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CULTIVABLE MICROALGAE FROM PEATLAND WATERS: MORPHOLOGICAL FEATURES AND PIGMENT PROFILES

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Abstract

Peatlands represent globally significant carbon reservoirs, and the microalgae inhabiting these sensitive ecosystems play an essential role in climate change mitigation. Peatland microalgae occur not only in surface waters but also within peat layers, contributing to carbon fixation through photosynthesis. This study investigates the diversity of cultivable microalgae from peatland waters and provides a preliminary characterisation based on pigment composition. Water samples were collected from two restored Romanian peatland sites, Avrig Peatbog and Mlaca Tătarilor Peatbog, selected due to their similar characteristics. Microalgal strains were isolated and cultivated under laboratory conditions in BG-11 medium. Identification was performed at the genus level using the microphotography method, an approach suitable for baseline diversity assessment studies. Photosynthetic pigments were extracted from the isolates and analysed using UV–Vis spectrophotometry. Ten cultivable microalgal strains were obtained, with higher diversity in Avrig Peatbog compared to Mlaca Tătarilor Peatbog. The combined morphological and pigment-based analysis revealed microalgal genera adapted to acidic, humic-rich peatland waters and differences in pigment profiles among isolates. These findings provide baseline data and support future molecular, ecological, and biotechnological investigations.

Key words: *cultivable microalgae, peatlands, pigments spectrophotometry.*

INTRODUCTION

Peatlands are dynamic ecosystems that provide essential ecological services, including biodiversity conservation, carbon storage, and water regulation. Although they cover only a small proportion of the Earth's land surface (about 3%), peatlands store a disproportionately large amount of global soil carbon (about 30%), making them key components of climate regulation and long-term carbon sequestration (Parish et al., 2008; Yu et al., 2010; Leifeld & Menichetti, 2018). The functioning of these ecosystems is strongly controlled by complex interactions between vegetation, hydrology, and microbial communities that regulate the balance between carbon fixation and decomposition.

In peatland environments, microorganisms play a fundamental role in carbon cycling processes. Among them, microalgae represent an important yet relatively understudied component of peatland biodiversity. These unicellular photoautotrophic microorganisms occur not only in surface waters but also within moist peat layers, where they contribute to primary production and carbon fixation through photosynthesis (Rydin & Jeglum, 2013; Yadav & Singh, 2020). Their ability to tolerate acidic conditions, high concentrations of humic substances, and nutrient-poor environments allows them to thrive in peatland habitats and makes them valuable indicators of environmental conditions (Bellinger & Sigee, 2015). In addition, microalgae are increasingly recognised as aquatic bioindicators with multiple ecological and applied research applications (Tsuraya et al., 2023).

The physicochemical characteristics and biodiversity of peatland surface waters are influenced by climatic and geographical factors but also by land management practices and anthropogenic pressures (Kowalik et al., 2014; Serafin et al., 2023). Changes in hydrology, drainage, or vegetation structure may alter water chemistry and light availability, thereby influencing the composition and ecological strategies of phototrophic microorganisms.

Romanian peatlands represent an important component of the national natural heritage and contribute significantly to biodiversity conservation and climate regulation. Among these ecosystems, Mlaca Tătarilor Peatbog was designated as a Site of Community Importance (ROSCI0112) in 2011 under the Natura 2000 network, primarily for the habitat type 7150 "Depressions on peat substrates of the Rhynchosporium" (Natura 2000 Reference Portal; Natura 2000 Viewer). Currently, the site is designated as a Special Area of Conservation (SAC) - ROSAC0112 under Government Decision No. 47/2024. The Avrig Peatbog overlaps with the Natura 2000 site ROSPA0098 Piemontul Făgăraș and was recently proposed as a Special Area of Conservation (SAC) in April 2024 (Ștefănuț et al., 2025) for habitats 7120 "Degraded raised bogs still capable of natural regeneration" and 7110* "Active raised bogs".

Following more than fifteen years of ecological monitoring, Mlaca Tătarilor Peatbog was selected in 2016 within the PeatRO project as a pilot area for demonstrating restoration methods applied to degraded peatland ecosystems. Similarly, the Avrig Peatbog, first described by Pop (1960) and rediscovered during the PeatRO project, was restored in 2023 through the PeatRO2 project. In both sites, the main factor responsible for the deterioration of peatland conservation status was long-term hydrological imbalance caused by drainage channels and the expansion of woody vegetation. Due to their comparable size, hydrological recovery, and restoration history, these two peatbogs provide suitable systems for comparative ecological investigations.

Despite their ecological importance, the diversity and ecological roles of microalgae inhabiting Romanian peatland waters remain poorly documented. Baseline studies based on the isolation and cultivation of microalgal strains are therefore important for understanding the composition of these communities and for exploring their physiological and biochemical characteristics.

