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Diversity and ecology of benthic diatoms in a mire with narrow pH gradient

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Abstract: Mires are specific freshwater habitats with great importance for global biodiversity. The present study investigated diatoms in different habitats of a Don Močvar mire and their relationship with environmental parameters, as well as the use of diatom indicator values and the German Red List for conservation purposes. The study was conducted monthly from January to November 2012. Diatoms were sampled simultaneously with water for physical and chemical properties in three different habitats. A total of 50 diatom taxa were identified. Based on their relative abundance and diversity, habitat—related environmental factors shaped the diatom community when seasonal dynamics were less important. Multivariate analysis showed that chemical oxygen demand (COD) and conductivity were the most significant environmental variables describing diatom communities. The indicator values characterized the diatoms as a typical mire community and proved to be a reliable starting point for future monitoring and management. Diatom classification by German Red List revealed a dominance of species with priority for conservation, suggesting that the Don Močvar should have conservation status. This study brings a better understanding of the response of diatoms to living in specific habitats and the basis for future studies on ecological characteristics of new or rare taxa.

Key words: algal flora, Bacillariophyceae, COD, community structure, conductivity, Đon Močvar, peatlands, pond, temporal dynamics

Introduction

Peatlands are special amphibious ecosystems characteristic of Europe, North America and Asia, where organic matter accumulates due to waterlogging and often poorly aerated conditions (RYDIN & JEGLUM 2013). They are a dominant landscape in the boreal zone of the Northern Hemisphere, while in Southeastern Europe they form small isolated habitats (Topić & Stančić 2006). Peatlands are classified into a few categories depending on their connection to groundwater, acidity, flooding, and vegetation cover (Rubec 2018). The gradient from bog through fen and swamp to marsh ranges from acidic to alkaline in terms of pH, from oligotrophic to eutrophic in terms of nutrients, and from ombrogenous to limnogenous in terms of hydrological regime. Due to the gradient, overlap is possible, resulting in habitat complexity even in a very small area (Shotyk, 1988). This has a strong influence on environmental conditions and on the biotic community (Gaiser & Ruhland 2010).

A particularly important living component in

mires is diatoms, and similar to habitat distribution, they are studied more in Central and Northern Europe (Poulíčková et al. 2004; Nováková 2005; Kulikovskiy 2009; Poulíčková et al. 2013a; Poulíčková et al. 2013b) than in Southeastern Europe (Klemenčič et al. 2010; Kapetanović et al. 2011; Vidaković et al. 2016).

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For diatoms, peatlands can be considered an extreme environment (GAISER & RUHLAND 2010). Recent studies have shown that diatom assemblages in peatlands with a wide acid range are primarily defined by pH (POULÍČKOVÁ et al. 2013b) and that the chemical gradient, together with moisture, influences the decrease in species richness and diversity from fen to bog (POULÍČKOVÁ et al. 2004). Nevertheless, pH was found to be less important in habitats with a short and uniform acid range, where other environmental factors are more important (BORICS et al. 2003). In addition to the physical and chemical properties of water, shading also reduces diatom diversity in peatlands (Nováková 2005), as they are autotrophs whose community structure depends on the photosynthetic rate, just as in other

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autotrophs (HULL 2008).

Diatoms occur in almost all aquatic habitats and are sensitive to light, moisture conditions, temperature, water velocity, salinity, pH, conductivity, oxygen and nutrients, which is why they have become one of the most important indicators of water quality, climate change, eutrophication and acidification (Passy 2006; SMOL & STOERMER 2010). Diatom ecology, particularly species-level ecological preferences, are best classified as indicator values in VAN DAM et al. (1994). This classification has recently been successfully used to assess anthropogenic influence and natural succession of aquatic habitats around the world, from mountain tops to Mediterranean streams (LAI et al. 2020; SALINAS-CAMARILLO et al. 2020). In addition to indicator taxa, German Red List (HOFMANN et al. 2018) is a widely accepted classification of diatoms for the protection of species and habitats.

Don Močvar has shown strong vegetation succession in a period of 100 years (Pevalek 1925; Alegro & Šegota 2008), and being one of the largest mires in Southeast Europe, it aroused the interest of scientists for studies of the biota and its conservation (Brigić et al. 2014; Vilenica et al. 2016; Brigić et al. 2017a; Brigić et al. 2017b).

In light of previous studies of diatoms in peatlands, we hypothesised that pH is not the primary environmental parameter affecting the diatom community due to the short acid gradient, and that diatoms can be used for future management and protection of the area. Therefore, in this study we systematically investigated the physical and chemical properties of water and diatoms in different aquatic habitats of the study area. The main objectives were: determination of seasonal diatom composition and in different habitats, description of environmental factors affecting diatom composition and diversity, testing VAN DAM et al. (1994) indicator taxa and recognizing threatened taxa according to the German Red List (HOFMANN et al. 2018) for conservation and management purposes.

MATERIALS AND METHODS

Study area. Don Močvar is the oldest and largest bog in Croatia (ALEGRO & ŠEGOTA 2008; ALEGRO & TOPIĆ 2017). The first description by PEVALEK (1925) states that its area was 40 ha at the beginning of the 20th century, but in recent times its area has been reduced to only 10 ha due to overgrowth of surrounding vegetation (ALEGRO & ŠEGOTA 2008; ALEGRO & TOPIĆ 2017). Don Močvar is located in central Croatia at an altitude of 130 m a.s.l. (Fig. 1). The silicate pebbles as the predominant geological substrate are impermeable to water and therefore a perfect place for the formation of mire, which require a constant and large water supply from underground springs and surface streams. The mire is mosaic of *Sphagnum* stands (dominated by *Sphagnum capillifolium* (Ehrh.) Hedw. and *Sphagnum palustre*

Table 1. Minimum, maximum and median values of environmental variables at all three sampling sites in Đon Močvar over the study period from January to November 2012. Abbraviations: (TN) total nitrogen; (SRP) soluble reactive phosphorus; (TP) total phosphorus; (COD–Cr) chemical oxygen demand; (TOC) total organic carbon; (SiO₂) silicates.

