

Contents lists available at ScienceDirect

Journal of Hydrology



journal homepage: www.elsevier.com/locate/jhydrol

Characterization of DOC in riparian wetland pore water and its interference in stable water isotope analysis of δ^2 H and δ^{18} O

Filippa Fredriksson^{a,*}, Kenneth Thorø Martinsen^b, Kai-Ying Liao^b, Tyler B. Coplen^c, Rasmus Jakobsen^d, Okke Batelaan^e, Søren Jessen^a

^a Department of Geosciences and Natural Resource Management, University of Copenhagen, Copenhagen, Denmark

^b Freshwater Biological Laboratory, Department of Biology, University of Copenhagen, Copenhagen, Denmark

^c U.S. Geological Survey, 431 National Center, Reston, VA, United States

^d Geological Survey of Denmark and Greenland, Øster Voldgade 10, 1350 Copenhagen, Denmark

e National Centre for Groundwater Research and Training, College of Science and Engineering, Flinders University, Bedford Park, South Australia, Australia

ARTICLE INFO

Keywords: Stable water isotopes Dissolved organic carbon Wetlands Dual-inlet isotope-ratio mass spectrometry Laser absorption spectrometry

ABSTRACT

Wetlands play an important role in carbon retention, which is greatly dependent on hydrological conditions. Therefore, the interest in wetland hydrology has increased over recent decades. In wetland hydrology, stable water isotopes (δ^2 H, δ^{18} O) have found increasing use, as laser-based instruments have become readily available and allow for faster analysis and lower costs. However, the use of laser-based determinations of stable hydrogen and oxygen isotopes in wetland pore waters might be challenging because of dissolved organic carbon (DOC), especially as wetland pore waters often contain elevated DOC concentrations. To study the potential interference of DOC on laser-based stable water isotopes determination, we determined concentration, fluorescence and absorbance characteristics of DOC in pore waters collected from six restored and six near-natural riparian wetlands. The water samples' δ^2 H and δ^{18} O values were determined by two laser absorption spectrometers (LAS) and, for reference, in parallel by dual-inlet isotope-ratio mass spectrometry (DI-IRMS). The two LAS methods showed significant deviations from the DI-IRMS-determinations. Variations in the specific UV absorbance index (SUVA254, the quotient of the absorbance at 254 nm and the DOC concentration), which indicates the aromaticity of the DOC, partially accounted for interference for $\delta^2 H$ measurements. Elevated SUVA₂₅₄ index values were especially linked to restored wetlands. Studies that investigate pore waters with high DOC aromaticity but where the DOC is not characterized, and which require a high accuracy of the δ^2 H-values for interpretation of the hydrological system, may be severely affected by DOC interference.

1. Introduction

Wetlands make up about three percent of the global terrestrial surface and are estimated to store up to almost a third of the global soil organic carbon (Yu et al., 2010; Mitsch & Gosselink, 2015; Leifeld & Menichetti, 2018; Xu et al., 2018). Wetland ecosystems are thus carbon cycling hotspots as they store disproportionately high quantities of carbon and are sites of high greenhouse gas (GHG) emissions. This has prompted new interest in wetland hydrology as increased GHG emissions largely depend on the hydrological conditions (Tiemeyer et al., 2020). In this respect, stable hydrogen (δ^{2} H) and oxygen (δ^{18} O) isotopes of the water molecule are useful tracers to differentiate sources of water in wetlands, having distinct isotopic signatures (Kværner & Kløve, 2008;

Rautio & Korkka-Niemi, 2015; Isokangas et al., 2017; Bam & Ireson, 2019; Cooper et al., 2019; Bugna et al., 2020; Marttila et al., 2021). These measurements provide a non-invasive, quantitative method that can give insight into the hydrological processes and spatiotemporal variability in wetlands. During the last decades, analysis of δ^2 H and δ^{18} O values has become less expensive and easier due to the use of laser absorption spectrometry (LAS) that determines the absorption of specific wavelengths related to specific isotopologues of the water molecule, in lieu of the traditional use of costly isotope-ratio mass spectrometry (IRMS), which measures the difference in mass over charge of isotopologues of water directly. Use of LAS has enabled sampling of water for determination of isotopic composition at higher frequency, resulting in improved interpretations based on transient variations in the isotopic

https://doi.org/10.1016/j.jhydrol.2025.133245

Received 19 December 2024; Received in revised form 28 March 2025; Accepted 3 April 2025 Available online 4 April 2025

^{*} Corresponding author. *E-mail address:* kjff@ign.ku.dk (F. Fredriksson).

^{0022-1694/© 2025} The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

signatures (Hong et al., 2022; Mattei et al., 2022).

Previous studies (Penna et al., 2010; Singleton et al., 2009; Hissler et al., 2024) have investigated the possible discrepancy in measurements of $\delta^{18}\mathrm{O}$ and $\delta^{2}\mathrm{H}$ in water between laser-based and IRMS instruments. Penna et al. (2010) explored both the performance of specific instruments, as well as the repeatability and reproducibility amongst different instruments. Although differences between the two methods have been identified and the precision of LAS can be poor, the usage of LAS has become an established norm. However, Singleton et al. (2009) observed that adding activated carbon granules to the sampled water before analysis, which absorbs dissolved organic carbon (DOC), could improve the precision of the LAS measurements, implying a potential interference of DOC on water isotope determinations by LAS. If high DOC concentrations or specific DOC characteristics cause significant interference in laser-based analysis of stable water isotopes, this would have implications specifically for the application of stable isotopes of water in studies of wetland hydrology, because wetland pore waters often contain dynamic (Rosset et al., 2020; Zhang et al., 2022) and high DOC concentrations of several tens or even a few hundreds of milligrams per liter. The underlying reasons for potential interference could be associated with the fact that DOC consists of large complex molecules of mostly unmapped structure, which may show unpredictable absorption at the wavelengths used to determine the specific isotope bearing molecules. Further, the recent availability of a 'salt liner', developed by Picarro Inc. as a follow-up on the study by Skrzypek & Ford (2014) on analysis of high-salinity samples, indicates that post-vaporization effects can occur in the analyzer. In our experience, the liners improve performance even for freshwater samples with much-below-sea salt concentrations, suggesting that DOC could also cause such problems.