In this context, the present study aimed to isolate cultivable microalgae from the surface waters of two restored Romanian peatbogs, Mlaca Tătarilor and Avrig, and to perform a preliminary characterization based on morphological features and pigment composition. Microalgal strains were identified using optical microscopy and the microphotography method, while pigment profiles were analysed using UV–Vis spectrophotometry. By integrating morphological observations with pigment-based characterization, this study provides baseline information on the diversity and physiological traits of microalgae inhabiting restored peatland ecosystems and supports future ecological, molecular, and biotechnological research.

MATERIALS AND METHODS

Sites` localisation and description

Both study sites lie in Sibiu County, Romania, within the Făgăraș Depression (northern sector of the Southern Carpathians, subalpine biogeographic region). The Mlaca Tătarilor Peatbog (SB-001; Figure 1) covers an area of 3.7 ha at an altitude of 548 m a.s.l. (45.425486°N, 24.3915012°E) and is administrated by the Arpașu de Sus town hall. The Avrig Peatbog (SB-013; Figure 2) covers 3.6 ha at 405 m a.s.l. (45.716282°N, 24.400133°E) and falls within the administrative boundaries of Avrig town. Their similar size and hydrological recovery after restoration make them ideal for parallel microalgal assessment.

Water sampling and physicochemical analysis

Water samples for microalgae identification were collected during the March 2025 field trip from zones with relevant water accumulation at both sites.

Physicochemical parameters of the peatland water were measured *in situ* at five points (P1-P5) per site (Figures 1 and 2), using a HI9829 multiparameter probe (Hanna Instruments). Parameters recorded included pH, oxidation-reduction potential (ORP), oxygen saturation (DO%), dissolved oxygen (DO), conductivity, total dissolved solids (TDS), salinity (PSU), turbidity, water temperature and water pressure (PSI).

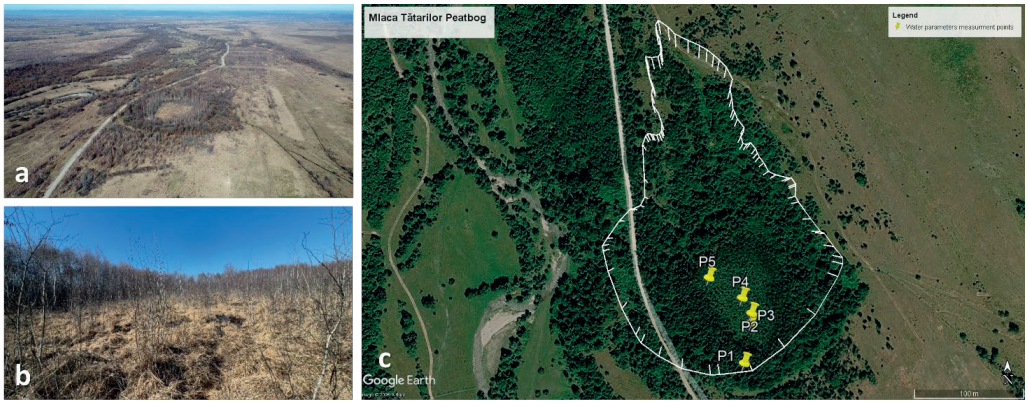


Figure 1. Mlaca Tătarilor Peatbog: (a) Drone view (Photo: Ștefănuț S., 10.03.2026); (b) Ground view (Photo: Moroșanu A.-M., 09.03.2026); (c) Site`s limits; marked points of water parameter measurements (Google Earth)

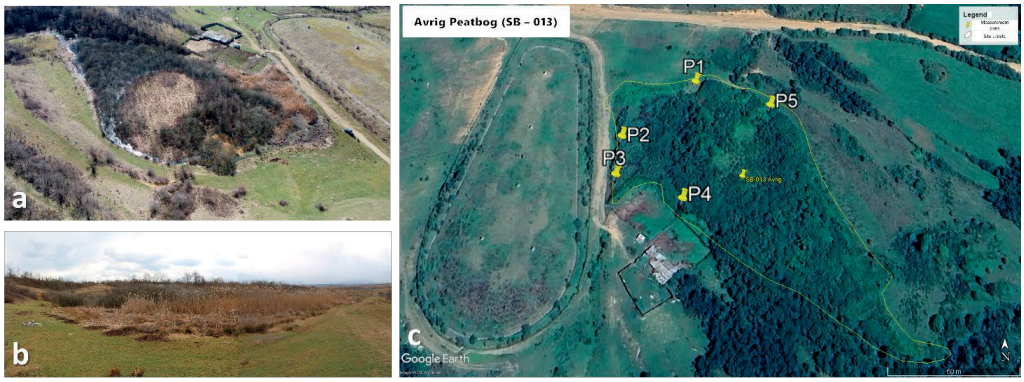


Figure 2. Avrig Peatbog: (a) Drone view (Photo: Ștefănuț S., 11.03.2026); (b) Ground view (Photo: Moroșanu A.M., 13.03.2025); (c) Site`s limits; marked points of water parameter measurements (Google Earth)

