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3 **Carbon forms, nutrients and water velocity filter hydrophyte and river-bank species**  
4 **differently: A trait-based study**

5  
6 **Running title:** Community assembly of macrophytes in rivers

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8 Balázs András Lukács<sup>1</sup>, Anna E-Vojtkó<sup>2,6#</sup>, Tibor Erős<sup>3</sup>, Attila Molnár V.<sup>4</sup>, Sándor Szabó<sup>5</sup>  
9 & Lars Götzenberger<sup>6</sup>

10  
11 <sup>1</sup> Department of Tisza Research, MTA Centre for Ecological Research-DRI, 4026 Debrecen,  
12 Hungary

13 <sup>2</sup> University of South Bohemia, České Budějovice, Czech Republic

14 <sup>3</sup> Department of Zoology, MTA Centre for Ecological Research-DRI, 8270 Tihany, Hungary

15 <sup>4</sup> Department of Botany, University of Debrecen, Debrecen, Hungary

16 <sup>5</sup> Department of Environmental Sciences, University of Nyiregyhaza, Nyiregyhaza, Hungary

17 <sup>6</sup> Institute of Botany, Czech Academy of Sciences, Třeboň, Czech Republic

18  
19 Correspondence

20 Balázs András Lukács, Department of Tisza Research, MTA Centre for Ecological Research-  
21 DRI, 4026 Debrecen, Hungary

22 Email: [lukacs.balazs@okologia.mta.hu](mailto:lukacs.balazs@okologia.mta.hu)

23  
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34

35 **Abstract**

36

37 **Questions:** The majority of theories of trait-based plant community assembly have been  
38 developed and tested predominantly in terrestrial ecosystems. Studies investigating the  
39 functional trait composition of aquatic plant communities and their relation to environmental  
40 determinants remain scarce. Macrophytes are essential components of aquatic ecosystems,  
41 and a more detailed knowledge of their trait-based assembly is crucial for their management.  
42 We identified how plant functional traits respond to environmental gradients in streams and  
43 rivers.

44 **Location:** Danube River Catchment, Hungary

45 **Methods:** We studied the processes governing community assembly along major  
46 environmental gradients related to carbon and nutrient limiting factors as well as physical  
47 strain. We used six continuous traits (leaf area, specific leaf area, leaf dry matter content,  
48 seed weight, seed shape, woodiness) and calculated community weighted mean and  
49 standardised effect size of functional diversity for each community. We then used stepwise  
50 regression analyses for each trait along the environmental gradients to test which  
51 environmental factors explain the changes in community weighted mean and functional  
52 diversity. All analyses were conducted for aquatic (hydro-helophyte) and riverbank species  
53 separately.

54 **Results:** We found that the effect of environmental filtering significantly increased toward  
55 higher pH, indicating the response of functional traits to carbon limitation. Our results  
56 showed trait convergence among riverbank species in rivers with higher productivity. Larger  
57 functional diversity (i.e. trait divergence) among hydro-helophyte species suggests an  
58 increase in the diversity of resource acquisition strategies under higher productivity.

59 **Conclusions:** Here we have shown that the functional trait distribution of aquatic and  
60 riverbank plant communities respond to major environmental drivers related to nutrient and  
61 carbon availability. The understanding of how community assembly mechanisms varied  
62 along environmental gradients might be useful when proposing future management and  
63 restoration plans and actions towards the conservation of the aquatic vegetation in streams  
64 and rivers.

65

66 **Keywords:** macrophytes, hydrophytes, functional traits, environmental filtering, community  
67 assembly

## 68 **Introduction**

69 There is a growing consensus that trait composition and diversity of communities explain  
70 functioning better than species richness per se, because filters operate on the traits of species,  
71 rather than on species themselves (McGill, Enquist, Weiher, & Westoby, 2006; Díaz et al.,  
72 2007). The trait-based approach provides more information about the functioning of species  
73 within and across communities, therefore it is a widely used tool to explore community  
74 assembly (Götzenberger et al., 2012). Plant community composition in a given site is a result  
75 of abiotic and biotic filters including dispersal limitation, environmental suitability and  
76 species interactions that determine which traits, and consequently which species, can persist  
77 at a site from an available species pool (Weiher & Keddy, 1995). It is generally accepted that  
78 on the community scale species coexistence is mainly driven by two distinct non-random  
79 processes: environmental filtering and niche differentiation. These community assembly  
80 processes are thought to shape the mean, spread and spacing of functional trait values  
81 differently within and among communities (Cornwell & Ackerly, 2009).

82 Theoretically, abiotic conditions act as filters selecting on species with the suitable  
83 functional traits that can persist in the given habitat. Thus, environmental filtering causes  
84 convergence in traits and reduces functional diversity, therefore, species tend to be more  
85 similar within a community (Kraft, Valencia, & Ackerly, 2008). Niche differentiation is  
86 supposed to prevail on a finer scale, where co-existing species are prevented from being too  
87 similar in their resource use strategies (i.e. have small overlap in functional niches), most  
88 commonly by competitive exclusion. This process leads to divergent traits and increased  
89 functional diversity, supporting the limiting similarity hypothesis (MacArthur & Levins  
90 1967, Stubbs & Wilson, 2004).

91 The distribution of aquatic plants in rivers is mainly determined by the prevailing  
92 environmental conditions mediated by the surrounding water. The most important factors are  
93 water chemical variables (alkalinity, nutrient content) and water physical variables (light,  
94 temperature, substrate characteristics, water movements; Riis, Sand-Jensen, & Vestergaard,  
95 2000; Lacoul & Freedman, 2006; Bornette & Puijalon, 2011). In river ecosystems  
96 environmental variables have a stronger effect on the trait composition of the community  
97 than on its species composition (Göthe et al., 2017). There have been only few studies carried  
98 out so far that investigated the influence of these environmental factors on the trait  
99 distribution of aquatic plant communities. These studies have been limited by the use of  
100 categorical traits (e.g. growth forms in Baattrup-Pedersen et al., 2015), studying single  
101 environmental factors, or not making use of the complementary framework to study

102 community trait means and functional diversity in combination (Göthe et al., 2017). We  
103 therefore, have a more detailed knowledge on functional trait responses only along indirect  
104 gradients of water depth, soil depth, and water availability gradients (Fu et al., 2014b;  
105 Baastrup-Spohr, Sand-Jensen, Nicolajsen, & Bruun, 2015; Rocarpin, Gachet, Metzner, &  
106 Saatkamp, 2016). Understanding the functional trait responses of aquatic plants to other  
107 relevant environmental variables of lakes and rivers, such as alkalinity, pH, water velocity  
108 and trophic conditions, as well as applying a more comprehensive approach regarding trait  
109 distributions, would refine our knowledge about the environment driven assembly of these  
110 communities (Moor et al., 2017).

111 The aim of this study was to investigate the effect of environmental filtering on the  
112 composition of species traits along the above mentioned environmental gradients in a set of  
113 aquatic vegetation samples of streams and rivers in the Danube River catchment. The stress-  
114 dominance hypothesis predicts that environmental filtering is important under stressful  
115 conditions, while competitive interactions will be more important in benign environments  
116 (Weiher & Keddy, 1995; Swenson & Enquist, 2007). According to that, we suggest that  
117 under nutrient limited and physically harsh conditions plant growth is limited, leading to a  
118 change in dominant traits and decrease of trait diversity among co-existing species, but under  
119 optimal conditions competition for light and nutrients becomes more intense. While our  
120 general aim was to detect the environmental gradients that filter species into local  
121 communities, we put forward a number of hypotheses predicting relationships between  
122 particular gradients and traits:

123

124 (1) Increased current velocity leads to a selection of species that are more resistant to physical  
125 stress through a more resistant structure, such as higher stem woodiness, stronger leaf tissues,  
126 and smaller leaf size (Puijalon et al., 2011).

127

128 (2) Submerged aquatic plants obtain carbon for photosynthesis through direct exchange with  
129 the surrounding water. In water carbon is available in three main inorganic forms (carbon-  
130 dioxide, bicarbonate and carbonate) that are transformed to each other along the pH gradient.  
131 Therefore, the availability of carbon changes with different pH levels (Pedersen et al., 2013).  
132 As for most plants carbon uptake is most efficient from carbon-dioxide, we hypothesize a  
133 convergence in CO<sub>2</sub> uptake strategies at lower pH (when this is the only available inorganic  
134 carbon form), whereas a higher diversity in traits related to carbon exchange, growth rate and  
135 nutrient acquisition with increasing pH (decreasing CO<sub>2</sub>, increasing HCO<sub>3</sub><sup>-</sup>).

136

137 (3) Higher nutrient content in the water as well as in the soil favour a higher number of  
138 strategies to exploit these resources (Reich et al., 2003). Therefore, we suggest higher  
139 diversity in traits related to nutrient acquisition and growth. At the same time, species in  
140 nutrient poor environments are constrained to a smaller range of these strategies.

141

142 (4) Although many aquatic plants have the ability to grow and reproduce clonally, they are  
143 still capable of long distance seed dispersal. In running waters, seed size and seed shape  
144 potentially determine seed dispersal ability: smaller and lighter seeds, or seeds with non-  
145 spherical shapes, being able to spread further (Sousa et al., 2007). We therefore, predict seeds  
146 with these characteristics to be dominant in aquatic communities.

147

148 Although our hypotheses concern aquatic plants in running waters in general, we divided the  
149 species in the sampled communities into “true” aquatic species (hydato-helophytes) and  
150 riverbank species, expecting that some of the postulated relationships will not hold, or do so  
151 weaker for the latter group.

152

## 153 **Materials and methods**

### 154 *Study sites*

155 We selected altogether 48 sampling sites in the Danube River catchment within the Pannon  
156 ecoregion, Hungary. Sites were selected using geoinformatic maps in relatively intact  
157 catchments in a way that large artificial barriers (e.g. large reservoir dams) do not constrain  
158 the dispersal of organisms.

159

### 160 *Environmental variables*

161 Site surveys were conducted from July to August 2013, during relatively low water level  
162 conditions. In streams, 6–15 transects (depending on the complexity of the habitat, for details  
163 see Erős, Takács, Specziár, Schmera, & Sály, 2017) were placed perpendicularly to the main  
164 channel at each sampling site to characterise physical features of the environment. A list of  
165 the environmental variables and their descriptive values can be found in Table 1.

