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Catchment Influence on the Seasonal Dynamics of Dissolved Organic Matter (DOM) Optical Properties in Small Lakes

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Abstract

Dissolved organic matter (DOM) plays an important role in biogeochemical cycling and water quality in lake ecosystems, and its composition is strongly influenced by catchment characteristics and anthropogenic pressure. Understanding seasonal variability in DOM is therefore important for assessing ecological processes in small lakes. The aim was to assess the optical properties and seasonal variability of DOM in small (<50 ha) lakes differing in morphometry and catchment characteristics. Samples were collected in spring, summer and autumn. The quality of the DOM was assessed using UV–Vis spectroscopy and synchronous scan fluorescence spectra (SSF), in addition to measurements of nutrients and basic physicochemical parameters. UV–Vis indices ($SUVA_{270}$, a^*_{355} and E_{250}:E_{365}) revealed a steady increase in aromaticity, chromophore content and degree of DOM humification throughout the season. In landscape lakes, fluorescence parameters followed a typical seasonal pattern: shifting from protein-like fractions in the spring to fulvic acid-like fractions in the summer and autumn. A different pattern of optical changes was observed in a lake subject to anthropogenic pressure, where a marked increase in protein-like fluorescence was recorded in the autumn, indicating an influx of fresh, reactive organic matter (DOM). These results demonstrate that optical properties of DOM are sensitive indicators of seasonal variability and differences among lakes with contrasting environmental characteristics. The combined optical approach provides useful information on changes in DOM quality and composition and may support the monitoring of ecological processes in small lake ecosystems.

Keywords: small lakes; UV-Vis absorbance; synchronous scan fluorescence spectra; humification; seasonal variability; catchment influence

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1. Introduction

Northern Europe is characterised by a large number of lakes with diverse hydromorphological and physical and chemical conditions resulting from Weichselian glaciation [1]. Poland has more than 7000 natural lakes larger than 1 ha [2], which are important for conserving water resources, maintaining biodiversity, and supporting the national economy [3]. However, in recent decades, marked changes in their physical, chemical and hydrological conditions have been observed, driven by increasing anthropogenic pressure and climate change. This has consequences for aquatic organisms [4–6]. These processes

have environmental and economic implications, including the deterioration of water quality and the decline of water resources [7].

Accelerated eutrophication, which is linked to rising nitrogen and phosphorus concentrations, is considered one of the major threats to lake water quality in Poland. Dissolved organic matter (DOM) is closely connected to the cycling of these elements and is a vital part of the conversion of matter and energy within aquatic ecosystems [8]. In inland waters, DOM is the dominant form of organic carbon, and in lakes it mainly originates from terrestrial sources, influencing food-web functioning [9–11]. Substantial amounts of oxygen are consumed and nutrients are released during DOM degradation, which affects water quality [12,13]. As DOM primarily enters lakes from their catchments, land use significantly impacts DOM quantity, nutrient concentrations, and trophic status [9]. Small lakes located in agricultural catchments are particularly vulnerable to these pressures. However, most studies focus on lakes larger than 50 hectares [8,12,14], despite the fact that more than 85% of Polish lakes are smaller [2]. Effective protection of these ecosystems requires comprehensive monitoring of trophic status and biogeochemical processes.

DOM is a complex mixture of compounds originating from both terrestrial and aquatic sources. In freshwater ecosystems, DOM undergoes continuous biotic and abiotic transformations, including microbial and photochemical degradation, sedimentation, and incorporation into higher trophic levels [15]. Natural DOM sources include autochthonous production by phytoplankton, bacteria, microorganisms, and zooplankton, as well as allochthonous inputs of terrestrial organic matter transported from the catchment through surface runoff and atmospheric deposition. In addition, anthropogenic activities may contribute substantial amounts of DOM to aquatic ecosystems. Lakes located near urban areas may receive DOM from municipal wastewater, urban stormwater runoff, and industrial discharges, whereas lakes situated farther from urban centres are generally influenced predominantly by natural terrestrial inputs, although agricultural land use may also contribute to DOM loading. In flow-through lakes, watercourses may additionally transport DOM and associated contaminants from upstream urbanised areas.

The quantity and composition of DOM are strongly influenced by its sources, hydrological conditions, and catchment land use [16–18]. Consequently, understanding the relationships between terrestrial inputs and in-lake processes is essential for interpreting DOM dynamics. The ecological significance of DOM depends on its origin, concentration, and chemical characteristics. As a key component of aquatic carbon cycling, DOM affects light penetration, nutrient availability, and microbial activity. Elevated inputs of organic matter and nutrients from catchments may stimulate eutrophication and alter water quality. Furthermore, anthropogenic DOM may act as a carrier of organic and inorganic contaminants, potentially affecting aquatic organisms and promoting shifts in community composition towards more pollution-tolerant taxa. A key feature of DOM is its ability to absorb solar radiation in the ultraviolet (UV) and visible (Vis) regions of the spectrum [19]. Many DOM components also exhibit fluorescence properties, making spectroscopic techniques particularly useful for their characterisation. UV-Vis absorbance and fluorescence spectroscopy provide sensitive, rapid, and non-destructive indicators of DOM sources, molecular characteristics, humification status, and transformation processes [20–23].

In small lakes, DOM properties are strongly affected by seasonal changes in primary production, hydrological conditions, and catchment-derived inputs [18,24]. Consequently, optical characterisation of DOM offers a valuable approach for tracking seasonal changes in organic matter composition, distinguishing natural variability from anthropogenic influences, and assessing the effects of catchment characteristics on DOM dynamics. These aspects are particularly important in lakes differing in morphometry, catchment structure, and exposure to human pressure.

This study investigates the influence of catchment type and seasonal biogeochemical variability on the quantity, composition and optical properties of DOM in lakes differing in morphometry and watershed features. Particular attention was paid to the usefulness of optical techniques, including UV-Vis absorbance and synchronous fluorescence spectroscopy (SFS), as sensitive indicators of trophic changes and catchment-related pressures. Together, these complementary methods provide a robust framework for detecting subtle changes in DOM composition and humification in small lake ecosystems.

2. Materials and Methods

2.1. Study Area

The study was carried out in three small lakes located in the Myślibórz Lake District, a post-glacial area in north-western Poland characterised by a large number of lakes, glacial landforms, and a mixture of agricultural land, forests, and wetlands (within the European Central Plains Ecoregion in Poland (Figure 1). The study lakes are located within the Central Plains Ecoregion of Poland, which is characterised by a temperate transitional climate resulting from the interaction of oceanic and continental air masses. This leads to marked seasonal variability in temperature and precipitation, with relatively mild winters and moderate summer conditions.

