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1 Macroinvertebrate communities along the main stem and tributaries of a
2 pre-Alpine river: composition responds to altitude, richness does not

3

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19 **Key words:** Macroinvertebrates; Alpine stream; diversity; metacommunity; longitudinal pattern

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34 **Abstract**

35 We collected quantitative macroinvertebrate samples and measured environmental and geographical
36 parameters at 13 sites: six along the main stem and seven in tributaries close to the main channel
37 over a 700 m gradient in altitude and 22 km longitudinal distance along the River Kokra in the
38 Slovenian Alps. Our objectives were 1) to compare longitudinal patterns in richness and community
39 composition between main stem and tributary sites, and 2) to determine the relative importance of
40 the replacement and richness difference component for overall beta diversity and of environmental
41 versus spatial distance on beta diversity among main stem and tributary sites.

42 In total 138 taxa were identified. There were no differences between main stem and
43 tributary sites in mean abundance or taxon richness (67 and 58, respectively). A nMDS and
44 ANOSIM based on Bray-Curtis similarities found no separation of main stem and tributary sites,
45 but that upper (≥ 880 m a.s.l) and lower sites (≤ 680 m a.s.l.) formed two different groups. In both
46 main stem and tributaries taxon richness increased only slightly going downstream while the
47 community composition (DCA1) was much better explained by altitude and distance from source.

48 Overall, beta diversity (Sørensen and Bray-Curtis dissimilarity) was similar for the two
49 groups, and total Sørensen dissimilarity was driven mainly by replacement in main stem (78 %) and
50 tributary sites (77 %). Mantel tests showed that main stem dissimilarities were significantly
51 correlated to environmental PCA distance, watercourse distance, overland distance and altitudinal
52 differences. Tributary dissimilarities were not correlated to any of these four factors. GLMs showed
53 that dissimilarity among main stem sites was explained only by altitude difference, while no factors
54 were significant among tributary sites, even though nearly so for environmental PCA distance.

55 The study illustrates the importance of measuring beta diversity along ecological gradients,
56 such as river continua and/or altitudinal gradients, where alpha diversity may fail to detect relatively
57 minor changes in assemblage composition. Such changes are likely to occur due to present and
58 future climate warming.

59

60 **1. Introduction**

61 Patterns in taxonomic richness and composition of natural communities along geographical and
62 environmental gradients are a fascinating topic in ecology. Stream ecologists have long been
63 interested in describing altitudinal and longitudinal patterns of macroinvertebrate communities. The
64 classic study of the Colorado Cement Creek by Allan (1975) demonstrated prominent community
65 and species replacement along the river, even over a relatively modest altitudinal gradient (2600-
66 3600 m). This was subsequently confirmed by studies covering other altitudinal gradients (see
67 references in Jacobsen and Dangles, 2017).

68 However, studies of longitudinal zonation along the same water course from mountain
69 headwaters to lowland river may obscure the effect of altitude itself because small streams and large
70 rivers, even at the same altitude, represent different habitat types (e.g. Finn et al., 2011; Richardson,
71 2019), and because stream size itself influences taxon richness (Brönmark et al., 1984; Malmqvist
72 and Hoffsten, 2000; Wiberg-Larsen et al., 2000). As opposed to longitudinal studies, clear
73 relationships between community parameters and altitude are usually not observed in multi-stream
74 studies of e.g. tributaries covering narrow altitudinal ranges; e.g. less than 500 m (e.g. Stoneburner,
75 1977; Miserendino, 2001). Studies covering very wide altitudinal gradients of comparable stream
76 types have mostly found near-linear decrease in local macroinvertebrate taxon richness with
77 increasing altitude. This pattern has been reported from Nepal (Suren, 1994), Ecuador (Jacobsen,
78 2004) and Colorado (Harrington et al., 2015). However, hump-shaped patterns with a peak in local
79 richness at some intermediate altitude have been found in the Yunnan mountains, China (Wang et
80 al., 2011), Switzerland (Altermatt et al., 2013) and in Nepal (Shah et al., 2015).

81 During the last decade focus has shifted towards understanding riverine ecosystems as
82 dendritic networks of metacommunities (e.g. Brown and Swan, 2010; Altermatt, 2013; Tonkin et
83 al., 2018), and on disentangling deterministic processes such as environmental filtering or species

84 sorting acting at a local scale from stochastic processes such as dispersal working at the regional
85 scale in shaping local communities and the distribution of stream biota. This is usually done by
86 analysing relationships between community metrics, environmental site characteristics and spatial
87 distance or connectivity between sites (e.g. Cauvy-Fraunié et al., 2015; Göthe et al., 2017; Schmera
88 et al., 2018;), and the relative importance of environmental versus spatial factors may change
89 among different habitat types (Heino et al., 2015; Sarremejane et al., 2017). Beta diversity, the
90 dissimilarity between communities, is the metric used to study these processes. Quantifying the two
91 components of beta diversity, namely turnover, which is the replacement of species, and difference
92 in richness, which in part may be derived from nestedness (Baselga, 2010; Podani and Schmera,
93 2011; Baselga and Laprieur, 2015), may further reveal mechanisms behind patterns in the
94 distribution of communities (e.g. Heino, 2011; Jamoneau et al., 2018).

