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9 **Ecological background of diatom functional groups: comparability of classification**
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28

29 **Abstract**

30

31 Functional classification based on species traits brought a revolution in community ecology,
32 and also boosted phytoplankton and in phytobenton (diatom) research. Several studies
33 stressed the usefulness of phytoplankton functional groups in ecological status assessment,
34 and there is also a strong emphasis to use combined traits in ecological assessments of diatom
35 assemblages. The Combined Eco-Morphological Functional Groups (CEMFGs) help to reveal
36 species-environmental correlations, which can be hidden, controlling traits separately.
37 Nowadays, there are three types of functional guild classifications simultaneously used in the
38 literature: (i) the original classification proposed by Passy (O); (ii) Passy's classification
39 complemented with a separated planktic guild (P); (iii) the refined guild classification by
40 Rimet and Bouchez, also containing the planktic guild (RB). One of the most important
41 criteria of the combined functional groups is the well-defined ecological frame of these
42 combined groups; thus it is vital to harmonise the classification of the taxa into guild based
43 combined eco-morphological functional groups (CEMFGs). In this study we tested the
44 similarities and dissimilarities of the correlations between environmental factors and
45 CEMFGs created in the (i)-(iii) classifications. Samples were collected in 138 sampling sites
46 on lowland rivers and streams in the Hungarian Lowland Region. Strong correlations were
47 anticipated between the functional groups and environmental factors due to the presence of
48 common and/or abundant planktic taxa. Our results validated the necessity of a separated
49 planktic guild in ecological assessments of diatom assemblages. Further relevant differences
50 between the correlations of CEMFGs-P or CEMFGs-RB and abiotic factors were
51 hypothesised in the case of those functional groups which contained reassigned taxa with high
52 frequency and/or abundance. Our results confirmed this hypothesis. Furthermore, the present
53 study also highlighted the relevance of well-defined trait classification. Abundant and/or
54 frequent taxa, which are able to change their life forms, can modify significantly the
55 relationship between the functional group containing them and environmental factors. In the
56 future, both field and laboratory studies should focus on revealing the circumstances, which
57 cause the mentioned changes in traits of diatoms.

58

59 **Keywords:** Combined Eco-Morphological Functional Groups, diatom traits, environmental
60 factors, unified classification method

61

62 **1. Introduction**

63

64 The definition of functional groups is based on the similarities in ecosystem functioning
65 (Tapolczai et al., 2016), i.e. species classified into a functional group have similar morpho-
66 physiological and/or ecological features. Functional classifications become widely used in
67 ecology and habitat quality assessment. Comparisons based on functional classifications (i)
68 helps in the joint analyses and evaluation of similar habitats with distinct species composition,
69 and (ii) provide an improved classification of habitats with a huge number of taxa and
70 problematic species groups compared to the taxonomic approach (Salmaso et al., 2015).
71 Moreover, the functional classification based approach is useful (iii) in water quality
72 assessment (B-Béres et al., 2016) and (iv) in the detection of overall changes in ecosystem
73 functions (Török et al., 2016).

74

75 The most widely known functional classification of diatoms is based on diatom guilds (Passy,
76 2007). Taxa are assigned to a guild based on similar resource use capability and disturbance
77 tolerance. There are three diatom guilds formed: the low profile, high profile and the motile
78 guild (Passy, 2007). Taxa in the low profile guild are resistant to high water flow conditions
79 and capable of colonising rapidly on bare surfaces and substrates. In contrast, high profile
80 taxa are sensitive to disturbances (Stenger-Kovács et al., 2013). The ability of motile guild
81 taxa to adapt to active movements allows them to select the most appropriate microhabitat
82 (Lengyel et al., 2015). Until now, the correlations of ecological guilds and environmental
83 factors and the usefulness of the functional approach in ecological status assessment were
84 only partially discussed (Berthon et al., 2011; Stenger-Kovács et al., 2013), but troubles with
85 this approach were also emphasized (B-Béres et al., 2014, 2016; Rimet and Bouchez, 2012;
86 Tapolczai et al., 2016). Rimet and Bouchez (2012) suggested a re-classification of taxa by the
87 creation of a fourth guild containing planktic diatoms, and also changed the guild-assignment
88 of several taxa. Studies proved that the joint use of the planktic guild, together with Passy's
89 original groups, can help to explain the dynamics of this guild in the case of a medium sized
90 lowland river or a lowland stream (B-Béres et al., 2014, 2016). But it has to be emphasized
91 that the guilds themselves do not seem to be robust enough (i) to display and explain highly
92 stochastic processes, like colonisation and re-colonisation after disturbance (B-Béres et al.,
93 2016), or (ii) to explain changes of taxa composition in harsh and unpredictably changing
94 physical and/or chemical environments (B-Béres et al., 2014; Tapolczai et al., 2016).

95

96 Not only ecological guilds but other easy-to-measure traits (e.g. cell size or biovolume) can
97 support the understanding of the relationship between environmental factors and diatom
98 assemblages. There are strong correlations between these single traits and (i) nutrient uptake
99 and efficiency (Tapolczai et al., 2016), (ii) trophic levels or organic pollution (Berthon et al.,
100 2011; Kókai et al., 2015; Lange et al., 2016), (iii) physical disturbances (Tapolczai et al.,
101 2016), or (iv) salinity and conductivity (Kókai et al., 2015). However, similarly to guilds,
102 single traits, like biovolume classes, are not robust enough by themselves to display stochastic
103 processes appropriately (B-Béres et al., 2016).

