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Phytoplankton Functional Group V *sensu* Reynolds in Shallow Macrophyte-Dominated Wetlands: Indications Beyond Stratified Lakes

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ABSTRACT

Phytoplankton Functional Group V *sensu* Reynolds in Shallow Macrophyte-Dominated Wetlands: Indications Beyond Stratified Lakes.

Functional Group V (FG V), which comprises anoxygenic phototrophic purple and green sulfur bacteria, has traditionally been associated with stratified lakes, where pronounced vertical redox gradients are typically described. Here, we report the repeated occurrence of anoxygenic phototrophic sulfur bacteria in two shallow riparian wetlands, suggesting that the ecological context of this functional group may extend beyond classical stratified systems. Environmental variables and phytoplankton functional structure were analysed in a lowland and a semi-mountain wetland characterized by dense macrophyte cover, high summer temperatures, elevated organic matter content, and low dissolved oxygen conditions. FG V, represented by *Chromatium okenii* and *Thiospirillum jenense*, was detected in all samples and episodically reached high relative abundance. It co-occurred within a functionally coherent assemblage of motile flagellates (W1, X2, Y), filamentous cyanobacteria (TC), and metaphytic diatoms (MP), typical of shallow, weakly mixed systems. Although direct redox measurements were not performed, the combination of low dissolved oxygen, high organic loading, and restricted mixing indicates the potential for reduced conditions and localized redox discontinuities. Our results are consistent with the interpretation that macrophyte-dominated shallow wetlands may locally provide environmental conditions functionally analogous to those of deeper stratified systems, allowing FG V to persist within phytoplankton communities. These findings support a process-based view of FG V ecology, emphasizing the role of redox-related conditions over water depth, and highlight shallow wetlands as potentially important but underexplored habitats for redox-dependent phototrophic communities.

KEY WORDS: Functional Group V, shallow wetlands, macrophytes, low oxygen, phytoplankton.

RESUMEN

Grupo Funcional V del fitoplancton* sensu *Reynolds en humedales someros dominados por macrófitos: indicios más allá de los lagos estratificados.

El Grupo Funcional V (FG V), que comprende bacterias fototróficas anoxigénicas púrpuras y verdes del azufre, ha sido tradicionalmente asociado con lagos estratificados, donde se describen gradientes verticales de óxido-reducción pronunciados. En este estudio, se documenta la presencia recurrente de bacterias fototróficas anoxigénicas del azufre en dos humedales ribereños someros, lo que sugiere que el contexto ecológico de este grupo funcional podría extenderse más allá de los sistemas estratificados clásicos. Las variables ambientales y la estructura funcional del fitoplancton fueron analizadas en un humedal de llanura y otro de media montaña, caracterizados por una densa cobertura de macrófitos, altas temperaturas estivales,

elevado contenido de materia orgánica y condiciones de bajo oxígeno disuelto. El FG V, representado por Chromatium okenii *y* Thiospirillum jenense*, fue detectado en todas las muestras y alcanzó ocasionalmente una alta abundancia relativa. Coexistió dentro de un ensamblaje funcionalmente coherente de flagelados móviles (W1, X2, Y), cianobacterias filamentosas (TC) y diatomeas metafíticas (MP), típicas de sistemas someros con mezcla débil. Aunque no se realizaron mediciones directas del potencial redox, la combinación de bajos niveles de oxígeno disuelto, alta carga orgánica y mezcla restringida indica el potencial desarrollo de condiciones reductoras y discontinuidades redox localizadas. Nuestros resultados son consistentes con la interpretación de que los humedales someros dominados por macrófitos pueden proporcionar localmente condiciones ambientales funcionalmente análogas a las de sistemas estratificados más profundos, permitiendo la persistencia del FG V en las comunidades fitoplanctónicas. Estos hallazgos respaldan una visión basada en procesos de la ecología del FG V, enfatizando el papel de las condiciones redox sobre la profundidad del agua, y destacan los humedales someros como hábitats potencialmente importantes, aunque poco explorados, para comunidades fototróficas dependientes del estado redox*

PALABRAS CLAVE: *Grupo Funcional V, humedales someros, macrófitos, bajo oxígeno, fitoplancton.*

INTRODUCTION

The phytoplankton functional group (FG) classification system proposed by Reynolds et al. (2002) and subsequently refined by Padisák et al. (2009) is widely applied as an integrative ecological framework linking species composition to key environmental drivers such as light climate, mixing regime, trophic state, and redox-related conditions. By grouping taxa with similar ecological strategies and habitat preferences, this approach enables a process-oriented interpretation of phytoplankton community structure across a wide range of aquatic ecosystems, including lakes, reservoirs, rivers, and wetlands (Salmaso et al., 2015, Török et al., 2016, Nagy-László et al., 2020).