The DOC characteristics in terms of chemical composition and structure can be determined by its fluorescence and absorbance properties, which describe the compounds' ability to fluoresce and absorb light when exposed to ultraviolet (UV) and visible light (Stedmon et al., 2003). Fluorescence characteristics and concentration of DOC depend on e.g. catchment land-use (Sankar et al., 2020), and are suggested to be used as hydrological tracers (Stedmon & Markager, 2005; Kristensen et al., 2018), making them highly relevant when investigating carbon cycling in aquatic ecosystems.

The present study is based on access to pore waters and stream water from temperate riparian wetland field sites with high DOC concentrations and different management (and restoration) histories. Our aim is to use stable isotopes of water in our study of the hydrology of wetlands. However, the knowledge on how laser-based determinations of δ^2 H and δ^{18} O are potentially affected by interference caused by the presence of DOC (concentration and/or characteristics) in the water samples is limited. To investigate potential interference, statistical analyses were performed using measurements of stable water isotopes (δ^2 H, δ^{18} O), DOC concentration, and DOC fluorescence and absorbance characteristics.

Accordingly, this study serves two aims: 1) to provide concentrations and characterization of the DOC in wetlands and investigate statistical relations to the wetland history, and 2) to test the hypothesis that DOC causes interference in laser-based determinations of δ^2 H and δ^{18} O in wetland groundwater and stream water associated with the wetlands. The conclusions of our study will be operational, as the data collected to fulfil these aims will enable any potential significant interference in isotope LAS measurements to be linked to specific DOC concentrations, characteristics, or hydrological compartments. Although the main focus in the present study is the potential interference of DOC on LAS isotope determination, the collected data, as an ancillary benefit, will contribute to the growing database on DOC concentrations, fluorescence and absorbance characteristics of various hydrological compartments (Fredriksson et al., 2025).

2. Methods

To investigate DOC as a possible source of interference in laser-based measurements, shallow (<1 m below ground level; mbgl) groundwater samples for DOC characterization and for stable water isotope analysis were collected from field sites located along three separate stream vallevs in the catchments of Omme stream (OM), Odense stream (OD) and Tryggevælde stream (TR) in Denmark (Fig. 1). The catchments are dominated by agriculture. DOC characterization was conducted for samples from 12 riparian wetlands, of which six are near-natural (NN) wetlands, while the other six sites were previously used for agricultural purposes but restored 12-17 years prior to sampling, referred to as restored (R) wetlands. Water samples for stable isotope analysis were collected from 10 of the field sites. Unfortunately, shallow groundwater in two restored sites in the TR catchment could not be sampled because of dry conditions at the time of sampling. In addition, stream water (SW) adjacent to the wetlands was collected, together with samples from deeper (3-8 mbgl), regional groundwater (RGW) wells inside or bordering three of the field sites. We define the four groups NN, R, SW and RGW as four distinct hydrological compartments, i.e.: groundwater sampled within the shallow peat of either near-natural (NN) or restored (R) wetlands; the deeper, regional groundwater (RGW); and stream water (SW).

The surface geology in the OM, OD and TR catchments are dominated, respectively, by sand, clay or chalk (Fig. 1). From each field site, six sediment cores of the uppermost 30 cm were collected, and analyses show that the average soil organic matter (SOM, %) content in the restored sites varies between 4-34 % and between 8-66 % in the nearnatural sites (Sørensen, 2024).

2.1. Well installation and sampling procedures

Fifty-nine shallow groundwater wells consisting of 1-m-long



Fig. 1. The 12 field sites (red dots) are located along the stream valleys (blue lines) of Omme (OM), Odense (OD) and Tryggevælde streams, in Denmark, with subsurface geology dominated by respectively sand (yellow), clay (brown) or chalk (green). Each stream valley hosts four sites: two restored and two nearnatural wetlands. Field sites by each stream valley catchment lie too close together to visually distinguish individually by red dots. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

polyethylene (PE) 54.6/50-mm OD/ID HDPE tubes (KIWA, Rotek A/S, Sdr. Felding, Denmark), with a 30-cm screen placed at 0.5-0.8 m below ground level, were installed using a 01.12.SA hand-operated bailer boring auger set (Eijkelkamp, Giesbeek, The Netherlands) with an Ø10-cm casing. The screens were gravel packed (0.9-1.6 mm quartz sand) and sealed with cement-bentonite (w/w 83/17 %, BC-2, Rotek A/S, Sdr. Felding, Denmark) from the top of the screen to the surface. The ten deep wells consisted of 1-inch galvanized steel pipes that were driven 3-8 m into the subsurface using a Makita HM1400 pneumatic hammer. The pipes were equipped with 10-cm screens near the lower end.

Water sampling for DOC and its fluorescence and absorbance characteristics took place during five sampling campaigns (September-November 2022; January-February 2023; April-May 2023; July 2023; October 2023), resulting in 267 samples in total, of which 209 samples were from shallow (<0.5-0.8 mbgl) groundwater, 33 samples from deeper (3-8 mbgl) groundwater and 25 samples from stream water. Sampling of water for stable isotopes only took place during the September-November 2022 campaign when 72 samples were collected, consisting of 53 shallow and 12 deeper groundwater samples, along with seven stream samples.

Using a peristaltic pump, the wells were first clean-pumped (three times their volume). The groundwater was then pumped into a 60-mL PE syringe and filtered (Sartorius Minisart CA, 0.20 µm) directly into the sample vials. Accordingly, all samples in this study were filtered prior to subsequent laboratory analyses. For $\delta^2 H$ and $\delta^{18} O$ analysis using DI-IRMS and LAS (on instrument denoted as 'Laser-1'), 20 mL of sample was collected in 30-mL borosilicate glass bottles. For the second laserbased determination (on the instrument denoted as 'Laser-2'), 1-1.5 mL samples were collected in 2-mL clear glass vials with silicone-PTFE septum (Chromacol 2-SVWST-CPK, Thermo Fisher). For DOC analysis, duplicate 15-mL samples in glass vials, cleaned prior to sampling by heating to 550 °C, were collected and preserved with 15 μL of 2M hydrochloric acid. For DOC fluorescence and absorbance characteristics analysis, duplicate 10-mL samples were filtered into polypropylene vials and stored in the dark. Stream samples were extracted using the syringe directly, i.e. without using the peristaltic pump. All samples were refrigerated until analysis.

