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

10 **systems**

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Abstract

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

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

1. Introduction

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

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

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

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

2. Materials and Methods

2.1. Sampling and measuring of environmental factors

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

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 μm^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 occured 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, NO_3^--N, NO_2^--N, NH_4^+-N, PO_4^{3-}-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), PO_4^{3-}-P (0.1845), NO_2^--N (0.1690) and NH_4^+-N

222 (0.1115) with the first axis; pH (0.2217) with the second axis; dissolved oxygen (DO; 0.1285)

223 and NO_3^--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), NO_3^--N (0.2204), NO_2^--N (0.1899), BOD (0.1667) and PO_4^{3-}-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), NO_3^--

258   N (0.2247), NO_2^--N (0.1896), PO_4^{3-}-P (0.1708), BOD (0.1611) and NH_4^+-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

8

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 filamnetous 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   *circulare* (Greville) C.A.Agardh, which prefers low water temperature (Stenger-Kovács et al.,

337   2013). *M. circulare* 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. *Achnanthidium*

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 share 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

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

378

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

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

401

5. Conclusions

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

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513

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 –ºC),

547 biological oxygen demand (BOD – mg L^{-1}), chloride ion (Cl⁻ – mg L^{-1}), ammonium-nitrogen

548     $(NH_4^+$- N – mg L^{-1}), nitrite-nitrogen $(NO_2^{--}N$ – mg L^{-1}), nitrate-nitrogen $(NO_3^{--}N$ – mg L^{-1}),

549   phosphate-phosphorus $(PO_4^{3--}P$ – 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

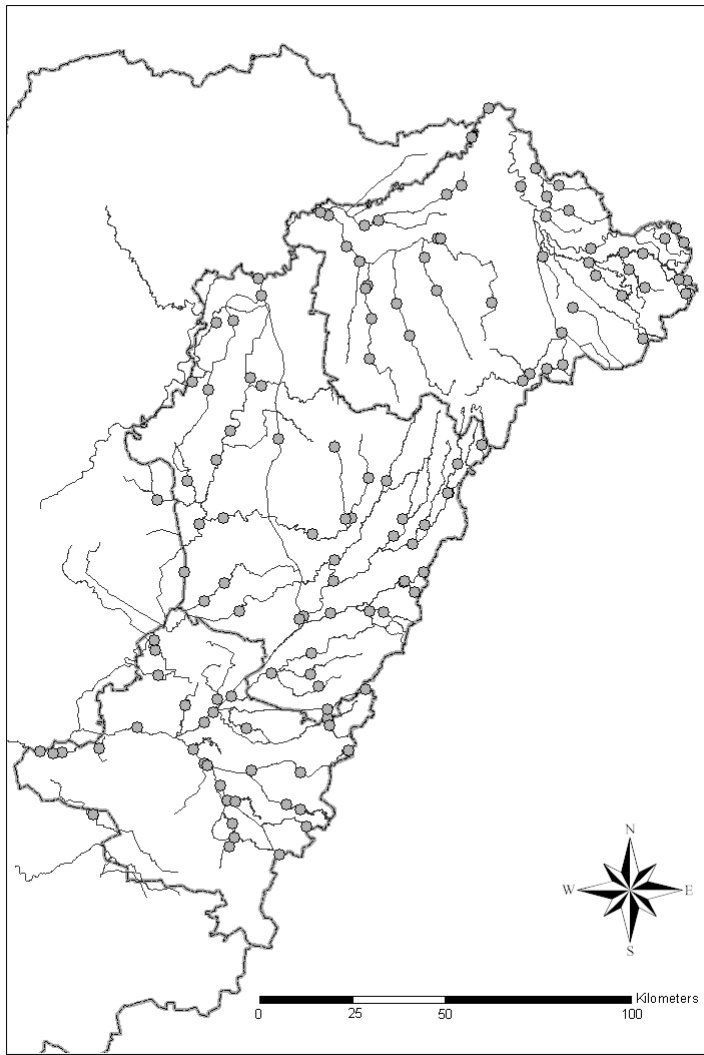555   based on the original guilds classification by Passy (CEMFGs-O).