Among the functional groups defined within this framework, FG V, which comprises phototrophic purple and green sulfur bacteria, remains one of the least documented (Padisák et al., 2009). This group is conceptually associated with habitats where pronounced redox gradients and persistent or seasonal anoxia are described, such as the metalimnion of stratified eutrophic lakes or the monimolimnion of meromictic systems (Reynolds et al., 2002, Padisák et al., 2009). The indicator value of FG V has been recognized and incorporated into ecological indices used for assessing water quality in large rivers and lakes (Padisák et al., 2006, Borics et al., 2007).

Despite its clear ecological definition and practical application, empirical field observations of FG V remain scarce, particularly outside deep stratified water bodies. In riverine communities, FG V has been reported only occasionally in specific river sections and time periods, and its ecological niche has been considered relatively narrow (Nagy-László et al., 2020). Its occurrence in shallow hypertrophic lakes has not been clearly linked to the spatial dimensions of the water bodies (Borics et al., 2016). In contrast, in a eutrophic lake with stable linear vertical stratification, FG V has been shown to dominate the aphotic layer during the summer period (Borics et al., 2015, T-Krasznai et al., 2024).

Recent studies indicate that shallow lakes and macrophyte-dominated wetlands are characterized by high habitat complexity and considerable functional diversity (Borics et al., 2016, Görgényi et al., 2024). Structural heterogeneity created by aquatic vegetation generates numerous microhabitats with locally varying light availability, oxygen concentration, and associated redox heterogeneity (Meerhoff & González-Sagrario, 2022). Within the functional group framework, such compressed environmental heterogeneity may allow groups typically associated with stratified systems to occur in shallow habitats under suitable conditions. In densely vegetated wetlands, macrophyte cover can substantially modify light availability and oxygen dynamics, potentially favouring the coexistence of oxygenic and anoxygenic phototrophs (Izaguirre et al., 2010, Somogyi et al., 2022).

In this context, the present study examines the occurrence and temporal variability of FG V in shallow riparian wetlands where anoxygenic phototrophic sulfur bacteria co-occur with cyanobacteria and eukaryotic phytoplankton func-

tional groups. These observations contribute to the discussion on the ecological context of FG V and highlight the potential importance of shallow vegetated wetlands for understanding phytoplankton functional diversity under low-oxygen conditions.

The aim of this study was to describe the functional structure of phytoplankton communities in two shallow riparian wetlands and to document the occurrence of FG V under non-stratified, low-oxygen conditions.

MATERIALS AND METHODS

Study sites

The Dragoman riparian wetland (hereafter Dragoman RW) is a karst marsh located in the Nishava River basin in western Bulgaria (42°56′ N, 23°01′ E; Fig. 1). It is a semi-mountain shallow wetland situated at 718 m a.s.l., with a maximum depth of approximately 1 m. The system is primarily fed by groundwater and precipitation. The surface area of Dragoman RW varies considerably during the year (2-4 km²) and partial desiccation may occur during the summer months. Dragoman RW supports a rich macrophyte community that covers about 65% of the wetland. Fifty macrophyte species have been recorded, with *Carex riparia*, *Typha latifolia*, *Schoenoplectus lacustris*, *Phalaris arundinacea*, and *Phragmites australis* being the most abundant (Vassilev et al., 2019). Submerged and floating hydrophytes include *Ceratophyllum submersum*, *Hydrocharis morsus-ranae*, and *Lemna trisulca*.

The Popovitsa riparian wetland (hereafter Popovitsa RW) is located in the Maritsa River floodplain in central Bulgaria (42°09′43″ N, 25°04′16″ E). It originated after the 1928 earthquake and represents a former river arm situated at 132 m a.s.l. The wetland is extremely shallow (maximum depth approximately 0.30 m; surface area