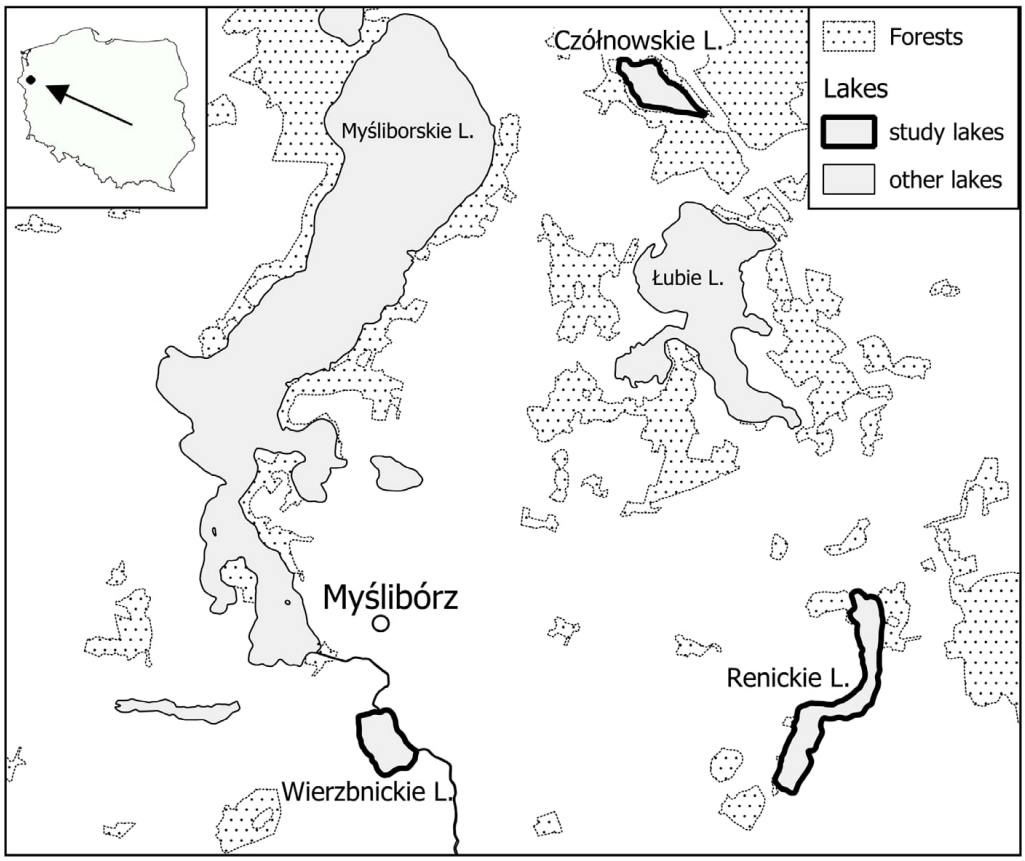


Figure 1. The map shows the location of the lakes in the Myślibórz Lake District, as well as the lakes that were included in the study. The arrow indicates the location of the sampling area from which water samples were collected.

Table 1 summarises the selected morphometric parameters and land-use patterns of the direct catchment areas of the three lakes studied.

Table 1. Morphometric parameters and land-use structure of the direct catchments of the lakes under study.

Lake	Area	Max Depth	Catchment Area	Forest	Arable Land	Meadows and Pastures	Anthropogenic Land
	(ha)	(m)	(ha)	(%)	(%)	(%)	(%)
Czółnowskie	28	4.3	192	28.4	52.3	17.0	2.4
Renickie	48	8.3	941	10.7	69.1	18.9	1.3
Wierzbnickie	23	3.2	325	0	74.7	16.4	8.9

The three lakes included in the study were selected to represent the variety of environmental conditions found in post-glacial landscapes, including differences in hydrological connectivity, morphometry, depth, sediment characteristics, and land use.

Situated in a relatively small catchment area dominated by arable land (52.3 per cent), Czółnowskie Lake is a small (28 ha), shallow (4.3 m) and heavily silted lake with extensive macrophyte growth. Deciduous forests also account for a significant proportion of the area (28.4 per cent). Renickie Lake, the largest and deepest of the analysed lakes, is 48 hectares in size and 8.3 metres deep. It is of the trough type and has a poorly defined anthropogenic system. It is characterised by a predominance of arable land (69.1 per cent) and an extensive shoreline. Wierzbnickie Lake, on the other hand, is a shallow through-flow lake. Despite its small size (23 ha), its catchment area is heavily modified, with a high proportion of agricultural land (74.7%) and a sewage treatment plant, which constitutes a significant source of anthropogenic pressure.

This selection provided a natural gradient of hydrological and ecological conditions, enabling the study of variability and potential sources of DOM across different lake types rather than within a single, homogeneous system. The diversity of morphometric parameters and land-use patterns in the direct catchment areas forms the basis for interpreting the observed differences in biogen concentrations and the optical properties of DOM in subsequent sections of the study.

2.2. Field Sampling and Water Quality Measurements

Field sampling was conducted during the spring, summer, and autumn (mid-April, mid-July, and mid-October). Water samples were collected from the surface layer (0–0.5 m below the water surface) in both the pelagic and littoral zones of each lake using a boat. Samples from the two zones were analysed separately in the laboratory. For each lake and sampling campaign, the values reported in the manuscript were calculated as the arithmetic mean of the results obtained for the pelagic and littoral zones. Therefore, the presented data characterise the overall surface-water properties of each lake rather than differences between the two sampling zones. All lakes were sampled on the same day during each sampling campaign. Samples were collected into sterile 500 mL bottles in accordance with the relevant ISO guidelines for water sampling. After collection, the samples were stored in cool conditions, protected from direct sunlight and heat during transport to the laboratory.

In situ measurements included water temperature, pH, specific electrical conductivity and dissolved oxygen concentration. Temperature, pH and specific electrical conductivity were measured using a portable multi-parameter meter (HQ40D, Hach, Loveland, Colorado, USA), whilst dissolved oxygen and BOD_5 (biochemical oxygen demand) were measured using a Multi 3400 multi-parameter meter fitted with a Cellox 323 oxygen sensor (WTW). The concentrations of nitrate nitrogen ($N–NO_3$), ammonium nitrogen ($N–NH_4$) and phosphate phosphorus ($P–PO_4$) were determined in the laboratory using colorimetric methods

with an automatic flow-through analyser (SkalarAnalytical B.V., Breda, The Netherlands). BOD_5 was measured as the difference between the oxygen content at the time the bottle was sealed and after 5 days of storage in the dark.

