3 **Carbon forms, nutrients and water velocity filter hydrophyte and river-bank species**

4 **differently: A trait-based study**

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

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Abstract

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

Location: Danube River Catchment, Hungary

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

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

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

Keywords: macrophytes, hydrophytes, functional traits, environmental filtering, community assembly

Introduction

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

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

The distribution of aquatic plants in rivers is mainly determined by the prevailing environmental conditions mediated by the surrounding water. The most important factors are water chemical variables (alkalinity, nutrient content) and water physical variables (light, temperature, substrate characteristics, water movements; Riis, Sand-Jensen, & Vestergaard, 2000; Lacoul & Freedman, 2006; Bornette & Puijalon, 2011). In river ecosystems environmental variables have a stronger effect on the trait composition of the community than on its species composition (Göthe et al., 2017). There have been only few studies carried out so far that investigated the influence of these environmental factors on the trait distribution of aquatic plant communities. These studies have been limited by the use of categorical traits (e.g. growth forms in Baattrup-Pedersen et al., 2015), studying single 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_2 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_2, increasing HCO_3^-).

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

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

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

Materials and methods

Study sites

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

Environmental variables

Site surveys were conducted from July to August 2013, during relatively low water level conditions. In streams, 6–15 transects (depending on the complexity of the habitat, for details see Erős, Takács, Specziár, Schmera, & Sály, 2017) were placed perpendicularly to the main channel at each sampling site to characterise physical features of the environment. A list of the environmental variables and their descriptive values can be found in Table 1.
The sampled lowland and highland rivers and streams can be ordered along a stream size gradient (see Schmera et al., 2017). Orders 1 and 2 refer to lowland and highland rivers respectively, while type 3 and 4 refer to lowland and highland streams respectively. We used 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 ± 0.8 m and a mean depth of 34.5 ± 19.1 cm,

172 and a catchment size <1000 km^2. Rivers (n = 21) had a mean width of 29.7 ± 32.2 m and a

173 mean depth of 84.6 ± 54.3 cm, and a catchment size >1000 km^2. Lowland sites (n = 23) were

174 located between 85 and 180 m a.s.l., and their proportion of coarse substrate was 1.87 ± 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 ± 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*

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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*

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We assessed the functional composition of the studied communities through their functional diversity and community weighted mean (Ricotta and Moretti 2011). Functional diversity was measured as standardised effect size of abundance weighted mean pairwise distances (MPD) between species for each trait (SES$_{MPD}$), i.e. as a deviation of the observed functional diversity from a null expectation. We used MPD as a measure of functional diversity because it has been shown to be independent of species richness even for low numbers of species (de Bello, Carmona, Lepš, Szava-Kovats, & Pärtel, 2016), which we observed for some of our sampled communities. Standardisation was achieved by randomising the trait data across the species pool 999 times and using the resulting standard deviation of the expected MPD values to standardise the difference between the observed and mean expected MPD, i.e.

$$SES_{MPD} = (MPD_{obs} - mean(MPD_{exp}))/sd(MPD_{exp}).$$

This allowed us to quantify if the co-existing species were more similar or more dissimilar in their traits than under the null expectation that the species traits are randomly distributed among the species. Positive SES$_{MPD}$ values indicate trait divergence, while negative values indicate trait convergence, as expected under environmental filtering.

The community weighted mean expresses the mean trait value of a community emphasising the importance of more abundant species: $CWM = \sum_{i=1}^{S} p_i x_i$, with S as the number of species in the community, and p_i and x_i being the relative abundance and trait value of the ith species, respectively.
Trait values for SLA, LDMC, LA and seed weight were log transformed before calculating SES$_{MPD}$ and CWM to improve normality.

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

We used a stepwise regression approach to select important environmental gradients for each trait's functional diversity and community weighted mean. Because of shortcomings related to collinearity and to performance of stepwise model selection with high numbers of explanatory variables, we conducted principal component analyses (PCA) for two sets of environmental variables to reduce the number of explanatory variables. The first set contained variables related to the chemical composition of the river water (concentration of 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^2s 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).