	Deep	Hollow	Rhynchosi	oretum stan	d	Pond		
Env. Variable	Min-Max	Median	Min-Max	Median	Min-Max	Median		
Temperature (°C)	7–12.8	11.3	0-33.8	13.4	0–23.6	10.7		
pH	5.60-6.18	5.71	5.62-6.49	6.23	5.55-6.20	6.05		
Conductivity (µS.cm ⁻¹)	15–38	21	25–55	37	17–51	35.5		
Alkalinity (mgCaCO ₃ .l ⁻¹)	8–19	11.5	7–26	15	8.5-32.0	16.5		
Oxygen $(mgO_2.1^{-1})$	5.7-8.2	6.6	5.8-10.1	7.8	1.7–10.9	6.1		
Saturation (%)	49.5–72.4	57.5	61.1–102.8	78.6	18.0-69.0	57.2		
Ammonia ($\mu g N.l^{-1}$)	5–84	33	5-1786	16	5-80	11		
Nitrites ($\mu g N.1^{-1}$)	1–4	3	1–220	3	1–3	1		
Nitrates ($\mu g N.l^{-1}$)	290–710	530	50-3010	580	50-50	50		
$TN \left(\mu g N.l^{-1}\right)$	650-3840	740	470–7003	1100	330-14960	650		
$SRP\left(\mu gP.l^{-l}\right)$	3–7	3	3–228	3	3–13	3		
$TP\left(\mu gP.l^{-1}\right)$	5–73	17	5-480	17	5-60	28		
$CODCr\ (mgO_2.l^{-1})$	1.6–7.5	3.8	1.1-8.6	4.5	9.6–54.9	20.5		
TOC (mg.l ⁻¹)	1.2-3.58	1.7	1.0-7.1	3.2	6.2–22.5	12		
SiO ₂ (mg.l ⁻¹)	6.6–9.3	7.7	2.0-9.3	7.7	6.6–13–3	7.8		

L. and six other species with lower abundances), stands of *Rhynchospora alba* (L.) Vahl, *Eriophorum latifolium* Hoppe and *Carex lasiocarpa* Ehrh., small water ponds with helophytes and stands of *Phragmites australis* (Cav.) Trin. ex Steud. and *Carex acutiformis* Ehrh. on the edges. The surrounding area is currently protected as Botanical Reserve since 1963 and included in the Natura 2000.

Three habitats as sampling sites and sampling through all seasons were chosen to cover as much surface water habitats as possible within the mire Don Močvar, so that the link of seasonal changes of environmental factors and diatom composition could be established. Those habitats are named: Deep Hollow (DH), Rhynchosporetum stand (R) and Pond (P) (Fig. 1). Sampling site DH, located in the southwest of the area (45°19'3.86"N, 15°54'21.51"E), is a vertical hole in the shape of a cylinder with a diameter of 30 cm and a depth of 150 cm, filled with water. It is surrounded by E. latifolium, Carex echinata Murray and C. lasiocarpa which overgrows the edge of the hole and whose dead leaves hang into the water. Sampling site R, in the south of Đon Močvar (45°19'2.806"N, 15°54' 29.531"E), is mosaic of shallow ponds with water up to 5 cm deep, spread between carpets of R. alba. In the eastern part (45°19'4.08"N, 15°54'33.37"E), there are several small ponds of a few square metres. This area is overgrown by sedges (mainly C. lasiocarpa and C. acutiformis), and of very edges of pools with Typha latifolia L. The third sampling site P was chosen in one of the ponds 50 cm deep. Sampling sites R and P can dry out during the hot and dry summer periods and regularly freeze over in winter, while sampling site DH has a constant water level throughout the year and never freezes over.

Sampling and sample analysis. Sampling was conducted from January till November 2012, except for September. Ten samples were collected at sampling site DH, while seven samples were collected at sampling sites R and P due to the thick ice cover and completely frozen water in January, respectively, and the dry period in July and August.

Diatoms were collected from available substrates: at sampling sites DH and P they were collected from submerged plants (*Carex lasiocarpa* and *Typha latifolia*), while at sampling site R the surface of soft sediment was collected. Samples were stored in plastic bottles and preserved with ethanol to a final solution of 20%. Subsamples were collected and all organic compounds were removed from the small volume of the subsample by acid digestion with KMnO₄/HCl. The excess of acid was rinsed by centrifugation with the addition

of distilled water and disposal of the supernatant until a neutral pH was reached. Diatom slides were prepared from the cleaned material and mounted in Naphrax®. The slides were analysed using a Nikon E–80i light microscope. A total of 400 diatom valves were identified and counted for relative abundance. Light microscopic images were captured using a Nikon Coolpix 600 digital camera. Identification was based on the relevant identification keys (Lange–Bertalot & Moser 1994; Krammer 2000; Lange–Bertalot 2001; Lange–Bertalot et al. 2011). Taxa names were updated according to the Algebase (Guiry & Guiry 2021).

Samples for water chemistry were collected at the same time as the diatoms and stored in special bottles at 4 °C until analysis, which was performed in the laboratory. Water temperature was measured in situ with a digital thermometer. Conductivity and pH were measured using electrodes of SevenMulti Modular Meter System (Mettler Toledo). Oxygen, alkalinity and chemical oxygen demand (COD) were analysed according to APHA (2005). Soluble reactive phosphorus (SRP), total phosphorus (TP) and silicates (SiO₂) were determined spectrophotometrically using a Perkin Elmer Lambda UV–VIS spectrometer. Ammonia (NH₄⁺–N), nitrites (NO₂⁻–N) and nitrates (NO₃⁻–N) were analysed using Dionex 3000 ion chromatograph. Total nitrogen (TN) and total organic carbon (TOC) were analysed using a Shimadzu TOC–VCPH equipped with an analyser for TN and TOC.

Data analysis. Alpha diversity was analysed using species richness and Shannon diversity index (H'). Beta diversity among diatom communities was analysed with Bray–Curtis similarity using one–way SIMPER analysis to examine dissimilarities within diatom communities among habitats and seasons. ANOSIM analysis was used to test the significance of habitat and seasonal differences in relative abundance of diatoms. Seasonal and spatial changes in environmental factors and diatom composition were analysed using PERMANOVA with two factors, months and habitats. These analyses were performed in Primer 6 software (Clarke & Gorley 2006). Diatoms were classified according to the German Red List (HOFMANN et al. 2018) and ecological indicator values (VAN DAM et al. 1994). Data were presented as a proportion of relative abundance in samples using Grapher (2019).

The Spearman correlation coefficient was used to examine correlations between relative abundance of indicator taxa according to VAN DAM et al. (1994) and environmental variables using IBM SPSS Statistics (IBM CORP. RELEASED 2013).

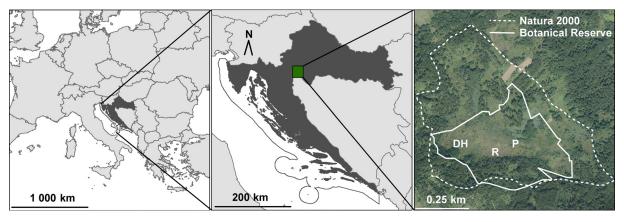


Fig 1. Location of the Don Močvar in Europe and Croatia with respective sampling sites Deep Hollow (DH), *Rhynchosporetum* stand (R) and Pond (P).

Detrended Correspondence Analysis (DCA) was used to identify and measure the relationships between diatoms and environmental variables. Analysis was conducted in Canoco 5 (TER Braak & ŠMILAUER 2012) on log transformed and centred relative abundance of diatom taxa and normalised environmental variables. Environmental variables that were autocorrelated were excluded from the analysis. Three sampling sites were coded as dummy variables to represent any differences between three different sampling sites that were not captured by the environmental variables. Results are presented as a triplot in which diatom taxa, samples, and environmental variables are plotted together.