166 The sampled lowland and highland rivers and streams can be ordered along a stream size  
167 gradient (see Schmera et al., 2017). Orders 1 and 2 refer to lowland and highland rivers  
168 respectively, while type 3 and 4 refer to lowland and highland streams respectively. We used  
169 the map and typological system of Hungarian running waters to distinguish these four

170 different running water types (Ministry of Environment and Water 2004). Stream sites (n =  
171 27) were wadeable and had a mean width of  $2.8 \pm 0.8$  m and a mean depth of  $34.5 \pm 19.1$  cm,  
172 and a catchment size  $<1000$  km<sup>2</sup>. Rivers (n = 21) had a mean width of  $29.7 \pm 32.2$  m and a  
173 mean depth of  $84.6 \pm 54.3$  cm, and a catchment size  $>1000$  km<sup>2</sup>. Lowland sites (n = 23) were  
174 located between 85 and 180 m a.s.l., and their proportion of coarse substrate was  $1.87 \pm 3.6$   
175 %. Highland sites (n = 25) were located between 109 and 261 m a.s.l., and their proportion of  
176 coarse substrate was  $35.1 \pm 19.2$  %.

177 Mean width of large rivers was measured using the landscape images from Google  
178 Earth, while mean velocity and water depth were measured along the sampling reach at 10–  
179 15 points. Visual estimates of percentage substratum cover were assessed following the  
180 AQEM protocol (AQEM Consortium, 2002). Conductivity and pH were measured with Hach  
181 Lange Q40D (Loveland, Colorado, USA) portable handheld meter, and the content of  
182 nitrogen forms (i.e., nitrite, nitrate and ammonium), calcium and phosphate were measured  
183 using field kits (Hanna Instruments Ltd, Leighton Buzzard, UK). Total phosphorous was  
184 determined by the acid molybdate method (MSZ EN ISO 6878:2004, 2004). Altitude was  
185 measured in the field using a GPS device (Garmin Montana 650, Olathe, Kansas, USA).

186

### 187 *Vegetation sampling*

188 During macrophyte survey we estimated the abundance of angiosperm and gymnosperm  
189 species. All submerged, free floating, amphibious and emerged plants, as well as individuals  
190 attached or rooted on parts of the bank substrate were surveyed. Species abundance of  
191 macrophytes was estimated visually according to a five-level descriptor scale (1, rare; 2,  
192 occasional; 3, frequent; 4, abundant; 5 very abundant) along a 100 m long transect (Kohler,  
193 1978). Streams were surveyed by wading the whole stream width; rivers were surveyed by  
194 wading along one shore and a grapnel was used to collect plant species from deeper regions.  
195 Macrophyte identification was done at the species level. Trees and shrubs were excluded  
196 from the analyses to avoid bias by the different life cycle and biomass allocation strategy of  
197 woody species. Species were differentiated to real aquatic (i.e. hydato-helophytes, thereafter  
198 HH) and river bank species (thereafter RB) according to the Raunkiær's life-form categories  
199 and species moisture index (i.e. Ellenberg's moisture indicator value adapted to the  
200 Hungarian flora: WB; Borhidi, 1993): HH = Hydato-Helophyte life-form; RB= WB > 6  
201 (excluding strictly aquatic species).

202

### 203 *Trait selection*

204 We chose six traits reflecting plant functions and strategies of growth, defence and dispersal  
205 capabilities along the various environmental gradients in rivers. The following trait data were  
206 obtained from the LEDA database (Knevel, Bekker, Bakker, & Kleyer, 2003; Kleyer et al.,  
207 2008):

208 (i) Leaf area (LA or leaf size) is strongly related to the energy and water balance of leaves  
209 (Cornelissen et al., 2003).

210 (ii) Specific leaf area (SLA, the ratio of leaf area to leaf dry mass) is part of the leaf  
211 economics spectrum (LES) and strongly correlated with photosynthetic capacity, relative  
212 growth rate, nitrogen content per leaf mass and leaf life span (Reich et al., 1999, Wright et  
213 al., 2004).

214 (iii) Leaf dry matter content (LDMC, the ratio of leaf dry mass to leaf fresh mass) reflects the  
215 average density of leaf tissues and a trade-off between the investments in structural tissues  
216 versus liquid-phase processes. LDMC is a key variable that governs the correlations among  
217 the traits in the leaf economics spectrum (LES), which is considered as a ‘hard trait’ (Roche,  
218 Díaz-Burlinson, & Gachet, 2004) and usually negatively correlated with relative growth rate  
219 (Weiher et al., 1999).

220 (iv) Seed weight (or seed size) is the oven dry mass of a seed. Large seeds are thought to  
221 have a better chance to establish seedlings. Seed weight also reflects the reproductive effort  
222 of a species; under harsh environmental conditions plants put more effort in stability and  
223 vegetative reproduction instead of seeds or produce smaller seeds (Leishmann, Wright,  
224 Moles, & Westoby, 2000). Seed weight is also correlated with competition ability (Burke &  
225 Grime, 1996).

226 (v) Seed shape is calculated from seed length, width and height (Bekker et al., 1998). Lower  
227 values of seed shape reflect more spherical seeds, while higher values reflect needle- and  
228 disc-shaped seeds. Seed shape is thought to reflect the dispersal ability of the species and the  
229 burial ability of the seeds in the seed-bank. Seed weight and seed shape are good predictors  
230 of seed persistence (‘hard trait’) in temperate-zone seed banks (Thompson, Band, &  
231 Hodgson, 1993).

232 (vi) Woodiness (or stem specific density) indicates the structural strength of the stem; the  
233 durability the plant needs to survive. It also reflects stem defensive ability against pathogens,  
234 herbivores or physical damage (See Appendix S1).

235

236 *Statistical analyses*

237 *Functional diversity and community weighted means*

238 We assessed the functional composition of the studied communities through their functional  
239 diversity and community weighted mean (Ricotta and Moretti 2011). Functional diversity  
240 was measured as standardised effect size of abundance weighted mean pairwise distances  
241 (MPD) between species for each trait ( $SES_{MPD}$ ), i.e. as a deviation of the observed functional  
242 diversity from a null expectation. We used MPD as a measure of functional diversity because  
243 it has been shown to be independent of species richness even for low numbers of species (de  
244 Bello, Carmona, Lepš, Szava-Kovats, & Pärtel, 2016), which we observed for some of our  
245 sampled communities. Standardisation was achieved by randomising the trait data across the  
246 species pool 999 times and using the resulting standard deviation of the expected MPD  
247 values to standardise the difference between the observed and mean expected MPD, i.e.  
248  $SES_{MPD} = (MPD_{obs} - mean(MPD_{exp}))/sd(MPD_{exp})$ .  
249 This allowed us to quantify if the co-existing species were more similar or more dissimilar in  
250 their traits than under the null expectation that the species traits are randomly distributed  
251 among the species. Positive  $SES_{MPD}$  values indicate trait divergence, while negative values  
252 indicate trait convergence, as expected under environmental filtering.

253 The community weighted mean expresses the mean trait value of a community  
254 emphasising the importance of more abundant species:  $CWM = \sum_{i=1}^S p_i x_i$ , with S as the  
255 number of species in the community, and  $p_i$  and  $x_i$  being the relative abundance and trait  
256 value of the  $i^{th}$  species, respectively.

257 Trait values for SLA, LDMC, LA and seed weight were log transformed before calculating  
258  $SES_{MPD}$  and CWM to improve normality.

259 To visualize the relationship between environmental variables for each trait's  
260 functional diversity and community weighted mean, we performed redundancy analyses  
261 (RDA) and plotted the results in biplots. We chose RDA over canonical correspondence  
262 analyses (CCA), because visual inspection of plots between community trait composition and  
263 studied environmental gradients generally indicated linear relationships.

264 We used a stepwise regression approach to select important environmental gradients  
265 for each trait's functional diversity and community weighted mean. Because of shortcomings  
266 related to collinearity and to performance of stepwise model selection with high numbers of  
267 explanatory variables, we conducted principal component analyses (PCA) for two sets of  
268 environmental variables to reduce the number of explanatory variables. The first set  
269 contained variables related to the chemical composition of the river water (concentration of  
270 nitrite, nitrate, ammonium, calcium, phosphorus, phosphate). We did not consider pH in this



271 PCA, as we wanted to retain it as a proxy for carbon dioxide, for which it is directly  
272 indicative. It was not strongly related to any of the other chemical parameters (correlation  
273 coefficients between -0.07 and 0.42). The second set was composed of the river substrate  
274 properties (proportions of marl, silt, sand, fine gravel, coarse gravel, stone, rock). From PCAs  
275 of both sets of variables we used the first two PCA axes scores as explanatory variables,  
276 together with stream size, pH and water velocity resulting in seven explanatory variables.  
277 From full models for each combination of trait, index ( $SES_{MPD}$ , CWM) and species pool (HH,  
278 RB), variables for the adequate model were selected using AIC as a criterion for retaining  
279 variables in the minimum adequate model. This can lead to variables being included,  
280 although their estimates are not statistically significant themselves. The  $R^2$ s of these models  
281 gives an indication of the strength of the relationship between the trait variation and the set of  
282 selected explanatory variables. Paired t-tests were conducted to reveal differences in  $SES_{MPD}$   
283 and CWM between HH and RB species.

284 All analyses were conducted in R version 3.2.4 (R Core Team, 2015), using packages  
285 *picante* (Kembel et al., 2010) and *vegan* (Oksanen et al., 2017).

286

287

## 288 **Results**

289 In total, we obtained trait and abundance data of 155 species in 48 sites (Appendix S1). The  
290 median and maximum numbers of species were higher for RB (median = 12, maximum = 44)  
291 than for the HH communities (median = 6, maximum = 20). Three samples, which contained  
292 only a single species were removed from the community data before conducting further  
293 analyses, because functional diversity calculations are not meaningful in this case.

294 Electronic appendix S2–3 shows the PCA plots of water chemical and substrate  
295 variables. For the water chemical variables, the first two PCA axes explained 59% of  
296 variability in the data. While the first axis was mainly related to nitrate and nitrite, the second  
297 axis was related to ammonium and calcium. Phosphorus and phosphate had lower loadings  
298 on the first two axes. For the PCA of substrate properties the explained variability of the first  
299 two PCA axes was 50%. The first PCA axis related to sandy silt, coarse and fine gravel, while  
300 marl, stone, rock, and sand loaded mainly on the second axis.