95 The detritic network approach has also been applied in altitudinal studies of richness and
96 beta diversity, demonstrating at a general level that centrality of sites works together with altitude in
97 determining patterns (Altermatt et al., 2013). A powerful design to disentangle the effect of factors
98 related to the longitudinal position (i.e. stream size and catchment area) from those related to
99 altitude (e.g. temperature) is to compare patterns among sites along a main stem with those in its
100 tributaries in a confined river system, due to systematic differences in connectivity and site
101 characteristics between the two categories of sites. Such studies are rare (but see Lujan et al., 2013),
102 in particular ones comprising the entire macroinvertebrate fauna and applying a high taxonomical
103 resolution.

104 We studied patterns in richness and composition of macroinvertebrate assemblages over a
105 modest 700 m gradient in altitude and 22 km longitudinal distance along the River Kokra in the
106 Slovenian Alps. We collected quantitative, benthic macroinvertebrate samples that were mostly
107 identified to species level, measured physico-chemical, and geographical parameters at 13 sites: six

108 along the main stem and seven in tributaries close to the main channel (Fig. 1). Our specific
109 objectives were 1) to compare longitudinal patterns in richness and community composition
110 between main stem and tributary sites, and 2) to determine the relative importance of the
111 replacement and richness difference component for overall beta diversity, and of environmental
112 versus spatial distance on beta diversity among main stem and tributary sites. Our main hypotheses
113 were 1) that taxonomic richness would increase and assemblage composition would change
114 systematically along the course of the river system, from high towards low altitude, in the main
115 stem as well as among tributaries, but with a more pronounced pattern along the main stem due to
116 the dual effect of both increasing water temperature and stream size, and 2) that environment and
117 spatial distance would be difficult to separate among main stem sites, and thus both contribute to
118 beta diversity with a considerable richness component, while beta diversity among tributary sites
119 would be clearly dominated by replacement and related to environmental species sorting.

120

121 **2. Material and methods**

122 2.1 Study stream

123 The fieldwork took place during daytime on May10-11 2000 and supplemented in 2011. The River
124 Kokra has its source at an altitude of about 1300 m in the Karawanke mountain range of the
125 Southern Limestone Alps close to the border between Slovenia and Austria. The river has a
126 catchment size of 221 km² with minimal human impact and land use of mostly forest, in particular
127 along the upper part. The total length of the river is 34 km and it flows into the Sava River in Kranj
128 located in the northwestern part of Slovenia. With a mean annual run-off of approx. 1200 mm year⁻¹
129 Kokra is among the Slovenian rivers with the highest relative discharge (Andjelov et al., 2016). We
130 included six sites along the main-stem and seven tributary sites over a 700 m gradient in altitude
131 (500 – 1250 m) and 22 km longitudinal distance (Fig. 1). Both River Kokra and its tributaries have

132 steep gradients (mean slope 30 m km⁻¹ in Kokra, higher in tributaries), high water velocity, and a
133 substrate dominated by boulders, cobbles and gravel. Further, all stream sites sampled were
134 generally shaded by riparian, mainly deciduous, forest.

135

136 2.2 Macroinvertebrate samples

137 Macroinvertebrates were sampled using a Surber sampler (area: 500 cm²; mesh size: 200 µm). At
138 each site, ten samples were taken stratified along one transect at the widest sites, or five at each of
139 two transects at narrow sites, in every case including all available substrate types. In the following
140 analyses, macroinvertebrate numbers from the ten samples were pooled for each site.

141 Macroinvertebrates were hand-sorted, identified to lowest possible taxonomic level, mostly species,
142 and counted (SM6). Although the high level of taxonomic resolution applied here is rare not all
143 groups could be identified to species level (e.g. we did not attempt to identify species of
144 Simuliidae), therefore true richness might be higher than reported here.

145

146 2.3 Environmental and geographical parameters

147 At each site, we recorded longitude, latitude and altitude using a GPS device. Flow connected
148 distances (*sensu* Tonkin et al., 2018) from the head of the River Kokra of tributaries and main stem
149 sites were estimated following the main river using the path function in Google Earth. Watercourse
150 and Euclidian distances between sampling sites (*sensu* Tonkin et al., 2017) were estimated using the
151 path and line function in Google Earth.

152 We measured stream width, depth, current velocity, and recorded substrate composition
153 along five representative transects 5-10 metres apart, the last three parameters at 10 cm intervals.
154 Velocity was measured at 0.6× depth (reflecting mean velocity of the water column) using an Ott
155 Kleinflügel anemometer. Mineral substrates were recorded as boulders, cobbles, pebbles, gravel,

156 sand/silt, and organic matter as LOD (Large Organic Debris) or POM (Particulate Organic Matter)
157 according to Swanson and Lienkaemper (1978). We used the salt dilution gauging to estimate
158 discharge (Hongve, 1987), and further measured pH, conductivity and oxygen at the deepest place
159 among transects using an YSI 6000 multi-probe. Water temperature was measured at 20 minutes
160 intervals over a 24-hour period on June 28-29 2011 at each site using TidbiT® V2 Temp Loggers
161 (UTBI-001) (substituting original spot measurements in 2000). Each logger was placed inside a
162 non-transparent plastic tube (to avoid direct solar radiation), open at both ends, and placed in the
163 main current. Even though these temperature measurements may not be representative of other
164 seasons, or on an annual basis, we assume that they represent maximum relative differences
165 between sites under the conditions when both the sampling (from 2000) and the temperature
166 measurements (from 2011) were carried out, namely on a warm and sunny summer day at baseflow
167 during mid-summer with discharge almost exclusively from ground water inflow.