104

105 Combining of various traits to explain changes in ecological processes and to characterise a
106 habitat type was proven a vital idea in phytoplankton research (Reynolds et al., 2002; Salmaso
107 and Padisák, 2007; Padisák et al., 2009), and several studies stressed for example the
108 usefulness of phytoplankton functional groups in ecological status assessment (Krasznai et al.,
109 2010); or in the detection of assembly changes following anthropogenic pollution (Bácsi et
110 al., 2016). The trait-based analyses for benthic algal and cyanobacterial assemblages started
111 very recently (simultaneous interpretation of different traits - Lange et al., 2016 and combined
112 eco-morphological functional groups of diatoms - B-Béres et al., 2016). One of the most
113 promising approaches is the combination of guilds with cell sizes by creating combined eco-
114 morphological groups (B-Béres et al., 2016). Using combined eco-morphological groups was
115 reported to be a powerful and robust method displaying quantitative and qualitative changes
116 in diatom assemblages (Tapolczai et al., 2016). However, the crucial points of the utility of
117 the combined groups are that (i) subgroups have to be clearly defined and their relationship
118 with the habitat environmental factors needs to be validated, and (ii) the functional role of
119 subgroups in a respective diatom assemblage has to be clearly specified (Tapolczai et al.,
120 2016).

121

122 Nowadays, there are three types of guild classification simultaneously used in the literature:
123 (i) the original classification proposed by Passy (2007); (ii) Passy's classification
124 complemented with a separated planktic guild (e.g. B-Béres et al., 2014); and (iii) the refined
125 classification by Rimet and Bouchez (2012), also containing the planktic guild. To reach a
126 generally useful practical application of diatom classifications, as with phytoplankton
127 functional group classification, it is important to compare the existing functional
128 classifications and to also use a unified system based on clear criteria and well-defined
129 functional groups. Therefore, it is vital to harmonise the classification of the taxa into guild

130 based combined eco-morphological functional groups (CEMFGs). In our study we compared
131 the three classification systems combined with cell sizes within the eco-morphological
132 functional group classification (CEMFGs) to test and validate their usability under the same
133 abiotic and biotic circumstances. Our final aim was to choose the ecologically most relevant
134 classification system of these three types and to recommend a unified classification method
135 for global use. We hypothesised the following: (i) Planktic taxa hypothesis: There is no
136 statistical validation of a planktic guild, although Rimet and Bouchez (2012) separated
137 planktic taxa into an independent guild, and many studies used an independent planktic guild.
138 We supposed that the presence of common and/or abundant planktic taxa strongly affect the
139 relationship of the functional groups and environmental factors. Thus, the planktic taxa should
140 be treated as a separated planktic guild in the functional-classification-based analyses.
141 (ii) CEMFGs hypothesis: We hypothesised that the widely accepted refinement by Rimet and
142 Bouchez (2012) on guilds affected the correlation between CEMFGs and abiotic factors only
143 for those particle groups which contained reassigned taxa with high frequency and/or
144 abundance.

145

146 **2. Materials and Methods**

147

148 *2.1. Sampling and measuring of environmental factors*

149

150 Altogether 865 diatom samples were collected in 138 sampling sites on small, medium and
151 large sized lowland rivers and streams in the Hungarian Lowland Region (Fig. 1) between
152 2007 and 2015 from the beginning of April to the end of October, usually at least twice a year
153 (spring and autumn). This is one of the largest datasets covering benthic diatom assemblages
154 ever collected and analysed for Central-Europe. There were 11 environmental factors
155 measured in all sampling points (Appendix 1). Conductivity (COND – $\mu\text{S cm}^{-1}$), pH,
156 dissolved oxygen concentration (DO – mg L^{-1}), and water temperature (T – $^{\circ}\text{C}$) were
157 measured with a portable-multiparameter digital meter (Multi 350i-WTW, Germany) in the
158 field. The water samples were kept at 4°C in a cooler bag during transportation to the
159 laboratory for further spectrophotometric analysis of NO_3^- -N, NO_2^- -N, NH_4^+ -N, Cl^- (mg L^{-1} ;
160 ISO 15923-1:2013) and PO_4^{3-} -P ($\mu\text{g L}^{-1}$; ISO 15923-1:2013), respirometric analysis
161 (Biological Oxygen Demand – BOD in mg L^{-1} ; MSZ EN 1899-1:2000, MSZ ISO 6060:1991)
162 and gravimetric analysis (Total Soluble Solids – TSS in mg L^{-1} ; MSZ 260-3:1973).

163

164 2.2. *Sample collection and preparation*

165

166 The European guideline (EN 13946) was used during the sampling and preservation. Diatom
167 valves were prepared by the hot hydrogen-peroxide method (EN 13946). Naphrax synthetic
168 resin was used for embedding. The Leica DMRB microscope with 1000–1600-fold
169 magnification was used for identification of diatom taxa. At least 400 diatom valves were
170 counted (EN 14407). We used Krammer and Lange-Bertalot (1997a, 1997b, 2004a, 2004b),
171 Potapova and Hamilton (2007) and Bey and Ector (2013) for diatom identification.