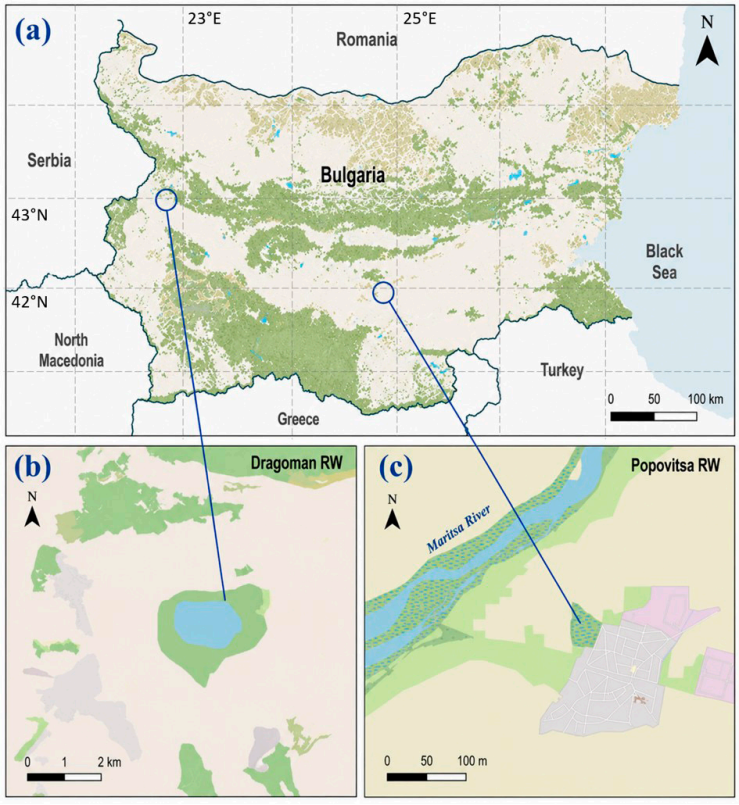

Figure 1. Location of the investigated riparian wetlands: (a) Bulgaria (overview map with two sites); (b) Dragoman RW; (c) Popovitsa RW. *Localización de los humedales ribereños investigados: (a) Bulgaria (mapa general con los dos sitios); (b) Dragoman RW; (c) Popovitsa RW.*

ca. 0.01 km²) and is mainly fed by groundwater and precipitation. Macrophytes occupy more than 80% of the water surface, predominantly *Lemna minor*, while emergent vegetation is dominated by *Phragmites australis* and *Typha angustifolia*.

Both wetlands are located within the temperate continental climatic zone. They are hydrologically isolated, wind protected, densely vegetated systems characterized by high organic matter accumulation, conditions conducive to oxygen depletion and redox-related heterogeneity.

Sampling, processing and analytical methods

Sampling was conducted four times during the warm period of 2020 (July–October). Samples were collected at a fixed sampling point at both sites from the upper 0–10 cm water layer after gently removing the overlying macrophyte cover. From each composite sample, two subsamples (250 mL each) were taken for phytoplankton analysis, one subsample (0.5 L) for chlorophyll *a* analysis, and one subsample (1 L) for chemical analyses.

Phytoplankton subsamples were preserved *in situ*: one with acidified Lugol's iodine solution and the other with formaldehyde (final concentration 4%). The main physical and chemical parameters (water temperature, pH, electrical conductivity, dissolved oxygen and oxygen saturation) were measured *in situ* using a multiparameter probe (WTW, Xylem Analytics Germany Sales GmbH & Co. KG). Measurements were taken between 10:00 and 12:00 at a depth of approximately 10 cm. Water transparency was determined using a 20-cm Secchi disk.

Subsamples for chemical analyses were stored in the dark at 4 °C and transported within 24 hours to a certified laboratory. Analyses were performed according to recognized standard methods: total phosphorus (EN ISO 11885), total nitrogen (EN ISO 11905-1), chlorophyll *a* (ISO 10260), and biological oxygen demand (EN 1899-2).

Taxonomic identification was performed to species level where possible using a Euromex light microscope (900× magnification) on formaldehyde-preserved samples. Phototrophic sulfur bacteria were identified based on cell morphology (shape and size), intracellular sulfur granules, and characteristic pigmentation under light microscopy. Representative micrographs of phototrophic sulfur bacteria are provided in Supplementary Figure S1 (available at https://www.limnetica.com/en/limnetica). Phytoplankton abundance, including phototrophic bacteria, was determined from Lugol-preserved samples using an inverted microscope (Olympus IX71) according to the Utermöhl method (Utermöhl, 1958). At least 400 algal units (cells, filaments, or colonies) were counted along transects in each sample. Rare and large taxa were additionally counted at 200× and 100× magnification. Species-specific biovolume was calculated using geometric formulas following Hillebrand et al. (1999), based on measurements of up to 30 individuals per taxon and multiplied by the observed abundance. Biomass was derived from biovolume using the conversion factor 1 mm³/L = 1 mg/L (Wetzel & Likens, 2000).

Phytoplankton taxa were assigned to FGs according to Reynolds et al. (2002) and Padisák et al. (2009). Functional groups representing more than 5% of the total phytoplankton biovolume in a given sample were considered dominant FGs.

Statistical methods

To explore patterns in the functional composition of the phytoplankton community, Principal Component Analysis (PCA) was performed using the relative biomass (%) of the dominant FGs. The analysis was based on the correlation matrix to standardize the contribution of the individual FGs. Differences in FG composition between the two wetlands were tested using one-way PERMANOVA based on Bray–Curtis dissimilarity with 9,999 permutations. Relationships between the first two PCA axes (sample scores) and the measured environmental variables were subsequently assessed using Spearman's rank correlation coefficient because of the limited sample size (n=8). In addition, relationships between the biomass of FG V and the measured environmental variables were evaluated using Spearman's rank correlation.

Principal Component Analysis (PCA), PERMANOVA and Spearman's rank correlation analyses were performed using PAST version 5

(Hammer et al., 2001).

RESULTS

Physical and chemical parameters

Due to dense macrophyte cover, shallow depth (Popovitsa RW: 0.3 m; Dragoman RW: 1.0 m), and limited mixing, both wetlands share several environmental characteristics, including high summer temperatures, hypertrophic conditions, and repeated low-oxygen conditions (Table 1).