Isolation, identification, and characterization of microalgae

Microalgae isolation and cultivation. Peat water samples were collected at each site from the upper layer of the water column directly into sterile 50 mL plastic bottles. The bottles were labelled and transported to the laboratory for further processing. The collected water samples were enriched in microalgae by mixing the water sample 1:1 with BG-11 medium (Allen & Stanier, 1968). The mixtures were incubated in Erlenmeyer flasks on a shelf under natural illumination at ambient temperature for at least one month, until algal growth became apparent. Well-grown algal cultures were streaked onto BG-11 agar plates, which were then incubated under aerobic conditions at 28 ± 2°C in a thermostatically controlled incubator. Cultures were illuminated with fluorescent lamps at an intensity of 16,500 lux under a 12:12 h light: dark cycle. A single colony was aseptically transferred and streaked onto fresh BG-11 agar plates, and the procedure was repeated until axenic algal cultures were obtained.

Morphological identification. Enriched microalgal samples and isolates grown on solid media were examined using a Zeiss AxioScope.A1 optical microscope (Carl Zeiss) equipped with an AxioCam ERc 5s camera. Identification was performed using the microphotography method based on detailed analysis of captured images. Microalgal cells were observed, documented, and morphologically identified to the genus level based on cell size and shape, chloroplast morphology, presence of pyrenoids, and the presence or absence of flagella, using standard microalgal identification keys (Prescott, 1962;

1964; Belcher & Swale, 1976; Serediak & Huynh, 2011; Bellinger & Sigee, 2015; Jeuck & Arndt, 2013).

Pigment extraction and characterization. For pigment extraction, 50 mL of each enriched culture (14–20 days old) was harvested by centrifugation at 7,500 rpm for 10 min to remove the water content. The resulting algal biomass was homogenized in 10 mL of 99% acetone and kept overnight in the dark at 4°C to ensure complete pigment extraction. The samples were then centrifuged at 13,000 rpm for 5 min, and the supernatant was collected. The pellet was re-extracted by repeated washing with 5 mL of 99% acetone until it became colorless. All extracts were pooled and evaporated under reduced pressure at 30°C, then stored at −20°C until pigment quantification.

UV-Vis characterization of the extracted pigments was recorded using a Specord 210 Plus spectrophotometer (Analytik Jena) over the wavelength range of 350-750 nm at room temperature.

The pigment concentrations were calculated using the equations of Lichtenthaler (1987) for 100% acetone at 470 nm, 661.6 nm and 644.8 nm. Pigment data were primarily interpreted using pigment ratios, namely chlorophyll a to chlorophyll b (Chl a/Chl b) and total carotenoids to total chlorophyll (Car/(Chl a+b)).

RESULTS AND DISCUSSIONS

The physico-chemical parameters

Mlaca Tătarilor Peatbog (SB-001; ROSCI0112)

Water parameters were measured at five points (P1-P5): four within the *Sphagnum*-dominated zone and one point at the entrance, all characterized by a high water table (Figure 2; Table 1). Measurements revealed pH values of 6.62-7.13 (slightly acidic to neutral), likely due to mineral influence or degradation, potentially favoring microalgae tolerant to slightly acidic to neutral conditions. ORP values were near-neutral to slightly reducing, indicating predominantly anoxic conditions that favor anaerobic processes typical to waterlogged peat bogs. Moderate-to-good DO% values reflected variable oxygenation, with hypoxic microsites (anaerobic processes) alongside oxygenated zones (aerobic decomposition); this variability reflects hydrological heterogeneity. Also, DO values confirm moderate oxygenation in the surface water of Mlaca Tătarilor, yet hypoxic conditions in some areas. Low conductivity values and TDS values confirmed oligotrophic conditions with minimal mineral input. Invariable PSU values indicated extremely pure freshwater, while turbidity values reflected extremely clear water, typical of oligotrophic peat bogs, favoring light penetration for microalgae and *Sphagnum* photosynthesis. Early spring water temperatures minimized organic matter decomposition, favoring carbon sequestration in waterlogged *Sphagnum* zones. A stable hydrology and an elevated water table were reflected by PSI values.

Overall, there is a high consistency between the physicochemical parameters at the five points (P1-P5), indicating typical oligotrophic conditions in a hydrologically stable ecosystem that favors microalgae and *Sphagnum* species development as well as active carbon sequestration.

Avrig Peatbog (SB-013)

Measurements were taken in water pools and drainage channels (Figure 2; Table 2). The water pH ranged between 6.61 and 7.47 (slightly acidic to neutral). The water pool showed the highest ORP and DO concentration, while the drainage channels exhibited had the highest TDS, salinity, conductivity, and turbidity values due to sediment accumulation. Water temperature and pressure were nearly similar across both location types, correlating with seasonal biochemical processes. ORP varied from positive (oxidation of organic matter) to negative (reducing reactions).

Overall, parameters indicated a gradient from well-oxygenated, low-mineralised reed-fringed pools (P2) to mineralised, drainage-influenced points (P3-P4) and highly reductive, hypoxic channels (P5), reflecting combined peatland hydrology and anthropogenic drainage influences.