2.3. Optical Analyses of DOM

To determine the dissolved organic carbon (DOC) and carry out spectral analyses followed by the determination of a set of optical parameters, the water samples were subjected to a two-stage filtration process to remove suspended particles and phytoplankton cells. Membrane filters with pore diameters of 0.2 µm and 0.45 µm were used, respectively. Following evaporation of the water sample to a constant mass, the DOC content was determined spectrometrically after 'wet' oxidation of the organic matter using $K_2Cr_2O_7$ by heating the sample for 20 min at 140 °C.

The optical properties of DOM were analysed using a set of complementary spectroscopic techniques, including UV–Vis absorbance and synchronous scan fluorescence (SSF) spectra. These methods enabled us to assess the quantity, quality and degree of transformation of organic matter, as well as identify signals related to catchment pressure and seasonal variability in biogeochemical processes.

2.3.1. UV-Vis Absorption

Absorbance spectra (200–800 nm) were recorded using a dual-beam UV-Vis-NIR spectrophotometer (Jasco 770) in 1 cm diameter quartz cuvettes. The following optical parameters were calculated:

- specific UV absorbance ($SUVA_{270}$) was calculated as the Napierian absorption coefficient at 270 nm, normalised to the concentration of DOC:

$$SUVA_{270} = \frac{a(\lambda = 270\ \text{nm})}{DOC},$$

and the coefficient $a(\lambda)$ was determined using the following equation:

$$a(\lambda) = 2.303 \cdot \frac{OD(\lambda)}{\beta},$$

where β—cuvette length.

$SUVA_{270}$ was interpreted as an indicator of the aromaticity and degree of humification of DOM.

- the specific absorption coefficient a^*_{355} was calculated as the Napierian absorption coefficient at 355 nm, normalised against DOC:

$$a^*_{355} = \frac{a(\lambda = 355\ \text{nm})}{DOC}$$

This parameter describes the quantitative level of chromophore-rich organic matter.

- the E_{250}:E_{365} ratio, calculated as the ratio of absorbance at $\lambda = 250$ nm to absorbance at $\lambda = 365$ nm:

$$E_{250} : E_{365} = \frac{a(\lambda = 250\ \text{nm})}{a(\lambda = 365\ \text{nm})}$$

Lower values of this ratio indicate a higher average molecular weight and a higher degree of DOM humification.

- the E_{270}:E_{405} ratio, calculated as the ratio of absorbance at $\lambda = 270$ nm to absorbance at $\lambda = 405$ nm:

$$E_{270} : E_{405} = \frac{a(\lambda = 270\ \text{nm})}{a(\lambda = 405\ \text{nm})}$$

- the E_{254}:E_{436} ratio was calculated as the quotient of absorbance at 254 nm and 436 nm:

$$E_{254} : E_{436} = \frac{a(\lambda = 254\ \text{nm})}{a(\lambda = 436\ \text{nm})}$$

It is commonly used as an indicator of DOM aromaticity and the relative contribution of low-molecular-weight versus more humified chromophores.

This index reflects the proportion of aromatic compounds and the degree of humification of organic matter.

2.3.2. Fluorescence Spectroscopy

Synchronous scan fluorescence spectra (SSF) were recorded using a fluorescence spectrometer (Hitachi F-7000, High-Technologies Corporation, Tokyo, Japan). These spectra were recorded in the wavelength range of 280–600 nm. The interval between the emission and excitation wavelengths was $\Delta\lambda = \lambda_{em} - \lambda_{ex} = 20$ nm. The monochromators of the excitation and emission slits were 5 nm and 10 nm, respectively, and the scan speed was 240 nm min^{-1}.

2.4. Statistical Analyses

Statistical analyses were carried out using the R programming environment, version 4.6.1 [25]. Prior to conducting the analyses, the normality of the data distribution was assessed using the Shapiro–Wilk test, and the homogeneity of variances between groups was assessed using Levene's test [26].

Spearman's rank correlation, implemented in the stats package [25], was used to assess the relationships between the analysed variables. This test was chosen due to the absence of the assumption of a linear nature of the relationships under investigation. Furthermore, using the ppcor package [27], partial Spearman's rank correlations were calculated between UV-Vis parameters and fluorescence parameters, with the study season included as a control variable.

To account for potential confounding by seasonal trends, partial Spearman rank correlations were calculated between environmental variables and selected optical parameters, with season included as a control variable. *p*-values were adjusted for multiple comparisons using the Benjamini–Hochberg procedure.

The effects of Lake and Season on the measured physicochemical, UV-Vis and fluorescence-derived parameters were assessed using additive two-factor linear models with Lake and Season as fixed factors. Because each Lake × Season combination was represented by a single observation, the interaction term could not be estimated independently and was therefore not included in the models. Analysis of variance (ANOVA) was performed using additive models, and Tukey-adjusted post hoc comparisons were applied where appropriate.

The PCA was performed using the prcomp() function on standardised variables, and the results were presented using a scatter plot showing the variable vectors, group ellipses and trajectories of site position changes.

3. Results

3.1. Physical and Chemical Properties of Water Samples

The physicochemical parameters of the water showed marked seasonal variation and differences between the lakes studied (Table 2). The water was slightly alkaline (pH 7.85–8.33), whilst the electrical conductivity (EC) ranged from 434 to 691 µS cm^{-1}, indicating variations in the mineralisation and ionic composition of the waters. The concentration of dissolved oxygen ranged from 6.39 to 10.36 mg L^{-1}, with the highest values

occurring in spring and autumn and lower values in summer, indicating seasonal dynamics in biological processes and gas exchange. The DOC concentration ranged from 5.26 to 11.78 mg L^{-1}, with the highest values observed in spring and the lowest in autumn in all lakes.

Table 2. Hydrochemical parameters of the waters in the studied lakes (values are presented as mean ± SD. Mean values were calculated as the arithmetic average of results obtained for the pelagic and littoral zones in a given season, while SD represents the corresponding standard deviation).