2.2. Laboratory analyses

2.2.1. Stable isotopes of water $(^{2}H, ^{18}O)$

The water samples were analyzed using two different DI-IRMS instruments, and by two LAS instruments (denoted 'Laser-1' and 'Laser-2').

In the Reston Stable Isotope Laboratory (RSIL) at the U.S. Geological Survey, for δ^2 H measurements by mass spectrometry, a dual-inlet VG Micromass 602 hydrogen isotope-ratio mass spectrometer was used (Révész and Coplen, 2008a). For δ^{18} O measurements of water by mass spectrometry, a DuPont double-focusing carbon dioxide dual-inlet isotope-ratio mass spectrometer was used (Révész and Coplen, 2008b).

Also in the RSIL, δ^2 H and δ^{18} O measurements were performed with a 2014 Los Gatos Research (LGR) Triple Isotope Water Analyzer (model number TIWA-45-EP), denoted as 'Laser-1'.

Determination of δ^2 H and δ^{18} O was also done by Cavity Ring-Down Spectroscopy (CRDS) at the water isotope laboratory at the Geological Survey of Denmark and Greenland, using a Picarro L2120-i, referred to as 'Laser-2'. The quantification was made using two internal standards (one depleted in ²H and ¹⁸O and one enriched in ²H and ¹⁸O) and a control between these, covering the range of samples normally encountered in the Danish rain and groundwater samples measured in the lab. The isotope values of the internal standards had been determined from International Atomic Energy Agency (IAEA) supplied standards, covering a range of water isotope values that can be encountered in natural systems. The internal standard depleted in ²H and ¹⁸O was made by filtering meltwater produced by deicing standard –20 °C freezers in the lab and the standard enriched in ²H and ¹⁸O was produced

by boiling MilliQ water. CRDS implies that instead of measuring absorption directly, the absorption is measured by the time it takes for the signal coming from a reflecting cavity to decay. The sample is introduced into the cavity as vapor, produced by a vaporizer from $\sim 2 \mu L$ of water. Normally, six injections of a sample or a standard are made, and the absorption is determined as an average of ~ 60 laser pulse decays. The results of the first two injections are discarded as they are affected by the previous sample (memory), and an average is taken of the remaining four, unless parameters determined for the measurement sequence (such as SD or slopes of the \sim 60 pulse decays) indicate a bad measurement, which is then discarded. If the spread in the value obtained from the remaining good measurements is >0.2 ‰ for $\delta^{18}\mathrm{O}$ and >1.0 % for δ^2 H, the sample was reanalyzed. During a run, the control and the two internal standards are measured at the start and after every five samples, and the values for the five samples are calculated using the average of the instrument response to the standards before and after the five samples.

2.2.2. DOC fluorescence and absorbance characteristics and concentration

Fluorescence and absorbance properties of water samples were analyzed within one month. The samples were filtered again (0.7 μ m nominal pore size Whatman GF/F filters) before measurements were performed in a 10-mm quartz cuvette using a fluorescence spectrometer (Aqualog UV-800-C, Horiba, Japan). Absorbance was measured between 240-800 nm with 5-nm intervals. Fluorescence was characterized by determining excitation-emission matrices (EEM), measured by excitation wavelengths of 240-800 nm with 5-nm increments and emission wavelengths of 247.81-830.20 nm with 4.66 nm increments. Samples were diluted using Milli-Q water if necessary. The DOC concentration was determined using a total organic carbon analyzer (Shimadzu, Japan), in accordance with Kragh & Søndergaard (2004).

2.3. Data processing and statistical analyses

The deviation of the δ^2 H and δ^{18} O results from the two LAS measurements of the 72 water samples were calculated by subtracting from the DI-IRMS-obtained values the corresponding LAS determined value. Thus, a negative difference indicates that the LAS measurement overestimated (more enriched) the δ -value, while a positive difference indicates an underestimated (more depleted) δ -value. The difference between the two values, or the deviation from the presumed 'true' DI-IRMS measurement, is represented by 'Diff. δ^2 H' and 'Diff. δ^{18} O'. We conducted a Kolmogorov-Smirnov test in R (R Core Team, 2024) to test if the deviations between the DI-IRMS determinations and the results from the two LAS analyses followed a normal distribution. A t-test was conducted for parameters that followed a normal distribution, followed by a Pearson Correlation analysis. A Wilcoxon Signed-Rank test was instead performed for the parameter with a non-normal distribution, together with Spearman's rank correlation. Analysis of variance (ANOVA) followed by post-hoc Tukey tests were as well performed in R.

Absorbance and fluorescence data were analyzed using the 'eemR' (Massicotte, 2022) and 'staRdom' (Pucher et al., 2019) R packages. For absorbance data, we performed a baseline correction using wavelengths between 680 and 700 nm and determined seven absorbance characteristics together with one additional index, SUVA₂₅₄ (quotient of the absorbance at 254 nm and the DOC concentration), using DOC measurements (Table S1; Helms et al., 2008). EEMs were post-processed by removing Rayleigh and Raman scattering, corrected for inner-filter effects, normalized to Raman units and finally interpolated. From the processed EEMs, we determined eight fluorescence characteristics (Table S1).

The principal component analysis (PCA) and hierarchical cluster analysis and visualizations were performed using Python packages 'seaborn' (Waskom, 2021), 'sklearn' (Pedregosa et al., 2011), and 'scipy' (Virtanen et al., 2020), in Python version 3.8 (Van Rossum and Drake, 2003). To provide the best statistical basis for the analysis of DOC

Journal of Hydrology 660 (2025) 133245

concentration and characteristics *vs.* water sample origin (i.e. hydrological compartment), the PCA of the absorbance and fluorescence characteristics, and the hierarchical cluster analysis were performed using all 267 samples. Fig. 4a accordingly makes use of 267 samples. The derived clustering for the 72 samples that were also measured for isotopes is shown in Fig. 4b-e and Fig. 5c-d.