RESULTS

Physical and chemical properties of water

The minimum, maximum and median values of physical and chemical properties of water are given in Table 1. Ice cover in the winter and drying out in the summer had an effect of wide range of water temperature during the study period at sampling sites R and P. On the contrary, sampling site DH had stable water temperature and a narrow range throughout the study period. The water was acidic at all three sampling sites with narrow pH gradient. Conductivity and alkalinity were low at all sampling sites, indicating soft water and low ion concentration. Oxygen concentration and saturation were lowest at sampling site P and highest at sampling site R, while it was quite stable at sampling site DH, like most parameters. Extreme values of nutrients were measured on different sampling sites, such as total phosphorus, ammonia and nitrates at sampling site R as well as extreme concentrations of total nitrogen at sampling sites P and R. Sampling site P was rich in organic matter (high COD, high TOC) compared to the other two sampling sites.

Floristic composition and diversity of diatoms

In an eleven-month study, a total of 50 diatom taxa were identified in 24 samples (Table 2) and typical ones are shown on Fig. 2. Sampling site DH was poorest in number of taxa (19), and sampling site P was richest (39), while sampling site R was intermediate (28). Two taxa were planktonic species, where Pantocsekiella costei was identified at sampling site P, while Aulacoseira alpigena (Fig. 2/1) was identified at sampling sites P and DH. All other taxa were benthic. A group of taxa that was present at all sampling sites consisted of Brachysira liliana (Fig. 2/14) (DH, R), Eunotia subarcuatoides (Fig. 2/7) (DH), Frustulia crassinervia (Fig. 2/22) (DH, P), Tabellaria flocculosa (Fig. 2/24), Chamaepinnularia mediocris, Eunotia nymanniana (Fig. 2/9), Pinnularia sinistra, P. subcapitata and P. viridiformis. The first three taxa can be considered dominant for Đon Močvar in general, due to high relative abundance at one or two sampling sites (indicated in parentheses). Taxa Achnanthidium neocryptocephalum, Encyonopsis cesatii, Eunotia spp. and Kurtkrammeira neoamphioxys (Fig. 2/13) were subdominant at sampling site R, while *Eunotia bilunaris* (Fig. 2/12), *E. exigua* (Fig. 2/6) and *T. flocculosa* (Fig. 2/24) were subdominant at sampling site P. Almost half of the taxa were found at single sites (44%). Those that were also found with a relative abundance of <5% and in only one or two samples are classified as rare. These are *Encyonema rostratum* and *Pinnularia microstauron* (Fig. 2/20) at sampling site DH, *Gomphonema micropus* and *Nitzschia pura* at sampling site R, and *Eunotia minor*, *Navicula viridula*, *Neidium bisulcatum* (Fig. 2/21), *Nitzschia alpina*, *P. costei*, *Pinnularia frequentis*, *P. gibba*, *P. rupestris* (Fig. 2/19), *Placoneis hambergii* and *Stauroneis gracilis* at sampling site P.

Alpha diversity, based on species richness (S) and Shannon diversity index (H'), differed on a temporal and spatial scale (Fig. 3). Both were lowest at sampling site DH and highest at sampling site P. The highest values at sampling sites R and P were in spring and shortly after the dry period in October and November, while at sampling site DH the highest values occurred at the beginning of spring with a sharp decline towards summer that lasted until the end of the study period.

According to Spearman correlation coefficient, both species richness and Shannon diversity index were significantly higher with high organic matter content (COD, TOC). Species richness decreased with lower nitrates, while conductivity also had a significant positive effect on Shannon diversity index. Other environmental parameters showed no significant correlation with species richness and Shannon diversity index.

To further test the seasonality of diatoms, a one—way SIMPER analysis was performed on relative abundance of all taxa. Bray-Curtis similarity showed low similarity between samples from the three different habitats (DH, R and P) in each month. The lowest similarity was 23.9% in October and the highest was 38.3% in February. ANOSIM analysis of seasonality showed that it had no significant effect on diatom composition (R = -0.373, p = 1.00, p > 0.05). On the spatial scale, the Bray–Curtis similarity after performing one-way SIMPER analysis, showed high similarity among samples within a sampling site, but also high dissimilarity among samples between different sampling sites, indicating high beta diversity. Analysis showed 68.5% similarity between samples at sampling site DH, 70.3% similarity at sampling site R, and 68.3% similarity at sampling site P. The descriptive taxa that contributed most to the > 5% similarity within sampling site DH were Frustulia crassinervia, Brachysira *liliana* and *Eunotia subarcuatoides*. At sampling site R these were B. liliana, Achnanthidium neocryptocephalum, E. subarcuatoides, Encyonopsis cesatii and Tabellaria flocculosa, while F. crassinervia, T. flocculosa, Eunotia exigua, Eunotia bilunaris and Encyonema lunatum contributed most to similarity at sampling site P. Sampling site P had the highest average dissimilarity with other sampling sites, 75.6% with sampling site R and 76.9% with sampling site DH. The average dissimilarity between sampling sites R and DH was 57.2%. ANOSIM

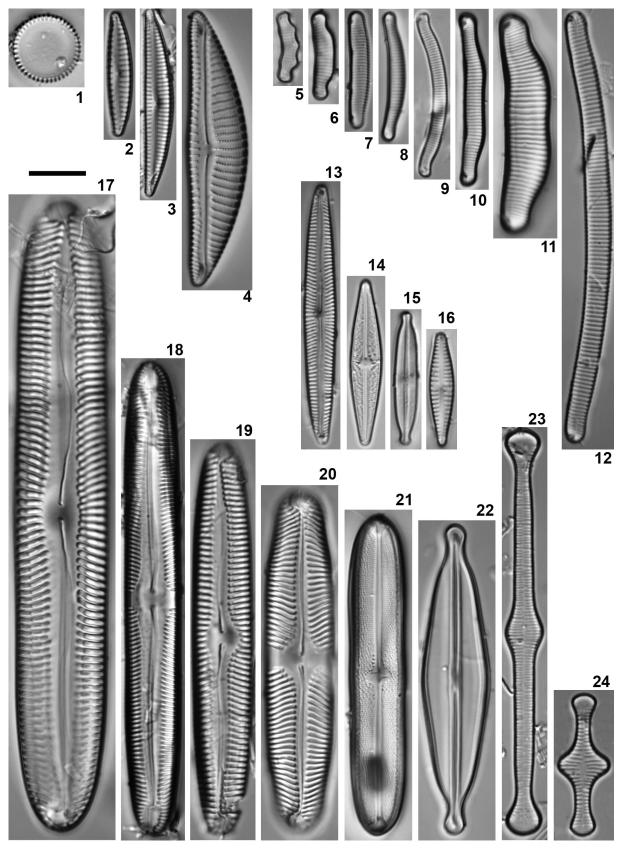


Fig. 2. LM micrographs of characteristic diatoms from Don Močvar: (1) Aulacoseira alpigena; (2) Encyonema perpusillum; (3) Encyonema lunatum; (4) Encyonema vulgare; (5) Eunotia paratridentula; (6) Eunotia exigua; (7) Eunotia subarcuatoides; (8) Eunotia pseudogroenlandica; (9) Eunotia nymanniana; (10) Eunotia implicata; (11) Eunotia islandica; (12) Eunotia bilunaris; (13) Kurtkrammeria neoamphioxys; (14) Brachysira liliana; (15) Kobayasiella parasubtilissima; (16) Gomphonema exilissimum; (17) Pinnularia substreptoraphe; (18) Pinnularia stomatophora; (19) Pinnularia rupestris; (20) Pinnularia microstauron; (21) Neidium bisulcatum; (22) Frustulia crassinervia; (23) Tabellaria fenestrata; (24) Tabellaria flocculosa. Scale bar 10 μm.

analysis of habitats showed that they were significantly different based on diatom composition (R = 0.994, p = 0.001, p < 0.05).