172

173 2.3. *Data processing and analyses*

174

175 Diatom taxa were classified to combined eco-morphological groups based on B-Béres et al.
176 (2016) using biovolume classes and diatom guilds.

177

178 (i) For the biovolume class assignment we used Berthon et al. (2011) where diatom taxa were
179 classified into five biovolume classes S1-S5 (S1: 5–99 μm^3 , S2: 100–299 μm^3 , S3: 300–599
180 μm^3 , S4: 600–1499 μm^3 , S5: $\geq 1500 \mu\text{m}^3$).

181

182 (ii) For the guild classification we used the following three well-known classification schemes
183 (See also details in Introduction and Appendix 2): - a) We classified diatom taxa into the
184 original low profile (L), high profile (H) and motile (M) guilds according to Passy (2007).
185 Combining this guild classification (Passy, 2007) with the biovolume classes (Berthon et al.,
186 2011) we created 15 combined eco-morphological functional groups (CEMFGs-O; LS1-LS5,
187 HS1-HS5, MS1-MS5 – Appendix 2, 3). - b) According to our knowledge, a separated planktic
188 guild firstly appeared in the work of Rimet and Bouchez (2012). Planktic taxa were taken out
189 from Passy's original guilds and were put together into a separated guild. The second type of
190 classification occurred when Passy's original guild classification was completed by this
191 planktic guild (B-Béres et al., 2014). Combining this guild classification (B-Béres et al.,
192 2014) with the biovolumes (Berthon et al., 2011) we created 20 combined eco-morphological
193 functional groups (CEMFGs-P; LS1-LS5, HS1-HS5, MS1-MS5, PS1-PS5 – Appendix 2, 4).-
194 c) Rimet and Bouchez (2012) not only created the planktic guild, but they revised Passy's
195 original classification. Some taxa were reclassified from one guild to another based on the
196 authors' experiences. This is the third type of classification of diatoms into guilds. The
197 combination of this guild classification (Rimet and Bouchez, 2012) with the five biovolume

198 classes (Berthon et al., 2011), resulted in 20 combined eco-morphological functional groups
199 (CEMFGs-RB; LS1-LS5, HS1-HS5, MS1-MS5, PS1-PS5 – Appendix 2, 5).

200

201 To analyse the relationship between CEMFGs-O, -P and -RB and eleven environmental
202 factors (COND, pH, DO, T, NO₃⁻-N, NO₂⁻-N, NH₄⁺-N, PO₄³⁻-P, Cl⁻, BOD and TSS)
203 Canonical Correspondence Analyses (CCA) was performed, where environmental factors
204 were added by weighted averages (ter Braak and Šmilauer, 2002).

205

206 **3. Results**

207

208 In the analysed 865 diatom samples, altogether 495 diatom taxa were identified. Among them,
209 481 taxa were identified at least at the species level, while only 14 taxa were identified at the
210 genus level. With the exception of the barely identifiable small centric diatoms in large or
211 medium sized rivers, the relative abundance of taxa identified at genus level did not exceed
212 2.9%.

213

214 *3.1. The relationship of environmental factors and CEMFGs-O*

215

216 The Canonical Correspondence Analysis (CCA) accomplished with the 11 environmental
217 factors and the 15 CEMFGs-O explained 83.3% of the variance of group-environment
218 relation in four axes. The Monte-Carlo permutation test indicated the pattern displayed by the
219 CCA differed significantly from a random pattern (N = 499; p = 0.002 for the first and p =
220 0.002 for all canonical axes). The CCA revealed that the factors with the highest correlation
221 were conductivity (0.3375), Cl⁻ (0.2420), PO₄³⁻-P (0.1845), NO₂⁻-N (0.1690) and NH₄⁺-N
222 (0.1115) with the first axis; pH (0.2217) with the second axis; dissolved oxygen (DO; 0.1285)
223 and NO₃⁻-N (-0.1772) with the third axis and water temperature (T; 0.1059) with the fourth
224 axis.

225

226 Most CEMFGs-O were negatively correlated with the environmental factors (Fig. 2). In
227 contrast, there were positive correlations: the medium sized and the largest sized motile
228 groups (MS3 and MS5) and the LS4 low profile group showed positive correlation with
229 nutrient content and salinity/conductivity factors (Fig. 2). Furthermore, MS4 and LS1 groups
230 correlated positively with pH, water temperature (T), and dissolved oxygen (DO).

231

232 *3.2. The relationship of environmental factors and CEMFGs-P*

233

234 There were significant correlations between the 11 environmental factors and the 20
235 CEMFGs-P based on the results of CCA analysis. The group and environmental correlation
236 was 85.5% in the four axes. The Monte-Carlo permutation test indicated a significant
237 difference from a random pattern for the pattern displayed by the CCA (N = 499; p = 0.002
238 for the first and p = 0.002 for all canonical axes). The relevant factors were Cl⁻ (0.3946),
239 conductivity (0.3621), NO₃⁻-N (0.2204), NO₂⁻-N (0.1899), BOD (0.1667) and PO₄³⁻-P
240 (0.1663) to axis one; pH (0.1967) to axis two; dissolved oxygen (DO; 0.1313) to axis three
241 and temperature (T; 0.1317) to axis four.