3. Results

3.1. Deviations of stable water isotopes and hydrological compartment

Precipitation in Denmark ranges seasonally between -11 % to -2 % for δ^{18} O, with an average precipitation-amount weighted δ^{18} O value around -8 % (Müller et al., 2017). The corresponding value for δ^2 H is around -53 % (Müller et al., 2017). Fig. 2a shows the observed isotopic compositions of the samples of groundwater and stream water, which correspond well to this precipitation input.

Fig. 2b-e show histograms of the differences between δ^2 H and δ^{18} O values measured by DI-IRMS vs. LAS. Values for Diff. δ^2 H and Diff. δ^{18} O for Laser-1 are slightly lower (more enriched; Fig. 2b and Fig. 2d), compared to the those of Laser-2 (Fig. 2c and Fig. 2e). This could be due to differences between in-house standards used or the operating principles of Laser-1 and Laser-2. The mean deviation between the DI-IRMS and LAS δ^2 H determinations was -0.35 ‰ (std. dev. 0.92 ‰) for

instrument Laser-1 (Fig. 2b), and +0.31 % (std. dev. 1.04 %) for Laser-2 (Fig. 2c). A *t*-test showed that the deviations were significant for both instruments (p = 0.002 for Laser-1, p = 0.01 for Laser-2). For δ^{18} O, the mean deviation was +0.03 % (std. dev. 0.20 %) for Laser-1 (Fig. 2d) and +0.15 % (std. dev. 0.20 %) for Laser-2 (Fig. 2e), but not significant for Laser-1 (p > 0.05). The measurements from Laser-2 displayed a non-normal distribution for δ^{18} O (Kolmogorov-Smirnov test, p = 0.006), but the deviation was significant according to a Wilcoxon Signed-Rank test (p < 0.001). Thus, henceforth, the following analyses will only include the measured δ^2 H of Laser-1 and Laser-2, and δ^{18} O values determined on Laser-2, while excluding the δ^{18} O values from Laser-1 from further analysis, as the deviation from the DI-IRMS determinations was not significant.

The two major hydrological compartments represented in Fig. 2 are related to wetland history and they comprise shallow groundwater of restored (R, red color coding) and near-natural (NN, blue) wetlands. As may be indicated by inspection of the stacked histograms of Fig. 2b-e, the observed differences between DI-IRMS and LAS measured δ -values did not differ markedly among these two compartments. Differences in Diff. δ -values of the hydrological compartments containing (the deeper) regional groundwater (RGW, beige) and stream water (SW, green) could neither be identified. Diff. δ^2 H of Laser-1 for stream water (Fig. 2b) may appear as an exception as the values appear to be more negative (more enriched) for δ^2 H, compared to the bulk of samples from other



Fig. 2. The results of the 72 stable water isotope samples. (a) Represents the 'true' values, from the DI-IRMS determination. The black solid line represents the Global Meteoric Water Line, and the grey solid line represents a Local Meteoric Water Line developed by Müller et al. (2017). (b-c) The deviations of the δ^{2} H measurements using LAS. (d-e) The deviations of the δ^{18} O measurements using LAS. Abbreviations: Restored (R), near-natural (NN), regional groundwater (RGW), stream water (SW), Omme stream catchment (OD), Tryggevælde stream catchment (TR). Data available in Fredriksson et al. (2025).

hydrological compartments. However, we could not identify a significant variation in Diff. δ^2 H values for either instrument, nor for δ^{18} O values measured on Laser-2, amongst the four hydrological compartments (ANOVA, p > 0.05).

3.2. Interference by concentration of DOC

Interference of DOC on laser-based isotope measurements would be most straightforwardly assessed in a plot of DOC concentration *vs.* Diff. δ -value. However, as the plots in Fig. 3 indicate, we could not identify a strong correlation between Diff. δ^{2} H of Laser-1 or Laser-2 (Fig. 3a), or Diff. δ^{18} O of Laser-2 (Fig. 3b) and the DOC concentration ($r < \pm 0.6$), when including all stable water isotope samples (n = 72). Likewise, no significant correlation (p > 0.05) was found when dividing the dataset based on hydrological compartment (i.e., shallow groundwater from restored or near-natural sites, regional groundwater, and stream water). The following sections therefore characterize DOC based on fluorescence and absorbance properties (Section 3.3), and then assess correlations between the derived DOC characteristics and the δ -value deviations (Section 3.4).

3.3. DOC characterization

In the principal component analysis (PCA) based on DOC fluorescence and absorbance properties (Fig. 4a), the first principal component (PC1) explained 41.3 % of the total variance, showing mainly high loadings of Peak T and DOC concentration. PC2 accounted for 20.25 % of the total variance, with high loadings of SR and HIX. PC3 accounted for 18.46 % of the total variance, and PC4 for 6.7 %. Previous work within our group (Liao, 2023), concluded that the pore waters mainly contained terrestrial humic-like substances. The factorial map in Fig. 4a displays three clusters (A, B, C) based on the two first components. A dendrogram displaying the water samples according to distance is provided in Fig. S1. Fig. 4b shows that most samples fell within cluster C, which is dominated by shallow groundwater samples from near-natural sites. Samples from restored sites were found in all three clusters, regional groundwater samples were found in clusters B and C, while stream water samples were only present in cluster C (Fig. 4b). The spatial origin (TR, OD, OM) of the samples, shown in Fig. 4c, does not generally show a clear division amongst the three clusters, although cluster B only contained samples from the sand-dominated catchment OM. OM-samples were also present in cluster C, but not in cluster A.

The DOC concentrations by the restored sites varied greatly, from 2.4 up to 148.1 mg L^{-1} (median: 22.3 mg L^{-1} , std. dev. 36.4 mg L^{-1} ; Fig. 4d). In contrast, the near-natural sites mostly displayed lower and less variable DOC concentrations with a median of 5.2 mg L^{-1} , but a

maximum of 112.2 mg L⁻¹ (std. dev. 24.3 mg L⁻¹). The deeper, regional groundwater (median: 6.4 mg L⁻¹, std. dev. 7.6 mg L⁻¹) and stream water (median: 6.7 mg L⁻¹, std. dev. 3.7 mg L⁻¹) showed the lowest DOC concentrations. Cluster A contained the most samples with higher DOC concentration (Fig. 4e), while samples in clusters B and C contained lower amounts of DOC.