PERMANOVA results showed no significant influence of seasonal changes when months were used as a factor for environmental data and diatom composition (p = 0.2978 and p = 0.9998; p > 0.05). Both environmental factors and diatom composition were significantly influenced by the habitat factor (p = 0.0001 and p = 0.0001; p < 0.05).

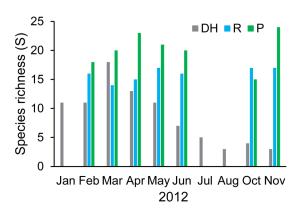
Environmental relations and diatoms

The ordination results of the diatom composition and environmental data of the DCA are shown on the ordination plot (Fig. 4). The environmental parameters were selected according to the correlation ranks, so that only one parameter was kept from the highly correlated parameters (Table 3). The excluded environmental parameters were as follows: dissolved oxygen, nitrites, soluble reactive phosphorus, total phosphorus, and total organic carbon. The eigenvalues of the first two axes are 0.872 and 0.740, respectively, and explained 52.8% of the variance of the diatom composition and environmental data. Axis 1 had the highest correlation with COD (R = 0.756), while axis 2 had the highest correlation with conductivity (R = 0.892).

The results again showed a clear separation of the sampling sites as three different habitats with individual characteristics and specific diatom community. The diatom community at sampling site DH was characterised by low pH, low conductivity and COD, while sampling site P had the highest level of organic matter (COD) and the highest alkalinity. Sampling site R was the warmest, the most oxygenated and the most nutrient-rich. The position of the three dominant taxa on DCA plot is consistent with the results of the SIMPER analysis of diatom composition and habitat factor as well as the data on environmental variables at the individual sampling sites (Table 1). Of the three dominant taxa, Brachysira liliana was dominant at sampling sites DH and R and in comparison to other two, tolerated the widest range of pH (5.60 - 6.49), temperature, and nutrient status. *Eunotia* subarcuatoides preferred stable conditions with colder water, the lowest pH, conductivity, and most nutrients at sampling site DH, while Frustulia crassinervia tolerated a wide range of organic matter, low pH, conductivity, and nutrients as being dominant at sampling site P.

Indicator values

Indicator values according to Van Dam et al. (1994) were applied to the relative abundance of diatoms (Fig. 5). Acidobiontic and acidophilic taxa were represented by only 50% of the total number of taxa, yet they were most abundant at all sampling sites (Fig. 5a). The most diverse proportion of relative abundance of pH classes was at sampling site P. Acidobiontic taxa were significantly more abundant at lower pH (Spearman correlation coefficient, $\rho = -0.451$, N = 24, $p \le 0.05$), while taxa



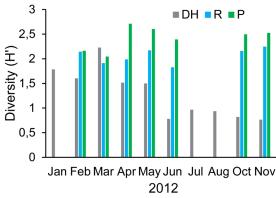


Fig. 3. Species richness (S) and Shannon diversity index (H') in Đon Močvar at sampling sites Deep Hollow (DH), *Rhynchosporetum* stand (R) and Pond (P) from January to November 2012.

from other categories had no statistically significant correlation with pH.

Apart from being the most represented in terms of number of taxa, aquatic to aerophilic taxa were most abundant at all sampling sites, especially at sampling sites DH and P (Fig. 5b). Sampling site R was characterized by the highest proportion of unclassified (unknown) taxa and the almost exclusive presence of occasionally aerophilic taxa. The latter showed a strong positive correlation to pH, conductivity and saturation (Spearman correlation coefficient, $\rho = 0.567$, $\rho = 0.557$ and $\rho = 0.533$, N = 24, $p \le 0.01$). Aquatic taxa preferred water with lower alkalinity (Spearman correlation coefficient, $\rho = -0.498$, N = 24, $p \le 0.05$) and aquatic to aerophilic taxa preferred water with lower conductivity (Spearman correlation coefficient, $\rho = -0.452$, N = 24, $p \le 0.05$).

The oxygen indicator values showed that most taxa were in the polyoxybiontic category and with their highest relative abundance (Fig. 5c). Only taxa with unknown oxygen preferences had a positive correlation with oxygen concentration (Spearman correlation coefficient, $\rho=0.502,\,N=24,\,p\leq0.05)$ and a very positive correlation with saturation (Spearman correlation coefficient, $\rho=0.654,\,N=24,\,p\leq0.01).$ Oxybiontic and moderate O_2 taxa preferred water rich in organic matter and showed a significant positive correlation with COD (Spearman correlation coefficient, $\rho=0.699,\,N=24,\,p\leq0.01$ and $\rho=0.438,\,N=24,\,p\leq0.05,$ respectively).

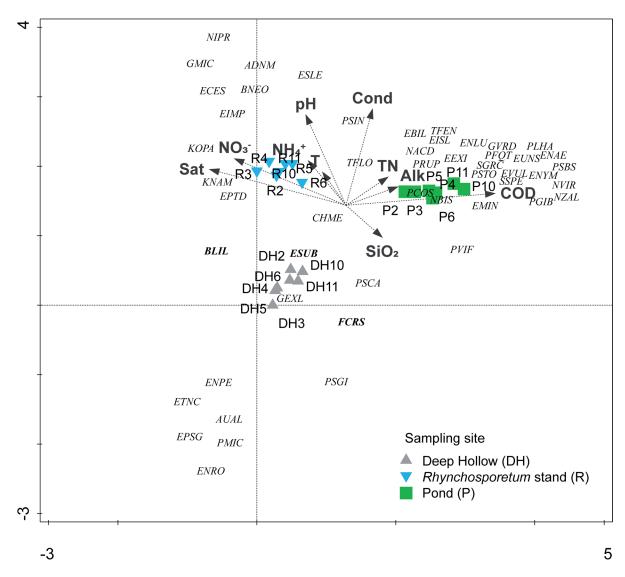


Fig. 4. DCA triplot showing environmental data, taxa, and samples from three different sampling sites. Dominant taxa are shown in bold letters. Diatom codes are the Omnidia codes (OC) listed in Table 2. Abbreviations: (T) temperature; (pH) pH; (Cond) conductivity; (Sat) saturation; (Alk) alkalinity; (NO_3^-) nitrates; (NH_4^+) ammonia; (SiO_2) silicates; (COD) chemical oxygen demand.