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

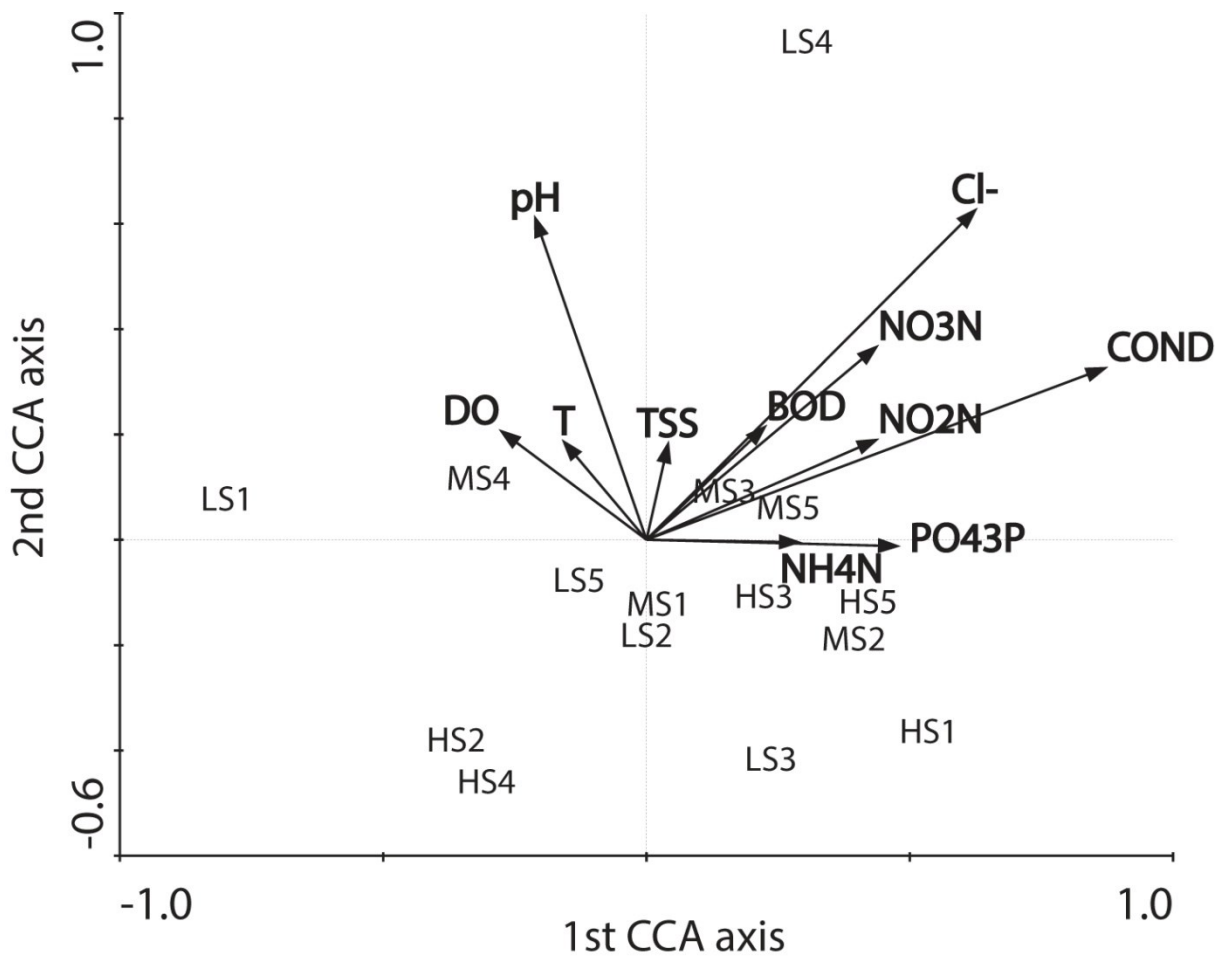561   based on the Passy's guilds classification modified by Rimet and Bouchez (CEMFGs-RB).