301 The correlation of environmental variables and  $SES_{MPD}$  and CWM values are shown  
302 in Figure 1-4. In case of HH species we found that the diversity of leaf related traits and seed  
303 shape had a positive correlation with nutrient rich fine sediment and showed negative  
304 correlation with pH, current velocity and elevation (i.e. at highland sites leaves had a lower

305 functional diversity) (Fig 1). The opposite trend was found for seed weight. In case of RB  
306 species the diversity of leaf related traits showed negative correlation with nutrient rich fine  
307 sediment and elevation (i.e. at lowland sites leaves had a lower functional diversity) (Fig. 2).  
308 Seed weight and woodiness showed positive correlation with pH and temperature, while seed  
309 shape showed the opposite trend.

310 Functional composition (i.e. CWM) of HH communities shifted to higher seed weight  
311 in lowland rivers (Stream Order 1) (Fig. 3) and to higher LDMC under higher pH. HH  
312 communities were characterised with higher woodiness and LDMC under higher velocity and  
313 in highland rivers (Stream Order 2) and lower LA and LDMC in nutrient rich fine sediment.  
314 Functional composition of RB communities shifted to higher seed weight and woodiness in  
315 lowland sites (Stream Order 1 and 3). The opposite trend was found for current velocity and  
316 highland sites (Stream Order 2 and 4). RB communities were characterised with higher LA  
317 under higher pH, but lower LA in nutrient rich fine sediment.

318 The significance of the single trait metric – environmental gradient relationships can  
319 be found in Table 2, and scatterplots for each relationship in Appendix S4-7.

320

#### 321 *Plant trait – stream size relationship*

322 We found a shift from trait convergence to trait divergence (i.e. from negative to positive  
323  $SES_{MPD}$  values) along the stream order gradient for SLA among HH and RB species (Table 2,  
324 Appendix S4-5). This suggests that we found decreasing trait convergence from lowland  
325 rivers to highland rivers and increasing trait divergence from lowland streams to highland  
326 streams. Woodiness became less converged along the stream size gradient among HH  
327 species.

328 The community weighted mean of LA increased, while seed weight and woodiness decreased  
329 significantly among HH species (Table 2, Appendix S6).

330

#### 331 *Plant trait – temperature relationship*

332 We found a significant shift from trait convergence to trait divergence (i.e. from negative to  
333 positive  $SES_{MPD}$  values) with increasing temperature for LDMC among HH species (Table 2,  
334 Appendix S3). The same trend was found for woodiness among RB species (Table 2,  
335 Appendix S4). Woodiness became less converged along the temperature gradient among HH  
336 species. We did not find any significant changes in the community weighted mean of the  
337 traits.

338

339 *Plant trait – water velocity gradient relationship*

340 Specific leaf area became more converged (i.e. more negative  $SES_{MPD}$  values) along the  
341 velocity gradient among RB species (Table 2, Appendix S5).

342 The community weighted mean of woodiness significantly increased along the velocity  
343 gradient among HH species (Table 2, Appendix S6), i.e. HH species tend to produce more  
344 resilient woody stems with increasing water velocity.

345

346 *Plant trait – pH gradient relationship*

347 We found a significant shift from trait divergence to trait convergence (i.e. from positive to  
348 negative  $SES_{MPD}$  values) with increasing pH in the case of LA, SLA, and LDMC among HH  
349 species (Table 2, Appendix S4). The same trend was found for SLA among RB species  
350 (Appendix S5). Woodiness became less converged (i.e.  $SES_{MPD}$  values were less negative)  
351 with increasing pH among HH species (Appendix S4).

352 The CWM of SLA significantly decreased with increasing pH among HH and RB  
353 species, which means that leaf tissue became on average denser towards higher pH (i.e.  
354 where bicarbonate is the main available form of carbon) (Table 2, Appendix S6-7). The  
355 CWM of LA significantly increased among HH species with increasing pH, while the same  
356 trend was found for LDMC among RB species.

357

358 *Plant trait – water chemical compound gradient relationship*

359 Along the PC1 axis of water chemical compounds (i.e. mostly related to nitrate content) we  
360 found a significant shift from trait convergence to trait divergence (i.e. shift from negative to  
361 positive  $SES_{MPD}$  values) in the case of LDMC among HH species (Table 2, Appendix S4).

362 We did not find any changes in the community weighted mean of the traits.

363

364 *Plant trait – substrate properties relationship*

365 Along the PC1 axis of substrate properties (i.e. nutrient rich, fine sediment) trait convergence  
366 became significantly weaker (i.e. less negative  $SES_{MPD}$  values) for SLA, while a significant  
367 shift from trait convergence to trait divergence in the case of LA among HH species could be  
368 observed (Table 2, Appendix S4). LA and SLA became more converged (i.e. more negative  
369  $SES_{MPD}$  values) along the substrate property gradient among RB species (Appendix S5).

370 Along the PC2 axis of substrate properties (i.e. nutrient poor, coarse sediment) we found a  
371 significant shift from trait divergence to trait convergence in the case of woodiness among  
372 RB species.

373 The community weighted mean of LDMC significantly decreased along the PC1 axis  
374 gradient among HH species (Table 2, Appendix S6), while the opposite trend was found for  
375 seed weight among RB species (Appendix S7). Community weighted mean of LA  
376 significantly decreased along the PC2 axis gradient among RB species.

377

378 *Differences between hydato-helophyte and riverbank species*

379 We found significantly higher functional diversity in RB communities than HH communities  
380 for all traits except for seed shape ~~We found significantly higher functional diversity in RB~~  
381 ~~communities than HH communities for LA, seed weight, and seed shape~~ (Fig. 5). River bank  
382 communities were characterised by significantly higher SLA, seed weight and woodiness  
383 than HH communities. The opposite trend was found for seed shape (Fig. 6).

384

## 385 **Discussion**

386 *The relative importance of environmental filtering and niche differentiation*

387 The growth and survival of aquatic plants is determined by various environmental factors  
388 (Sand-Jensen, 1989). Our study assessed the effect of environmental variables on functional  
389 traits of river plants. The results suggest that functional convergence due to environmental  
390 filtering acts along the studied environmental gradients. Although our analyses showed trait  
391 divergence in some cases, these patterns are unlikely the result of limiting similarity.  
392 Previous local scale studies have demonstrated that competition and limiting similarity (niche  
393 differentiation) can both play a role in aquatic ecosystems under high productivity  
394 (Engelhardt & Ritchie, 2001; Fu et al., 2014a). In our study, however, plot sizes were  
395 insufficient for investigating the effect of competitive interactions, which occur on a much  
396 finer scale (Weiher & Keddy 1995). Moreover, according to our results, when SES of  
397 functional diversity were positive, relationships with the nutrient gradients were weak. Trait  
398 divergence in our data is more likely a consequence of small scale environmental  
399 heterogeneity of the river environment than of limiting similarity, as a result of sampling  
400 across the entire river transect (Kraft & Ackerly, 2010).

401 Overall, we found significant differences in the case of both functional diversity and  
402 dominant trait values between HH and RB communities, which underpins their different  
403 resource use strategies and adaptations to occupy different habitats. We found the greatest  
404 and most consistent changes of trait composition and diversity along the stream size, pH and  
405 substrate property gradients, which underpins the importance of the leaf economic spectrum  
406 (LA, SLA, LDMC) and two key resources: carbon and nutrients.

407

408 *Traits response to physical properties*

409 The trait based structure of macrophyte communities changed significantly with stream size.  
410 We found a shift from convergence to divergence in SLA along the stream size gradient.  
411 Considering that streams have higher water velocity to channel width ratio than rivers,  
412 streams can represent a harsher environment, and act as a physiological (via substrate  
413 characteristics and light depletion) and mechanical stress for plants (Bornette & Puijalon,  
414 2011; Puijalon et al., 2011; Read & Stokes, 2006). Exposure to currents or waves can result  
415 in reduced plant biomass and height (dwarfed growth form), reduced leaf area and a greater  
416 allocation to below-ground organs (Doyle, 2001; Strand & Weisner, 2001). Conversely, the  
417 obtained convergence of SLA in rivers and divergence of SLA in streams (irrespective of  
418 its highland or lowland position) might contradict the stress-dominance hypothesis (i.e. trait  
419 convergence in harsher and trait divergence in benign conditions). Differences in the  
420 competition for light in river and stream habitats might be affected by the homogeneity of  
421 environmental conditions. Compared to rivers, streams offer a more heterogeneous range of  
422 niches (from open to shaded), therefore, they are favoured by plants with more diverse light-  
423 use strategies.

424

425         Although we cannot directly compare the continuous traits in our study and  
426 categorical trait attributes (e.g. meristem position) used by others (e.g. Willby, Abernethy, &  
427 Demars, 2000), our findings corroborate results of Baattrup-Pedersen et al. (2015), who also  
428 found that stream size influenced the abundance weighted trait characteristics of macrophyte  
429 communities. Although we didn't find changes in the CWM of SLA along the stream size  
430 gradient, Baattrup-Pedersen et al. (2015) described that plant communities in small streams  
431 are characterised by a higher abundance of light-demanding species (having meristems with  
432 single apical growth point). Overall, we can conclude that the size of the river habitat can  
433 affect not only the growth-form composition of aquatic plant communities (i.e. categorical  
434 trait attributes) but the size and composition of specific plant organs.

435         The increasing functional diversity of woodiness among HH species indicates the  
436 decreasing importance of environmental filtering related to mechanical durability and  
437 defences of the stems against water movement in streams compared to rivers. On the other  
438 hand, the increasing CWM of woodiness among HH species indicates their ability to resist  
439 mechanical fragmentation.

440

441 *Traits response to carbon limitation*

442 The pH of the sampled streams and rivers was between 7.62 and 9.07, which lies in the  
443 middle of the section of the pH gradient where the relative distribution of the three main  
444 inorganic carbon types (carbon-dioxide, bicarbonate and carbonate) is transformed because  
445 they are converted into each other (Pedersen et al., 2013). Below pH 6, dissolved inorganic  
446 carbon is present as CO<sub>2</sub>. In general, this carbon form is more readily used for underwater  
447 photosynthesis than bicarbonate. However, above pH 8, CO<sub>2</sub> gradually disappears from  
448 waters, because between pH 7 and 10 it is converted into bicarbonate (HCO<sub>3</sub><sup>-</sup>). Bicarbonate is  
449 an additional carbon source among most of the aquatic plants except for pteridophytes and  
450 mosses.