242

243 With the exception of PS4, planktic groups correlated positively with pH, T, and DO (Fig. 3).
244 Small and large sized low profile groups (LS1, LS4 and LS5) correlated negatively with
245 conductivity and nutrient content (Fig. 3). Medium and the largest sized high profile groups
246 (HS3 and HS5) correlated positively with these environmental factors. Other high profile
247 groups showed positive (HS1) or contrariwise, negative (HS3 and HS4) correlation to water
248 temperature (T) and dissolved oxygen (DO). With the exception of MS4, the motile groups
249 correlated positively to the nutrient content (Fig. 3).

250

251 *3.3. The relationship of environmental factors and CEMFGs-RB*

252

253 Significant correlation was indicated by CCA between the 11 environmental factors and the
254 20 CEMFGs-RB. The group and environmental correlation was 86% in the four axes. The
255 Monte-Carlo permutation test indicated a significant difference from a random pattern for the
256 pattern displayed by the CCA (N = 499; p = 0.002 for the first and p = 0.002 for all canonical
257 axes). The factors with the highest correlation were Cl⁻ (0.3946), conductivity (0.3780), NO₃⁻-
258 N (0.2247), NO₂⁻-N (0.1896), PO₄³⁻-P (0.1708), BOD (0.1611) and NH₄⁺-N (0.1034) with the
259 first axis. The most important factor to axis two was pH (0.1776), while dissolved oxygen
260 (DO; 0.1703) and temperature (T; 0.1182) showed the highest correlation to axis three and
261 axis four, respectively.

262

263 With the exception of LS4 and HS4, the distribution of the groups along environmental
264 factors was similar to those CEMFGs-RB which were classified by the original guilds
265 completed by planktic guild (Fig. 4). The differences were that LS4 showed the strongest

266 negative correlation with water temperature (T) and pH, HS4 showed a weaker negative
267 correlation with these factors (T and pH) and a stronger negative correlation with nutrient
268 content and conductivity (Fig. 4).

269

270 **4. Discussion**

271

272 *4.1. Relevance of a separated planktic guild*

273

274 In the original guild classification by Passy (2007) planktic taxa were classified mostly in low
275 or high profile guilds. But planktic taxa are not steady members of benthic assemblages.
276 According to Rimet and Bouchez (2012) these taxa are morphologically adapted not to
277 benthic but to lentic environments that make them able to resist sedimentation. In the benthic
278 assemblages they can appear even in relatively high proportion (i) after floods (B-Béres et al.,
279 2014); (ii) in sections of watercourses close to reservoirs (Szabó et al., 2004); (iii) in the late
280 successional stages of matured biofilm (Stevenson et al., 1996); and (iv) they can settle down
281 due to reduced flow rate. Thus, the presence of planktic guild taxa in benthic assemblages is
282 not affected by the same abiotic and biotic factors as the members of the other guilds.

283

284 Rimet and Bouchez (2012) argued that formation of a separated planktic guild was necessary
285 because planktic taxa are usually not adapted to those circumstances (disturbances, nutrients,
286 etc.), which basically determine the presence of the other three guilds in benthic assemblages.
287 However, the differences between the original classification (without planktic guild) and the
288 classifications with a separated planktic guild were not statistically analysed and validated
289 before. Our results supported the hypothesis that the planktic taxa should be treated as a
290 separated planktic guild in the functional-classification-based analyses. Most of the planktic
291 groups showed positive correlation with temperature and/or nutrient content (Fig. 3,4).
292 Classifying highly abundant planktic taxa into low or high profile groups may hide the real
293 ecological character of these benthic groups (Fig. 2-4). In our study, the total number of
294 planktic taxa was relatively high (~8% of total taxa number). The most common and
295 relatively abundant planktic species was only the S4 sized *Cyclotella meneghiniana* Kützing.
296 In the case of the other planktic taxa, only local accumulation was observed. Thus, only the
297 removal of *C. meneghiniana* from LS4 to PS4 had significant effect on the correlation of
298 CEMFGs and environmental factors (Figs. 2-4). The halophilic character of this taxon (Van
299 Dam et al., 1994) explained the positive correlation of LS4 and chloride ion in the case of

300 CEMFGs-O classification (Fig. 2). In contrast, the removal of the taxon from LS4 resulted in
301 negative correlation of this low profile group with chloride ion in the case of CEMFGs-P and
302 CEMFGs-RB (Fig. 3,4). The removal of small and medium sized (PS1 - PS3) or large (PS5)
303 centric diatoms from low or high profile guilds to planktic ones did not change significantly
304 the position of CEMFGs in the matrix (Fig. 2-4). This phenomenon can be explained by the
305 low abundance of these taxa. Namely, it is recommended to use separated planktic functional
306 groups, especially when planktic taxa are abundant in the samples.

307

308 4.2. Similarities and discrepancies between the guild classification systems

309

310 We hypothesised that the correlation between groups and abiotic factors change only in the
311 case of those groups, which contain reassigned taxa with high frequency and/or abundance.
312 Our findings supported this hypothesis. The most pronounced differences between CEMFGs-
313 P and CEMFGs-RB were the positions of LS4 and HS4 in the matrix (Fig. 3,4). It was
314 basically due to the removal of tube-forming taxa (e.g. *Encyonema mesianum* (Cholnoky)
315 D.G. Mann, *Encyonema silesiacum* (Bleisch in Rabh.) D.G. Mann) from low profile to high
316 profile guild (Rimet and Bouchez, 2012). The group containing them showed negative
317 correlation with inorganic nutrients (LS4 – Fig. 3, or HS4 – Fig. 4). These tube forming taxa
318 are often abundant and common in the studied watercourses and they are able to produce
319 extracellular enzymes similarly to motile taxa (Tapolczai et al., 2016). This ability offers
320 advantage in inorganic nutrient poor environments. Namely, this ability allows the
321 mobilization of other nitrogen and phosphorus-forms due to the lack of inorganic ones for
322 these taxa. But this phenomenon should be proved by measurements of organic nitrogen and
323 phosphorus forms, so to ascertain the real importance of extracellular enzymes in diatom
324 assemblages needs further field and laboratory investigations.