Table 1. Water parameter values - Mlaca Tătarilor Peatbog

Parameter	P1 45,71449°N, 24,65014°E	P2 45,71491°N, 24,65023°E	P3 45,71483°N, 24,65023°E	P4 45,71504°N, 24,65012°E	P5 45,71521°N, 24,64972°E
pH	6.62	6.63	7.10	6.77	7.13
ORP (mV)	-41.9	-41.6	2.6	-6.1	33.7
DO (%)	43.3	75.2	84.0	75.7	71.7
DO (mg/L)	4.80	8.62	9.56	8.62	9.00
Conductivity (µS/cm)	50	48	53	34	47
TDS (mg/L)	25	24	26	26	24
PSU (unit)	0.02	0.02	0.02	0.02	0.02
Turbidity (NTU)	0.0	4.7	14.9	0.0	0.0
Water temperature (°C)	6.77	6.58	7.75	7.50	3.51
PSI (mbar)	13.914	13.904	13.938	13.944	13.914

Table 2. Water parameter values - Avrig Peatbog

Parameter	**Avrig-P1**: NE water pool bog access point	**Avrig-P2**: Reed-fringed pool bog access point	**Avrig-P3**: Drainage channel dam near the access path	**Avrig-P4**: Reed-fringed pool crosswise path	**Avrig-P5**: Channel over pool, opposite the path
pH	6.68	7.19	7.47	7.35	6.61
ORP (mV)	41.4	-25	-21.4	-39.1	-95.6
DO (%)	10.4	28.6	14	10.4	5.1
DO (mg/L)	1.16	3.23	1.61	1.17	0.57
Conductivity (µS/cm)	287	1	954	740	412
TDS (mg/L)	143	0	478	370	218
PSU (unit)	0.14	0	0.47	0.36	0.19
Turbidity (NTU)	122	15	22.9	15.6	15.8
Water temperature (°C)	7.89	7.52	6.19	7.03	6.73
PSI (mbar)	13.765	13.749	13.744	13.745	13.73

Our slightly acidic-neutral pH (6.6-7.5), low conductivity/TDS, and variable ORP/DO values in both studied restored peatbogs align with post-restoration recovery trends in European minerotrophic peatlands (Andrews et al., 2025). Mlaca Tătarilor Peatbog`s stable anoxia resembles typical waterlogged peatbog conditions, while Avrig`s hydrological gradient reflects anthropogenic disturbance, creating microalgae niches. These patterns indicate incipient hydrological recovery post-restoration.

The physicochemical characteristics of peatland waters provide the environmental context in which phototrophic microorganisms develop. In order to further investigate the physiological traits of the cultivable microalgae associated with these environments, pigment extracts obtained from enriched cultures were analysed using UV–Vis spectrophotometry.

Pigment profiles of enriched microalgal cultures

Pigment extracts obtained from enriched microalgal cultures originating from the two studied peatland sites were analysed using UV-Vis spectrophotometry in order to investigate the physiological characteristics of the cultivable phototrophic microorganisms.

The recorded spectra (Figure 3) showed the characteristic absorption bands of photosynthetic pigments. Strong absorption maxima were observed in the blue region of the spectrum (Soret bands) and in the red region (Qy bands), corresponding to electronic transitions of chlorophyll molecules, while the spectral region between 400 and 500 nm corresponded to the main absorption domain of carotenoids. These pigments play essential roles in light harvesting and photoprotection and are widely used as indicators of physiological status and environmental adaptation in microalgae (Lichtenthaler, 1987; Falkowski & Raven, 2007).

Pigment ratios calculated from the spectra revealed variability among enriched cultures obtained from both peatlands (Table 3), suggesting differences in physiological acclimation of the cultivated microalgae.

In the cultures originating from Avrig Peatbog, the Chl a/Chl b ratio varied widely between 0.64 and 3.16. Higher ratios observed in several cultures (e.g., Avrig-2 and Avrig-3) indicate a relative predominance of chlorophyll a and may

reflect acclimation to environments with higher or more variable irradiance. Reduced antenna size associated with increased Chl a/Chl b ratios is commonly interpreted as an adaptive strategy in microalgae exposed to elevated light conditions or fluctuating illumination regimes (Falkowski & Raven, 2007; Bellinger & Sigee, 2015).

Conversely, lower Chl a/Chl b ratios, such as those observed in Avrig-1, suggest a relatively higher contribution of chlorophyll b and may indicate adaptation to shaded or optically filtered environments. Such conditions are typical for peatland waters where high concentrations of dissolved humic substances significantly modify the spectral composition and penetration of light in the water column (Rydin & Jeglum, 2013).

The Car/(Chl a+b) ratio also varied among the analysed cultures. Elevated carotenoid proportions recorded in some Avrig samples (e.g., Avrig-4 and Avrig-5) may reflect increased photoprotective investment. Carotenoids are known to play a key role in protecting the photosynthetic apparatus against oxidative stress and excessive irradiance, particularly in shallow or dynamic aquatic habitats (Falkowski & Raven, 2007).