451         Decreasing functional diversity of SLA, LA, and LDMC among HH species, and SLA  
452 among RB species along the pH gradient suggests that the effect of environmental filtering  
453 significantly increased toward higher pH (i.e. CO<sub>2</sub> limitation). Moreover, the observed  
454 pattern of functional diversity does support our expectation of a stronger filtering in HH  
455 communities, compared to RB. On the contrary, HH communities became less converged on  
456 woodiness (i.e. SES values of functional diversity became less negative) along the pH  
457 gradient, indicating weaker environmental filtering toward higher pH. Overall, the observed  
458 variation of leaf and woodiness traits along the pH gradient suggests that HH communities  
459 have only a small range of leaf „structure”, which can be characterised with high LDMC and  
460 low SLA (i.e. tough leaf syndrome, details see later) under higher concentration of  
461 bicarbonate, while woodiness (and physical resistance) became less important. In that way  
462 species can reallocate nutrients and energy from the stem to the leaves with increasing pH,  
463 which indicates a functional shift from resistance into photosynthesis.

464         Regarding CWM of leaf traits, SLA significantly decreased among HH and RB  
465 species, LDMC significantly increased among RB species, whereas LA significantly  
466 increased among HH species. These trends indicate denser leaf tissue (i.e. tough leaf  
467 syndrome) towards higher pH, where only the bicarbonate form of inorganic carbon is  
468 available. Aquatic plants with the ability to use bicarbonate have major competitive  
469 advantage over obligate CO<sub>2</sub> users under CO<sub>2</sub> limited conditions (Maberly & Madsen, 2002).  
470 Our results suggest a negative correlation between SLA and bicarbonate use ability (or  
471 efficiency) among HH species. Moreover, these results indicate different adaptive  
472 mechanisms for higher pH between HH and RB species. Hydato-helophyte species attain low  
473 SLA by producing larger and denser leaves, while RB species tend to invest more only in  
474 tissue density, i.e. produce denser leaves under higher pH conditions. This is confirmed by

475 the fact that LDMC converged to higher values in both HH and RB species, but LA  
476 converged to higher values only among HH species.

477 According to Poorter, Niinemets, Poorter, Wright, & Villar (2009) high SLA is  
478 typical for aquatic plants, as investment in supportive structures counteracting gravity is not  
479 needed in aquatic plants. However, Pierce, Brusa, Sartori, & Cerabolini (2012) and Lukács et  
480 al. (2017) demonstrated that not all aquatic plants lie at the acquisitive end of the leaf  
481 economics spectrum. In general, species with low SLA are geared for the conservation of  
482 acquired resources (Cornelissen et al., 2003). Due to the higher dry matter content they are  
483 characterised by lower growth rates, higher concentration of cell walls and secondary  
484 metabolites; overall, their leaves contain more carbon and are more resistant. Therefore, low  
485 SLA in aquatic plants might reflect the dominance of bicarbonate users on the community  
486 level..

487

#### 488 *Traits response to nutrient limitation*

489 Changes in trait patterns along the first axis of substrate property support our second  
490 hypothesis for HH, but not for RB communities. For HH communities, we found significant  
491 changes of the functional diversity of LA and SLA along the PC1 substrate property gradient,  
492 with higher diversity in these traits in finer, more nutrient rich sediments. For RB  
493 communities, the pattern was opposite, with less diversity of LA and SLA in communities  
494 towards nutrient enrichment.

495 There are contrasting views in the literature regarding how the strength of trait  
496 divergence and convergence varies along productivity gradients. The trends depend mainly  
497 on the studied traits (Bernard-Verdier et al., 2012; Spasojevic & Suding, 2012) and the size  
498 of the gradient (Bernard-Verdier et al., 2012). Some authors (Pakeman, 2011; e.g. Mason et  
499 al., 2012; Carboni, et al. 2014) found increasing trait convergence toward higher productivity,  
500 others (Lhotsky et al., 2016) found the opposite trend, while Navas & Violle (2009) argued  
501 that trait convergence is expected at both ends of the productivity gradient. Here, we found  
502 decreasing convergence of SLA and LA towards higher nutrient content among HH, and the  
503 opposite, increasing convergence of SLA and LA among RB species along the same gradient.  
504 These results would suggest that more productive aquatic habitats enable and maintain a  
505 higher diversity in growth rate and nutrient acquisition strategies (Cornelissen et al., 2003),  
506 supporting the idea of stronger filtering under more stressful conditions, in this case, low  
507 nutrient levels (Weiher & Keddy, 1995; Swenson & Enquist, 2009). Changes in CWM of  
508 SLA and LA along the nutrient gradients, however, were not significant, indicating that the

509 diversified strategies in nutrient richer environments are achieved from similar “average  
510 communities” for these traits.

511 On the other hand, the trait convergence of RB species are in line with Grime’s (2006)  
512 hypothesis that higher productivity leads to trait convergence. However, this interpretation  
513 needs to be made with caution, since the used sample scale is not the most appropriate to  
514 infer competition based patterns, and smaller scale studies would be needed to clarify this  
515 issue.

516

### 517 **Conclusion**

518 In this study, we identified how functional traits of macrophytes respond to the carbon  
519 (related to pH), soil nutrient and current velocity gradients in streams and rivers and how  
520 these relationships vary between HH and RB communities. The variation in communities’  
521 functional composition in terms of functional diversity ( $SES_{MPD}$ ) and dominant traits (CWM)  
522 mirrored significant trends and adaptation mechanisms to nutrient and carbon sources among  
523 macrophytes, with strength and direction largely depending on the specific trait. Overall,  
524 traits showed stronger associations with the carbon (i.e. pH) gradient compared to nutrient  
525 gradients. We can therefore conclude that mechanisms underlying changes in stream plant  
526 communities are related mostly to light capture and utilization and not to nutrient  
527 preferences. This clearly underpins the results of Baattrup-Pedersen et al. (2015) who  
528 detected similar trends through the composition of growth-forms. Our use of more precise  
529 continuous traits and specific relevant gradients has led to an improved understanding of  
530 aquatic community assembly in river habitats.

531

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537 The authors have no conflict of interest to declare.

538

### 539 **Authors’ contributions**

540 The study was planned by BAL; fieldwork was organized and performed mostly by BAL,  
541 AEV and AMV; statistical analyses were performed by LG; the manuscript was written by  
542 BAL, LG and AEV, all other authors made essential contributions to revise the text.



543

544 **Data accessibility**

545 Data used in the analyses are to be deposited in the Dryad repository.

546

547 **References**

- 548 AQEM Consortium (2002). Manual for the application of the AQEM method. A  
549 comprehensive method to assess European streams using benthic macroinvertebrates,  
550 developed for the purpose of the Water Framework Directive. Version 1.0.
- 551 Baastrup-Spohr, L., Sand-Jensen, K., Nicolajsen, S.V. & Bruun, H.H. (2015). From soaking  
552 wet to bone dry: predicting plant community composition along a steep hydrological  
553 gradient. *Journal of Vegetation Science*, 26, 619–630.
- 554 Baattrup-Pedersen, A., Göthe, E., Larsen, S.E., O'Hare, M., Birk, S., Riis, T. & Friberg, N.  
555 (2015). Plant trait characteristics vary with size and eutrophication in European lowland  
556 streams. *Journal of Applied Ecology*, 52, 1617–1628.
- 557 Bekker, R. M., Bakker, J. P., Grandin, U., Kalamees, R., Milberg, P., Poschlod, P.,  
558 Thompson K. & Willems, J. H. (1998). Seed size, shape and vertical distribution in the soil:  
559 indicators of seed longevity. *Functional Ecology*, 12, 834–842.
- 560 Bernard-Verdier, M., Navas, M.-L., Vellend, M., Violle, C., Fayolle, A. & Garnier, E.  
561 (2012). Community assembly along a soil depth gradient: contrasting patterns of plant trait  
562 convergence and divergence in a Mediterranean rangeland. *Journal of Ecology*, 100, 1422–  
563 1433.
- 564 Borhidi, A. (1993). Social behaviour types of the Hungarian flora, its naturalness and relative  
565 ecological indicator values. University of Janus Pannonius. 39 pp.
- 566 Bornette, G. & Puijalon, S. (2011). Response of aquatic plants to abiotic factors: a review.  
567 *Aquatic Sciences*, 73, 1–14.
- 568 Burke, M.J.W. & Grime, J.P. (1996). An experimental study of plant community invasibility.  
569 *Ecology*, 77, 776–790.
- 570 Carboni, M., de Bello, F., Janeček, Š., Doležal, J., Horník, J., Lepš, J., Reitalu, T. &  
571 Klimešová, J. (2014). Changes in trait divergence and convergence along a productivity  
572 gradient in wet meadows. *Agriculture, Ecosystems and Environment*, 182, 96–105.
- 573 Cornelissen, J.H.C., Lavorel, S., Garnier, E., Díaz, S., Buchmann, N., Gurvich, D.E., Reich,  
574 P.B., ... Poorter, H. (2003). Handbook of protocols for standardized and easy measurement  
575 of plant functional traits worldwide. *Australian Journal of Botany*, 51, 335–380.

576 Cornwell, W.K. & Ackerly, D.D. (2009). Community assembly and shifts in plant trait  
577 distributions across an environmental gradient in coastal California. *Ecological*  
578 *Monographs*, 79, 109–126.

579 de Bello, F., Carmona, C.P., Lepš, J., Szava-Kovats, R. & Pärtel, M. (2016). Functional  
580 diversity through the mean trait dissimilarity: resolving shortcomings with existing  
581 paradigms and algorithms. *Oecologia*, 180, 933–940.

582 Díaz, S., Lavorel, S., de Bello, F., Quétier, F., Grigulis, K. & Robson T.M. (2007).  
583 Incorporating plant functional diversity effects in ecosystem service assessments.  
584 *Proceedings of the National Academy of Sciences of the United States of America*, 104,  
585 20684–20689.

586 Doyle, R.D. (2001). Effects of waves on the early growth of *Vallisneria americana*.  
587 *Freshwater Biology*, 46, 389–397.

588 Engelhardt, K.A.M. & Ritchie, M.E. (2001). Effects of macrophyte species richness on  
589 wetland ecosystem functioning and services. *Nature*, 411, 687–689.

590 Erős, T., Takács, P., Specziár, A., Schmera, D., Sály, P. (2017). Effect of landscape context  
591 on fish metacommunity structuring in stream networks. *Freshwater Biology*, 62, 215–228.

592 Fu, H., Zhong, J., Yuan, G., Ni, L., Xie, P. & Cao, T. (2014a). Functional traits composition  
593 predict macrophytes community productivity along a water depth gradient in a freshwater  
594 lake. *Ecology & Evolution*, 4, 1516–1523.