Season	pH	DOC (mgC L^{-1})	Temperature (°C)	BOD_5	EC (µS cm^{-1})	$N\text{-}NO_3$ (mg L^{-1})	$N\text{-}NH_4$ (mg L^{-1})	$P\text{-}PO_4$ (mg L^{-1})
Czółnowskie Lake								
spring	8.00 ± 0.01	11.78 ± 0.23	18.8 ± 0.35	6.44 ± 0.23	472.2 ± 0.3	0.225 ± 0.002	0.202 ± 0.071	0.048 ± 0.018
summer	8.11 ± 0.04	8.52 ± 0.79	21.6 ± 0.00	4.81 ± 0.73	666.5 ± 0.7	1.730 ± 0.325	0.120 ± 0.000	0.005 ± 0.002
autumn	7.85 ± 0.06	5.83 ± 0.18	8.6 ± 0.07	5.71 ± 0.04	555.5 ± 0.7	0.155 ± 0.044	0.215 ± 0.021	0.006 ± 0.007
Renickie Lake								
spring	8.06 ± 0.04	11.56 ± 0.41	17.1 ± 0.75	6.59 ± 0.09	539.5 ± 0.4	0.905 ± 0.611	0.127 ± 0.042	0.075 ± 0.090
summer	8.06 ± 0.49	8.38 ± 0.09	21.6 ± 0.07	4.96 ± 0.49	691.3 ± 14.1	1.340 ± 0.445	0.107 ± 0.028	0.022 ± 0.012
autumn	8.02 ± 0.07	5.92 ± 0.01	9.0 ± 0.07	6.41 ± 0.54	567.7 ± 2.1	0.217 ± 0.042	0.290 ± 0.042	0.095 ± 0.006
Wierzbnickie Lake								
spring	8.33 ± 0.00	11.78 ± 1.33	17.4 ± 0.49	6.76 ± 0.22	434.0 ± 1.4	0.745 ± 0.442	0.167 ± 0.049	0.052 ± 0.008
summer	8.03 ± 0.04	10.02 ± 0.44	21.6 ± 0.21	2.37 ± 0.37	546.0 ± 15.6	1.550 ± 0.374	0.295 ± 0.035	0.006 ± 0.005
autumn	7.95 ± 0.16	5.26 ± 0.05	8.5 ± 0.21	7.05 ± 0.67	492.0 ± 2.8	0.355 ± 0.049	0.210 ± 0.000	0.039 ± 0.004

The concentrations of $N\text{-}NO_3$, $N\text{-}NH_4$ and $P\text{-}PO_4$ also showed seasonal variation between the lakes, with particularly high variability observed for nitrates, which reached their highest values in summer. The results obtained indicate varied physical, chemical and biogeochemical conditions in the studied lakes, providing an important context for interpreting the quantity and properties of DOM.

3.2. UV–Vis Absorbance–Based Indices

All the UV-Vis indices analysed showed a clear and consistent seasonal trend in DOM quality in the lakes studied (Table 3). Both $SUVA_{270}$ and a^*_{355} increased systematically from spring to autumn, indicating increasing aromaticity and humification of the chromophoric fraction of DOM during the growing season. $SUVA_{270}$ values increased on average from 4.9 to 6.2 (spring) to 9.8–11.0 L mg C^{-1} m^{-1} (autumn), whilst a^*_{355} values increased from 0.87 to 1.19 to 1.92–1.96 L mg C^{-1} m^{-1}. Because both parameters are normalised to DOC, these changes should be interpreted as reflecting an increasing chromophoric and aromatic character of DOM per unit carbon, rather than an increase in the total amount of CDOM. The consistent increase in both indicators suggests progressive changes in DOM quality and an increasing contribution of humified chromophoric components during the growing season [28–30].

Differences in DOM quality were also observed among the studied lakes. The Czółnowskie Lake was characterised by the lowest $SUVA_{270}$ and a^*_{355} values, indicating the most autochthonous and least humified DOM. In contrast, DOM in Lake Renickie exhibited intermediate values, typical of systems receiving a moderate input of allochthonous organic matter. The highest $SUVA_{270}$ values (up to 11.0) and a^*_{355} values (up to 1.92) were recorded in Lake Wierzbnickie, indicating the strongest aromatic and humified DOM signature relative to DOC, consistent with substantial catchment-derived organic matter inputs. Because both indices are normalised to DOC concentration, they describe DOM optical properties per unit carbon rather than the total abundance of chromophoric material. This variation

is fully consistent with the hydrological and trophic characteristics of the lakes [23,30,31]. The absorbance ratios E_{250}:E_{365}, E_{254}:E_{436} and E_{270}:E_{405} did not exhibit a uniform seasonal trend across the studied lakes (Table 3). Their values varied between lakes and seasons, reflecting differences in DOM sources and degrees of humification. In general, higher values of these ratios indicate a greater contribution of low-molecular-weight and less-condensed chromophores, whereas lower values reflect more humified and structurally condensed DOM [18,28].

Table 3. UV-Vis-derived optical indices of dissolved organic matter (DOM) in the studied lakes during the growing season.

Season	a^*_{355} ($L\ mg\ C^{-1}\ m^{-1}$)	$SUVA_{270}$ ($L\ mg\ C^{-1}\ m^{-1}$)	E_{270}:E_{405}	E_{254}:E_{436}	E_{250}:E_{365}
Czółnowskie Lake					
spring	0.94	4.9	13.4	23.8	8.8
summer	1.23	6.3	13.0	22.8	8.5
autumn	1.94	9.8	12.2	21.7	8.4
Renickie Lake					
spring	1.19	6.2	14.5	27.6	8.8
summer	1.54	8.2	14.2	26.0	9.0
autumn	1.96	10.5	13.8	25.0	9.2
Wierzbnickie Lake					
spring	0.87	4.9	15.7	29.1	9.7
summer	1.20	6.4	13.2	23.6	8.9
autumn	1.92	11.0	14.6	25.8	9.9

In Czółnowskie Lake, the E_{270}:E_{405} ratio decreased from spring to autumn, suggesting a gradual shift towards more humified DOM. In Renickie Lake, E_{250}:E_{365} increased only slightly from 8.8 to 9.2, indicating relatively stable DOM characteristics throughout the study period. In Wierzbnickie Lake, both E_{250}:E_{36}5 and E_{254}:E_{436} fluctuated (9.7–8.9–9.9 and 29.1–23.6–25.8, respectively), which may reflect episodic inputs of fresh DOM associated with river inflow and anthropogenic pressure.