Enriched cultures originating from Mlaca Tătarilor Peatbog displayed somewhat narrower Chl a/Chl b variation (0.85-1.79), suggesting a more balanced contribution of chlorophyll a and b compared with some of the Avrig cultures. These intermediate values may reflect adaptation to relatively stable light conditions within *Sphagnum*-dominated peatland microhabitats, where vegetation structure and water colour create diffuse light environments typical for bog ecosystems (Rydin & Jeglum, 2013).

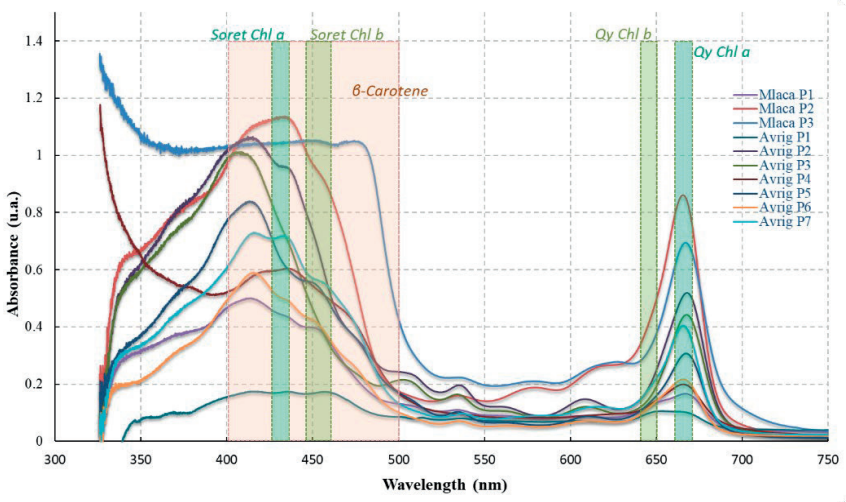


Figure 3. UV-Vis absorption spectra (300-750 nm) of pigment extracts from the analyzed microalgal cultures. The Soret bands (blue region) and Qy bands (red region) correspond to characteristic electronic transitions of chlorophylls. The 400-500 nm region represents the main carotenoid absorption range, overlapping with the chlorophyll Soret bands

Table 3. Pigment ratios of the analyzed microalgal enriched cultures

Sample	Mlaca-1	Mlaca-2	Mlaca-3	Avrig-1	Avrig-2	Avrig-3	Avrig-4	Avrig-5	Avrig-6	Avrig-7
Chl a/Chl b	0.85	1.79	1.78	0.64	2.74	3.16	1.13	1.91	1.46	1.92
Car/(Chl a+b)	0.20	0.13	0.37	0.09	0.20	0.15	0.44	0.33	0.26	0.26

The Car/(Chl a+b) ratio also showed variability among Mlaca samples, with the highest value observed in Mlaca-3. Increased carotenoid proportions are frequently associated with microalgal responses to environmental stress factors such as fluctuating irradiance or oxidative stress (Falkowski & Raven, 2007). Conversely, lower carotenoid values recorded in

Mlaca-1 and Mlaca-2 may indicate more stable microhabitat conditions where photoprotective requirements are reduced.

Although the physicochemical parameters were measured at representative monitoring points rather than at the exact locations of each enrichment culture, they provide a useful characterization of the environmental conditions prevailing within the studied peatland waters.

When interpreted together, the pigment patterns observed in the enriched cultures suggest that the cultivated microalgae exhibit considerable physiological plasticity, enabling them to cope with the heterogeneous environmental conditions typical of peatland ecosystems.

Microalgae identification and ecological interpretation

In addition to pigment analysis, microscopic examination of enriched cultures revealed several dominant microalgal morphotypes corresponding to common freshwater green algal and cyanobacterial genera (Figure 4). The observed taxa included representatives of *Chlorella*, *Chlorococcum*, *Scenedesmus*, *Closterium*, *Stichococcus*, *Chlamydocapsa*, *Gonium*, *Palmellopsis*, *Aphanocapsa*, and *Nostoc*.

The observed morphotypes correspond well with the pigment signatures detected in the UV–Vis spectra, which suggested the presence of chlorophyll-containing green algae and cyanobacteria in the enriched cultures.

It should be noted that enrichment cultivation may selectively favor fast-growing taxa. Consequently, the microalgal assemblages described in this study represent the cultivable fraction of the natural community rather than the complete *in situ* diversity.

During the isolation procedure, several strains were also transferred to solid BG-11 medium in order to obtain axenic cultures. Although these pure cultures were not the primary focus of the present study, their establishment ensures the availability of well-defined strains for future physiological, molecular, and biotechnological investigations. In the present work, however, pigment analysis was performed on enriched cultures in order to better reflect the dominant phototrophic components of the original peatland microbial assemblages.