595 Fu, H., Zhong, J., Yuan, G., Xie, P., Guo, L., Zhang, X., ... Ni, L. (2014b). Trait-based  
596 community assembly of aquatic macrophytes along a water depth gradient in a freshwater  
597 lake. *Freshwater Biology*, 59, 2462–2471.

598 Göthe, E., Baattrup-Pedersen, A., Wiberg-Larsen P., Graeber, D., Kristensen, E.A. & Friberg,  
599 N. (2017). Environmental and spatial controls of taxonomic versus trait composition of  
600 stream biota. *Freshwater Biology*, 62, 397–413.

601 Götzenberger, L., de Bello, F., Bråthen, K.A., Davison, J., Dubuis, A., Guisan, A., ... Zobel,  
602 M. (2012). Ecological assembly rules in plant communities—approaches, patterns and  
603 prospects. *Biological Reviews*, 87, 111–127.

604 Grime, J.P. (2006). Trait convergence and trait divergence in herbaceous plant communities:  
605 mechanisms and consequences. *Journal of Vegetation Science*, 17, 255–260.

606 Kembel, S.W., Cowan, P.D., Helmus, M.R., Cornwell, W.K., Morlon, H., Ackerly, D.D.,  
607 Blomberg, S.P. & Webb, C.O. (2010). Picante: R tools for integrating phylogenies and  
608 ecology. *Bioinformatics*, 26, 1463–1464.

609 Kleyer, M., Bekker, R.M., Knevel, I.C., Bakker, J.P., Thompson, K., Sonnenschein, M., ...  
610 Peco, B. (2008). The LEDA Traitbase: a database of life-history traits of the Northwest  
611 European flora. *Journal of Ecology*, 96, 1266–1274.

612 Knevel, I.C., Bekker, R.M., Bakker, J.P. & Kleyer, M. (2003). Life-history traits of the  
613 Northwest European flora: the LEDA database. *Journal of Vegetation Science*, 14, 611–  
614 614.

615 Kohler, A. (1978) Methoden der Kartierung von Flora und Vegetation von  
616 Süßwasserbiotopen. *Landschaft und Stadt*, 10, 73–85.

617 Kraft, N.J.B. & Ackerly, D.D. (2010). Functional trait and phylogenetic tests of community  
618 assembly across spatial scales in an Amazonian forest. *Ecological Monographs*, 80, 401–  
619 422.

620 Kraft, N.J.B., Valencia, R. & Ackerly D.D. (2008). Functional traits and niche-based tree  
621 community assembly in an amazonian forest. *Science*, 322, 580–582.

622 Lacoul, P. & Freedman, B. (2006): Environmental influences on aquatic plants in freshwater  
623 ecosystems. *Environmental Reviews*, 14, 89–136.

624 Leishmann, M.R., Wright, I.J., Moles, A.T. & Westoby, M. (2000). *The evolutionary ecology*  
625 *of seed size*. In: Fenner, M. (eds): *Seeds: the ecology of regeneration in plant communities*  
626 2<sup>nd</sup> Edition. CABI publishing, UK, pp 31–57.

627 Lhotsky, B., Kovács, B., Ónodi, G., Cseceserits, A., Rédei, T., Lengyel, A., Kertész, M. &  
628 Botta-Dukát Z. (2016). Changes in assembly rules along a stress gradient from open dry  
629 grasslands to wetlands. *Journal of Ecology* 104: 507–517.

630 Lukács, B.A., Vojtkó, A.E., Mesterházy, A., Molnár, V.A., Süveges, K., Végvári, Z., ...  
631 Cerabolini, B.E.L. (2017). Growth-form and spatiality driving the functional difference of  
632 native and alien aquatic plants in Europe. *Ecology and Evolution*, 7, 950–963.

633 Maberly, S.C. & Madsen, T.V. (2002). Freshwater angiosperm carbon concentrating  
634 mechanisms: processes and patterns. *Functional Plant Biology*, 29, 393–405.

635 MacArthur, R.H. & Levins, R. (1967). The limiting similarity, convergence and divergence  
636 of coexisting species. *American Naturalist*, 101, 377–385. <http://dx.doi.org/10.1086/282505>

637 Mason, N.W.H., Richardson, S.J., Peltzer, D.A., de Bello, F., Wardle, D.A. & Allen, R.B.  
638 (2012). Changes in coexistence mechanisms along a long-term soil chronosequence  
639 revealed by functional trait diversity. *Journal of Ecology*, 100, 678–689.

640 McGill, B.J., Enquist, B.J., Weiher, E. & Westoby, M. (2006). Rebuilding community  
641 ecology from functional traits. *Trends in Ecology and Evolution*, 21, 178–185.

642 Ministry of Environment and Water (2004). Departmental Order 31/2004 (XII. 30.) on the  
643 Rules of Assessment and Evaluation of Surface Waters in Hungary [In Hungarian].  
644 Moor, H., Rydin, H., Hylander, K., Nilsson, M.B., Lindborg, R. & Norberg J. (2017).  
645 Towards a trait-based ecology of wetland vegetation. *Journal of Ecology*, 105, 1623–1635.  
646 MSZ EN ISO 6878:2004, 2004. Water quality. Determination of phosphorus. Ammonium  
647 molybdate spectrometric method (ISO 6878:2004).  
648 Navas, M. & Violle, C. (2009). Plant traits related to competition: how do they shape the  
649 functional diversity of communities? *Community Ecology*, 10, 131–137.  
650 Oksanen, J., Blanchet, F.J., Friendly, M., Kindt, R., Legendre, P., McGlenn, D., ... Wagner,  
651 H. (2017). vegan: Community Ecology Package. R package version 2.4-5. [https://CRAN.R-](https://CRAN.R-project.org/package=vegan)  
652 [project.org/package=vegan](https://CRAN.R-project.org/package=vegan).  
653 Pakeman, R.J. (2011). Functional diversity indices reveal the impacts of land use  
654 intensification on plant community assembly. *Journal of Ecology*, 99, 1143–1151.  
655 Pedersen, O., Colmer, T.D. & Sand-Jensen, K., (2013). Underwater photosynthesis of  
656 submerged plants – recent advances and methods. *Frontiers in Plant Science*, 4, 1–19.  
657 Pierce, S., Brusa, G., Sartori, M. & Cerabolini, B.E.L. (2012). Combined use of leaf size and  
658 economics traits allows direct comparison of hydrophyte and terrestrial herbaceous adaptive  
659 strategies. *Annals of Botany*, 109, 1047–1053.  
660 Poorter, H., Niinemets, Ü., Poorter, L., Wright, I.J. & Villar, R. (2009). Causes and  
661 consequences of variation in leaf mass per area (LMA): a meta-analysis. *New Phytologist*,  
662 182, 565–588.  
663 Puijalon, S., Bouma, T.J., Douady, C.J., van Groenendael, J., Anten, N.P.R., Martel, E. &  
664 Bornette, G. (2011). Plant resistance to mechanical stress: evidence of an avoidance–  
665 tolerance trade-off. *New Phytologist*, 191, 1141–1149.  
666 R Core Team (2015). R: A language and environment for statistical computing. *R Foundation*  
667 *for Statistical Computing*. Vienna, Austria. Available at [www.r-project.org](http://www.r-project.org)  
668 Ricotta, C. & Moretti, M. (2011). CWM and Rao's quadratic diversity: A unified framework  
669 for functional ecology. *Oecologia*, 167, 181–188.  
670 Read, J. & Stokes, A. (2006). Plant biomechanics in an ecological context. *American Journal*  
671 *of Botany*, 93, 1546–1565.  
672 Reich, P.B., Ellsworth, D.S., Walters, M.B., Vose, J.M., Gresham, C., Volin, J.C. &  
673 Bowman, W.D. (1999). Generality of leaf trait relationships: a test across six biomes.  
674 *Ecology*, 80, 1955–1969.

675 Reich, P.B., Wright, I.J., Cavender-Bares, J., Craine, J.M., Oleksyn, J., Westoby, M., &  
676 Walters, M.B. (2003). The evolution of plant functional variation: traits, spectra, and  
677 strategies. *International Journal of Plant Science*, 164, S143–S164.

678 Riis, T., Sand-Jensen, K. & Vestergaard, O. (2000). Plant communities in lowland Danish  
679 streams: species composition and environmental factors. *Aquatic Botany*, 66, 255–272.

680 Rocarpin, P., Gachet, S., Metzner, K. & Saatkamp, A. (2016). Moisture and soil parameters  
681 drive plant community assembly in Mediterranean temporary pools. *Hydrobiologia*, 781,  
682 55–66.

683 Roche, P., Díaz-Burlinson, N. & Gachet, S. (2004). Congruency analysis of species ranking  
684 based on leaf traits: which traits are the more reliable? *Plant Ecology*, 174, 37–48.

685 Sand-Jensen, K. (1989). Environmental variables and their effect on photosynthesis of  
686 aquatic plant communities. *Aquatic Botany*, 34, 5–25.

687 Schmera, D., Árva, D., Boda, P., Bodis, E., Bolgovics, Á., Borics, G., ... Erős, T. (2017).  
688 Does isolation influence the relative role of environmental and dispersal-related processes  
689 in stream networks? An empirical test of the network position hypothesis using multiple  
690 taxa. *Freshwater Biology*, 63, 74–85.

691 Sousa, W.P., Kennedy, P.G., Mitchell, B.J. & Ordonez, B.M. (2007). Supply-Side Ecology in  
692 Mangroves: Do Propagule Dispersal and Seedling Establishment Explain Forest Structure?  
693 *Ecological Monographs*, 77, 53–76.

694 Spasojevic, M.J. & Suding, K.N. (2012). Inferring community assembly mechanisms from  
695 functional diversity patterns: the importance of multiple assembly processes. *Journal of*  
696 *Ecology*, 100, 652–661.

697 Strand, J.A. & Weisner, S.E.B. (2001). Morphological plastic responses to water depth and  
698 wave exposure in an aquatic plant (*Myriophyllum spicatum*). *Journal of Ecology*, 89, 166–  
699 175.

700 Stubbs, W.J. & Wilson, J.B. (2004). Evidence for limiting similarity in a sand dune  
701 community. *Journal of Ecology*, 92, 557–567.

702 Swenson, N.G. & Enquist, B.J. (2007). Ecological and evolutionary determinants of a key  
703 plant functional trait: wood density and its community-wide variation across latitude and  
704 elevation. *American Journal of Botany*, 94, 451–459.