In Wierzbnickie Lake, high $SUVA_{270}$ and a^*_{355} values indicate the presence of highly aromatic and humified DOM, whereas elevated E_{250}:E_{365} and E_{254}:E_{436} ratios reflect a concurrent contribution of low-molecular-weight, more labile chromophores [18,28]. This combination suggests a mixed DOM pool influenced by both allochthonous humified inputs and fresher, more reactive organic matter [32]. All UV-Vis indices form a consistent, unambiguous pattern of changes in DOM quality. DOM in the studied lakes showed an increase in the proportion of chromophoric fractions, increased aromaticity, and a higher degree of humification over the course of the season. However, the magnitude of these changes differed among the lakes, reflecting differences in catchment characteristics, trophic conditions, and the degree of anthropogenic pressure.

3.3. Synchronous-Scan Fluorescence Characteristics of DOM

The SSF spectra revealed two dominant fluorescence regions: (I) a protein-like band (PLF, $\lambda \approx 280$ nm) and (IIA and IIB) a fulvic-like band with a double peak (FLF, $\lambda \approx 315$ nm and $\lambda \approx 350$ nm) (Figure 2). The (III) humic-like (HLF, $\lambda \approx 470$ nm) band was faint, indicating a low proportion of highly aromatic DOM in the surface layer [33].

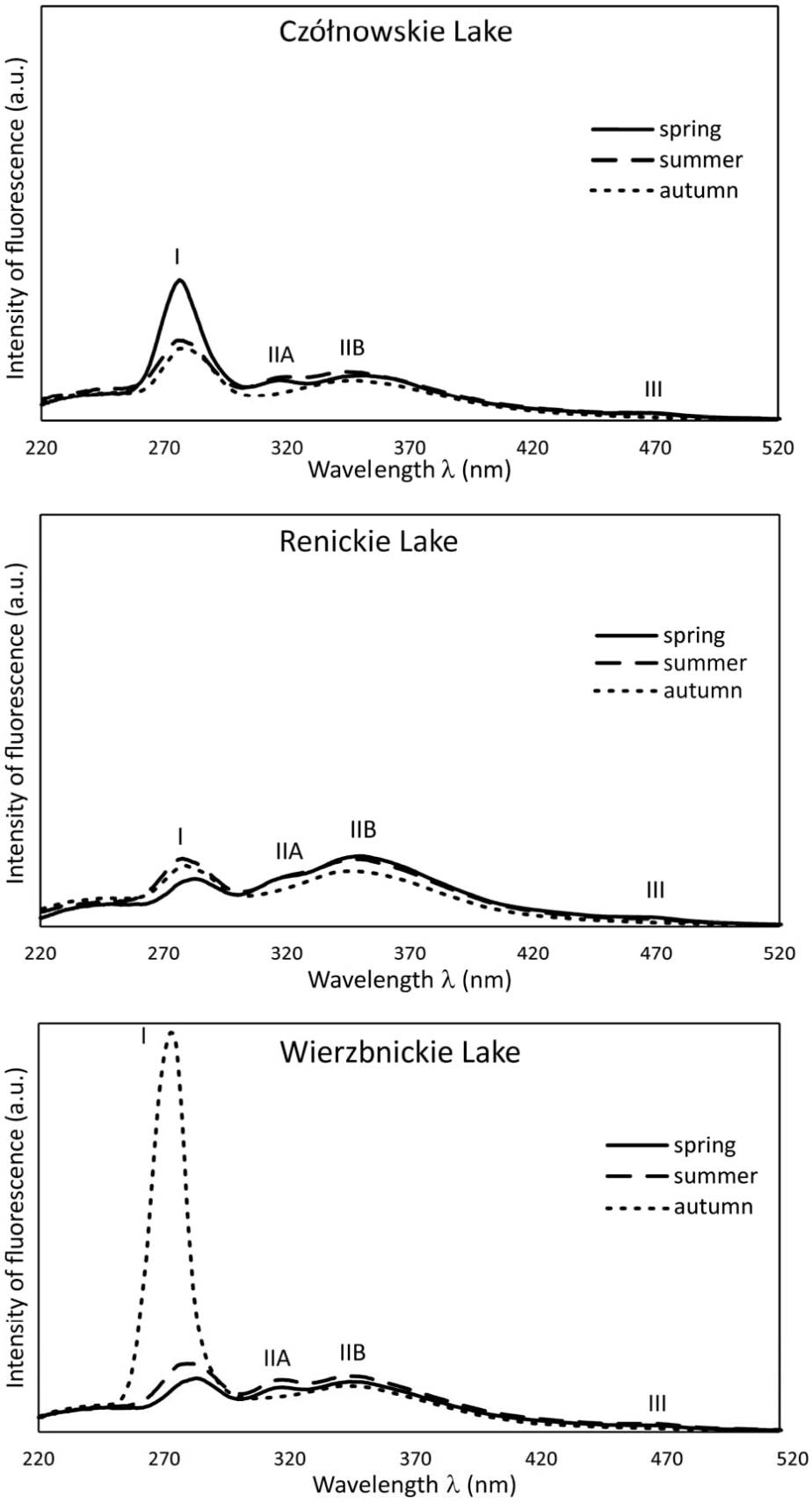


Figure 2. Synchronous scan fluorescence spectra. I (λ = 280 nm)—protein-like signal; IIA (λ = 315 nm)—fulvic-like with a lower molecular weight; IIB (λ = 350 nm)—humic-like with a medium molecular weight; III (λ = 470 nm)—humic-like with high aromaticity.

In spring, the protein-like fraction (PLF = 32.4%) dominated in Czółnowskie Lake, indicating fresh, autochthonous DOM (Table 4). In summer, the PLF proportion decreased, whilst in autumn it stabilised alongside a simultaneous increase in FLF fluorophores. The HLF band remained weak.

Table 4. Values of SSF indices used to characterise DOM composition and humification.

