006. World Soil Resources Reports No. 103. FAO, Rome. <<ftp://ftp.fao.org/agl/agll/docs/wsr103e.pdf>> (Accessed 24.03.2014).
- Jeong, J.-J., Bartsch, S., Fleckenstein, J.H., Matzner, E., Tenhunen, J.D., Lee, S.D., Park, S.K., Park, J.-H., 2012. Differential storm responses of dissolved and particulate organic carbon in a mountainous headwater stream, investigated by high-frequency, in situ optical measurements. *J. Geophys. Res.* 117, 1–13. <http://dx.doi.org/10.1029/2012JG001999>.
- Koehler, A.-K., Murphy, K., Kiely, G., Sottocornola, M., 2009. Seasonal variation of DOC concentration and annual loss of DOC from an Atlantic blanket bog in South Western Ireland. *Biogeochemistry* 95, 231–242. <http://dx.doi.org/10.1007/s10533-009-9333-9>.
- Korshin, G.V., Li, C.-W., Benjamin, M.M., 1997. Monitoring the properties of natural organic matter through UV spectroscopy: a consistent theory. *Water Res.* 31, 1787–1795. [http://dx.doi.org/10.1016/S0043-1354\(97\)00006-7](http://dx.doi.org/10.1016/S0043-1354(97)00006-7).
- Langer, S., 2012. The tracer potential of nutrient elements for lateral matter transport in a boreal forest-peatland-landscape. Diploma Thesis, University of Hamburg.
- Langergraber, G., Fleischmann, N., Hofstädter, F., 2003. A multivariate calibration procedure for UV/VIS spectrometric quantification of organic matter and nitrate in wastewater. *Water Sci. Technol.* 47, 63–71.
- Langergraber, G., Fleischmann, N., Hofstaedter, F., Weingartner, A., 2004. Monitoring of a paper mill wastewater treatment plant using UV/VIS spectroscopy. *Water Sci. Technol.* 49, 9–14.
- Lauerwald, R., Hartmann, J., Ludwig, W., Moosdorf, N., 2012. Assessing the nonconservative fluvial fluxes of dissolved organic carbon in North America. *J. Geophys. Res. Biogeosci.* 117, G01027. <http://dx.doi.org/10.1029/2011JG001820>.
- McKnight, D.M., Harnish, R., Wershaw, R.L., Baron, J.S., Schiff, S., 1997. Chemical characteristics of particulate, colloidal, and dissolved organic material in Loch Vale Watershed, Rocky Mountain National Park. *Biogeochemistry* 36, 99–124. <http://dx.doi.org/10.1023/A:1005783812730>.
- Miller, J.N., Miller, J.C., 2010. *Statistics and Chemometrics for Analytical Chemistry*, sixth ed. Prentice Hall, UK, 278p.
- Pastor, J., Solin, J., Bridgman, S.D., Updegraff, K., Harth, C., Weishampel, P., Dewey, B., 2003. Global warming and the export of dissolved organic carbon from boreal peatlands. *Oikos* 100, 380–386. <http://dx.doi.org/10.1034/j.1600-0706.2003.11774.x>.
- Pereira, R., Isabella Bovolenta, C., Spencer, R.G.M., Hernes, P.J., Tipping, E., Vieth-Hillebrand, A., Pedentchouk, N., Chappell, N.A., Parkin, G., Wagner, T., 2014. Mobilization of optically invisible dissolved organic matter in response to rainstorm events in a tropical forest headwater river. *Geophys. Res. Lett.* 41. <http://dx.doi.org/10.1002/2013GL058658>.
- Peuravuori, J., Pihlaja, K., 1997. Molecular size distribution and spectroscopic properties of aquatic humic substances. *Anal. Chim. Acta* 337, 133–149. [http://dx.doi.org/10.1016/S0003-2670\(96\)00412-6](http://dx.doi.org/10.1016/S0003-2670(96)00412-6).
- Pokrovsky, O., Dupré, B., Schott, J., 2005. Fe–Al–organic colloids control of trace elements in peat soil solutions: results of ultrafiltration and dialysis. *Aquat. Geochim.* 11, 241–278. <http://dx.doi.org/10.1007/s10498-004-4765-2>.
- Pokrovsky, O., Schott, J., Dupre, B., 2006. Trace element fractionation and transport in boreal rivers and soil porewaters of permafrost-dominated basaltic terrain in Central Siberia. *Geochim. et Cosmochim. Acta* 70, 3239–3260. <http://dx.doi.org/10.1016/j.gca.2006.04.008>.
- Pokrovsky, O., Viers, J., Shirokova, L.S., Shevchenko, V.P., Filipov, A.S., Dupré, B., 2010. Dissolved, suspended, and colloidal fluxes of organic carbon, major and

- trace elements in the Severnaya Dvina River and its tributary. *Chem. Geol.* 273, 136–149. <http://dx.doi.org/10.1016/j.chemgeo.2010.02.018>.
- Rieger, L., Langergraber, G., Thomann, M., Fleischmann, N., Siegrist, H., 2004. Spectral in-situ analysis of NO₂, NO₃, COD, DOC and TSS in the effluent of a WWTP. *Water Sci. Technol.* 50, 143–152.