Water temperature in the lowland Popovitsa RW ranged between 24.5 and 33.9 °C, whereas considerably lower values were recorded in the semi-mountain Dragoman RW (14.0–19.7 °C). Water transparency remained low throughout the study period, particularly in Popovitsa RW, where Secchi depth reached only 0.1 m. The pH ranged from 6.2 to 8.0, with most values indicating slightly acidic to neutral conditions. Electrical conductivity in Popovitsa RW (1380–1796 µS/cm) was approximately three times higher than in Dragoman RW (456–693 µS/cm). Dissolved oxygen saturation was consistently low in all samples from both wetlands: ≤15% in Popovitsa RW and ≤31% in Dragoman RW. High values of biological oxygen demand (15–42 mg/L) were recorded in both systems.

Total phosphorus (0.16–1.36 mg/L), total nitrogen (1.40–14.15 mg/L), and chlorophyll *a* (75–270 µg/L) indicated hypertrophic conditions according to Nürnberg (1996), with the exception of the last two samples from Dragoman RW.

Although redox potential was not directly measured, the combination of very low dissolved oxygen saturation, high biological oxygen demand, and dense macrophyte cover is consistent with the potential presence of reduced microzones within the water column and at the sediment-water interface.

Phytoplankton taxonomic composition and FGs in riparian wetlands

A total of 90 taxa belonging to eight taxonomic groups were identified in the investigated wetlands, including anoxygenic phototrophic sulfur bacteria, cyanobacteria, and eukaryotic algae: Proteobacteria (2), Cyanobacteria (18), Chlorophyta (13), Bacillariophyta (37), Ochrophyta (1), Euglenophyta (13), Cryptophyta (5), and Dino-

Table 1. Physical and chemical parameters, nutrients and chlorophyll *a* measured in the investigated riparian wetlands. WT-Water temperature, SD-Secchi depth, EC-Electrical conductivity, O_2-Dissolved oxygen, O_2-sat-Oxygen Saturation, TN-Total nitrogen, TP-Total phosphorus, BOD-Biological oxygen demand, Chl *a*-chlorophyll *a*. *Parámetros físicos y químicos, nutrientes y clorofila* a *medidos en los humedales ribereños investigados. WT-Temperatura del agua; SD-Profundidad de Secchi; EC-Conductividad eléctrica; O_2-Oxígeno disuelto; O_2-sat-Saturación de oxígeno; TN-Nitrógeno total; TP-Fósforo total; BOD-Demanda biológica de oxígeno; Chl* a-*Clorofila* a.

Name	Date sampling	WT °C	SD m	pH	EC µS/ cm	O_2 mg/ L	O_2-sat %	TN mg/L	TP mg/L	BOD mg/L	Chl *a*, µg/L
Popovitsa RW	19.07.2020	31.2	0.1	7.5	1670	1.3	15	4.47	0.16	30.8	271.8
	10.08.2020	32.5	0.1	8.0	1796	0.1	1.4	6.92	0.31	42.0	101.5
	29.08.2020	33.9	0.1	6.3	1397	0.7	10	13.1	1.31	34.0	144.0
	21.09.2020	24.5	0.1	6.2	1380	1	12	14.15	0.85	31.2	75.0
Dragoman RW	27.07.2020	19.7	0.5	7.1	693	1.1	12	1.40	1.36	23.5	26.8
	28.08.2020	19.7	0.5	6.3	456	1.6	17	1.40	0.90	32.1	42.9
	20.09.2020	18.4	0.3	6.2	481	2.9	31	2.04	0.98	27.12	18.6
	10.10.2020	14.0	0.3	6.4	512	2.3	23	2.02	0.97	15.24	5.4

flagellata (1). These taxa were assigned to 16 phytoplankton FGs, of which 11 FGs were shared by both sites: V, J, K, MP, S1, W1, X1, X2, Y, YPh, and TC. The complete taxa list and their assignment to functional groups are provided in Supplementary Table S1a (available at https://www.limnetica.com/en/limnetica). Biomass (BM) and relative biomass (Rel. BM, %) of phototrophic microorganisms are presented in Supplementary Table S1b (available at https://www.limnetica.com/en/limnetica). The relative contribution (%) of all functional groups and the identification of dominant groups (>5% biomass) are summarized in Supplementary Table S2 (available at https://www.limnetica.com/en/limnetica).

Phytoplankton communities in both wetlands were characterized by six dominant FGs in Popovitsa RW and eight dominant FGs in Dragoman RW (Fig. 2 a, b).