705 Swenson, N. G. & Enquist, B. J. (2009). Opposing assembly mechanisms in a Neotropical  
706 dry forest: implications for phylogenetic and functional community ecology. *Ecology*, 90,  
707 2161–2170.

- 708 Thompson, K., Band, S.R. & Hodgson, J.G. (1993). Seed size and shape predict seed  
709 persistence in the soil. *Functional Ecology*, 7, 236–241.
- 710 Weiher, E. & Keddy, P. (1995). Assembly rules, null models, and trait dispersion: new  
711 questions from old patterns. *Oikos*, 74, 159–164.
- 712 Weiher, E., van der Werf, A., Thompson, K., Roderick, M., Garnier, E. & Eriksson, O.  
713 (1999). Challenging Theophrastus: a common core list of plant traits for functional ecology.  
714 *Journal of Vegetation Science*, 10, 609–620.
- 715 Willby, N.J., Abernethy, V.J. & Demars, B.O.L. (2000). Attribute-based classification of  
716 European hydrophytes and its relationship to habitat utilization. *Freshwater Biology*, 43,  
717 43–74.
- 718 Wright, I.J., Reich, P.B., Westoby, M., Ackerly, D.D., Baruch, Z., Bongers, F., ... Villar, R.  
719 (2004). The worldwide leaf economics spectrum. *Nature*, 428, 821–827.

720

**Tables**

721 **Table 1:** Ranges of environmental variables in the studied running waters. Minimum,  
 722 maximum and mean values with standard errors are indicated.

<b>Variable</b>	<b>Min-Max value</b>	<b>mean <math>\pm</math> S.E.</b>
<i>Water chemistry variables</i>		
pH	7.62–9.07	8.33 $\pm$ 0.34
Conductivity ( $\mu\text{S} / \text{cm}$ )	142.40–1432.00	658.55 $\pm$ 347.53
Nitrite ( $\mu\text{g.l}^{-1}$ )	9.02–270.10	62.92 $\pm$ 40.87
Nitrate ( $\text{mg.l}^{-1}$ )	1.14–22.05	5.92 $\pm$ 4.77
Ammonium ( $\text{mg.l}^{-1}$ )	00–99.90	3.08 $\pm$ 15.37
Calcium ( $\text{mg.l}^{-1}$ )	200-600	409.04 $\pm$ 132.25
Phosphorus ( $\mu\text{g.l}^{-1}$ )	0.12–200.00	146.23 $\pm$ 70.14
Phosphate ( $\text{mg.l}^{-1}$ )	0.00–2.50	1.00 $\pm$ 0.87
<i>Instream habitat variables</i>		
Marl (%)	0–100	9.40 $\pm$ 24.87
Silt (%)	0–100	40.19 $\pm$ 35.31
Sand (%)	0–60	12.78 $\pm$ 14.57
Fine gravel (%)	0–60	18.53 $\pm$ 19.20
Coarse gravel (%)	0–80	15.64 $\pm$ 19.74
Stone (%)	0–30	1.98 $\pm$ 5.23
Rock (%)	0–40	1.48 $\pm$ 6.52
<i>River habitat variables</i>		
Wetted width (cm)	1.60–155.00	14.44 $\pm$ 25-89
Water depth (cm)	1.50–250.00	57.99 $\pm$ 48.06
Current velocity ( $\text{cm.s}^{-1}$ )	2.00–85.00	24.80 $\pm$ 22.45
Altitude (m a.s.l.)	85.00–261.00	142.31 $\pm$ 47.34
Temperature ( $^{\circ}\text{C}$ )	16.90-30.90	22.46 $\pm$ 3.65

723

724

725 **Table 2.** Results of stepwise variable selection for environmental variables and their  
726 relationship with traits community weighted mean (CWM) and functional diversity (FD) for  
727 hydato-helophyte (HH) and riverbank (RB) communities. Plus and minus signs indicate the  
728 direction of the relationship between environmental variables and community trait structure.  
729 In the case of categorical variables (i.e. stream order) “yes” means it was retained in the  
730 adequate model. Functional diversity was measured by standardised effect size of mean  
731 pairwise dissimilarity (SES<sub>MPD</sub>) of species.

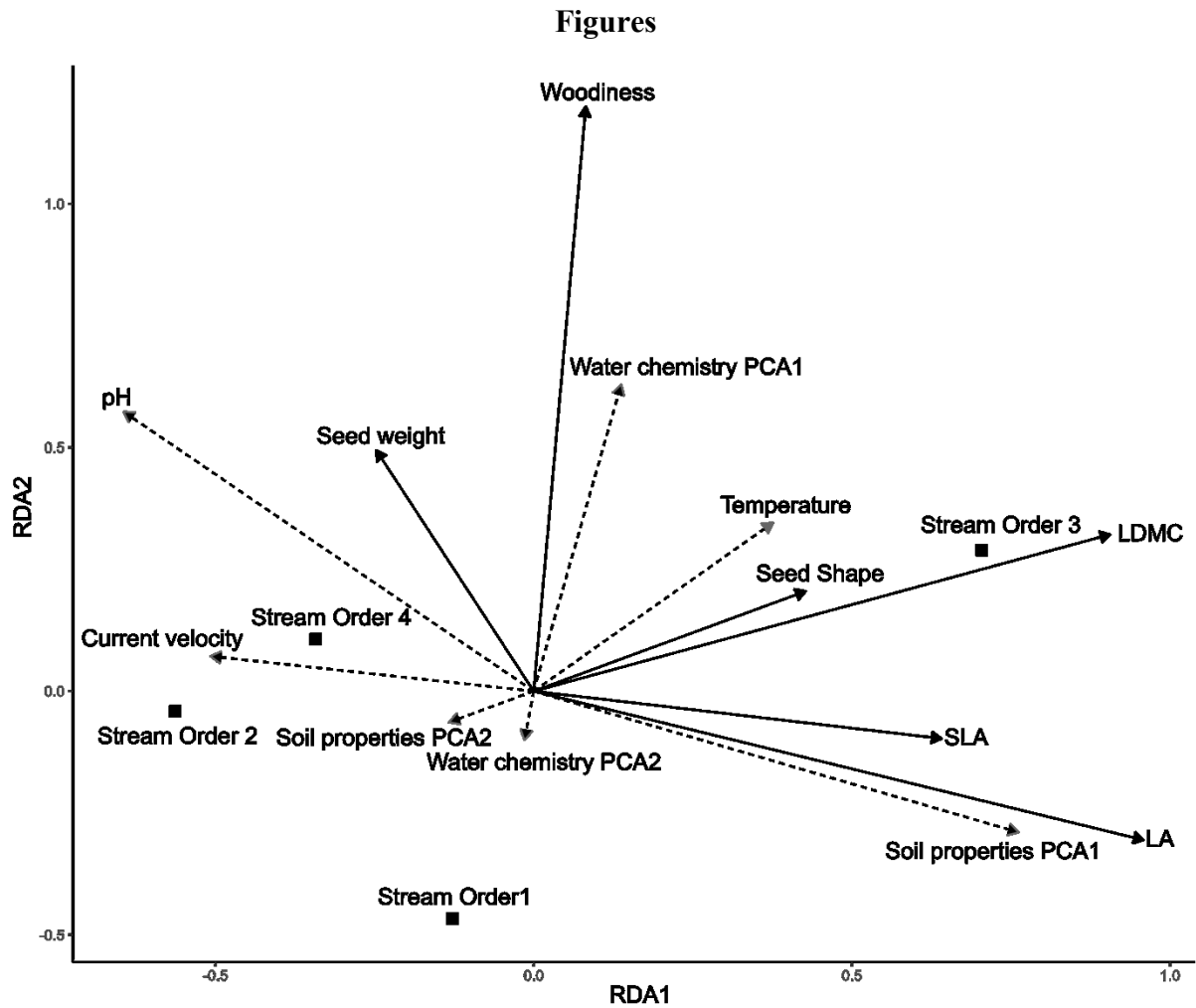
732 Abbreviations: CWM = community weighted mean, FD = functional diversity, SLA =  
733 specific leaf area, LDMC = leaf dry matter content, LA = leaf area. \* p<0.05; \*\* p<0.01; \*\*\*  
734 p<0.001, NS – not significant.

Trait	Stream order	Temperature	pH	Current velocity	Water chem. PCA1	Water chem. PCA2	Soil prop. PCA1	Soil prop. PCA2	R <sup>2</sup>
SLA	CWM RB		_*						0.12*
	CWM HH		_*	-		-			0.16*
	FD RB	yes***	_*	_*			_*	-	0.45***
	FD HH	yes**	_*				***	-	0.32***
LDMC	CWM RB		+*						0.11*
	CWM HH		+				_*	-	0.24**
	FD RB	yes	-				-		0.06
	FD HH		+*	_*		***		+	0.31**
LA	CWM RB		+	-				_*	0.13*
	CWM HH	yes*	-	+***					0.37***
	FD RB	yes	-	-			_*		0.20**
	FD HH	yes	-	_*			+*	-	0.39***
Seed weight	CWM RB	yes	-				***	-	0.23**
	CWM HH	yes**							0.15**
	FD RB		+						NS
	FD HH		+	+					0.12*
Seed shape	CWM RB								NS
	CWM HH								NS
	FD RB		-						NS
	FD HH		+	-					NS
Stem woodiness	CWM RB	yes					+	-	0.14*
	CWM HH	yes**	-	***		-			0.28**
	FD RB		+*	+				_*	0.20**
	FD HH	yes**	***	***		+			0.43***