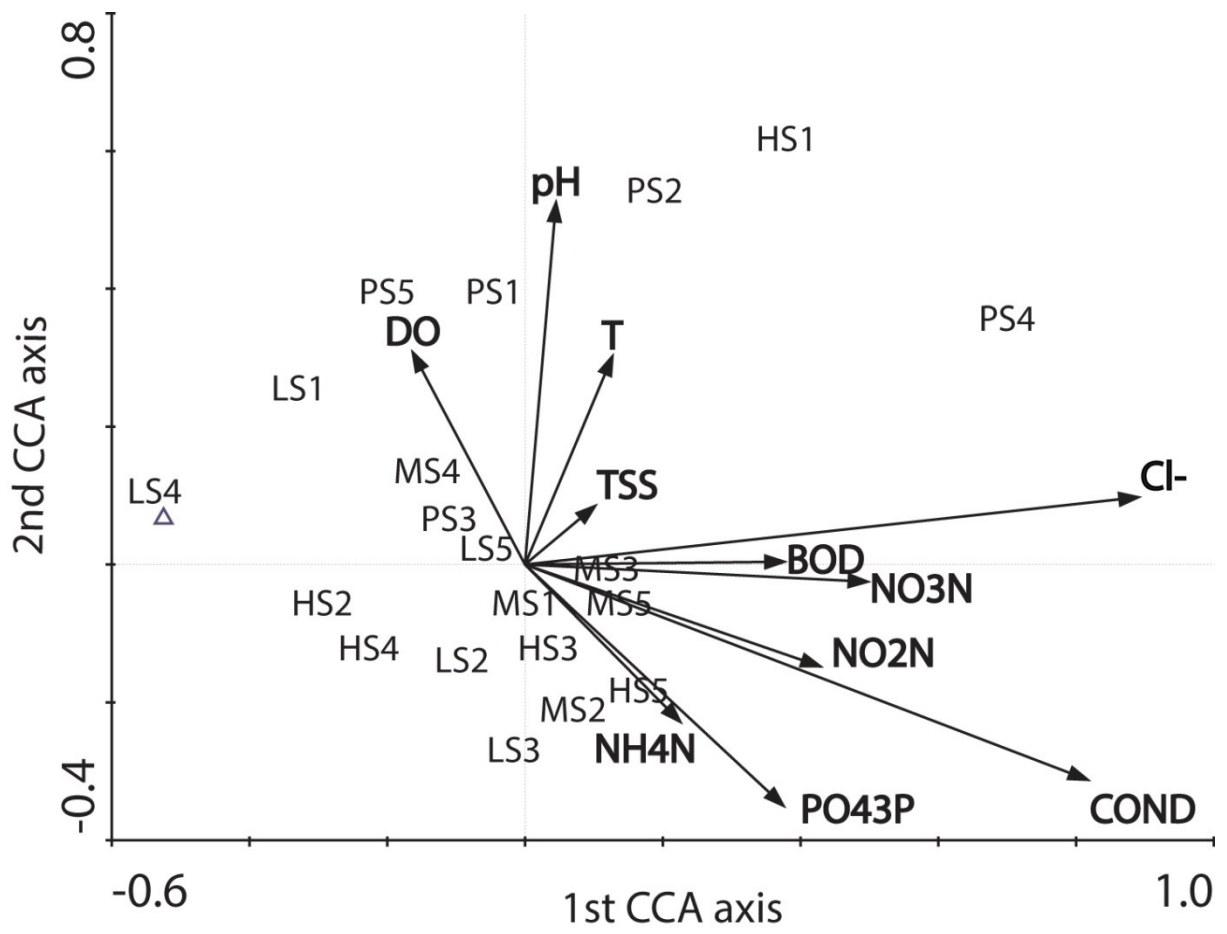
562

563   Fig. 1

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565

566   Fig. 2

569   Fig. 3