Most taxa were classified as oligosaprobic and dominated at sampling site DH, while sampling sites R and P had a higher proportion of beta-mesosaprobic taxa in addition to oligosaprobic (Fig. 5d). Beta-mesosaprobic and alpha-mesosaprobic taxa were significantly more abundant in waters with higher organic matter where they showed a strong positive correlation (Spearman correlation coefficient, $\rho = 0.674$, N = 24, p \leq 0.01 and ρ = 0.704, N = 24, p \leq 0.01, respectively), while oligosaprobic taxa showed no significant correlation with COD. Taxa tolerant of very low concentrations of organically bound nitrogen (21 sensitive N-autotrophic taxa) dominated with number of taxa and abundance at all sampling sites (Fig. 5e). No significant correlation was found between sensitive or tolerant N-autotrophic taxa and nitrogen compounds.

Based on the trophic categories of VAN DAM et al. (1994), trophic status is high at all sampling sites (Fig. 5f). Oligotrophic taxa are most abundant and represented

by the greatest number of representatives at all sampling sites, especially at sampling site P. Oligo–mesotrophic and mesotrophic taxa were represented with low relative abundance at sampling sites DH and R, while lower trophic categories were rare or absent. No significant correlation with phosphorus or nitrogen concentration was found for any of the trophic categories. On the other hand, oligo–mesotrophic and mesotrophic taxa showed positive correlation with conductivity (Spearman correlation coefficient, $\rho=0.659,\,N=24,\,p\leq0.01$ and $\rho=0.427,\,N=24,\,p\leq0.05$) and COD (Spearman correlation coefficient, $\rho=0.525$ and $\rho=0.625,\,N=24,\,p\leq0.01$). Oligotrophic taxa were significantly more abundant at lower conductivity (Spearman correlation coefficient, $\rho=-0.631,\,N=24,\,p\leq0.01$).

Conservation status of diatoms

Analysis of German Red List (HOFMANN et al. 2018) revealed different proportions of diatoms at three sampling

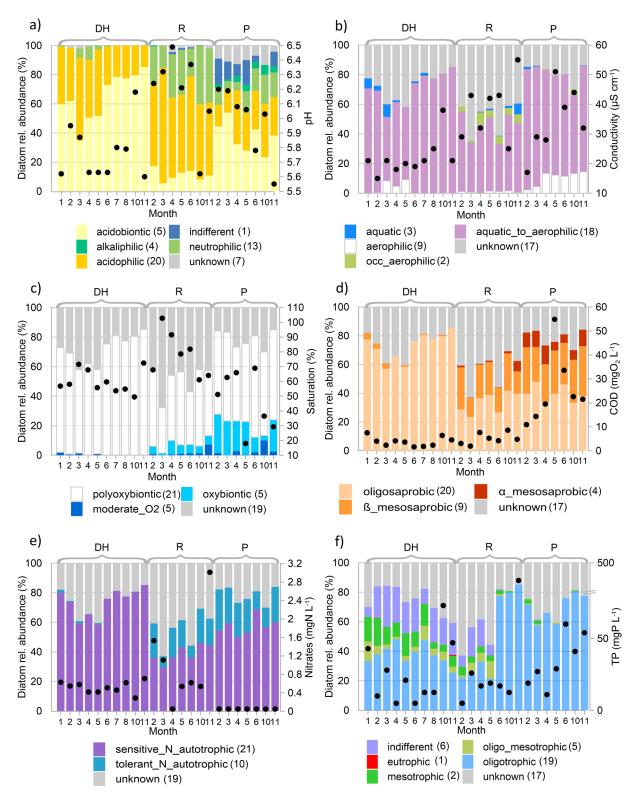


Fig. 5. Proportion of relative abundance of diatoms classified according to the indicators of Van Dam et al. (1994) in Đon Močvar at sampling sites Deep Hollow (DH), *Rhynchosporetum* stand (R) and Pond (P) from January to November 2012 together with relevant environmental parameters marked with black dots. (a) pH preferences with pH; (b) moisture with conductivity; (c) oxygen requirements with saturation; (d) saprobity with chemical oxygen demand; (e) nitrogen uptake with nitrates and (f) trophic state with total phosphorus. Numbers in brackets indicate number of taxa classified to a certain category.

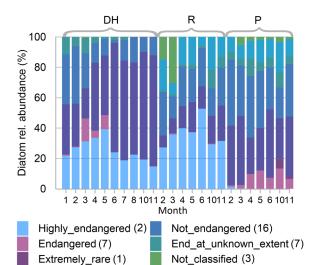


Fig. 6. Proportion of relative abundance of diatoms classified according to the German Red List (HOFMANN et al., 2018) in Đon Močvar at sampling sites Deep Hollow (DH), *Rhynchosporetum* stand (R) and Pond (P) from January to November 2012.

Unsufficient_data (5)

sites depending on the conservation status (Fig. 6). Data were most consistent at sampling site DH, where near threatened was indicated as the most abundant. Only two taxa representing the highly endangered category were abundant at sampling sites DH and R, with proportions up to 50%, while endangered taxa were most abundant at sampling sites DH and P.

DISCUSSION

Near_threatened (9)

This paper presents new data on the diversity, different habitat and seasonal composition of diatoms, and detailed environmental factors shaping the community in the Đon Močvar. In this study we covered different habitats within one mire to get more reliable view on those aspects (Szigyártó et al. 2017). For future management and conservation of this area, VAN DAM et al. (1994) indicator taxa and German Red List (HOFMANN et al. 2018) are used and their importance explained. Earlier studies of mires in this area were focused more on listing species of various autotrophs (desmids, bryophytes and angiosperms) (PEVALEK 1925). More recently, animals such as beetles and ants (BRIGIĆ et al. 2017b), terrestrial isopods (Brigić et al. 2017a), and mayflies (VILENICA et al. 2016), have been the focus of interest, while algae and detailed water chemistry have remained unexplored.

Characteristics of the habitat

Previous biological studies classified Don Močvar as a peat bog (Brigić et al. 2014; VILENICA et al. 2016; Brigić et al. 2017a; Brigić et al. 2017b), but these studies did not analyse detailed water chemistry to support the classification. In this study, water chemistry was analysed in

three very different habitats selected for a better overview of diatoms, thus providing three specific sets of environmental conditions. According to the pH gradient of 5.5 to 6.5 and sporadically high nutrient concentration (Table 1, Fig. 5), Don Močvar occasionally shows characteristics of bog, fen and marsh (RYDIN & JEGLUM 2013). The low alkalinity supports the characteristics of a bog, suggesting that there is no connectivity of surface pools with groundwater (BENDELL-YOUNG 2003). The results of high silica concentration indicate a possible influence of groundwater (SHOTYK 1988). The constant water level, the temperature very similar to the average temperature of the area of 10.3 °C (Mesić 2000), and the low saturation indicate that the DH sampling site is filled with groundwater. Based on the entire dataset and the confirmed groundwater at the DH sampling site, the results of this study indicate that the majority of features classify Don Močvar as a mire.