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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 axis 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 axis 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

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

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

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

Plant trait – stream size relationship

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

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

Plant trait – temperature relationship

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

Plant trait – water velocity gradient relationship

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

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

Plant trait – pH gradient relationship

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

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

Plant trait – water chemical compound gradient relationship

Along the PC1 axis of water chemical compounds (i.e. mostly related to nitrate content) we found a significant shift from trait convergence to trait divergence (i.e. shift from negative to positive SES$_{MPD}$ values) in the case of LDMC among HH species (Table 2, Appendix S4). We did not find any changes in the community weighted mean of the traits.

Plant trait – substrate properties relationship

Along the PC1 axis of substrate properties (i.e. nutrient rich, fine sediment) trait convergence became significantly weaker (i.e. less negative SES$_{MPD}$ values) for SLA, while a significant shift from trait convergence to trait divergence in the case of LA among HH species could be observed (Table 2, Appendix S4). LA and SLA became more converged (i.e. more negative SES$_{MPD}$ values) along the substrate property gradient among RB species (Appendix S5). Along the PC2 axis of substrate properties (i.e. nutrient poor, coarse sediment) we found a significant shift from trait divergence to trait convergence in the case of woodiness among RB species.

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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.

Traits response to physical properties

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

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

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

Traits response to carbon limitation

The pH of the sampled streams and rivers was between 7.62 and 9.07, which lies in the middle of the section of the pH gradient where the relative distribution of the three main inorganic carbon types (carbon-dioxide, bicarbonate and carbonate) is transformed because they are converted into each other (Pedersen et al., 2013). Below pH 6, dissolved inorganic carbon is present as CO_2. In general, this carbon form is more readily used for underwater photosynthesis than bicarbonate. However, above pH 8, CO_2 gradually disappears from waters, because between pH 7 and 10 it is converted into bicarbonate (HCO_3^-). Bicarbonate is an additional carbon source among most of the aquatic plants except for pteridophytes and mosses.

Decreasing functional diversity of SLA, LA, and LDMC among HH species, and SLA among RB species along the pH gradient suggests that the effect of environmental filtering significantly increased toward higher pH (i.e. CO_2 limitation). Moreover, the observed pattern of functional diversity does support our expectation of a stronger filtering in HH communities, compared to RB. On the contrary, HH communities became less converged on woodiness (i.e. SES values of functional diversity became less negative) along the pH gradient, indicating weaker environmental filtering toward higher pH. Overall, the observed variation of leaf and woodiness traits along the pH gradient suggests that HH communities have only a small range of leaf „structure", which can be characterised with high LDMC and low SLA (i.e. tough leaf syndrome, details see later) under higher concentration of bicarbonate, while woodiness (and physical resistance) became less important. In that way species can reallocate nutrients and energy from the stem to the leaves with increasing pH, which indicates a functional shift from resistance into photosynthesis.

Regarding CWM of leaf traits, SLA significantly decreased among HH and RB species, LDMC significantly increased among RB species, whereas LA significantly increased among HH species. These trends indicate denser leaf tissue (i.e. tough leaf syndrome) towards higher pH, where only the bicarbonate form of inorganic carbon is available. Aquatic plants with the ability to use bicarbonate have major competitive advantage over obligate CO_2 users under CO_2 limited conditions (Maberly & Madsen, 2002). Our results suggest a negative correlation between SLA and bicarbonate use ability (or efficiency) among HH species. Moreover, these results indicate different adaptive mechanisms for higher pH between HH and RB species. Hydato-helophyte species attain low SLA by producing larger and denser leaves, while RB species tend to invest more only in tissue density, i.e. produce denser leaves under higher pH conditions. This is confirmed by

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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

Conclusion

517

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 at 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

538

Authors' contributions

539

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

Data accessibility

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

546

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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.