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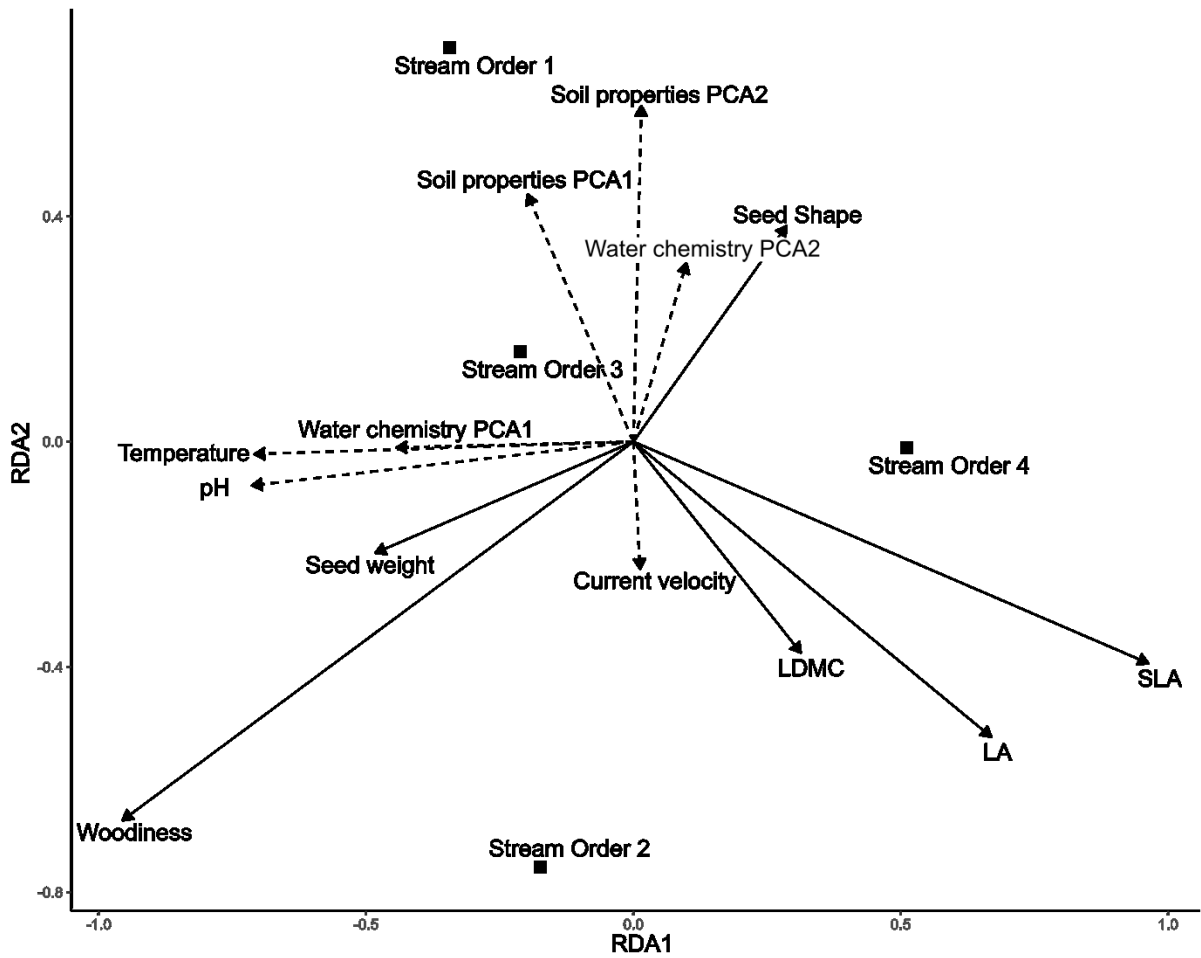




738

739 **Fig. 1.** Biplot of redundancy analysis of hydato-helophyte (HH) species functional diversity  
 740 (FD). Functional diversity is based on the mean pairwise distances between species (MPD).  
 741 Environmental variables are indicated with dashed arrows and black squares, traits are  
 742 indicated with solid arrows. Abbreviations: LA = leaf area; SLA = specific leaf area, LDMC  
 743 = leaf dry matter content. Stream Order 1 = lowland river; Stream Order 2 = highland river;  
 744 Stream Order 3 = lowland stream, Stream Order 4 = highland stream.

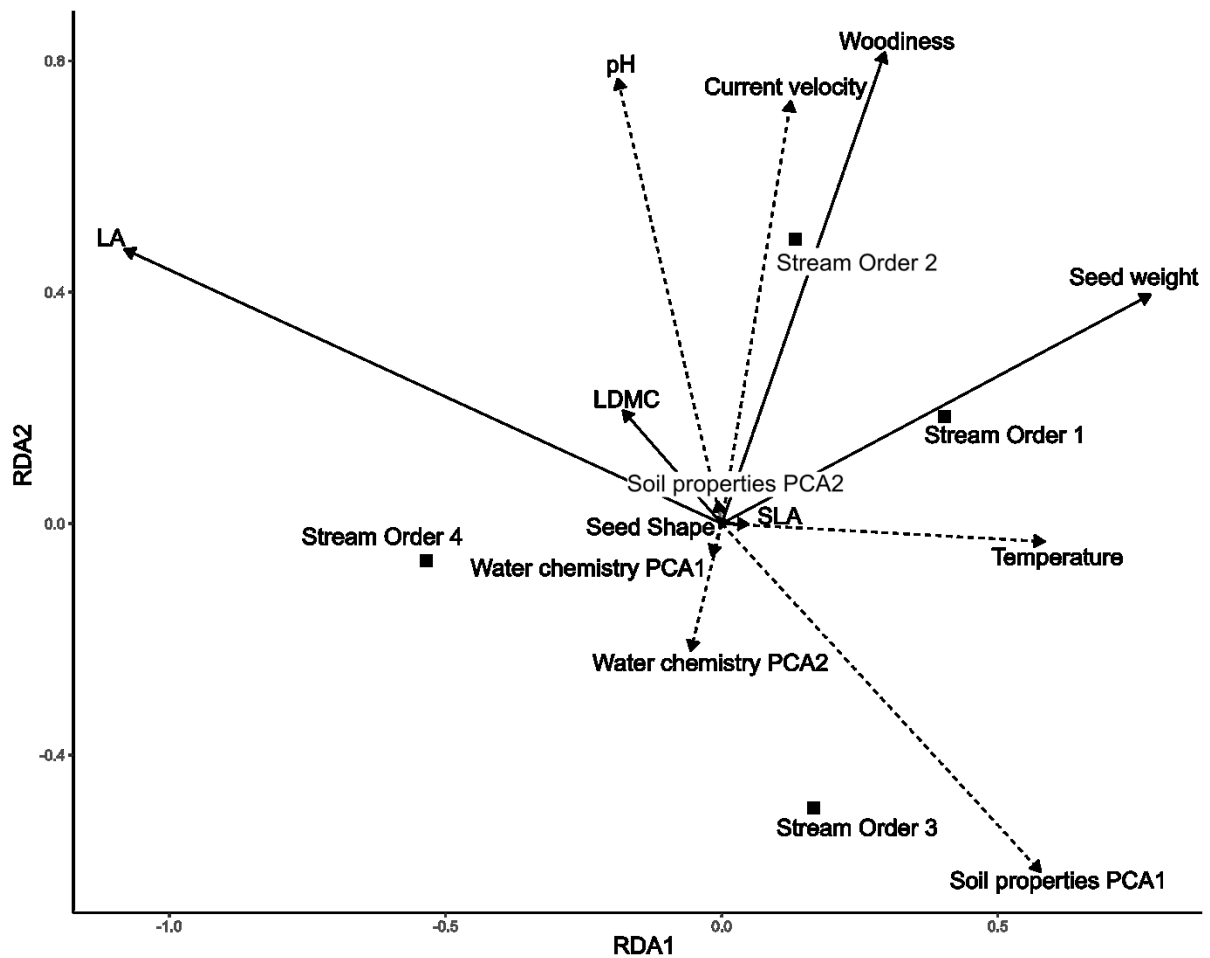
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746

747 **Fig. 2.** Biplot of redundancy analysis of riverbank (RB) species functional diversity (FD).  
 748 Functional diversity is based on the mean pairwise distances between species (MPD).  
 749 Environmental variables are indicated with dashed arrows and black squares, traits are  
 750 indicated with solid arrows. Abbreviations: LA = leaf area; SLA = specific leaf area, LDMC  
 751 = leaf dry matter content. Stream Order 1 = lowland river; Stream Order 2 = highland river;  
 752 Stream Order 3 = lowland stream, Stream Order 4 = highland stream.

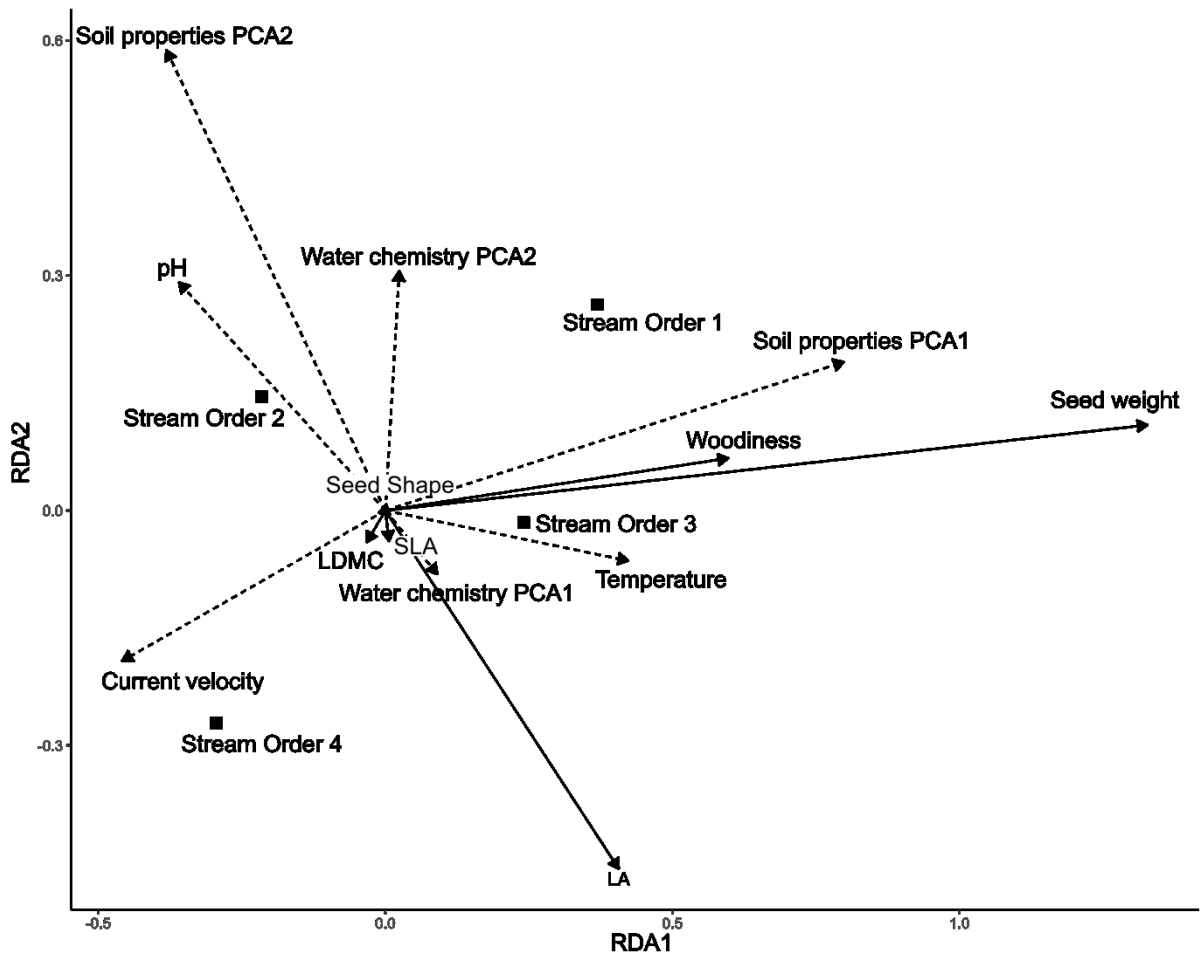
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755 **Fig. 3.** Biplot of redundancy analysis of hydato-helophyte (HH) species community weighted  
 756 mean trait values (CWM). Environmental variables are indicated with dashed arrows and  
 757 black squares, traits are indicated with solid arrows. Abbreviations: LA = leaf area; SLA =  
 758 specific leaf area, LDMC = leaf dry matter content. Stream Order 1 = lowland river; Stream  
 759 Order 2 = highland river; Stream Order 3 = lowland stream, Stream Order 4 = highland  
 760 stream.