- Runkle, B.R.K., Wille, C., Gažovič, M., Wilmking, M., Kutzbach, L., 2014. The surface energy balance and its drivers in a boreal peatland fen of northwestern Russia. *J. Hydrol.* 511, 359–373. <http://dx.doi.org/10.1016/j.jhydrol.2014.01.056>.
- Sandford, R.C., Bol, R., Worsfold, P.J., 2010. In situ determination of dissolved organic carbon in freshwaters using a reagentless UV sensor. *J. Environ. Monit.* 12 (9), 1678–1683. <http://dx.doi.org/10.1039/c0em00060d>.
- Schneider, J., Kutzbach, L., Wilmking, M., 2012. Carbon dioxide exchange fluxes of a boreal peatland over a complete growing season, Komi Republic, NW Russia. *Biogeochemistry* 1111, 485–513. <http://dx.doi.org/10.1007/s10533-011-9684-x>.
- Schwalger, B., Spitz, A., 2009. Separation of natural organic colloids with a PALL tangential flow filtration system. *Water Sci. Technol. Water Supply* 9, 583–590.
- Shvartsev, S.L., Serebrennikova, O.V., Zdvizhkov, M.A., Savichev, O.G., Naimushina, O.S., 2012. Geochemistry of wetland waters from the lower Tom basin, Southern Tomsk oblast. *Geochem. Int.* 50, 367–380.
- Strohmeier, S., Knorr, K.-H., Reichert, M., Frei, S., Fleckenstein, J.H., Peiffer, S., Matzner, E., 2013. Concentrations and fluxes of dissolved organic carbon in runoff from a forested catchment: insights from high frequency measurements. *Biogeosciences* 10, 905–916. <http://dx.doi.org/10.5194/bgd-9-11925-2012>.
- Thacker, S.A., Tipping, E., Gondar, D., Baker, A., 2008. Functional properties of DOM in a stream draining blanket peat. *Sci. Total Environ.* 407, 566–573. <http://dx.doi.org/10.1016/j.scitotenv.2008.09.011>.
- Thurman, E.M., 1985. *Organic Geochemistry of Natural Waters*. Springer Science & Business.
- Tipping, E., Hilton, J., James, B., 1988. Dissolved organic matter in Cumbrian lakes and streams. *Fresh. Biol.* 19, 371–378. <http://dx.doi.org/10.1111/j.1365-2427.1988.tb00358.x>.
- Tipping, E., Corbishley, H.T., Koprivnjak, J., Lapworth, D.J., Miller, M.P., Vincent, C.D., Hamilton-Taylor, J., 2009. Quantification of natural DOM from UV absorption at two wavelengths. *Environ. Chem.* 6, 472–476.
- Ulanowski, T.A., Branfireun, B.A., 2013. Small-scale variability in peatland pore-water biogeochemistry, Hudson Bay Lowland, Canada. *Sci. Total Environ.* 454–455, 211–218. <http://dx.doi.org/10.1016/j.scitotenv.2013.02.087>.
- Varmuza, K., Filzmoser, P., 2009. *Introduction to Multivariate Statistical Analysis in Chemometrics*. CRC Press, Taylor and Francis Group.
- Wallage, Z.E., Holden, J., 2010. Spatial and temporal variability in the relationship between water colour and dissolved organic carbon in blanket peat pore waters. *Sci. Total Environ.* 408, 6235–6242. <http://dx.doi.org/10.1016/j.scitotenv.2010.09.009>.
- Waterloo, M.J., Oliveira, S.M., Drucker, D.P., Nobre, A.D., Cuartas, L.A., Hodnett, M.G., Langedijk, I., Jans, W.W.P., Tomasella, J., de Araújo, A.C., Pimentel, T.P., Múnera Estrada, J.C., 2006. Export of organic carbon in run-off from an Amazonian rainforest blackwater catchment. *Hydrol. Process.* 20, 2581–2597. <http://dx.doi.org/10.1002/hyp.6217>.
- Weishaar, J.L., Aiken, G.R., Bergamaschi, B.A., Fram, M.S., Fujii, R., Mopper, K., 2003. Evaluation of specific ultraviolet absorbance as an indicator of the chemical composition and reactivity of dissolved organic carbon. *Environ. Sci. Technol.* 37, 4702–4708. <http://dx.doi.org/10.1021/es030360x>.
- Worrall, F., Burt, T.P., Jaeban, R.Y., Warburton, J., Shedden, R., 2002. Release of dissolved organic carbon from upland peat. *Hydrol. Process.* 16, 3487–3504. <http://dx.doi.org/10.1002/hyp.1111>.
- Worrall, F., Burt, T.P., Adamson, J., 2008. Long-term records of dissolved organic carbon flux from peat-covered catchments: evidence for a drought effect? *Hydrol. Process.* 22, 3181–3193. <http://dx.doi.org/10.1002/hyp.6907>.
- Xiao, Y.-H., Sara-Aho, T., Hartikainen, H., Vähätalo, A.V., 2013. Contribution of ferric iron to light absorption by chromophoric dissolved organic matter. *Limnol. Ocean.* 58, 653–662. <http://dx.doi.org/10.4319/lo.2013.58.2.0653>.