Morphological characterization of the observed morphotypes

Figure 4 (a-c) shows solitary spherical cells measuring approximately 11-12 µm in diameter, which may tentatively be assigned to the genus *Chlorella* based on their morphological characteristics.

Figure 4 (d) shows solitary cylindrical cells with rounded ends, measuring approximately 11-12 µm in length and 5-6 µm in width. Considering the observed morphology and the habitat of origin, the isolate can be provisionally assigned to the genus *Stichococcus*.

In Figure 4 (e), a solitary elongated cell slightly curved with gradually tapered ends can be observed, measuring approximately 25 µm in length and 2.85 µm in width.

The high length-to-width ratio gives the cell a filiform appearance typical of small desmids. The observed morphology is consistent with the genus *Closterium*, a taxon frequently encountered in acidic oligotrophic ecosystems such as peatlands.

Figure 4 (f) illustrates cells with an ovoid morphology forming grape-like colonies composed of approximately eight cells embedded within a gelatinous sheath. The cells measure approximately 8-9.5 µm in length and 5.5-6.5 µm in width, and a pyrenoid is visible. Based on these characteristics, the colony may tentatively be assigned to the genus *Chlamydocapsa*, which is commonly reported from freshwater environments.

In Figure 4 (g), filamentous structures composed of spherical cells measuring approximately 3-6 µm in diameter are observed. Based on their morphology, these filaments most likely belong to the genus *Nostoc*.

Figure 4 (h) shows a flat plate of spherical cells embedded in a mucilaginous matrix. The colony consists of approximately 16 well-spaced cells and measures around 15 µm in length. The observed morphology is consistent with the genus *Gonium*.

In Figure 4 (i), cells embedded within a mucilaginous matrix forming a compact irregular colony can be observed. The cells are ovoid to ellipsoidal, measuring approximately 2-3.5 µm in width and 5-8 µm in length, and contain a distinct chloroplast with a visible pyrenoid. This palmelloid organization suggests affiliation with the genus *Palmellopsis*.