The source of the higher nutrient concentration at the DH sampling site, as well as at other sampling sites, may be from agricultural land in the watershed or the excrement of a permanently present wild boar. As mires are wetlands whose soils and water consist almost entirely of organic matter derived from the remains of dead and decomposing plant material (CRAFT 2016), this is consistent with our measurements of high levels of COD and TOC. This is particularly emphasised at sampling site P, where additional plant material consisting of filamentous charophyte algae and helophytes is much more decomposed in contrast to the other two sampled habitats. The lower oxygen concentration and saturation reflect this higher organic load at sampling site P (VIESSMAN et al. 2009).

Community structure and diversity

Novel studies of diatoms in peatlands, their diversity and ecological preferences are of utmost importance as there are still unresolved questions as to what are the driving environmental factors for diatoms in different peatland types, which can serve as a starting point for assessing anthropogenic influence or natural habitat succession (Gaiser & Ruhland 2010). The results of this study of generally low diatom diversity are consistent with those of previous studies on diatoms (CANTONATI et al. 2011) and desmids (ŠTĚPÁNKOVÁ et al. 2008; ŠTĚPÁNKOVÁ et al. 2012) in similar habitats. Higher species richness and diversity were often found only in studies that were large either in terms of study area, number of sampling sites or habitats (Borics et al. 2003; Kulikovskiy 2009; VIDAKOVIĆ et al. 2016). Among the prominent dominant benthic taxa, only two typical planktonic taxa were identified in habitats P and DH, which appear to be deep enough to support a planktonic community. Aulacoseira alpigena was found at both sites, especially at the DH sampling site in high 60% of the samples. Most studies, such as Turkia & Lepistö (1999) and Dunck et al. (2012) classify it as a planktonic species, even when it is found on sediments (CHEN et al. 2012). According to

Table 2. List of diatoms with their Omnidia code (OC), frequency of occurrence (Freq.) in samples, minimum (Min) and maximum (Max) relative abundance at all three sampling sites in Đon Močvar from January to November 2012.

		Deep Hollow				Rhyn	chospo stand	retum	Pond		
No.	Taxon	ОС	Freq.	Min	Max	Freq.	Min	Max	Freq.	Min	Max
1.	Achnanthidium neocryptocepha- lum (Grunow) Novais et Van de Vijver	ADNM				100%	6.0	21.5	14%	0.8	0.8
2.	Aulacoseira alpigena (Grunow) Krammer	AUAL	60%	1.3	8.5				14%	0.5	0.5
3.	Brachysira liliana Lange–Bertalot	BLIL	100%	14.8	39.3	100%	26.0	51.8	14%	1.3	1.3
4.	Brachysira neoexilis Lange–Bertalot	BNEO				100%	1.3	8.0			
5.	Chamaepinnularia mediocris (Krasske) Lange–Bertalot et Krammer	CHME	30%	0.8	1.5	71%	0.5	1.5	57%	0.3	3.3
6.	Encyonema lunatum (W. Smith) Van Heurck	ENLU				43%	0.3	2.0	100%	2.0	8.8
7.	Encyonema perpusilum (A. Cleve) D.G. Mann	ENPE	40%	0.5	2.8	14%	0.5	0.5			
8.	Encyonema rostratum Krammer	ENRO	10%	0.5	0.5						
9.	Encyonema silesiacum (Bleisch) D.G. Mann	ESLE				71%	0.3	7.3	14%	1.5	1.5
10.	Encyonema vulgare Krammer	EVUL							100%	1.0	6.7
11.	Encyonopsis cesatii (Rabenhorst) Krammer	ECES				100%	2.8	15.5			
12.	Eunotia bilunaris (Ehrenberg) Schaarschmidt	EBIL				57%	0.3	1.5	86%	4.3	17.5
13.	Eunotia exigua (Brébisson ex Kützing) Rabenhorst	EEXI				29%	0.3	1.3	100%	3.3	12.5
14.	Eunotia girdle view	EIMP				29%	14.8	30.5	14%	1.0	1.0
15.	Eunotia implicata Nörpel– Schempp. Alles et Lange–Ber- talot	EISL				29%	1.0	2.8	100%	1.3	7.3
16.	Eunotia islandica Østrup	EMIN							100%	1.0	6.7
17.	Eunotia minor (Kützing) Grunow	ENAE							14%	0.3	0.3
18.	Eunotia naegelii Migula	ENYM							71%	1.8	7.3
19.	Eunotia nymanniana Grunow	EPTD	40%	0.8	4.5	86%	0.3	3.3	14%	0.3	0.3
20.	Eunotia paratridentula Lange– Bertalot et Kulikovskiy	EPSG	40%	0.8	8.0						
21.	Eunotia pseudogroenlandica Lange–Bertalot et Tagliaventi	EUNS							57%	0.3	2.8
22.	Eunotia subarcuatoides Alles. Nörpel–Schempp et Lange–Bertalot	ESUB	100%	1.3	33.8	100%	4.0	12.8	71%	0.3	2.0
23.	Eunotia trinacria Krasske	ETNC	30%	3.3	7.3	14%	0.8	0.8			