325

326 Usually, abundance of HS4 group containing *Diatoma*, *Eunotia* and *Fragilaria* taxa was high
327 in early spring or in autumn. Autumn is the slow flowing period of the studied watercourses,
328 so it ensures "low disturbance-circumstances" for colonial taxa (mentioned above).
329 Furthermore, Rimet et al. (2015) also presented the dominance of these high profile taxa in
330 early spring. But they emphasized the effects of low nutrient availability as a key factor.
331 Namely, these colonial and/or filamentous taxa are able to extend the biofilm thickness
332 contributing to the nutrient access into the mat. Completion of HS4 group with tube-forming
333 taxa in CEMFGs-RB increased the negative correlation with nutrient and conductivity in our

334 study. In addition, LS4 group without tube-forming taxa in CEMFGs-RB showed strong
335 negative correlation with temperature (Fig. 4). This was due to one species, *Meridion*
336 *circularis* (Greville) C.A.Agardh, which prefers low water temperature (Stenger-Kovács et al.,
337 2013). *M. circularis* was present in 16% of the samples with high abundance (maximum
338 relative abundance was 41%). This species is usually common in winter or in spring in the
339 watercourses, it tolerates high disturbance well (Stenger-Kovács et al., 2013). Based on the
340 detailed comments above, reclassification of tube-forming taxa from LS4 to HS4 seems to be
341 ecologically justified.

342

343 Similarly to the tube-forming HS4 taxa, the motile group with the same size (MS4) also
344 showed negative correlation with nutrient content (Fig. 3,4). One reason could be the ability
345 of tube-forming HS4 and MS4 taxa mobilizing organic nutrient forms, but it needs further
346 confirmations (see above). Furthermore, it should also be taken into account that certain tube-
347 forming taxa (e.g. *E. silesiacum*) are able to change their life form character and they can be
348 motile also (Rimet and Bouchez, 2012; Tapolczai et al., 2016). So it is possible, that the
349 strong negative correlation of S4 groups containing tube forming taxa with inorganic nutrients
350 may be due to changing their life form to motile. Therefore, the strong negative correlation of
351 S4 groups with inorganic nutrient content needs further investigations.

352

353 In the case of S1-S3 and S5 groups, there were no significant differences in classification
354 between CEMFGs-P and CEMFGs-RB. Due to this fact, these groups correlated similarly
355 with environmental factors (Fig. 3,4). LS1 correlated negatively with nutrient content in both
356 cases (Fig. 3,4). This group includes the attached and/or pioneer taxa (e.g. *Achnanthydium*
357 *minutissimum* (Kutz) Czarnecki, *Amphora pediculus* (Kutzing) Grunow; Rimet and Bouchez,
358 2012; B-Béres et al., 2016). Due to this pioneer character (high disturbance tolerance, and
359 high efficiency to nutrient uptake) the position of this group was expected. There was strong
360 positive correlation between temperature and HS1: these taxa (e.g. *Pseudostaurosira*
361 *parasitica* (W.Smith) Morales var. *subconstricta* (Grunow) E.Morales,) were usually
362 abundant at the end of spring or at the beginning of autumn in the studied watercourses, when
363 the low biotic or abiotic pressures (low grazing in spring, or low shade effects in autumn) did
364 not suppress their populations' growth. With the exception of HS2, S2 and S3 groups
365 correlated negatively to dissolved oxygen and water temperature, but the correlation of these
366 groups with nutrient content was rather positive (Fig. 3,4). Positive correlation of S2 and S3
367 groups with different nutrient forms were also justified by Berthon et al. (2011).

368

369 Although the correlation between MS1 and environmental factors was weak, the position of
370 this group connected to nutrients (Fig. 3,4). The strongest correlation appeared between
371 nutrients and MS2-MS3 groups (Fig. 3,4). Motile taxa have many adaptation advantages to
372 dominate the assemblages in nutrient rich environment (e.g. secretion ability of extracellular
373 enzymes, nutrient storages, ability to choose their microhabitats by motility, Berthon et al.,
374 2011). Due to their motile character, these groups are able to find and reach faster the well-
375 resourced habitats than the other groups. Our data confirmed again, that taxa belonging to the
376 same biovolume categories could be separated from each other by their other features, like
377 relation to nutrients and disturbances (belonging to guilds; B-Béres et al., 2016).