Fig. 4b-e include only the 72 samples that were subjected to both DOC characterization and stable isotope analysis of water, to secure transparency regarding conclusions on DOC's interference on laserbased isotope measurements. However, the diagrams in Fig. 4b-e appeared nearly identical when drawn for all 267 samples used for DOC characterization, as shown in Fig. S2. For example, the distribution of hydrological compartments is very similar (Fig. S2b), and except for one regional groundwater sample from the TR catchment, cluster B still only contained samples from the sandy OM catchment (Fig. S2c). Also, most DOC-rich samples came from restored sites (Fig. S2d), which mainly plotted in cluster A (Fig. S2e). Accordingly, the analysis of DOC interference does not appear to be biased by the lower number of samples.

3.4. Identifying relationships between DOC characteristics and LAS

Across all samples (n = 72), there was no strong correlation between Diff. δ^2 H of Laser-1 or Laser-2, or Diff. δ^{18} O of Laser-2, vs. the directly observed DOC absorbance and fluorescence indices ($r < \pm 0.6$). However, for shallow groundwater samples from restored sites only (n = 24), we observed a strong positive correlation (r = 0.65, p < 0.001) for Laser-2 between Diff. δ^2 H and the derived SUVA₂₅₄ index (specific UV absorbance, $L mg^{-1} m^{-1}$), and a moderate positive correlation for Laser-1 (r = 0.59, p = 0.002; Fig. 5a). We could not identify the same relationship for Diff. δ^{18} O of Laser-2 (r < 0.6). As presented in Fig. 5b, the restored sites contained the highest measured SUVA₂₅₄ values. We performed an analysis of variance, which yielded a significant variation in SUVA₂₅₄ amongst cluster groups (ANOVA, p < 0.001). A post-hoc Tukey test showed that cluster B, which contained samples with the highest SUVA₂₅₄ index, displayed a significant difference from cluster A and cluster C (p = 0.02, p < 0.001, respectively), with no significant difference between the latter two clusters (Fig. 5c).

Further, we conducted the same analysis for Diff. δ^2 H amongst cluster groups for both instruments (ANOVA, p < 0.001 for Laser-1, p = 0.002 for Laser-2), which showed that Diff. δ^2 H values in cluster B were significantly different from the values in both cluster A (p = 0.01 and p = 0.007 for Laser-1 and Laser-2, respectively) and cluster C (p = 0.001, p = 0.004, respectively). Cluster B contained most of the positively deviating samples, i.e. samples where the LAS determined δ^2 H-values were more depleted than the corresponding DI-IRMS measurements (Fig. 5d). There was no significant difference in Diff. δ^2 H between



Fig. 3. No correlation was found between the DOC concentration and the determined (a) Diff. $\delta^2 H$ or (b) Diff. $\delta^{18}O$ values. The grey dashed line indicates 0 ‰ deviation from the DI-IRMS determinations. Data available in Fredriksson et al. (2025).



Fig. 4. (a) Distribution of DOC samples within the principal component and hierarchical cluster analyses (n = 267). (b-e) Distribution of hydrological compartments (b), catchment origin (c), DOC concentration (d) and cluster division (e) for water samples analyzed for stable hydrogen and oxygen isotopes (n = 72). Abbreviations: Restored (R), near-natural (NN), regional groundwater (RGW), stream water (SW), samples included in the PCA, but not in the isotope dataset (Other), Omme stream catchment (OM), Odense stream catchment (OD), Tryggevælde stream catchment (TR). See Table S1 for description of absorbance and fluorescence indices. Data available in Fredriksson et al. (2025).



Fig. 5. (a) With increased SUVA₂₅₄ index, Diff. δ^2 H becomes more positive on both instruments, for shallow groundwater samples from restored sites. (b) Samples from restored shallow groundwater show the greatest SUVA₂₅₄ index. (c) Samples within cluster B display an elevated SUVA₂₅₄ index. (d) Samples within cluster B display more positive Diff. δ^2 H values. Grey dashed line indicates 0 ‰ deviation from the DI-IRMS measurements. Abbreviations: Restored (R), near-natural (NN), regional groundwater (RGW), stream water (SW). Data available in Fredriksson et al. (2025).

clusters A and C (p > 0.05). As previously noted in Fig. 2, the means of Diff. δ^2 H for Laser-1 are slightly lower (more enriched) compared to the mean values of Laser-2 (Fig. 5d), across the three clusters.

4. Discussion

The significant differences between DI-IRMS and LAS measured δ -values did not appear to be controlled by the particular hydrological compartment from which the samples originated (Fig. 2b-e), nor by DOC

concentration (Fig. 3). Therefore, one must look for other controlling factors for the observed Diff. δ -values. The results indicate that the DOC characteristics, specifically increased SUVA₂₅₄ index, may have an effect on the δ^2 H measurements from both LAS instruments. At a given DOC concentration, the SUVA₂₅₄ index increases with increasing 254 nm-absorption. Higher SUVA₂₅₄ index indicates increased aromaticity and molecular weight of the DOC (Weishaar et al., 2003), making it a useful proxy for general chemical properties in wetlands.

Most of the positive Diff. δ^2 H-values (i.e., more depleted LAS determined δ^2 H-values relative to the assumed 'true' value determined by DI-IRMS) from shallow groundwater samples from restored sites (Fig. 5a), as well as samples in cluster B consisting of shallow and deep groundwater samples that all originated from the sandy OM catchment (Fig. 5d), coincided with increased SUVA₂₅₄ index. Combining the results shown in Fig. 4b-e and Fig. 5b-d altogether, indicates that more positive Diff. δ^2 H-values are observed in restored wetlands of the sandy OM catchment where the DOC concentrations are below roughly 30 mg L⁻¹, which is relatively low compared to the frequently much higher DOC concentration observed in restored wetlands (Fig. 4d). Accordingly, when the DOC concentrations were in the relatively low range for restored wetlands, the DOC had relatively high aromaticity.