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

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Trait		Stream order	Temperature	pH	Current velocity	Water chem. PCA1	Water chem. PCA2	Soil prop. PCA1	Soil prop. PCA2	R^2
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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Figures
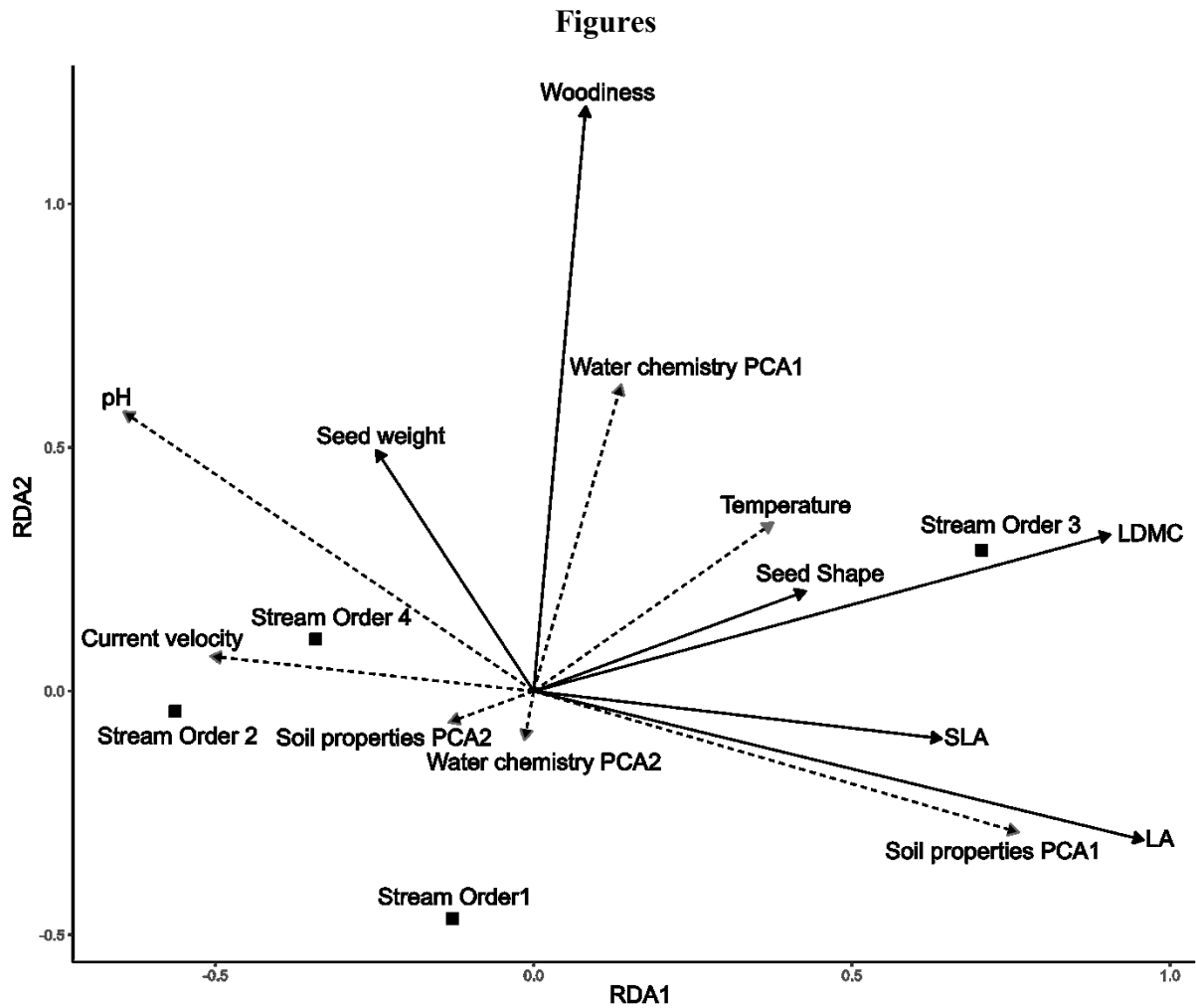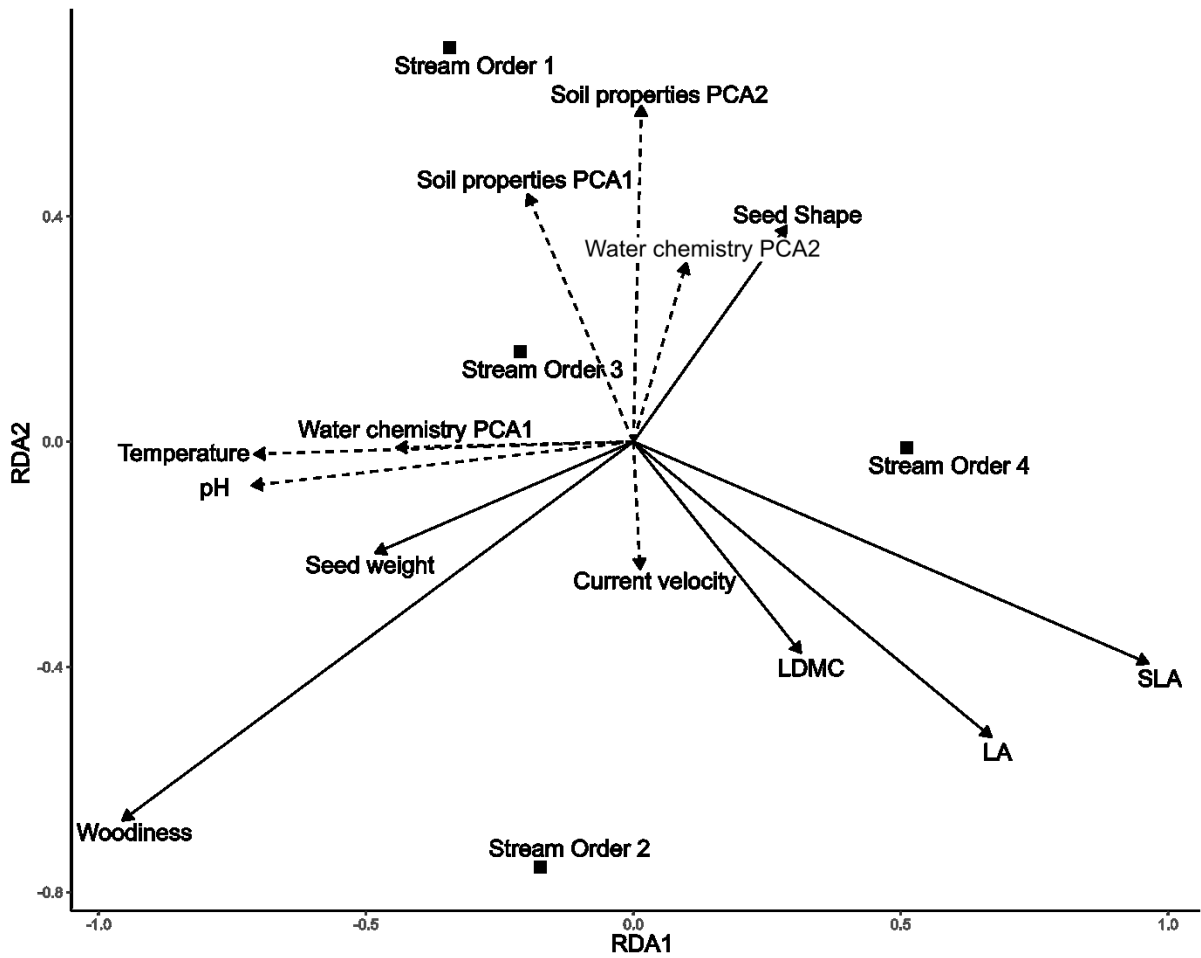