378

379 Among groups composed of large species, LS5 showed negative correlation with inorganic
380 nutrient content, while HS5 and MS5 correlated positively to inorganic nutrients in both
381 CEMFGs-P and CEMFGs-RB (Fig. 3,4). Representatives of LS5 group are common and
382 abundant adnate taxa in the studied watercourses (e.g. varieties of *Cocconeis placentula*
383 Ehrenberg). Despite adnate taxa showing strong positive correlation to total nitrogen content
384 (B-Béres et al., 2014), they have to adapt or tolerate the strong interaction caused by high
385 profile guild in thick biofilm (Tapolczai et al., 2016). So, their real nutrient preference is
386 masked in thick biofilm, and appears only in physically disturbed environments. But these
387 two key factors (high nutrient content and high physical disturbance together) are not
388 common in the studied watercourses. It has to be emphasized, the advantages of adnate taxa
389 could appear in the presence of physical disturbance if two differently disturbed segments of
390 the same river were studied (so there were similar nutrient regime; B-Béres et al., 2014).

391

392 Both HS5 and MS5 groups have abilities, which provide them advantages in nutrient rich
393 environments. The position of high profile taxa in the upper part of biofilm allows them to
394 access faster the dissolved nutrients than the other groups, especially the low profile taxa
395 positioned in the bottom layer (Stenger-Kovács et al., 2013; Tapolczai et al. 2016). In turn,
396 motile taxa are able to appear quickly in the most appropriate microhabitats (high nutrient
397 content and moderate disturbance – Tapolczai et al., 2016). It seems that the key factor, which
398 will effect to the ratio of these large sized taxa is the physical disturbance in lowland
399 watercourses. This requires further investigation focused more on the role of physical
400 disturbance among nutrient rich circumstances.

401

402 **5. Conclusions**

403

404 The statistical analyses of the differences between the original guild classification of diatoms
405 (CEMFGs-O) and the classifications with separated planktic guild supported the ecological
406 importance of the separate planktic guild in assessment of diatom assemblages. The most
407 pronounced differences between the CEMFGs-P and CEMFGs-RB were the relation of LS4
408 and HS4 to environmental factors. It was basically due to the removal of common and usual
409 dominant tube-forming taxa from LS4 to HS4 groups. Considering the ecological characters
410 of these differently classified morpho-functional groups, we propose the general use of
411 CEMFGs-RB for classification of diatoms. This study also highlighted that abundant and/or
412 frequent taxa, which are able to change their life forms can modify significantly the relation
413 of the functional group containing them with environmental factors. In the future, both field
414 and laboratory studies should focus on revealing the circumstances which cause the changes
415 in traits of diatoms.

416

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418

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423

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425

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513

514 **Figure legends**

515

516 **Fig. 1** The study area on Trans-Tisza region of Hungary: (a) localization of the study area
517 (marked with grey borders); (b) sampling sites on the rivers and channels marked with dots,
518 grey lines: borders of the study area, black lines: the rivers and channels.

519

520 **Fig. 2** Relation of the Combined Eco-Morphological Functional Groups based on the original
521 classification proposed by Passy (CEMFGs-O) and the environmental variables displayed by
522 CCA based on groups' abundances. Combining the original guild classification with
523 biovolume classes 15 combined eco-morphological functional groups were created (LS1-LS5,
524 HS1-HS5, MS1-MS5). Cumulative percentage variance of the species-environment relation
525 was 34.3 and 57.9 for the first and second axis, respectively.

526

527 **Fig. 3** Relation of the Combined Eco-Morphological Functional Groups based on the original
528 classification proposed by Passy complemented with a separated planktic guild (CEMFGs-P)
529 and the environmental variables displayed by CCA based on groups' abundances. Combining
530 this guild classification with biovolume classes 20 combined eco-morphological functional
531 groups were created (LS1-LS5, HS1-HS5, MS1-MS5, PS1-PS5). Cumulative percentage
532 variance of the species-environment relation was 38.0 and 62.2 for the first and second axis,
533 respectively.

534

535 **Fig. 4** Relation of the Combined Eco-Morphological Functional Groups based on the refined
536 guild classification by Rimet and Bouchez, also containing the planktic guild (CEMFGs-RB)
537 and the environmental variables displayed by CCA based on groups' abundances. Combining
538 this guild classification with biovolume classes 20 combined eco-morphological functional
539 groups were created (LS1-LS5, HS1-HS5, MS1-MS5, PS1-PS5). Cumulative percentage
540 variance of the species-environment relation was 38.7 and 64.1 for the first and second axis,
541 respectively.

542

543 **Supporting Information**

544

545 **Appendix 1** Summary of the data set for the eleven tested chemical and physical parameters:
546 dissolved oxygen (DO – mg L⁻¹), conductivity (COND – μS cm⁻¹), pH, temperature (T – °C),
547 biological oxygen demand (BOD – mg L⁻¹), chloride ion (Cl⁻ – mg L⁻¹), ammonium-nitrogen

548 $(\text{NH}_4^+ - \text{N} - \text{mg L}^{-1})$, nitrite-nitrogen $(\text{NO}_2^- - \text{N} - \text{mg L}^{-1})$, nitrate-nitrogen $(\text{NO}_3^- - \text{N} - \text{mg L}^{-1})$,
549 phosphate-phosphorus $(\text{PO}_4^{3-} - \text{P} - \text{mg L}^{-1})$, and total soluble solids (TSS – mg L⁻¹).

550

551 **Appendix 2** Taxa classification into the three different Combined Eco-Morphological
552 Functional Groups.

553

554 **Appendix 3** Relative abundances of the 15 Combined Eco-Morphological Functional Groups
555 based on the original guilds classification by Passy (CEMFGs-O).