Season	PLF (%)	FLF (%)	HLF (%)	PLF/FLF	PLF/HLF	FLF/HLF	I/IIA	I/IIB	I/III	IIB/III
Czółnowskie Lake										
spring	32.4	38.4	1.5	0.84	21.36	25.31	3.13	2.78	17.65	6.36
summer	23.1	43.6	1.6	0.53	14.34	27.11	1.76	1.60	10.98	6.87
autumn	24.6	42.2	0.9	0.59	26.56	45.27	2.44	1.79	22.70	12.68
Renickie Lake										
spring	12.8	56.3	2.0	0.23	6.59	28.88	0.93	0.66	5.28	8.05
summer	17.8	52.0	1.6	0.34	11.30	32.99	1.32	0.99	9.01	9.14
autumn	18.8	49.8	1.0	0.38	18.44	48.79	1.58	1.08	15.48	14.33
Wierzbnickie Lake										
spring	16.8	50.4	1.7	0.33	9.91	29.62	1.15	1.02	7.88	7.71
summer	19.3	48.8	1.8	0.40	10.47	26.45	1.32	1.23	8.24	6.72
autumn	54.4	26.2	0.5	2.08	106.75	51.43	6.45	5.11	72.53	14.19

The percentage contributions of the PLF, FLF and HLF fractions reflect the integrated fluorescence within predefined wavelength ranges, which differ in width among the fractions. Consequently, percentage values do not directly correspond to the position or intensity of fluorescence maxima. The seasonal trend described in this study therefore relates to shifts in fluorescence maxima, indicating an increasing contribution of FLF- and HLF-associated fluorescence towards autumn, rather than a transition from PLF-dominated to FLF- dominated DOM.

In Czółnowskie Lake, the proportion of the PLF (32.4%) was highest in spring, suggesting a relatively greater contribution of autochthonous DOM during spring compared with the other sampling periods (Table 4). During summer, the PLF contribution decreased, while in autumn it remained relatively stable and was accompanied by an increase in the contribution of FLF fluorophores. The HLF signal remained low throughout the study period.

Renickie Lake was characterised by a consistently low contribution of PLF (12.8–18.8%) and a predominance of FLF (49.0–56.3%) throughout the study period, suggesting relatively stable DOM composition and limited seasonal variability.

In contrast, Wierzbnickie Lake exhibited a marked seasonal shift, with PLF increasing to 54.4% in autumn, accompanied by declining contributions of FLF and HLF. This pattern indicates a greater relative contribution of protein-like fluorophores to the fluorescence signal in autumn. However, the specific sources of this material cannot be identified based on the available data. An interesting observation is the disappearance in autumn of the maximum at ~315 nm in all lakes, which reflects the photodegradation of moderately humified DOM and a shift in fluorescence towards more condensed structures (350 nm) (Figure 2).

Similarly, the faint fluorescence peak at around 470 nm, characteristic of the highly humified humic-like fraction, decreased in autumn in all lakes. This decrease reflects a seasonal shift in the ratio between highly humified DOM and more reactive fulvic-like

fractions, as well as the optical masking of long-wavelength emission by stronger protein-like and fulvic-like bands.

The calculated ratios (I/IIA, I/IIB, I/III, IIB/III) confirmed the dominance of fresh DOM in spring and its gradual transformation towards fulvic-like fractions in autumn (Table 4). In Lakes Czółnowskie and Renickie, the FLF/HLF ratios increased in autumn, indicating a predominance of moderately humified DOM. In Wierzbnickie Lake, the protein-like component dominated in autumn, which distinguishes this reservoir from the others and indicates a strong influence of anthropogenic pressure.

4. Discussion

The seasonal variability of DOM in the studied lakes reflects the interplay of processes occurring within the catchment area and in the water column [32]. In spring, inputs from soils and accumulated organic matter in the surface layer dominate, alongside the first signs of autochthonous production. In summer, intra-lake processes predominate, including intensive photosynthesis, photodegradation and mineralisation. Autumn is a period of intense DOM transformation, characterised by catchment inflows, the decomposition of organic matter, and the complete mixing of the water column [34].

All UV–Vis parameters ($SUVA_{270}$, a^*_{355}, E_{250}:E_{365}, and E_{270}:E_{405}) exhibited consistent seasonal patterns. From spring to autumn, DOM became more aromatic, exhibiting a higher molecular weight and greater humification. An increase in $SUVA_{270}$ and a^*_{355} indicates rising levels of chromophoric DOM and humic fraction content, which is characteristic of small lakes with catchment-derived inflows and intensive autochthonous production [34–36].

The SSF analysis revealed distinct differences in DOM structure between the lakes, reflecting their varying catchment pressures and morphometry [37]. In Czółnowskie Lake, fresh, autochthonous DOM predominates in spring, while moderate humification is observed in summer and autumn. Renickie Lake is characterised by the stable dominance of the fulvic-like fraction, which is typical of deeper lakes with moderate catchment pressure. However, in Wierzbnickie Lake, a strong protein-like surge appears in autumn, indicating an inflow of fresh DOM associated with anthropogenic pressure. In small inland lakes, it is common for a significant proportion of the DOM pool to consist of terrestrial matter, as confirmed by numerous studies indicating the dominance of allochthonous carbon sources [9]. At the same time, hydrological variability further modulates the input and transformation of DOM [3,38,39].

The ecological interpretation is supported by the results of the statistical analyses. Amongst other things, the additive two-factor model revealed a significant effect of season on $SUVA_{270}$ values ($F_{2,4} = 53.86$; $p = 0.0013$), while there was no significant effect of lake ($F_{2,4} = 3.48$; $p = 0.133$). Model-estimated mean $SUVA_{270}$ values increased from spring (5.33) through summer (6.97) to autumn (10.43); Tukey-adjusted post hoc comparisons showed that SUVA270 values were significantly higher in autumn than in spring ($p = 0.0012$) and summer ($p = 0.0051$), whereas the difference between spring and summer was not significant ($p = 0.0659$). These results suggest that changes in DOM quality were primarily seasonal, with a smaller contribution of spatial variation. Similar conclusions were drawn by Du et al. [40], who demonstrated that seasonal changes were the dominant factor in shaping the composition and transformation of DOM components in a hydrologically isolated aquatic continuum. The authors also observed that different groups of DOM signals exhibit distinct environmental control, which is consistent with the separation of fluorescence and UV–Vis indices seen in the present study.

Spearman's rank correlations were used to assess relationships among the optical parameters because seasonal trends may generate apparent correlations partial Spearman correlations controlling for season were additionally performed (Figure 3). The analysis

revealed two distinct sets of optical parameters (Figure 3). The first and largest set comprises $SUVA_{270}$ and a^*_{355}, together with the fluorescence parameters (FLF, HLF, FLF/HLF, IIB/III and I/III). All of these parameters exhibit strong positive correlations. These parameters describe a common process related to DOM humification and aromaticity. The second set consists of UV–Vis structural indices (E_{250}:E_{365}, E_{254}:E_{436} and E_{270}:E_{405}), which are strongly intercorrelated, but show weak or no relationships with $SUVA_{270}$ or the fluorescence parameters. These indices reflect a different aspect of DOM properties, being associated with absorption structure and chromophore proportions rather than aromaticity itself. After controlling for season, the relationships between $SUVA_{270}$ and FLF/HLF ($\rho = 0.803$, p_adj $= 0.046$) and between $SUVA_{270}$ and l_{350}/l_{470} ($\rho = 0.812$, p_adj $= 0.046$) remained significant, whereas the other $SUVA_{270}$–fluorescence relationships were no longer significant.