Fig. 1. Biplot of redundancy analysis of hydato-helophyte (HH) species functional diversity (FD). Functional diversity is based on the mean pairwise distances between species (MPD). Environmental variables are indicated with dashed arrows and black squares, traits are indicated with solid arrows. Abbreviations: LA = leaf area; SLA = specific leaf area, LDMC = leaf dry matter content. Stream Order 1 = lowland river; Stream Order 2 = highland river; Stream Order 3 = lowland stream, Stream Order 4 = highland stream.
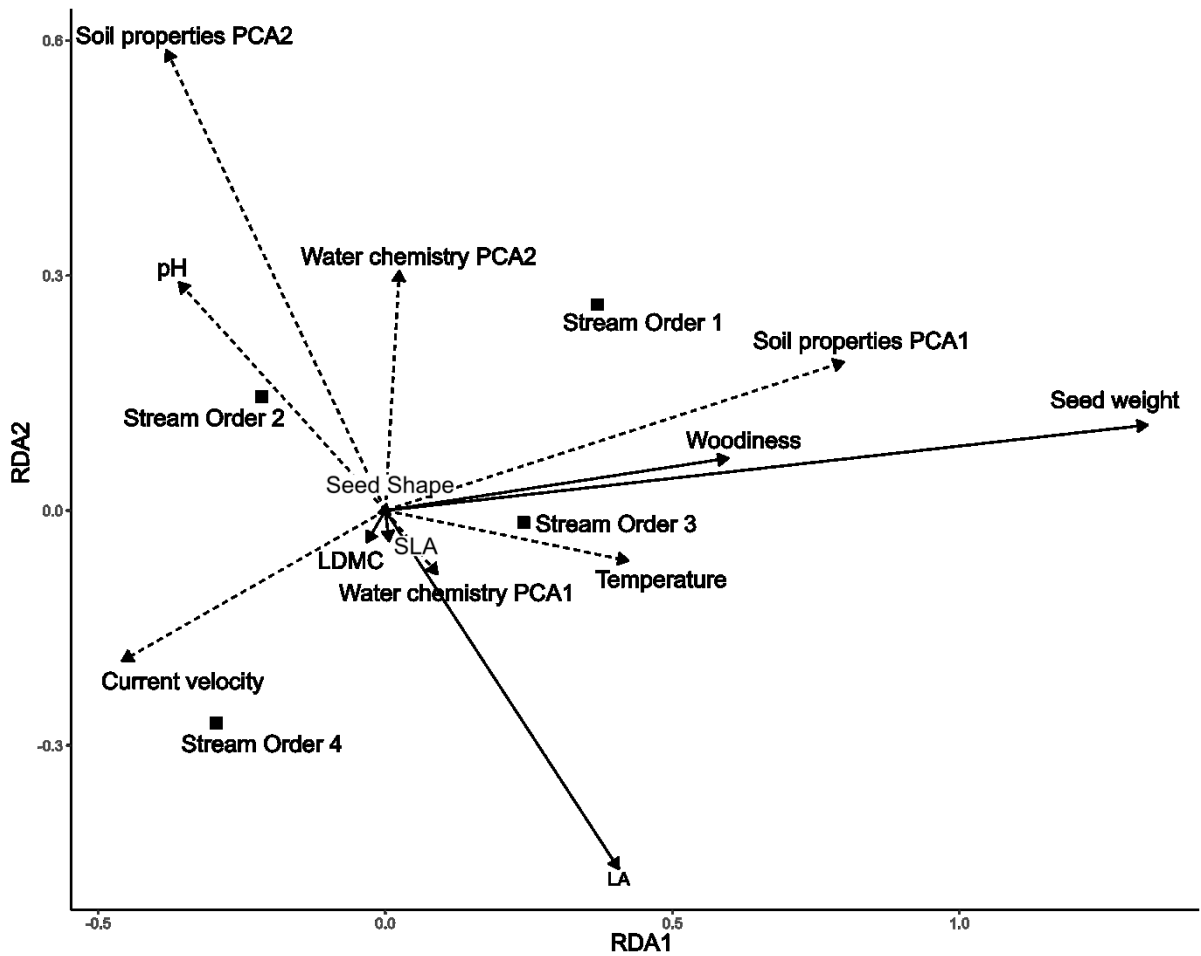
Fig. 2. Biplot of redundancy analysis of riverbank (RB) species functional diversity (FD). Functional diversity is based on the mean pairwise distances between species (MPD). Environmental variables are indicated with dashed arrows and black squares, traits are indicated with solid arrows. Abbreviations: LA = leaf area; SLA = specific leaf area, LDMC = leaf dry matter content.Stream Order 1 = lowland river; Stream Order 2 = highland river; Stream Order 3 = lowland stream, Stream Order 4 = highland stream.