Popovitsa RW

The dominant FGs in Popovitsa RW included anoxygenic phototrophic sulfur bacteria (V), euglenophytes (W1), filamentous cyanobacteria (TC), green flagellates (X2), metaphytic diatoms (MP), and centric diatoms (C). These groups co-occurred in all four analysed samples, although their relative contributions varied considerably. In the first sample, FG V (*Chromatium okenii*, *Thiospirillum jenense*) accounted for 73% of the total community biomass. Representatives of X2 (*Carteria*, *Nephroselmis*), TC (*Oscillatoria*), and W1 (*Euglena*, *Monomorphina*) were also present but at lower relative abundance. In the second sample, filamentous cyanobacteria of the TC group (*Oscillatoria* spp.) dominated the community, contributing 79% of the total biomass, accompanied by metaphytic diatoms of

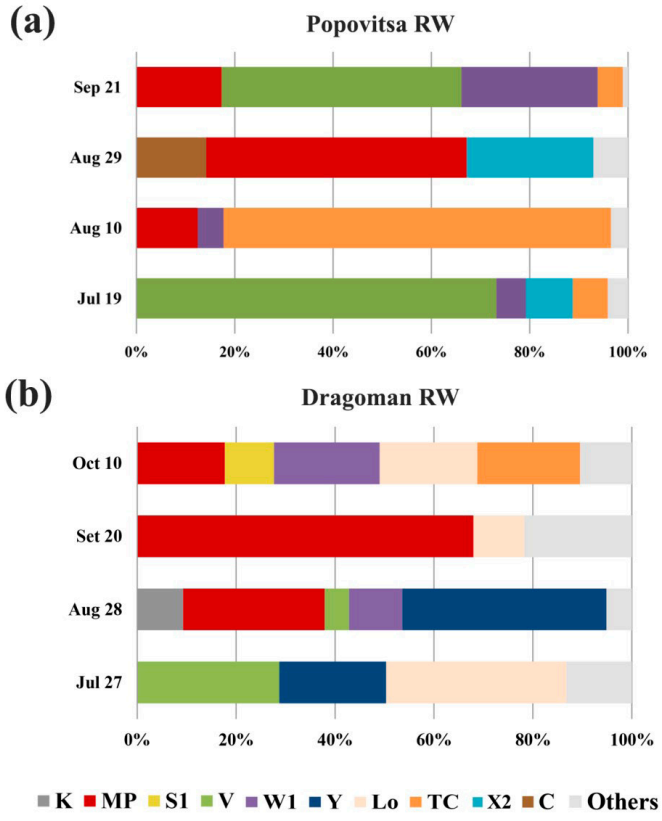

Figure 2. Relative contribution (%) of dominant phytoplankton functional groups (FGs; >5% of total biomass) in the investigated riparian wetlands: (a) Popovitsa RW; (b) Dragoman RW. Minor functional groups are presented in Supplementary Table S2. *Contribución relativa (%) de los grupos funcionales dominantes del fitoplancton (FG; >5% de la biomasa total) en los humedales ribereños investigados: (a) Popovitsa RW; (b) Dragoman RW. Los grupos funcionales minoritarios se presentan en la Tabla Suplementaria S2*.

the MP group (*Cymbella*, *Craticula*). In the third sample, metaphytic diatoms of the MP group (*Craticula ambigua*, *Gomphonema acuminatum*) accounted for more than half of the total biomass (53%), followed by green flagellates of the X2 group (*Chlamydomonas pyrenodiscus*). In the final sample, FG V again represented approximately half of the total community biomass (49%), co-occurring with euglenophytes of W1 (*Euglena texta*), metaphytic diatoms of MP (*Craticula*, *Rhopalodia*), and filamentous cyanobacteria of TC (*Oscillatoria*, *Phormidium*). FG V dominated only at the beginning and at the end of the study period, although the anoxygenic phototrophic sulfur bacteria *Chromatium okenii* and *Thiospirillum jenense* were present in all samples (Supplementary Table S1b).

Dragoman RW

Eight dominant phytoplankton FGs were recorded in Dragoman RW, four of which were also dominant in Popovitsa RW: anoxygenic phototrophic sulfur bacteria (V), euglenophytes (W1), filamentous cyanobacteria (TC), and metaphytic diatoms (MP). In addition, cryptophytes (Y), small colonial cyanobacteria (K, LO), and filamentous cyanobacteria (S1) were identified as dominant groups in Dragoman RW. The relative abundance of FG V in Dragoman RW was lower than in Popovitsa RW and ranged between 5 and 29%. The anoxygenic phototrophic sulfur bacteria *Chromatium okenii* and *Thiospirillum jenense* were among the dominant taxa only in the first two samples, although they were present throughout the study period (Supplementary Table S1b). In the first sample, FG V co-dominated with cyanobacteria of the LO group (*Woronichinia compacta*) and cryptophytes of the Y group (*Cryptomonas* spp.). In the second sample, flagellated taxa of the Y group (*Gymnodinium*, *Cryptomonas*) dominated the community, contributing 41% of the total biomass, followed by metaphytic diatoms of the MP group (*Epithemia*, *Cocconeis*). In the third sample, metaphytic and epiphytic diatoms of the MP group (*Cocconeis*, *Lemnicola hungarica*) contributed 68% of the total biomass, accompanied by cyanobacteria of the LO group (*Woronichinia*, *Coelosphaerium*), which accounted for approximately 10%. In the final sample, euglenophytes (W1: *Euglena*, *Lepocinclis*), filamentous cyanobacteria (TC: *Tenebriella curviceps*, formerly *Oscillatoria curviceps*), and colonial cyanobacteria (LO: *Woronichinia*, *Coelosphaerium*) contributed comparably to the total community biomass.