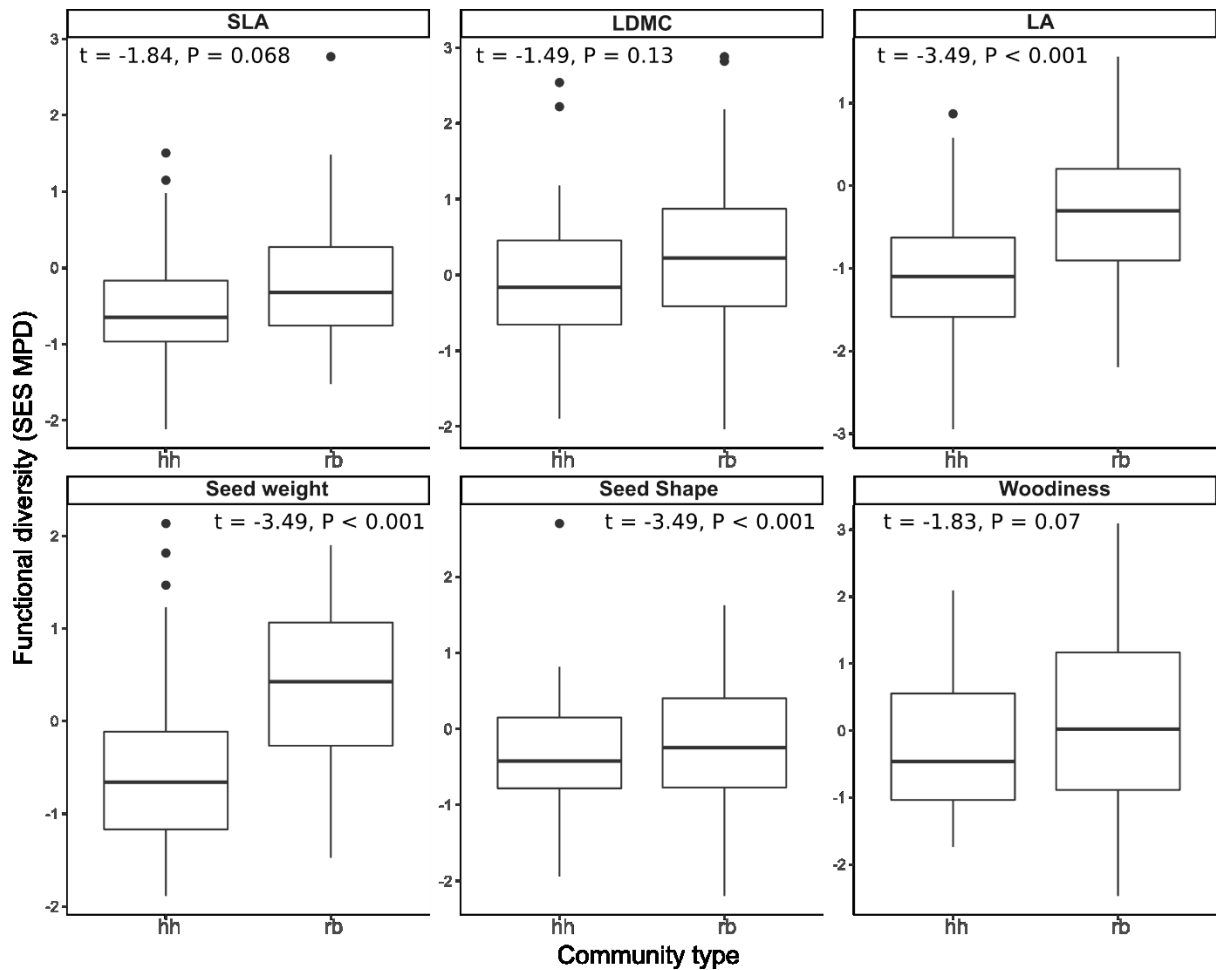
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763 **Fig. 4.** Biplot of redundancy analysis of riverbank (RB) species community weighted mean  
 764 trait values (CWM). Environmental variables are indicated with dashed arrows and black  
 765 squares, traits are indicated with solid arrows. Abbreviations: LA = leaf area; SLA = specific  
 766 leaf area, LDMC = leaf dry matter content. sStream Order 1 = lowland river; Stream Order 2  
 767 = highland river; Stream Order 3 = lowland stream, Stream Order 4 = highland stream.

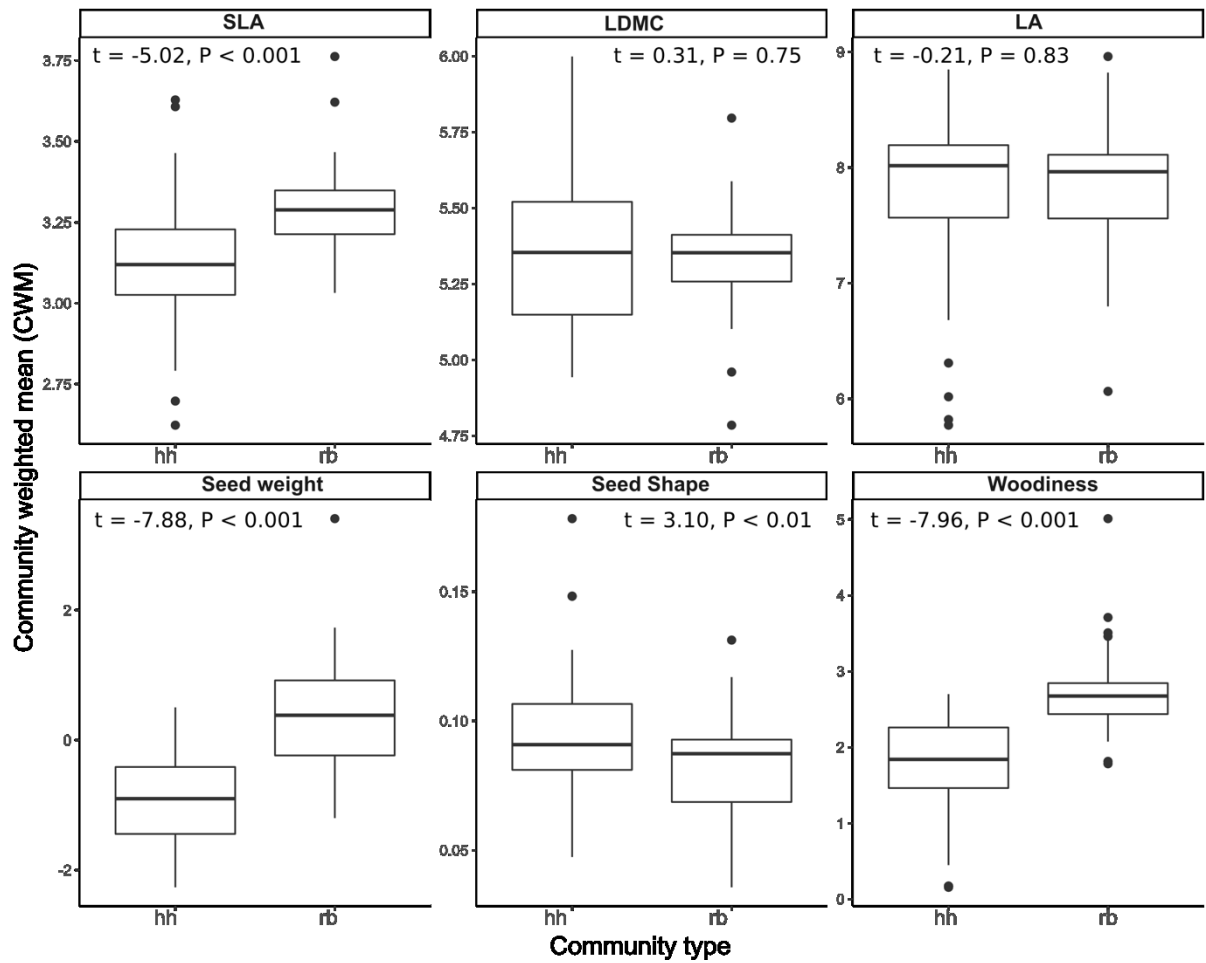
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770 **Fig. 5.** Boxplots of differences in functional diversities per trait between riverbank (RB) and  
 771 hydato-helophyte (HH) communities. Functional diversity expressed as standardised effect  
 772 size of abundance weighted mean pairwise distances (MPD) between species for each trait  
 773 (SES<sub>MPD</sub>). Significant differences between hydato-helophyte and riverbank communities  
 774 revealed by t-tests. Abbreviations: SLA = specific leaf area, LDMC = leaf dry matter content,  
 775 LA = leaf area; hh = hydato-helophyte, rb = river bank species.

776



777

778 **Fig. 6.** Boxplots of differences in community weighted means (CWM) per trait between  
 779 riverbank (RB) and hydato-helophyte (HH) communities. Significant differences between  
 780 hydato-helophyte and riverbank communities revealed by t-tests. Abbreviations: SLA =  
 781 specific leaf area, LDMC = leaf dry matter content, LA = leaf area; hh = hydato-helophyte, rb  
 782 = river bank species.