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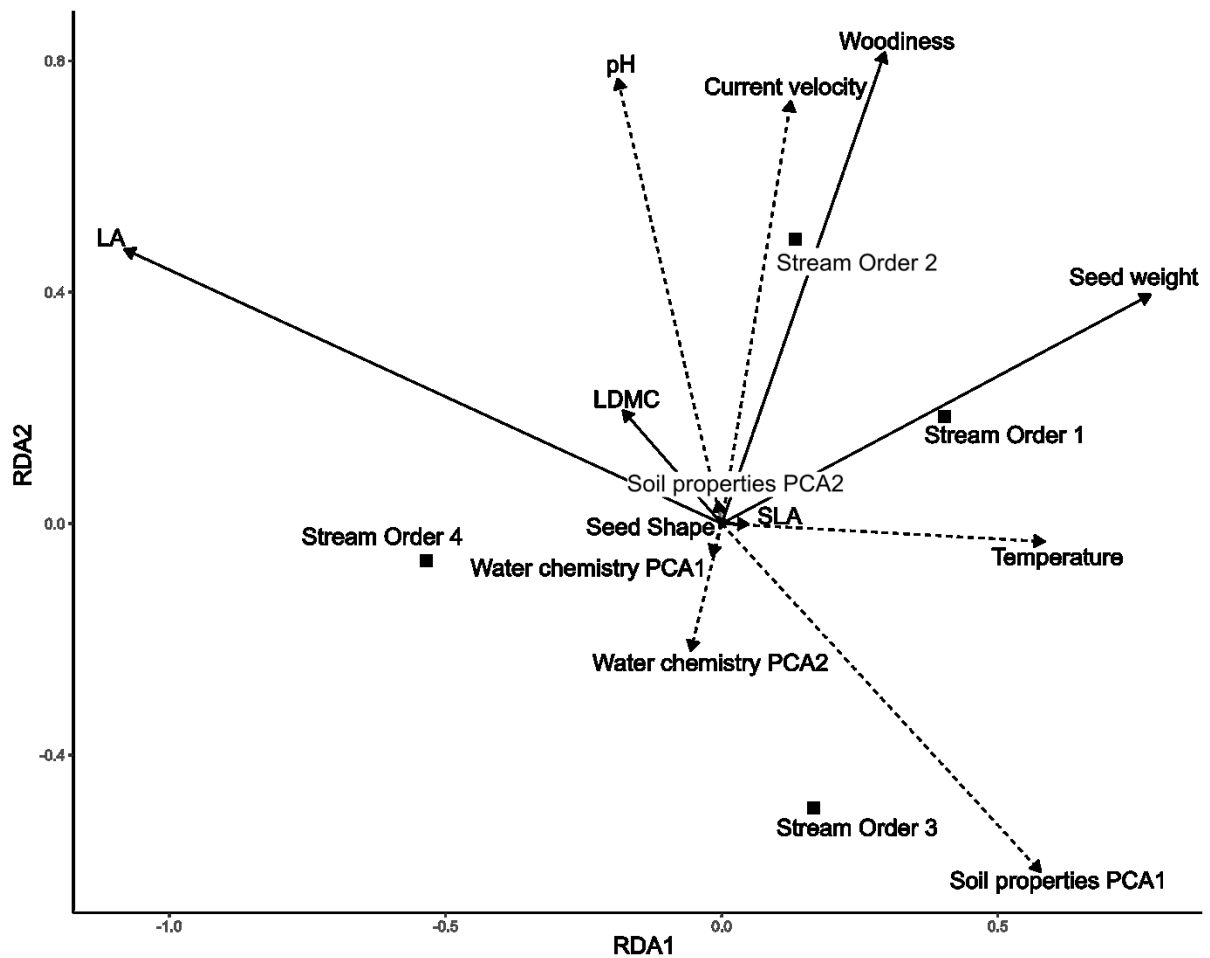
Fig. 3. Biplot of redundancy analysis of hydato-helophyte (HH) species community weighted mean trait values (CWM). Environmental variables are indicated with dashed arrows and black squares, traits are indicated with solid arrows. Abbreviations: LA = leaf area; SLA = specific leaf area, LDMC = leaf dry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