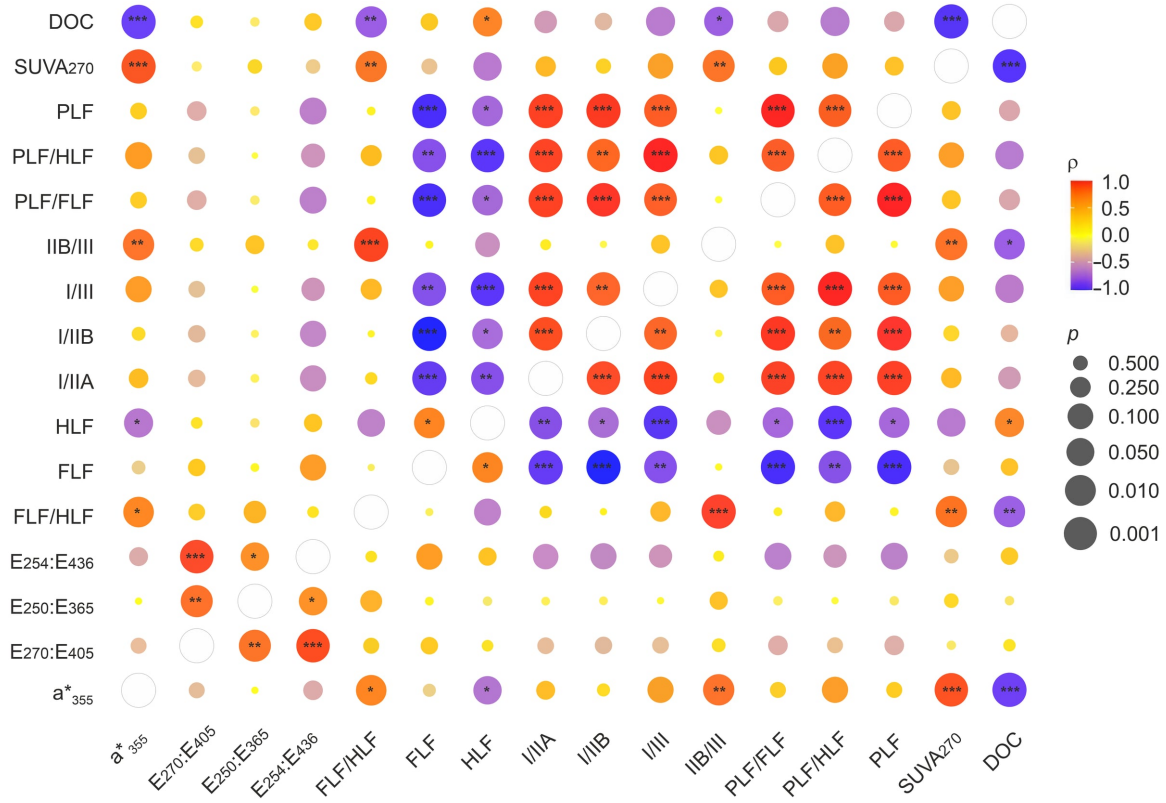


Figure 3. Spearman's correlations between optical parameters that characterise DOM. Each observation represents one lake × season combination ($n = 9$; three lakes × three seasons). Significance levels: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***).

Fluorescence analysis revealed an additional dimension of DOM variability that was independent of UV–Vis indices. The strongest and statistically significant correlations occurred among the fulvic-like and humic-like components: FLF, HLF, and FLF/HLF formed a coherent group of parameters associated with processed, stable organic matter. High FLF and FLF/HLF values were positively correlated with $SUVA_{270}$ and a^*_{355}, indicating a parallel increase in aromaticity and the proportion of humified fractions (Figure 3). In contrast, the protein-like component (PLF and PLF/FLF) showed weaker or insignificant correlations with UV–Vis parameters, but responded strongly to catchment pressure. This confirms the influx of fresh DOM into Wierzbnickie Lake in autumn. Significant positive correlations between HLF, FLF/HLF, and the fluorescence ratios I/III and IIB/III demonstrate that fluorescence signals are particularly sensitive to the degree of humification and the sources of DOM. Therefore, fluorescence parameters not only mirror $SUVA_{270}$, but also provide additional information regarding fluorophore structure, DOM freshness and processing

history. Similar relationships between fluorescence signals and the degree of humification have been documented in previous studies investigating the redox properties of DOM [37] and its biodegradability [34].

The seasonal increase in $SUVA_{270}$ was accompanied by parallel changes in DOM fluorescence parameters. Strong positive correlations were observed between $SUVA_{270}$ and FLF/HLF ($\rho = 0.803$; $p = 0.046$) and between $SUVA_{270}$ and IIB/III ($\rho = 0.812$; $p = 0.046$), indicating the co-occurrence of absorption and fluorescence signals associated with DOM humification and aromaticity. $SUVA_{270}$ also remained strongly correlated with a^*_{355} ($\rho = 0.929$; $p = 0.003$), confirming that both indices describe the same aspect of organic matter quality—the proportion of aromatic and humified fractions. The varied behaviour of the UV–Vis and fluorescence indices is consistent with previous observations that aromaticity and the particle size of organic matter are affected by degradation and photochemical processes [30,33].

Unlike the clear optical correlations, the relationships between UV-Vis parameters and the physical and chemical properties of water were weak and statistically insignificant after correction for multiple comparisons. When seasonality was taken into account, partial Spearman's rank correlations showed that all relationships between $SUVA_{270}$ and environmental parameters disappeared. This indicates that the earlier correlations were the result of a common seasonal gradient rather than direct ecological relationships. This confirms that $SUVA_{270}$ is primarily a seasonal characteristic of DOM rather than an indicator of changes to the water's physical and chemical properties. Similarly, analysing the correlations between fluorescence parameters and parameters characterising the properties of the water in the studied lakes revealed no significant relationships.