Comparative functional structure

Despite differences in altitude, depth, and conductivity, both wetlands exhibited similar functional structure of the phytoplankton communities. This structure was characterized by the recurrent co-occurrence of anoxygenic phototrophic sulfur bacteria (FG V), motile flagellates (W1, X2, Y), filamentous cyanobacteria (TC), and metaphytic diatoms (MP). FG V exceeded 5% of total biomass in half of the samples, reaching dominance at the beginning and at the end of the study period in Popovitsa RW and contributing up to 29% of total community biomass in Dragoman RW.

Statistical analyses

Principal Component Analysis (PCA) explained 49.2% of the total variation in functional group (FG) composition by the first two principal components (PC1=27.7%, PC2=21.5%) (Fig. 3). The ordination distinguished three major assemblages of FGs. Along PC1, FGs V, W1, S1, TC and LO were separated from FGs C, MP and X2, whereas PC2 further separated FGs K and Y from the remaining assemblages.

Spearman's rank correlation analysis revealed no statistically significant relationships between the PCA axes and the measured environmental variables (all $p>0.05$). Likewise, no significant correlations were detected between the biomass of FG V and any individual environmental variable. Nevertheless, PC2 showed comparatively stronger, although statistically non-significant, correlations with Secchi depth (RSP=0.62), electrical conductivity (RSP=–0.57) and total nitrogen (RSP=–0.47). In contrast, no comparable relationships were observed for PC1.

The PCA did not reveal a clear separation between samples from the two wetlands, and PERMANOVA likewise indicated no statistically significant differences in FG composition between

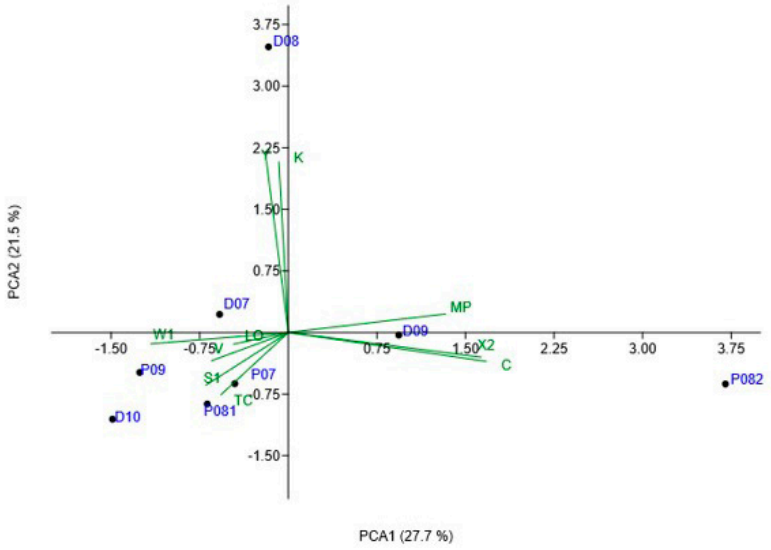

Figure 3. Principal Component Analysis (PCA) biplot of phytoplankton functional groups based on relative biomass (%) in the eight investigated samples. The analysis was performed using the correlation matrix. Samples are shown as points and functional groups as loading vectors. The first two principal components explained 49.2% of the total variance (PC1=27.7%, PC2=21.5%). Sample abbreviations consist of the initial letter of the respective wetland (P=Popovitsa RW; D=Dragoman RW), followed by the two-digit sampling month (07=July; 08=August; 09=September; 10=October). For Popovitsa RW, two samples collected in August are distinguished as P081 and P082. Biplot del análisis de componentes principales (ACP) de los grupos funcionales del fitoplancton basado en la biomasa relativa (%) d*e las ocho muestras analizadas. El análisis se realizó utilizando la matriz de correlación. Las muestras se representan como puntos y los grupos funcionales como vectores de carga. Los dos primeros componentes principales explicaron el 49.2% de la varianza total (CP1=27.7%, CP2=21.5%). Las abreviaturas de las muestras consisten en la letra inicial del humedal correspondiente (P=Popovitsa RW; D=Dragoman RW), seguida del mes de muestreo expresado con dos dígitos (07=julio; 08=agosto; 09=septiembre; 10=octubre). En Popovitsa RW, las dos muestras recolectadas en agosto se distinguen como P081 y P082.*

wetlands (Pseudo-F=1.088, p=0.369). Instead, the ordination showed considerable variation among samples within each wetland.