										Tab	le 2 Cont.
24.	Frustulia crassinervia (Brébisson) Lange–Bertalot et Krammer	FCRS	100%	16.5	73.3	100%	0.5	6.0	100%	16.0	42.5
25.	Gomphonema exilisimum (Grunow) Lange–Bertalot et E. Reichardt	GEXL	40%	0.8	8.8	71%	1.5	4.0	57%	0.8	3.0
26.	Gomphonema micropus Kützing	GMIC				14%	0.8	0.8			
27.	Gomphonema varioreduncum Jüttner. Ector. E.Reichardt. Van de Vijver et E.J. Cox	GVRD				14%	1.5	1.5	57%	4.0	6.7
28.	Kobayasiella parasubtilissima (H.Kobayasi et T.Nagumo) Lan- ge–Bertalot	KOPA	40%	0.5	2.0	100%	0.5	4.3			
29.	Kurtkrammeira neoamphioxys (Krammer) Bahls	KNAM	60%	0.5	4.5	86%	0.8	11.3			
30.	Navicula viridula (Kützing) Ehrenberg	NVIR							14%	0.3	0.3
31.	Neidium bisulcatum (Lagerstedt) Cleve	NBIS							14%	0.8	0.8
32.	Nitzschia acidoclinata Lange– Bertalot	NACD				29%	0.8	1.8	86%	1.3	8.3
33.	Nitzschia alpina Hustedt	NZAL							29%	0.8	3.3
34.	Nitzschia pura Hustedt	NIPR				29%	1.3	4.3			
35.	Pantocsekiella costei (Druart et Straub) Kiss et Ács	PCOS							14%	1.8	1.8
36.	Pinnularia frequentis Krammer	PFQT							29%	1.0	2.3
37.	Pinnularia gibba Ehrenberg	PGIB							29%	0.5	3.3
38.	Pinnularia microstauron (Ehrenberg) Cleve	PMIC	20%	0.8	0.8						
39.	Pinnularia rupestris Hantzsch	PRUP							14%	2.0	2.0
40.	Pinnularia sinistra Krammer	PSIN	10%	0.5	0.5	43%	0.5	2.8	57%	0.3	2.0
41.	Pinnularia stomatophora (Grunow) Cleve	PSTO							57%	0.3	1.3
42.	Pinnularia subcapitata Gregory	PSCA	40%	0.8	1.3	29%	0.8	1.0	57%	0.3	2.3
43.	Pinnularia subgibba Krammer	PSGI	30%	0.5	0.8				14%	1.3	1.3
44.	Pinnularia substreptoraphe Krammer	PSBS							29%	2.3	6.7
45.	Pinnularia viridiformis Krammer	PVIF	20%	0.8	0.8	14%	1.3	1.3	100%	0.3	6.7
46.	Placoneis hambergii (Hustedt) Bruder	PLHA							14%	1.0	1.0
47.	Stauroneis gracilis Ehrenberg	SGRC							14%	1.0	1.0
48.	Staurosira sp (girdles)	SSPE							86%	1.5	4.3
49.	Tabellaria fenestrata (Lyngbye) Kützing	TFEN				29%	1.5	5.5	100%	1.0	7.8
50.	Tabellaria flocculosa (Roth) Kützing	TFLO	50%	0.8	2.8	100%	2.5	7.3	100%	8.5	16.7

Buczkó et al. (2010), it is a species characteristic of peat bogs, which is supported by our results that give it a benthic character in this specific habitat. In contrast, Pantocsekiella costei was found in only one sample and being a typical representative of the plankton of karstic lakes (GLIGORA UDOVIČ et al. 2016), it would imply to be an accidental species. However, according to recent studies by HALIUC et al. (2020) P. costei is more indicative of climate change and is found in more diverse habitats. Our results on species richness and diversity have proven to be useful descriptive parameters for the community, as they clearly reflect the main differences in diatom assemblages over time in the three habitats, which differ by morphological features such as depth and type of available substrate, as well as by environmental parameters. The highest diversity and species richness at sampling site P, especially in April and May, together with a strong positive correlation with organic matter (COD and TOC), suggest that additional plant material consisting of filamentous charophyte algae had a much stronger positive effect on diatom diversity from organic loading than a negative effect from possible shading or inhibition with allelochemicals, as TROCHINE et al. (2010) suggest as a possibility for phytoplankton. Spring maxima of species richness and diversity in open habitats (R and P) resemble the seasonality of phytoplankton and diatoms as the main component in temperate meso-eutrophic lakes (Munawar & Munawar 1986), in contrast to the

decrease of diversity in closed habitats (DH), which starts in spring, clearly with the growth of vegetation and the increase of shading. Nováková (2005) reported that shading decreased the number of species in the mires of Czech Republic, which is consistent with our results of increased richness and diversity in the open habitats R and P and decreased diversity in the closed and more shaded habitat DH. Although the diversity of diatoms in the Đon Močvar showed seasonal patterns, statistical analysis confirmed that habitat characteristics such as the substrate or other environmental factors shape the diatom community more strongly than the effect of seasonality. These results are also confirmed by statistical tests based on species composition.

In general, the species composition of diatoms sampled from different substrates often differs significantly, as certain species are better adapted to one substrate than the other (FISHER & DUNBAR 2007). The choice of substrates in our study was specific because the substrates sampled were the only ones available: submerged plants in DH and P and soft sediment in R. Townsend & Gell (2005) reported that diatom communities on macroalgal and macrophyte substrates were highly variable compared to other substrates, suggesting that the differences in diatom communities between habitats in the Đon Močvar originated there. The positive correlation of COD and TOC with richness and diversity suggests that diatoms can be used to assess water quality even in such

Table 3. Draftsman's plot correlations of environmental variables. Abbreviations: (TN) total nitrogen; (SRP) soluble reactive phosphorus; (TP) total phosphorus; (COD–Cr) chemical oxygen demand; (TOC) total organic carbon; (SiO₂) silicates.

	Temperature (°C)	Hď	Conductivity (µS.cm ⁻¹)	Alkalinity (mgCaCO ₃ .I ⁻¹)	Oxygen (mgO ₂ .l ⁻¹)	Saturation (%)	COD-Cr (mgO ₂ ,1 ⁻¹)	Amonnia (mgN.F¹)	Nitrites (mgN.l-¹)	Nitrates (mgN.F¹)	TN (mgN.I ⁻¹)	SRP (mgP.I ⁻¹)	TP (mgP.l ⁻¹)	TOC (mg.l ⁻¹)	SiO2 (mg.l ⁻¹)
Temperature (°C)															
pH	0.379														
Conductivity ($\mu S.cm^{-1}$)	0.420	0.492													
Alkalinity (mgCaCO ₃ .l ⁻¹)	0.763	0.225	0.521												
Oxygen (mgO ₂ .l ⁻¹)	-0.131	0.192	-0.307	-0.413											
Saturation (%)	0.472	0.389	-0.020	0.057	0.804										
$CODCr\ (mgO_2.l^{-1})$	0.146	0.016	0.454	0.516	-0.727	-0.575									
Amonnia (mgN.l-1)	-0.099	0.064	0.482	-0.018	0.084	0.005	-0.074								
Nitrites (mgN.l-1)	-0.124	0.082	0.458	-0.063	0.134	0.033	-0.105	0.997							
Nitrates (mgN.l-1)	-0.154	0.079	0.307	-0.180	0.453	0.281	-0.407	0.809	0.827						
TN (mgN.l-1)	-0.282	0.010	0.418	-0.139	-0.153	-0.275	0.181	0.257	0.257	0.127					
SRP (mgP.l ⁻¹)	-0.113	0.080	0.476	-0.043	0.096	0.006	-0.067	0.998	0.998	0.812	0.263				
TP (mgP.l ⁻¹)	-0.128	0.048	0.513	-0.025	0.062	-0.024	-0.025	0.983	0.980	0.769	0.302	0.979			
TOC (mg.l ⁻¹)	0.087	0.007	0.525	0.447	-0.712	-0.585	0.959	0.067	0.043	-0.316	0.315	0.075	0.122		
SiO2 (mg.l ⁻¹)	0.217	-0.185	-0.224	0.327	-0.030	0.093	0.193	-0.625	-0.630	-0.490	-0.188	-0.642	-0.584	0.111	

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a specific habitat (strong influence of *Sphagnum* moss as substrate, lower pH, conductivity and alkalinity than in other surface waters of the area, icing in winter, drying in summer), and not only traditionally in rivers and lakes (Martín & Fernandez 2012). However, specific habitat related species and type–specific conditions must be taken into account (WFD 2000; Taylor et al. 2007).