556

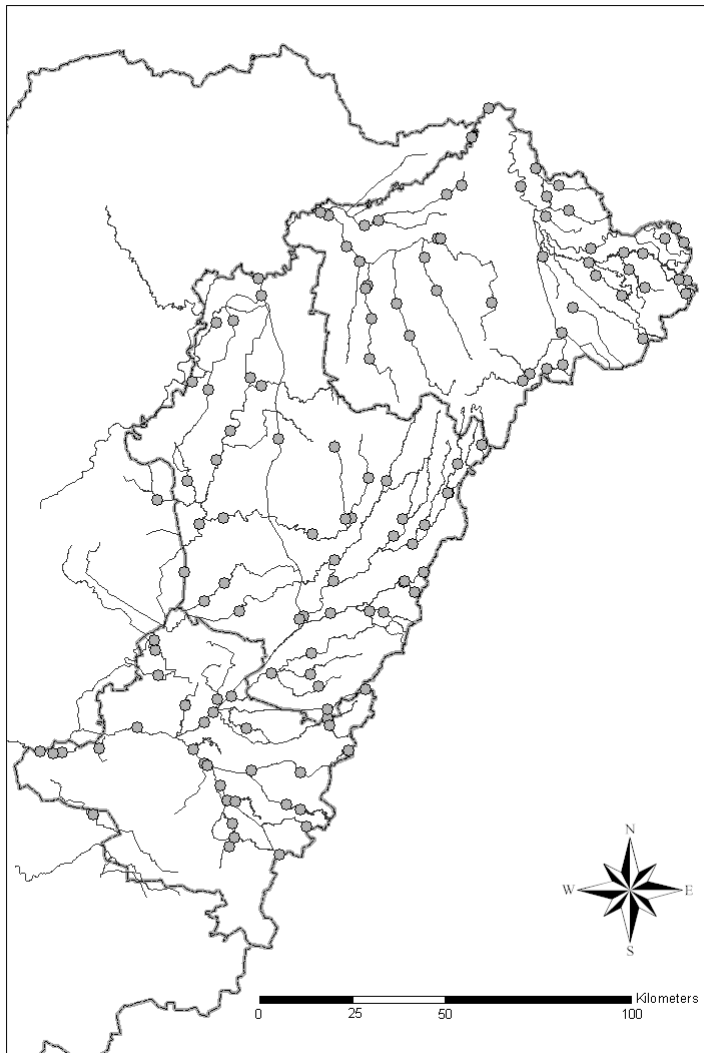
557 **Appendix 4** Relative abundances of the 20 Combined Eco-Morphological Functional Groups
558 based on the Passy's guilds classification completed with planktic groups (CEMFGs-P).

559

560 **Appendix 5** Relative abundances of the 20 Combined Eco-Morphological Functional Groups
561 based on the Passy's guilds classification modified by Rimet and Bouchez (CEMFGs-RB).