DISCUSSION

Classical limnological models associate anoxygenic phototrophic sulfur bacteria assigned to FG V with pronounced redox gradients in stratified lakes (Reynolds et al., 2002, Padisák et al., 2009). In meromictic and seasonally stratified lakes, steep light and redox gradients generate finely stratified phototrophic communities, in which distinct microbial populations occupy narrow ecological niches around the chemocline (Rodrigo et al., 1999, 2000, Camacho et al., 2000). These communities exhibit pronounced vertical organization and diel migrations driven by changes in light availability, oxygen concentration, and sulfide distribution. Among phototrophic sulfur bacteria, *Chromatium* is considered particularly well adapted to such dynamic conditions, because active flagellar motility enables cells to rapidly track favourable combinations of light and sulfide, providing a competitive advantage over taxa that rely primarily on buoyancy regulation (Rodrigo et al., 1999, Camacho et al., 2000). This conceptual framework has long shaped the ecological interpretation of FG V in phytoplankton studies.

Recent studies, however, suggest that this classical ecological framework does not fully encompass the diversity of habitats occupied by phototrophic sulfur bacteria. An illustrative example is provided by Cabello-Yeves et al. (2025), who described a persistent bloom of a novel *Thiocapsa* species in a shallow gypsum-karst lake. During seasonal mixing, the population occupied the entire water column, whereas following the re-establishment of stratification it became restricted to the anoxic–microaerophilic layer. The authors attributed this remarkable ecological behaviour to the combined effects of sulfur-rich hydrogeochemistry and the exceptional metabolic versatility of the newly described species. These

findings indicate that phototrophic sulfur bacteria are capable of occupying a broader range of ecological settings than predicted by the classical model, although the underlying mechanisms may differ substantially among habitats.

The present study provides further evidence that anoxygenic phototrophic sulfur bacteria may represent a recurrent component of phytoplankton communities in shallow macrophyte-dominated wetlands. Although phototrophic sulfur bacteria have previously been reported from vegetated shallow lakes (Mentes et al., 2018, Somogyi et al., 2022), these observations have not been interpreted within the phytoplankton functional groups framework proposed by Reynolds et al. (2002) and refined by Padisák et al. (2009). In this context, our findings support a process-based interpretation of the habitat template of FG V, suggesting that the occurrence of suitable redox-related habitats depends primarily on local ecological conditions rather than on lake morphometry itself.

Macrophyte cover may provide the key local mechanism through which suitable conditions for FG V develop in shallow systems. Recent studies on macrophyte-dominated shallow systems (e.g., Lake Kolon and Lake Neusiedl) demonstrate that dense vegetation profoundly alters the physical and chemical environment by increasing dissolved organic carbon (DOC) and coloured dissolved organic matter (CDOM) concentrations, modifying the underwater light spectrum enriched in red and infrared radiation, and reducing oxygen availability (Mentes et al., 2018, Somogyi et al., 2022). These changes promote a shift from photoautotrophic to chemoorganotrophic processes and favour the development of photoheterotrophic and bacteriochlorophyll-containing bacteria (Somogyi et al., 2022). Although these systems lack classical vertical stratification, they exhibit pronounced spatial heterogeneity and the formation of localized anoxic and low-light microhabitats. Thus, gradients in shallow macrophyte-dominated systems may be structurally mediated and spatially compressed, yet functionally analogous to those in deeper stratified lakes (Mentes et al., 2018).

Extensive macrophyte cover in the studied semi-mountain and lowland wetlands, Dragoman RW and Popovitsa RW (65% and >80% cover, respectively), provides favourable conditions for the development of physical and chemical heterogeneity during the warm season, despite water depths not exceeding 1 m. High water temperatures (24.5-33.9 °C), elevated concentrations of organic matter (BOD up to 42.0 mg L-1), limited water mixing, and low dissolved oxygen levels (≤31% saturation) indicate the potential for reduced conditions and localized redox discontinuities. Such environmental conditions are known to favour the development of anoxygenic phototrophic sulfur bacteria (Kushkevych et al., 2021). Despite the contrasting geographical settings of the two wetlands, persistent populations of FG V, represented by *Chromatium okenii* and *Thiospirillum jenense*, were recorded in all samples. In half of the samples, FG V ranked among the dominant FGs, episodically reaching high relative biomass (29% in Dragoman RW and 73% in Popovitsa RW).

In the studied communities, FG V consistently co-occurred with oxygenic phytoplankton FGs characteristic of shallow systems with pronounced spatial heterogeneity (Borics et al., 2016). These co-occurring FGs exhibited complementary ecological strategies, including active motility (FGs W1, X2, Y), tolerance to reduced light conditions (FGs S1, TC), benthic-pelagic coupling (FG MP), and close association with macrophyte habitats (FG TC). Additional metabolic flexibility was provided by mixotrophic nutrition (FGs W1, Y) and by the capacity for oxygenic photosynthesis under sulfidic conditions, previously demonstrated for *Oscillatoria*, *Phormidium* and *Pseudanabaena*, representatives of FGs TC and MP (Cohen et al., 1975, Camacho & Vicente, 1998, Klatt et al., 2016, Lumian et al., 2021). Collectively, these complementary adaptive strategies may enable the community to respond rapidly to the mosaic of locally contrasting environmental conditions generated by dense macrophyte stands (Meerhoff & González-Sagrario, 2022).