In restored wetlands, oxic conditions prompted by drainage as part of the previous agricultural use, promotes degradation of SOM resulting in elevated DOC production (Holden et al., 2004). Further, SUVA₂₅₄ values have been observed to increase after drainage, when more labile (more easily biodegraded) DOC of lower molecular weight is removed, leading to increased aromaticity of the DOC (Xu et al., 2021). Prior investigations of the same studied field sites by Kiær et al. (2024), revealed that the restored sites experienced both lower annual mean water level and higher water level amplitude, compared to their near-natural counterparts. Thus, periods of dryer conditions were still ongoing in the restored wetlands post-restoration, potentially facilitating further DOC production and subsequent increase of DOC aromaticity. Consequently, possible variations in DOC fluorescence and absorbance characteristics and concentration amongst field sites, should be considered, as they seem to affect the deviation from the DI-IRMS-determined δ^2 Hvalues.

Our study found that Diff. δ^2 H determined on the two LAS instruments ranged from -2.4 ‰ to +4.9 ‰ (Fig. 2b-c), which could partially be attributed to interference by DOC with high SUVA₂₅₄ index, in shallow groundwater from restored wetlands. Many studies make use of larger δ^2 H-variations in isotopic signature in order to distinguish different sources of water for interpretation of the hydrological system. However, studies where the analyzed pore water contains DOC of high aromaticity, and in which hydrological conclusions are based on interpretations that make use of variations in isotopic signature within the observed range for Diff. δ^2 H, may be severely affected. As above-described, measurements of DOC concentration alone did not provide an indication of DOC interference (Fig. 3). Therefore, to quantify the effect of DOC interference in a given hydrogeological system, DOC characteristics and in particular the SUVA₂₅₄ index, must be evaluated. Increasing the number of samples will not improve the usability of the δ^2 H-values, if all pore water samples' DOC characteristics (increased aromaticity) cause interference with the LAS determinations.

Fig. 6 displays the data of Fig. 5a, but where Diff. δ^2 H instead is a function of the SUVA₂₅₄ index, with linear regression models superimposed. In Fig. 6a, the regression model for Laser-1 suggests that δ^2 Hvalues of shallow pore water in restored sites need to be corrected for SUVA₂₅₄ by Diff. δ^2 H (‰) = 0.050 × SUVA₂₅₄ – 0.97 (with SUVA₂₅₄ in units of L mg⁻¹ m⁻¹). The corresponding correction for SUVA₂₅₄ for δ^2 Hvalues measured with Laser-2 is Diff. δ^2 H (‰) = 0.075 × SUVA_{254} -0.59 (Fig. 6b). This relation between SUVA₂₅₄ and Diff. δ^2 H may have important implications. For example, the regression model in Fig. 6b, indicates that water samples with a δ^2 H-value of -45 ‰, as determined by Laser-2, and a SUVA₂₅₄ index of 40 L mg⁻¹ m⁻¹, would have a true δ^2 H-value of -42.6 ‰, i.e., some Diff. δ^2 H = +2.4 ‰ higher than the LAS-determined value. If the same water samples had a true *d*-excess = $\delta^2 H - 8 \times \delta^{18} O$ of 10 ‰ and a corresponding true $\delta^{18} O$ -value of -6.58 %, then the *d*-excess value calculated using the LAS-determined δ^2 Hvalue would equal $-45 - 8 \times (-6.58) = 7.6$ ‰. In a common dual isotope plot, these samples may therefore appear slightly evaporated. In conclusion, when pore water DOC is not characterized and the SUVA₂₅₄ index is unknown and cannot be corrected for, the interference may thus be significant for the hydrological interpretation.

5. Conclusions

- The present study investigated the DOC concentrations and fluorescence and absorbance characteristics of groundwater and stream water of 12 temperate riparian wetland sites, and tested for potential interference in laser-based stable isotope (δ^2 H and δ^{18} O) analysis of water.
- DOC with high SUVA₂₅₄ index appeared to partially explain the observed significant deviations from the DI-IRMS-determined δ^2 H-values, in shallow groundwater samples from restored wetlands, calling for further research.



Fig. 6. The impact of increasing SUVA₂₅₄ index on Diff. δ^2 H for shallow groundwater samples from restored wetlands, for (a) Laser-1 and (b) Laser-2. Data available in Fredriksson et al. (2025).

- The DOC interference was most prominent amongst samples from restored wetlands in a sandy catchment, with up to roughly 30 mg DOC L⁻¹.
- Studies that analyze pore waters with high DOC aromaticity and require a high level of accuracy of the δ^2 H-determinations for interpretation of hydrological systems, may be severely affected by the potential DOC interference.

CRediT authorship contribution statement

Filippa Fredriksson: Writing – review & editing, Writing – original draft, Visualization, Software, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. Kenneth Thorø Martinsen: Writing – review & editing, Writing – original draft, Software, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. Kai-Ying Liao: Writing - review & editing, Writing - original draft, Software, Methodology, Investigation, Formal analysis, Data curation. Tyler B. Coplen: Writing - review & editing, Writing - original draft, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. Rasmus Jakobsen: Writing - review & editing, Writing - original draft, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. Okke Batelaan: Writing - review & editing, Writing - original draft, Supervision, Formal analysis. Søren Jessen: Writing review & editing, Writing - original draft, Supervision, Project administration. Methodology, Investigation, Formal analysis, Conceptualization.

Declaration of competing interest

Chair of Editors-in-Chief Journal of Hydrology - O.B. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was part of the project *Supporting climate and biodiversity by rewetting low-lying areas*, and has received funding from the Independent Research Fund Denmark (Danmarks Frie Forskningsfond), grant number 0217-00112B.

Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhydrol.2025.133245.

Data availability

Data for this study are publicly available (Fredriksson et al., 2025).

References

- Bam, E.K., Ireson, A.M., 2019. Quantifying the wetland water balance: A new isotopebased approach that includes precipitation and infiltration. J. Hydrol., 570, 185–200. https://doi.org/10.1016/j.jhydrol.2018.12.032.
- Bugna, G.C., Grace, J.M., Hsieh, Y., 2020. Sensitivity of using stable water isotopic tracers to study the hydrology of isolated wetlands in North Florida. J. Hydrol., 580, 124321. https://doi.org/10.1016/j.jhydrol.2019.124321.
- Cooper, D.J., Sueltenfuss, J., Oyague, E., Yager, K., Slayback, D., Caballero, E.M., Argollo, J., Mark, B.G., 2019. Drivers of Peatland Water Table Dynamics in the Central Andes. Bolivia and Peru. Hydrol. Process., 33 (13), 1913–1925. https://doi. org/10.1002/hyp.13446.
- Fredriksson, F., Martinsen, K.T., Liao, K.Y., Coplen, T.B., Jakobsen, R., Batelaan, O., Jessen, S., 2025. Measurements of concentration, fluorescence, and absorbance characteristics of DOC and stable hydrogen and oxygen isotope analysis of pore

water from riparian wetlands in Denmark from September 2022 to October 2023: U. S. Geological Survey data release, https://doi.org/10.5066/P1YLUZ4V.