Spatial distribution and ecological preferences

High similarity of diatom composition within a habitat and high dissimilarity between different habitats, suggests that there is a stable diatom community within each habitat but as already stated, different from the others due to different environmental factors. Principles of monopolization hypothesis (DE MEESTER et al. 2002; DE MEESTER et al. 2016) support the results of our study with environmental factors as the most important driving factor for diatom composition and beta diversity. Priority effects of niche pre–emption have most likely occurred in the past, while priority effects of niche modification are an ongoing process in constant trade–off between direct and indirect competitive effects (Fukami 2015; Weidlich et al. 2021), depending on the availability of food and other resources.

Previous studies in peatlands suggested that pH is one of the driving factors for diatoms, especially where there is a larger gradient (Poulíčková et al. 2004; Poulíčková et al. 2013a). The same importance of pH has been observed in other protists in similar habitats such as desmids (ŠTĚPÁNKOVÁ et al. 2008; ŠTĚPÁNKOVÁ et al. 2012) and testate amoebae (JIROUŠEK et al. 2013). In our study, pH had no significant effect on diatom diversity and composition, just as in an extensive study by Borics et al. (2003), suggesting that other elements of the environment are important when there is a narrow acidity gradient. Furthermore, Soininen (2007) concluded for diatoms in general that conductivity and pH are the most important variables determining their composition. In the later study Soininen et al. (2016) concluded that diatom communities are highly spatially structured, as the variability of these parameters changes significantly at local and continental scales. Our results show that conductivity with COD are the most important variables determining diatom composition. We find these results highly important they bring a new perspective to the understanding of the diatom community in a new and specific bog habitat.

Known ecology of dominant taxa *Frustulia* crassinervia and *Eunotia subarcuatoides* was confirmed by CCA analysis. Both preferred low conductivity, pH and nutrients (LANGE–BERTALOT et al. 2017). These results support *F. crassinervia* as an indicator of good water quality. Its dominance in mires is also known from previous studies (CANTONATI et al. 2011). The preference of *E. subarcuatoides* for low pH (ALLES et al. 1991) and its use to assess acidification in surface waters (Passy 2006) is supported by our results. The ecology of *Brachysira liliana* is not well known, only

that it prefers oligotrophic (Lange–Bertalot et al. 2017) and calcareous waters (Kennedy & Allott 2017). It is morphologically similar to species such as *Brachysira vitrea* (Grunow) R.Ross, *Brachysira neoexilis* Lange–Bertalot, *Brachysira microcephala* (Grunow) Compère and *Brachysira neglectissima* Lange–Bertalot, so that confusion could occur in the past (Kennedy & Allott 2017). Therefore, our finding of *B. liliana* as one of three dominant species in the acidic environment adds significantly to our knowledge of the ecological preferences of this species.

Application of indicator values

Indicator values (Van Dam et al. 1994) have been used in many studies in the past, including the development of indices to assess anthropogenic impact in different water bodies (SALINAS-CAMARILLO et al. 2020) to simple analysis of diatom community and, for example, nutrient enrichment (LAI et al. 2020). The use of these indicator values in our study also suggests a successful application to the specific peatland community, as there are a number of significant correlations of certain categories with relevant parameters, such as significantly more abundant acidobionts at lower pH, more taxa tolerating less oxygen in conditions of high COD and TOC where oxygen is used for the decomposition of organic matter (VIESSMAN et al. 2009) and so on. In the case of trophic state, the correlation with nutrients was not significant, but an indirect link was established by more abundant oligotrophic taxa with lower conductivity and more abundant mesotrophic taxa with higher conductivity, COD and TOC. It is well known that peatlands have a high proportion of aerophilic flora due to frequent subaerial conditions (Buczkó 2006; Cambra 2015). Our finding of a high number of taxa with occasional aerophilic to aerophilic character agrees with the results of these studies, indicating an adaptability of the diatom community to the surface drying of the Đon Močvar in the summer months. After freezing or desiccation events, diatoms recover from either vegetative or resting cells, with a higher recovery rate in terrestrial taxa (Souffreau et al. 2013). Although none of the diatoms from this study are classified as terrestrial taxa, the results of high diversity after desiccation at sampling sites P and R suggest that aerophilic and aquatic to aeophilic taxa also readily recover after such stressful events in Đon Močvar.

Overall, the results of this study suggest that the classification of diatoms into different categories of indicator values can be used as a starting point for the future development of indices for the purpose of monitoring and managing management of this protected area.

Conservation aspects

The area of the Đon Močvar has decreased four times in the last hundred years (Pevalek 1925; Alegro & Šegota 2008; Modrić Surina 2011), therefore its conservation is one of the main interests of all recent studies on the bog itself (VILENICA et al. 2016; Brigić

et al. 2017a; Brigić et al. 2017b) or on the target species living there (Brigić et al. 2014). Since most faunas have been studied only recently, we consider our results on diatoms, which are present in almost all aquatic habitats (SMOL & STOERMER 2010), to be extremely important for the conservation of this and other similar habitats. Our results followed the principle of species abundance distribution models (SADs) with more than 50% of taxa having low relative abundance (McGILL et al. 2007). These were also not in any conservation priority group according to German Red List (HOFMANN et al. 2018). Therefore, taxa in the conservation priority categories dominated with their abundance mainly in typical mire habitats (DH and R), which is consistent with the results of Mašić et al. (2018), who found the greatest number of rare and endangered diatoms in mountain peatlands rather than in a variety of other habitats studied. The German Red List has also been successfully applied in past studies from the Mediterranean (FALASCO et al. 2019) to the Alps (Fell et al. 2018). The application in the Đon Močvar suggests diatoms as a group of organisms that should be included in the future management, regular monitoring and protection of the area. However, for practical application this list should be modified according to the geographic region which leaves an open space for regional studies on unclassified taxa due to the high number of taxa with unknown conservation status.

CONCLUSION SENTENCES

The findings of endangered diatoms support the holistic approach to conservation of the mire as a whole proposed by Brigić et al. (2017b). Diatoms are known to be a very good indicator in river and lake monitoring (Charles et al. 2020), but this study shows that they are a group of organisms that need to be considered in the further protection of a unique habitat in the Southeastern Europe, such as the Đon Močvar.

The results of our study indicate that the hypothesis has been proven correct and that pH is not a driving factor for the diatom community in short gradient acidic habitats. Our results help to better understand complex habitats such as the Don Močvar and possibly similar habitats in the future. We suggest that these results can be used as a starting point for future studies of other aquatic organisms as well as paleobotanical studies of diatoms. Indicator taxa and the German Red List have been successfully tested on the diatom assemblage in mire, leading to a better understanding of diatom ecology in specific habitats, future assessment of anthropogenic influence, and protection of habitats potentially threatened by climate change or human activities (SHOTYK 1988; GAISER & RUHLAND 2010).

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