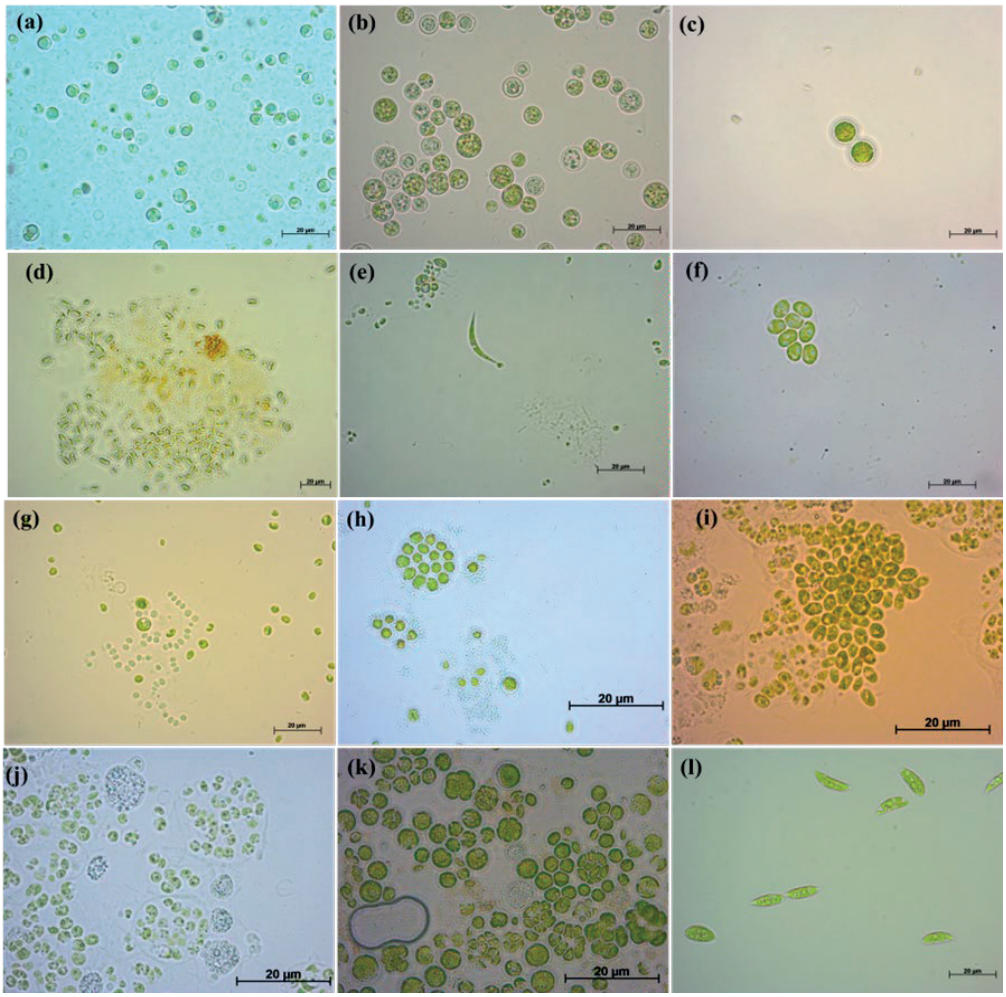


Figure 4. Representative microalgal morphotypes observed in enriched cultures derived from peatland water samples. (a-c) *Chlorella*-like cells; (d) *Stichococcus* sp.; (e) *Closterium* sp.; (f) *Chlamydocapsa* sp.; (g) *Nostoc* sp.; (h) *Gonium* sp.; (i) *Palmellopsis* sp.; (j) *Aphanocapsa* sp.; (k) *Chlorococcum* sp.; (l) *Scenedesmus* sp. Images were captured using bright-field microscopy. Scale bar = 20 µm

Figure 4 (j) shows a group of small cells embedded within a wide mucilaginous sheath resembling colonies of *Aphanocapsa*. Cells appear spherical and reach up to 4 µm in diameter. Species of *Aphanocapsa* typically form gelatinous colonies composed of spherical cells measuring approximately 1-5.5 µm depending on the species.

In Figure 4 (k), unicellular spherical cells with smooth cell walls are observed, occurring both as solitary individuals and in small colonies. These characteristics are consistent with the genus *Chlorococcum*.

Figure 4 (l) shows solitary unicellular organisms with elongated fusiform to ellipsoidal shapes measuring approximately 13-18 µm in length and 5.2-7 µm in width. Cells contain a parietal chloroplast with a visible central pyrenoid, a morphology consistent with the genus *Scenedesmus*, a taxon commonly reported from nutrient-rich freshwater environments.

The observed morphotypes reflect the taxonomic diversity of cultivable phototrophic microorganisms present in the studied peatland waters.

Distribution of microalgal genera across sampling sites

The distribution of the identified genera across sampling sites provides additional insight into the ecological characteristics of the studied peatland habitats and is summarized in Table 4. Overall, the results indicate the presence of a diverse assemblage of green algae and cyanobacteria in both peatland systems.

In Mlaca Tătarilor Peatbog, several colonial and mucilage-producing taxa were detected, including *Chlamydocapsa, Palmellopsis* and *Aphanocapsa*. These genera are commonly associated with freshwater environments rich in dissolved organic matter and are frequently reported from peatland pools where extracellular mucilage provides protection against environmental stress and facilitates colony formation (Bellinger & Sigee, 2015).

The presence of *Gonium*, a colonial volvocalean alga, suggests the existence of shallow water microhabitats with sufficient illumination for colonial flagellate algae. Volvocalean taxa are commonly observed in nutrient-poor freshwater environments where they contribute to planktonic primary production (Bellinger & Sigee, 2015).

Table 4. Dominant microalgal morphotypes observed in enriched cultures obtained from peatland water samples.

Sampling site	Observed microalgal and cyanobacterial genera	Dominant morphotype	Cell morphology	Approx. cell size (µm)
Mlaca P1	*Chlorella* sp. *Chlamydocapsa* sp.	*Chlamydocapsa* sp.	ovoid morphology forming grape-like colonies	8-9.5 µm in length 5.5–6.5 µm in width
Mlaca P2	*Aphanocapsa* sp. *Palmellopsis s*p.	*Aphanocapsa* sp.	spherical ovoid to ellipsoidal	3-4 µm in length 2-3.5 µm in width
Mlaca P3	*Chlorella* sp. *Gonium* sp.	*Gonium* sp.	mucilaginous matrix with spherical cells	15 µm
Avrig-P1	*Chlorella* sp.	*Chlorella* sp.	solitary spherical cells	11-12 µm
Avrig-P2	*Chlorella* sp. *Scenedesmus sp.* *Chlorococcum* sp.	*Scenedesmus sp.*	fusiform to ellipsoidal	13-18 µm
Avrig-P3	*Chlorella* sp.	*Chlorella* sp.	solitary spherical cells	11-12 µm
Avrig-P4	*Chlorella* sp. *Nostoc* sp.	*Chlorella* sp.	solitary spherical cells	11-12 µm
Avrig-P5	*Chlorococcum* sp. *Chlorella* sp. *Closterium* sp.	*Closterium* sp.	solitary elongated cell, slightly curved	25 µm in length 2.85 µm in width
Avrig-P6	*Stichococcus* sp.	*Stichococcus* sp.	solitary cylindrical cells with rounded ends	11-12 µm length 5-6 µm in width
Avrig-P7	*Chlorella* sp. *Chlorococcum* sp.	*Chlorococcum* sp.	spherical	5-8 µm

The frequent occurrence of *Chlorella*-like cells in Mlaca samples further indicates the presence of ecologically versatile microalgae capable of colonizing a wide range of aquatic habitats. Species of *Chlorella* are widely distributed in freshwater ecosystems and are known for their high physiological tolerance to variations in nutrient availability, light conditions and mineralization (Bellinger & Sigee, 2015).