- Helms, J.R., Stubbins, A., Ritchie, J.D., Minor, E.C., Kieber, D.J., Mopper, K., 2008. Absorption spectral slopes and slope ratios as indicators of molecular weight, source, and photobleaching of chromophoric dissolved organic matter. L&O 53 (3), 955–969. https://doi.org/10.4319/lo.2008.53.3.0955.
- Hissler, C., Klaus, J., Barnich, F., Guignard, C., Louis, L., Zuecco, G., Angeli, N. 10-12 September 2024. Analyzing O and H water isotopes in organic enriched solutions [Abstract]. WATSON Final Action Online Conference. https://watson-cost.eu/wpcontent/uploads/2024/09/WATSON-FOAC-Book-of-Abstracts_finalv1.pdf.
- Holden, J., Chapman, P.J., Labadz, J.C., 2004. Artificial drainage of peatlands: Hydrological and hydrochemical process and wetland restoration. PPG: Earth and Environ. 28 (1), 95–123. https://doi.org/10.1191/0309133304pp403ra.
- Hong, Z., Ding, S., Zhao, Q., Geng, Z., Qiu, P., Zhang, J., Wang, A., Zhang, P., 2022. Relative contribution of multi-source water recharge to riparian wetlands along the lower Yellow River. JEM 321, 115804. https://doi.org/10.1016/j. ienyman.2022.115804.
- Isokangas, E., Rossi, P.M., Ronkanen, A., Marttila, H., Rozanski, K., Kløve, B., 2017. Quantifying spatial groundwater dependence in peatlands through a distributed isotope mass balance approach. Water Resour. Res., 53 (3), 2524–2541. https://doi. org/10.1002/2016wr019661.
- Kjær, J.E., Baumane, M., Fredriksson, F., Jessen, S., Thorø Martinsen, K., Skjærlund, T., Krogh Norrild, A., Rath Hansen, M.E., Sand-Jensen, K., Bruun, H.H., Baastrup-Spohr, L., 2024. Effects of riparian wetland restoration and grazing on CH₄ and CO₂ exchange more than a decade after rewetting. Manuscript submitted.
- Kragh, T., Søndergaard, M., 2004. Production and bioavailability of autochthonous dissolved organic carbon: Effects of mesozooplankton. AME 36 (1), 61–72. https:// doi.org/10.3354/ame036061.
- Kristensen, E., Madsen-Østerbye, M., Massicotte, P., Pedersen, O., Markager, S., Kragh, T., 2018. Catchment tracers reveal discharge, recharge and sources of groundwater-borne pollutants in a novel lake modelling approach. BG 15, 1203–1216. https://doi.org/10.5194/bg-15-1203-2018.
- Kværner, J., Kløve, B., 2008. Generation and regulation of summer runoff in a Boreal Flat fen. J. Hydrol., 360 (1–4), 15–30. https://doi.org/10.1016/j.jhydrol.2008.07.009.
- Leifeld, J., Menichetti, L., 2018. The underappreciated potential of peatlands in global climate change mitigation strategies. Nat. Commun., 9. https://doi.org/10.1038/ s41467-018-03406-6.
- Liao, K.Y., 2023. Characteristics of groundwater dissolved organic carbon in wetlands with different management strategies in Denmark. Master thesis. University of Copenhagen.
- Marttila, H., Aurela, M., Büngener, L., Rossi, P.M., Lohila, A., Postila, H., Saari, M., Penttilä, T., Kløve, B., 2021. Quantifying groundwater fluxes from an AAPA mire to a riverside esker formation. Hydrol. Res., 52 (2), 585–596. https://doi.org/10.2166/ nh.2021.064.
- Massicotte, P., 2022. Tools for Pre-Processing Emission-Excitation-Matrix (EEM) Fluorescence Data. R Package Version 1.0.1. https://CRAN.R-project.org/ package=eemR.
- Mattei, A., Huneau, F., Garel, E., Sorba, L., Orsini, S., Santoni, S., 2022. Isotope hydrology to provide insights into the behaviour of temporary wetlands as a basis for developing sustainable ecohydrological management strategies in Mediterranean regions. Ecohydrol., 15 (5). https://doi.org/10.1002/eco.2411.

Mitsch, W., Gosselink, J., 2015. Wetlands, (5th edition). Wiley & Sons Inc.

Müller, S., Stumpp, C., Sørensen, J.H., Jessen, S., 2017. Spatiotemporal variation of stable isotopic composition in precipitation: Post-condensational effects in a humid area. Hydrol. Process., 31 (18), 3146–3159. https://doi.org/10.1002/hyp.11186.