The co-occurring phototrophic FGs also possess physiological adaptations that make them effective competitors for light under dense macrophyte cover. In vegetated shallow systems, the underwater light climate is substantially reduced and shifted towards longer wavelengths, favour-

ing anoxygenic phototrophic sulfur bacteria capable of utilizing red and near-infrared radiation (Somogyi et al., 2022). Nevertheless, filamentous cyanobacteria (FGs S1, TC) are well adapted to low-light environments owing to their filamentous morphology and complementary chromatic adaptation mediated by phycobiliproteins (Borics et al., 2012, Singh et al., 2015). Likewise, metaphytic diatoms (FG MP) efficiently exploit modified light spectra through the accessory pigment fucoxanthin. In addition, the mixotrophic capabilities of several motile FGs (W1, Y) reduce their dependence on photosynthesis by enabling the utilization of dissolved organic substrates (Salmaso & Padisák, 2007). The occurrence of colourless euglenoids (*Menoidium pellucidum*) further supports the ecological importance of facultative heterotrophic metabolism in these wetlands.

The recurrent pattern of co-occurring FGs observed in both wetlands suggests that these assemblages are unlikely to represent a random association. Rather, they may constitute a niche-specialized functional assemblage adapted to the highly heterogeneous environmental conditions characteristic of shallow macrophyte-dominated wetlands. Comparable niche partitioning has been described in stratified lakes, where sharp redox transitions occur over very small spatial scales (Gasol et al., 1991, Rodrigo et al., 1999, T-Krasznai et al., 2024). In shallow macrophyte-dominated wetlands, however, these gradients are more likely to be organized as a dynamic mosaic of localized microhabitats rather than as a single persistent vertical chemocline.

This ecological interpretation is further supported by the multivariate analyses, which indicated no statistically significant differences in functional group composition between the two wetlands despite their contrasting geographical settings. Principal Component Analysis (PCA) distinguished three major assemblages. The first assemblage (FGs V, W1, S1, TC and LO) may represent a shared functional response to shallow, macrophyte-dominated, organic-rich and weakly mixed conditions common to both wetlands. In contrast, the second (FGs C, MP, X2) and third (FGs K, Y) assemblages appear to reflect habitat-specific characteristics of Popovitsa RW and Dragoman RW, respectively. Importantly, none of the measured environmental variables explained the observed functional organization of the community. This finding suggests that community structure is more likely shaped by the combined influence of multiple interacting processes operating throughout the growing season across fine spatial and temporal scales. Given the limited number of samples, however, these multivariate analyses should be regarded as exploratory and interpreted with appropriate caution.

The ecological interpretation proposed here should be considered in light of several limitations. This study is based on a limited number of sites and a single growing season, and no targeted microbiological or molecular analyses of anoxygenic phototrophic bacterial communities were performed. Furthermore, the applied sampling design does not resolve fine-scale spatial gradients within the studied wetlands, and no measurements of redox potential or reduced sulfur compounds (e.g., sulfide) were available. Nevertheless, our interpretation is consistent with previous studies conducted in both shallow macrophyte-dominated wetlands (Mentes et al., 2018, Somogyi et al., 2022) and stratified lakes (T-Krasznai et al., 2024), where redox-related conditions were likewise inferred indirectly from oxygen availability, organic carbon dynamics, and microbial community composition. Future studies combining phytoplankton functional group analyses with high-resolution measurements of underwater light climate, redox conditions, sulfide concentrations, and microbial community composition will be essential for testing and refining the conceptual framework proposed here.

The present study highlights the ecological importance of shallow riparian wetlands for understanding phytoplankton functional diversity under low-oxygen conditions. Such systems remain underrepresented in monitoring programmes, yet they may support complex functional assemblages shaped by macrophyte influence and spatially heterogeneous environmental conditions. The recurrent seasonal occurrence of FG V observed in this study suggests that anoxygenic phototrophic sulfur bacteria may represent an under-recognized functional component of primary production and biogeochemical cycling in wetland ecosystems.

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DATA AVAILABILITY

The datasets (in Excel format) generated and analysed during the current study, including phytoplankton taxa lists and functional group composition data, are available in the Zenodo repository: https://doi.org/10.5281/zenodo.19133740

AUTHOR CONTRIBUTIONS

D.B.: Conceptualization, Data curation, Formal analysis, Writing – original draft; M.B.: Data curation, Formal analysis, Writing – review & editing; M.I.: Investigation, Visualization; M.S.: Investigation, Visualization, Supervision, Validation.

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