In Avrig Peatbog, a broader morphological diversity was observed, including *Chlorococcum, Scenedesmus, Closterium, Stichococcus* and the cyanobacterium *Nostoc*. Several of these taxa are commonly reported from freshwater environments characterized by fluctuating nutrient concentrations and dynamic hydrological conditions.

The occurrence of *Scenedesmus* sp. and *Chlorococcum* sp. suggest the presence of microhabitats influenced by increased nutrient availability or environmental disturbance, as these genera are well known for their ability to rapidly colonize nutrient-enriched aquatic environments (Bellinger & Sigee, 2015).

In contrast, the presence of *Closterium* sp., a representative of desmid algae, indicates the occurrence of microhabitats typical of peatland ecosystems. Desmids are frequently associated with oligotrophic, slightly acidic waters rich in dissolved organic matter and are considered characteristic components of bog and fen algal communities (Coesel & Meesters, 2007).

The detection of *Nostoc* sp., a filamentous cyanobacterium capable of atmospheric

nitrogen fixation, may further indicate localized nitrogen limitation in certain peatland microhabitats. Nitrogen-fixing cyanobacteria are known to play an important ecological role in nutrient-poor aquatic systems by contributing biologically available nitrogen (Falkowski & Raven, 2007).

When interpreted together, the pigment data and the morphological identification provide complementary information regarding the ecological strategies of the cultivable microalgae inhabiting the studied peatland waters.

Ecological significance of cultivable microalgal assemblages

The ecological characteristics and indicator value of the most frequently identified genera are summarized in Table 5. Several of these taxa are widely used as indicators of water quality and trophic conditions in freshwater ecosystems (Bellinger & Sigee, 2015).

Table 5. Ecological characteristics and indicator value of the main microalgal genera

Genus	Typical habitat	Indicator value
Chlorella	freshwater, planktonic	mesotrophic–eutrophic waters
Scenedesmus	planktonic green algae	eutrophic conditions
Closterium	oligotrophic acidic waters	peatland/desmid flora
Nostoc	benthic or colonial cyanobacteria	nitrogen-limited environments
Stichococcus	aerophytic / oligotrophic	tolerant to stress

Ecologically versatile genera such as *Chlorella, Chlorococcum* and *Scenedesmus* are commonly reported as early colonizers of newly formed or disturbed aquatic habitats. Their presence in several enriched cultures suggests the ability of these taxa to rapidly establish populations in peatland pools and shallow water accumulations, contributing to primary production and the initial development of microbial food webs.

At the same time, the occurrence of taxa typically associated with oligotrophic peatland habitats, such as *Closterium* and *Stichococcus*, indicates the persistence of microhabitats characterized by low nutrient concentrations and high levels of dissolved organic matter.

Colonial and mucilage-producing forms such as *Palmellopsis, Chlamydocapsa* and *Aphanocapsa* may represent adaptive strategies to humic-rich waters where extracellular mucilage provides mechanical protection and facilitates colony stability.

Together, these observations suggest that the cultivable microalgal assemblages reflect the environmental heterogeneity of restored peatland ecosystems, where variations in hydrology, light availability and nutrient concentrations generate a mosaic of ecological niches for phototrophic microorganisms.

Similar patterns of microalgal colonization have been described in other peatland systems, where phototrophic microorganisms play an important role in primary production and in the re-establishment of microbial communities following ecological disturbance or restoration (Yadav & Singh, 2020).

These findings contribute to the baseline knowledge of peatland microbial phototroph diversity in Romanian wetlands and provide a starting point for future ecological and molecular investigations.

Future studies integrating molecular identification techniques and seasonal monitoring would further improve our understanding of the ecological dynamics of microalgal communities in restored peatlands.

CONCLUSIONS

The present study provides a first overview of the cultivable microalgal diversity inhabiting the surface waters of two restored Romanian peatlands (Avrig Peatbog and Mlaca Tătarilor Peatbog).

The physicochemical measurements revealed a clear environmental gradient across sampling points in Avrig Peatbog, ranging from well-oxygenated pools to mineralized drainage channels and reductive, hypoxic microhabitats. In contrast, Mlaca Tătarilor Peatbog exhibited more uniform, stable oligotrophic conditions with predominant anoxia. Both sites shared slightly acidic-neutral pH and low mineralization favoring non-acidophilic microalgae and carbon sequestration, with incipient hydrological recovery post-restoration.

Pigment analysis of enriched cultures indicated variations in photosynthetic pigment composition, suggesting physiological acclimation of microalgal strains to local environmental conditions such as light availability, mineralization, and redox status.

Microscopic observations revealed several representative freshwater microalgal genera, including *Chlorella*, *Chlorococcum*, *Scenedesmus*, *Closterium*, and *Stichococcus*, whose ecological preferences are consistent with the physicochemical characteristics of peatland waters.

These findings provide baseline information on the cultivable fraction of peatland microalgal communities and contribute to a better understanding of the microbial diversity associated with restored peatland ecosystems.

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