562

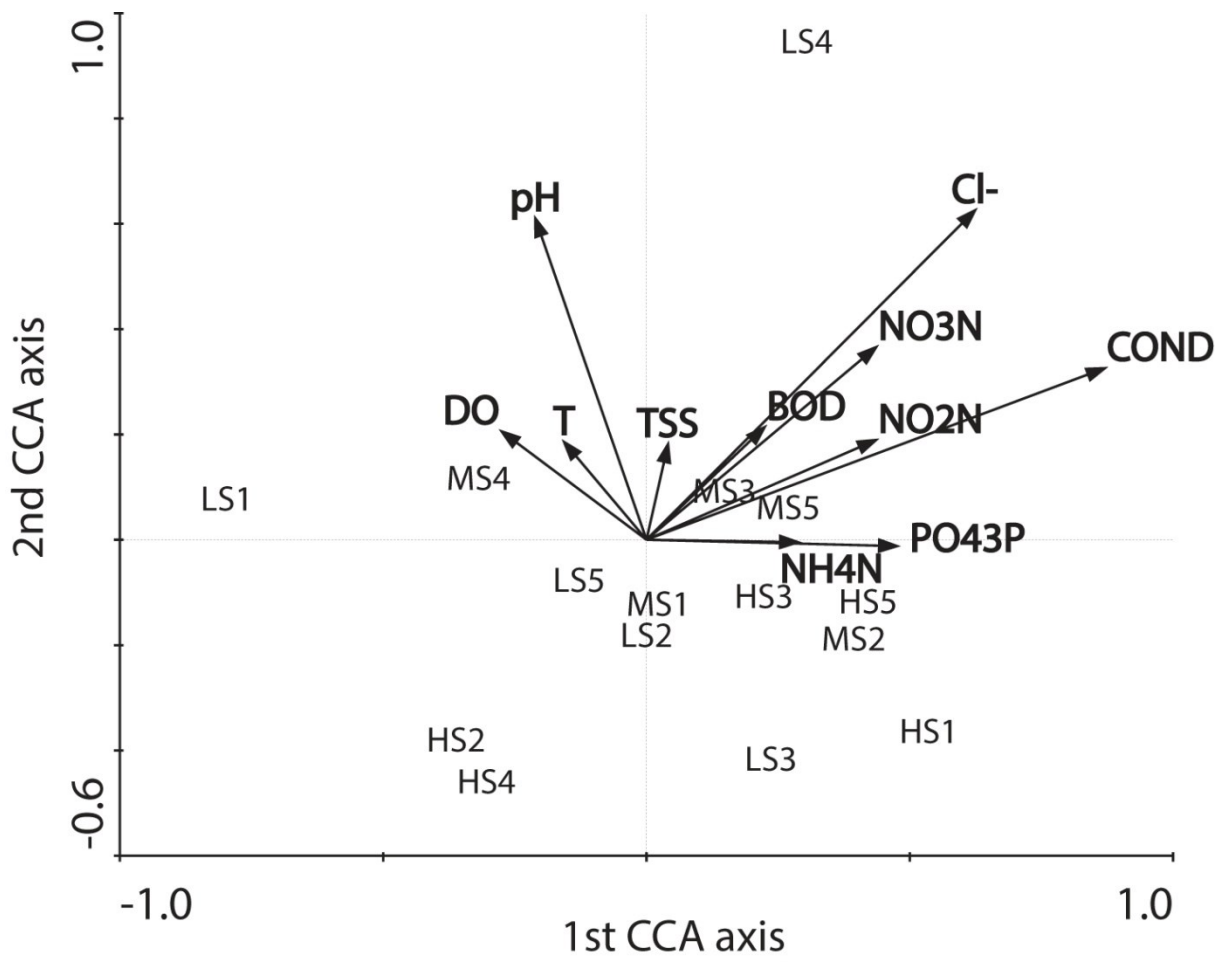
563 Fig. 1



564

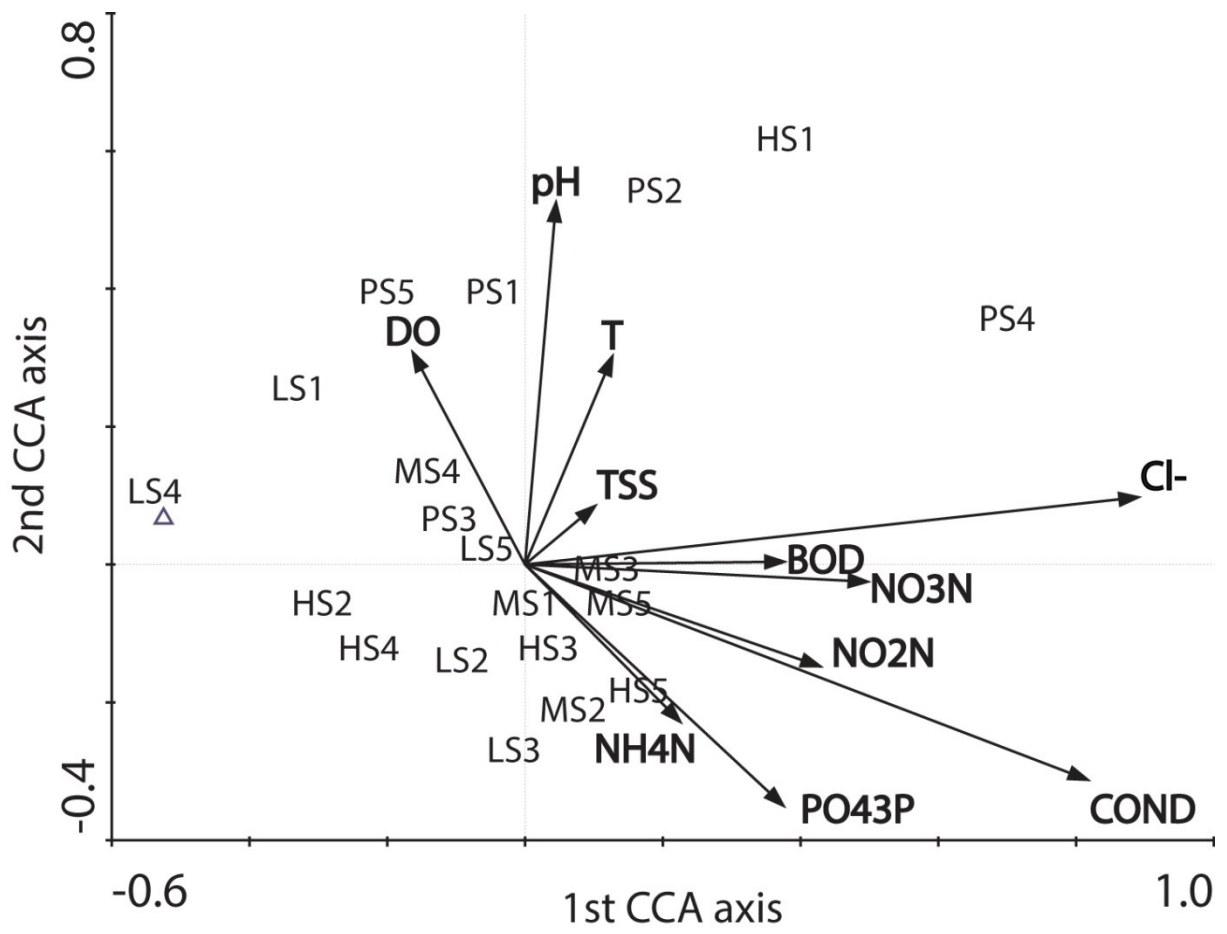
565

566 Fig. 2



567
568

569 Fig. 3



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571