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

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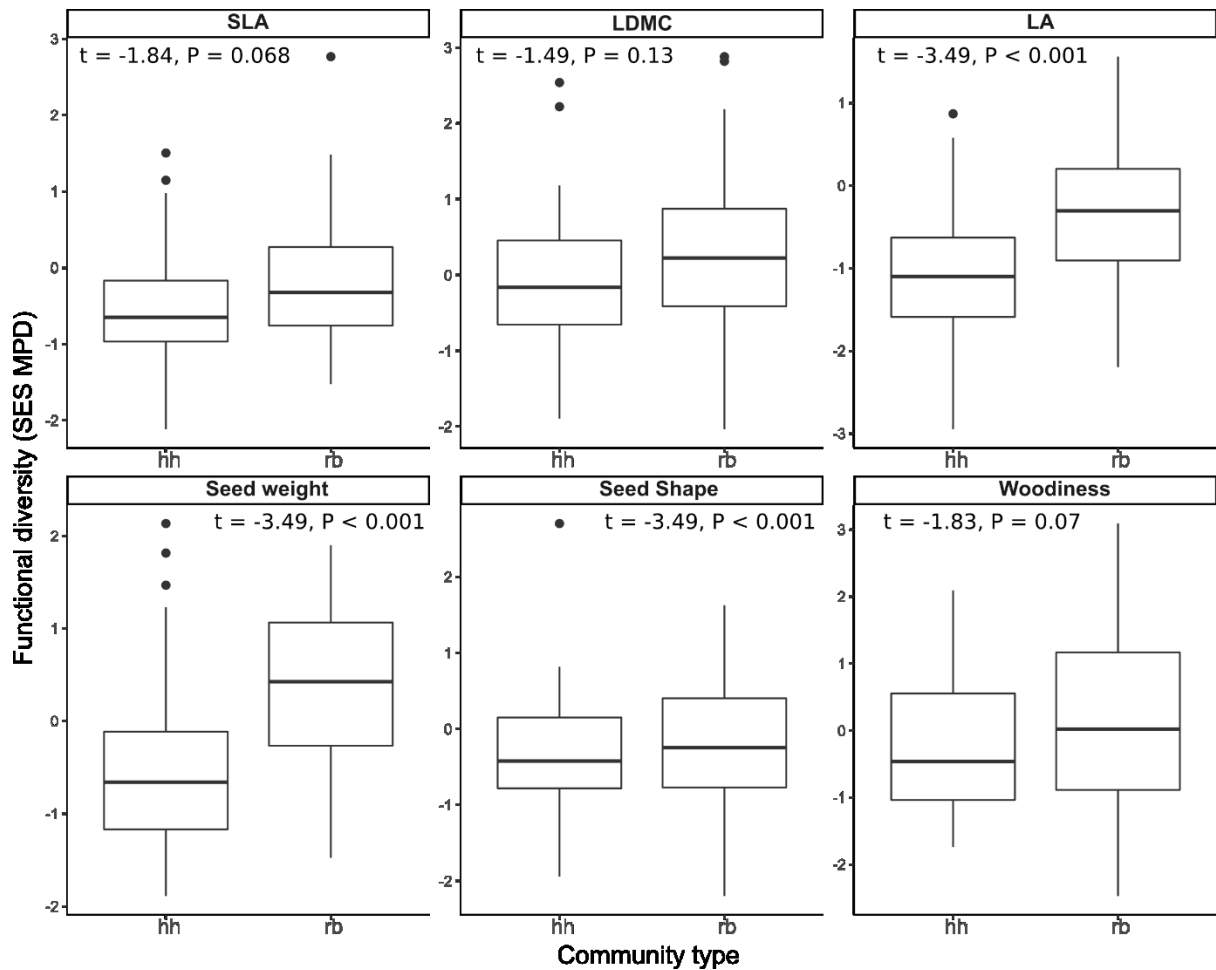
Fig. 5. Boxplots of differences in functional diversities per trait between riverbank (RB) and hydato-helophyte (HH) communities. Functional diversity expressed as standardised effect size of abundance weighted mean pairwise distances (MPD) between species for each trait (SES_{MPD}). Significant differences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