- Pedregosa, F., Varoquaux, G., Gramfort, A., Michel, V., Thirion, B., Grisel, O., Blondel, M., Prettenhofer, P., Weiss, R., Dubourg, V., Vanderplas, J., Passos, A., Cournapeau, D., Brucher, M., Perrot, M., Duchesnay, E., Louppe, G., 2011. Scikitlearn: Machine Learning in Python. JMLR 12, 2825–2830.
- Penna, D., Stenni, B., Šanda, M., Wrede, S., Bogaard, T.A., Gobbi, A., Borga, M., Fischer, B.M., Bonazza, M., Chárová, Z., 2010. On the reproducibility and repeatability of laser absorption spectroscopy measurements for δ²H and δ¹⁸O isotopic analysis. HESS 14 (8), 1551–1566. https://doi.org/10.5194/hess-14-1551-2010.
- Pucher, M., Wünsch, U., Weigelhofer, G., Murphy, K., Hein, T., Graeber, D., 2019. staRdom: Versatile Software for Analyzing Spectroscopic Data of Dissolved Organic Matter in R. Water 11 (11), 2366. https://doi.org/10.3390/w11112366.
- Rautio, A., Korkka-Niemi, K., 2015. Chemical and isotopic tracers indicating groundwater/surface-water interaction within a Boreal Lake catchment in Finland. Hydrogeol. J., 23 (4), 687–705. https://doi.org/10.1007/s10040-015-1234-5.
- R Core Team, 2024. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria https://www.R-project.or
- Révész, K., Coplen, T.B., 2008a. Determination of the ô(2H/1H) of water: RSIL lab code 1574, chap. C1 of Révész, K., & Coplen, T.B., eds., Methods of the Reston Stable Isotope Laboratory: U.S. Geological Survey Techniques and Methods 10–C1, 27 p. http://pubs.usgs.gov/tm/2007/tm10c1/.
- Révész, K., Coplen, T.B., 2008b. Determination of the 8(180/160) of water: RSIL lab code 489, in Révész, K., & Coplen, T.B., eds., Methods of the Reston Stable Isotope Laboratory: U.S. Geological Survey Techniques and Methods, book 10, chap. C2, 28 p. http://pubs.usgs.gov/tm/2007/tm10c2/.
- Rosset, T., Binet, S., Antoine, J.-M., Lerigoleur, E., Rigal, F., Gandois, L., 2020. Drivers of seasonal- and event-scale Doc Dynamics at the outlet of mountainous peatlands revealed by high-frequency monitoring. BG 17 (13), 3705–3722. https://doi.org/ 10.5194/bg-17-3705-2020.
- Sankar, M.S., Dash, P., Lu, Y., Mercer, A.E., Turnage, G., Shoemaker, C.M., Chen, S., Moorhead, R.J., 2020. Land use and land cover control on the spatial variation of

dissolved organic matter across 41 lakes in Mississippi. USA. Hydrobiolog., 847 (4), 1159–1176. https://doi.org/10.1007/s10750-019-04174-0.

- Singleton, G., Coplen, T., Qi, H., Lorenz, J.M., 2009. Laser-based stable hydrogen and oxygen analyses: How reliable can measurement results be? [Abstract]. EGU General assembly 2009. Geophysical Research Abstracts 11, EGU2009-3290. https://meeting organizer.copernicus.org/EGU2009/EGU2009-3290.pdf.
- Skrzypek, G., Ford, D., 2014. Stable isotope analysis of saline water samples on a cavity ring-down spectroscopy instrument. Environ. Sci. Technol., 48 (5), 2827–2834. https://doi.org/10.1021/es4049412.
- Stedmon, C.A., Markager, S., 2005. Resolving the variability in dissolved organic matter fluorescence in a temperate estuary and its catchment using PARAFAC analysis. L&O 50 (2), 686–697. https://doi.org/10.4319/10.2005.50.2.0686.
- Stedmon, C.A., Markager, S., Bro, R., 2003. Tracing dissolved organic matter in aquatic environments using a new approach to fluorescence spectroscopy. Mar. Chem., 82 (3–4), 239–254. https://doi.org/10.1016/s0304-4203(03)00072-0.

Sørensen, V.R.M., 2024. Effects of long-term drainage on wetlands. Master thesis. University of Copenhagen.

- Tiemeyer, B., Freibauer, A., Borraz, E.A., Augustin, J., Bechtold, M., Beetz, S., Beyer, C., Ebli, M., Eickenscheidt, T., Fiedler, S., Förster, C., Gensior, A., Giebels, M., Glatzel, S., Heinichen, J., Hoffmann, M., Höper, H., Jurasinski, G., Laggner, A., Drösler, M., 2020. A new methodology for organic soils in national greenhouse gas inventories: Data synthesis, derivation and application. Ecol. Indic., 109, 105838. https://doi.org/10.1016/j.ecolind.2019.105838.
- Van Rossum, G., Drake, F.L., 2003. The Python Language Reference Manual. Network Theory Ltd, Massachusetts.
- Virtanen, P., Gommers, R., Oliphant, T.E., Haberland, M., Reddy, T., Cournapeau, D., Burovski, E., Peterson, P., Weckesser, W., Bright, J., van der Walt, Brett, M.,

Wilson, J., Millman, K.J., Mayorov, N., Nelson, A.R., Jones, E., Kern, R., Larson, E., Carey, C.J., Polat, İ., Feng, Y., Moore, E.W., VanderPlas, J., Laxalde, D., Perktold, J., Cimrman, R., Henriksen, I., Quintero, E.A., Harris, C.R., Archibald, A.M., Ribeiro, A. H., Pedregosa, F., van Mulbregt, P., SciPy, 1.0 Contributors, 2020. SciPy 1.0: Fundamental algorithms for scientific computing in python. Nat. Methods 17 (3), 261–272. https://doi.org/10.1038/s41592-019-0686-2.

- Waskom, M.L., 2021. seaborn: statistical data visualization. JOSS 6 (60), 3021. https:// doi.org/10.21105/joss.03021.
- 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. https://doi.org/10.1021/es030360x.
- Xu, J., Morris, P.J., Liu, J., Holden, J., 2018. PEATMAP: Refining estimates of global peatland distribution based on a meta-analysis. CATENA 160, 134–140. https://doi. org/10.1016/j.catena.2017.09.010.
- Xu, X., Lu, K., Wang, Z., Wang, M., Wang, S., 2021. Effects of drainage on dissolved organic carbon (DOC) characteristics of surface water from a mountain peatland. Sci. Total Environ., 789, 147848. https://doi.org/10.1016/j.scitotenv.2021.147848.
- Yu, Z., Loisel, J., Brosseau, D.P., Beilman, D.W., Hunt, S.J., 2010. Global peatland dynamics since the Last Glacial Maximum. GLR 37 (13). https://doi.org/10.1029/ 2010gl043584.
- Zhang, Y., Huang, X., Zhang, Z., Blewett, J., Naafs, B.D.A., 2022. Spatiotemporal dynamics of dissolved organic carbon in a subtropical wetland and their implications for methane emissions. Geoderma 419, 115876. https://doi.org/10.1016/j. geoderma.2022.115876.