PCA further confirmed the significant influence of season on the optical properties of DOM (Figure 4). The first two principal components together explained 87.1% of the variation in the data, with autumn samples clearly separated from spring and summer samples along the PC1 axis. $SUVA_{270}$, FLF/HLF and IIB/III were associated with positive PC1 values, highlighting the seasonal increase in the proportion of humified fractions. PC2 differentiated the samples in terms of variability in the E_{250}:E_{365} and E_{270}:E_{405} indices. These indices formed a distinct system of co-varying UV–Vis parameters that were weakly correlated with $SUVA_{270}$.

Additionally, the PCA biplot shows that fluorescence and UV–Vis parameters influence sample distribution in different ways. The fluorescence variables (FLF, HLF, FLF/HLF, IIB/III and I/III) are strongly aligned with PC1, indicating that seasonal changes in humification and aromaticity are the dominant drivers of variation in DOM properties. In contrast, the UV–Vis structural indices (E_{250}:E_{365}, E_{254}:E_{436} and E_{270}:E_{405}) primarily load on PC2, forming an independent axis of variation related to chromophore composition and absorption structure. The separation of these two parameter systems in the PCA space confirms that the fluorescence parameters provided a more sensitive and discriminating view of DOM quality than the structural UV–Vis indices alone, capturing aspects of processing and source characteristics that the latter do not reflect. The sensitivity of small lakes to catchment pressures has been well documented in various climatic zones [8,10,38,39]. Our results are consistent with these patterns, showing that shifts in DOM quality and optical properties reflect the interplay between catchment inputs, autochthonous production, and hydrological dynamics.

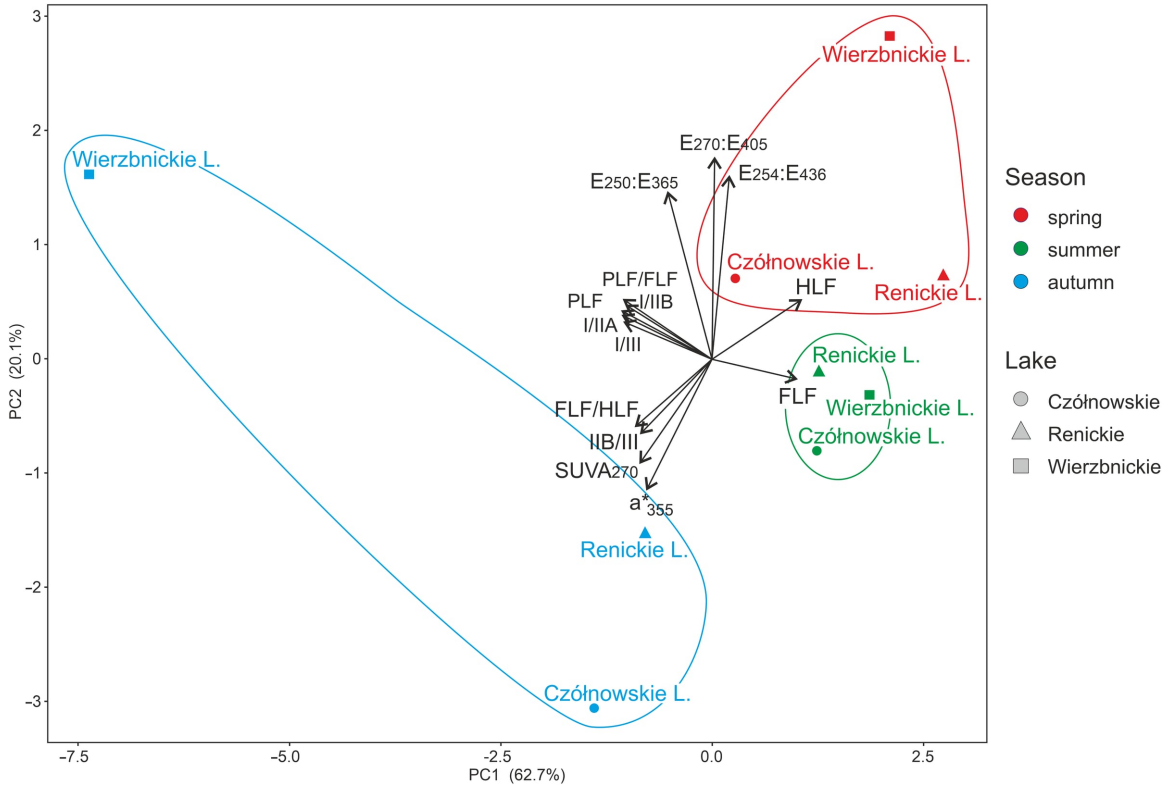


Figure 4. The principal component analysis (PCA) of the optical parameters of DOM in the studied lakes. Each observation represents one lake × season combination (n = 9; three lakes × three seasons).

Taken together, the results of the univariate, correlation and ordination analyses provide a coherent interpretation. In the lakes studied, the key factor shaping the optical properties of DOM was its seasonal production, degradation and transformation, whereas differences between lakes were of limited significance. Both UV–Vis and fluorescence parameters proved to be sensitive indicators of seasonal changes in DOM quality, with the strongest responses observed for $SUVA_{270}$ and selected fluorescence indices (FLF/HLF, IIB/III and PLF). These results confirm the usefulness of optical methods for monitoring the short-term dynamics of organic matter in small lake ecosystems.

5. Conclusions

The quality of DOM in the studied lakes showed a clear seasonal trend: a predominance of protein-like fractions in spring, followed by an increase in fulvic- and humic-like components in autumn. This pattern was consistent across the natural lakes, whereas the lake characterised by stronger anthropogenic influence exhibited an additional increase in protein-like fluorescence in autumn.

SSF spectra revealed differences in DOM fluorescence characteristics, showing a predominance of fulvic-like fractions in the natural lakes and a distinct increase in PLF in the anthropogenically impacted lake in autumn. Fluorescence spectroscopy provided additional insight into DOM composition and seasonal changes in its optical properties.

Statistical analyses showed that season was the main factor affecting the optical properties of DOM, while differences between lakes were insignificant. $SUVA_{270}$ and selected fluorescence parameters were particularly sensitive to short-term changes in DOM quality. Combining UV-Vis and fluorescence methods provides an effective approach for monitoring DOM variability in small lake ecosystems. The combined optical framework provides complementary information on seasonal changes in DOM quality and composition and may be useful in studies of lakes exposed to different environmental conditions.

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