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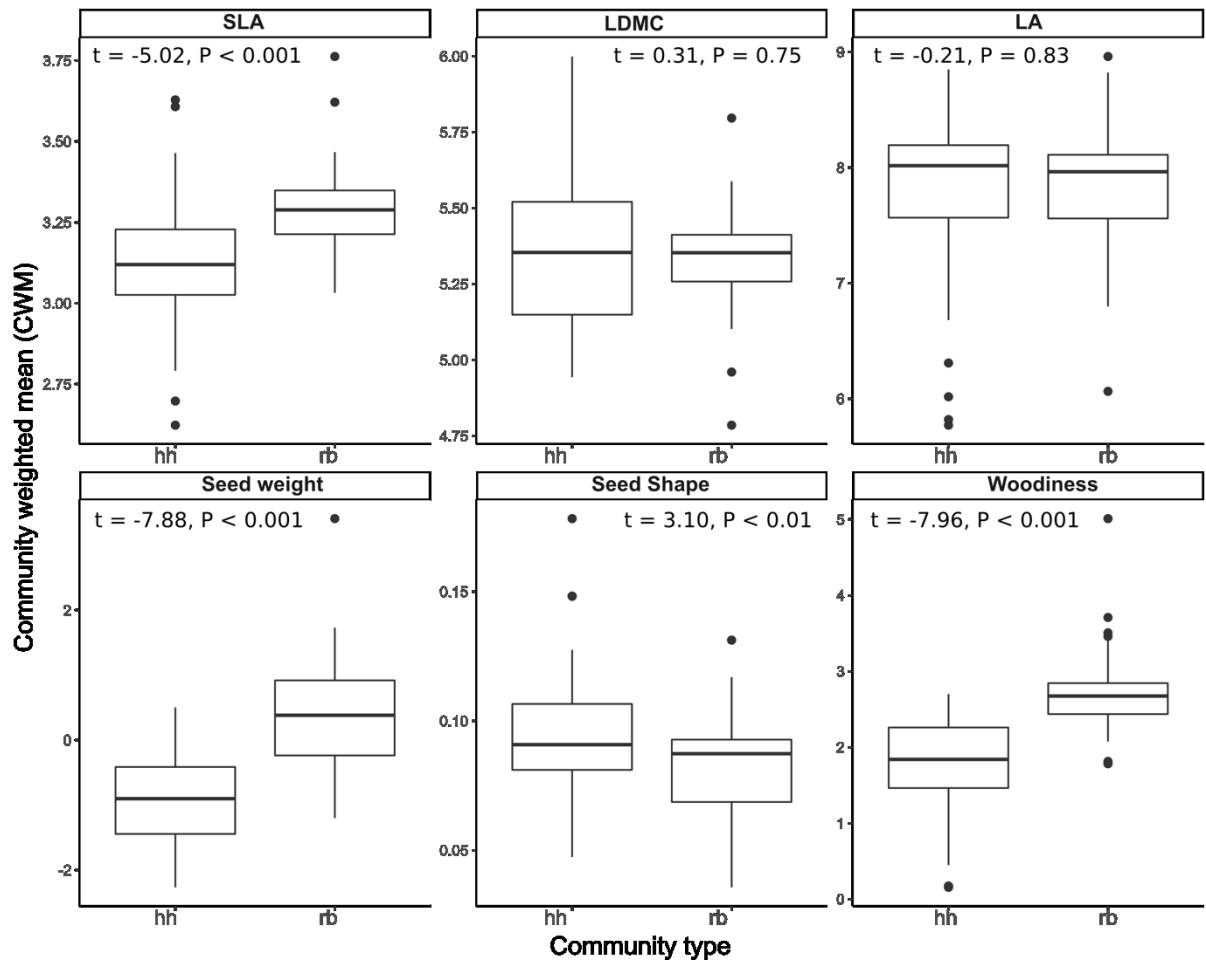
Fig. 6. Boxplots of differences in community weighted means (CWM) per trait between riverbank (RB) and hydato-helophyte (HH) communities. Significant differences between hydato-helophyte and riverbank communities revealed by t-tests. Abbreviations: SLA = specific leaf area, LDMC = leaf dry matter content,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