168

169 2.4 Data analyses

170 Substrate composition was described by three metrics; a weighted average particle size (proportion
171 of cover * mean particle diameter) of mineral substrates (sizeWA), the Pielou evenness index
172 (PielouSub) , and the Hill's N2 diversity index (HillN2_sub), the two latter using both mineral and
173 organic substrate categories. We performed two Principal Components Analyses (PCA) to extract
174 main environmental gradients among main stem and tributary sites using all environmental
175 parameters except the geographical ones altitude and distance from source, and selecting among
176 highly correlated parameters ($r > 0.7$). Data were $\log(x+1)$ transformed prior to analysis, performed
177 in PC-Ord (McCune and Mefford, 2011).

178 For macroinvertebrate samples, we calculated local taxon richness (alpha diversity), total
179 abundance and Fisher's alpha index as a comparable measure of richness between samples with

180 varying number of individuals. A correlation matrix between all environmental and/or biological
181 variables was produced. As none of these relationships appeared to be clearly non-linear this was
182 done using Pearson correlations. To test for differences in mean values of fauna metrics between
183 main stem and tributary sites we used t-tests (after $\log(x+1)$ transformation of data and checking for
184 normality). These analyses were performed in SigmaPlot version 13 (SYSTAT).

185 We analysed taxon composition between sites using nonmetric Multi-Dimensional Scaling
186 (nMDS) based on quantitative Bray-Curtis similarities and Primer software (PRIMER-E version
187 6.1.14). To down weigh very abundant taxa, data were $\log(x+1)$ transformed prior to analyses. A
188 two-way ANOSIM was used to test for statistical significant differences between main stem sites
189 versus tributaries, and upper (located ≥ 880 m a.s.l.) versus lower sites (located ≤ 680 m a.s.l.).
190 SIMPER was used to determine which taxa were responsible for differences between groups. The
191 use of the Primer package is described by Clarke and Warwick (2001). In addition, we did a
192 Detrended Correspondance Analysis (DCA) on $\log(x+1)$ transformed abundances to obtain a
193 relative measure of assemblage composition among sites, and to compare gradient lengths between
194 main stem and tributary sites using PC-ord.

195 We used the Sørensen's index, based on presence/absence data, as a measure of the overall
196 compositional dissimilarity, or betadiversity, β_{SOR} , between the 15 pairwise combinations of main
197 stem sites and the 21 combinations of tributary sites (Baselga and Leprieur, 2015). To compare
198 effects of environmental and spatial factors on patterns in taxonomic composition in main stream
199 and tributary sites these pairwise Sørensen dissimilarities were plottet as a function of
200 environmental differences as euclidian distances from the PCA, watercourse distances, overland
201 euclidian distances and altitudinal differences between pairs. To test the effect of these four factors
202 on pairwise dissimilarities we performed Mantel tests using a Pearson's correlation analysis. All
203 four explicative factors were treated as Euclidian distances, and the coefficient r was calculated

204 with 5000 permutations. Mantel tests are suitable for monotonic comparisons between the values of
205 two distance matrices (Legendre and Fortin, 2010), and were carried out in PAST version 2.17. In
206 addition, to account for possible inter-correlations between these environmental and geographic
207 factors, we also used General Linear Models (full factorial, type III sum of squares) on main stem
208 and tributary sites separately entering all four explanatory factors as co-variates, performed in SPSS
209 version 25.

210 To identify which of the two components of community dissimilarity replacement (β_{REPL})
211 and difference in richness (β_{RICH}) were the main responsible for the overall beta diversity among
212 main stem and tributary sites, we quantified these pairwise according to Podani and Schmera
213 (2011).

214

215 **3. Results**

216 3.1 Environmental variables

217 All measured environmental and geographical parameters are shown in SM1. Along the main stem
218 width, depth, mean current velocity and discharge all positively correlated with distance from
219 source ($p < 0.05$), whereas mean temperature and substrate variables were not significantly
220 correlated with distance from source ($p > 0.05$). Among the tributaries, in contrast, none of the
221 environmental variables showed systematic patterns, i.e. correlations with their position along the
222 main stem.

223 For the PCA's on main stem (SM 2A) and tributary sites (SM 2B) we included the 11
224 environmental variables: width, depth, mean velocity, mean and diel temperature range, oxygen %,
225 pH, conductivity, and the substratum metrics size WA, Pielou S and Hills N2, excluding highly
226 correlated variables ($r > 0.7$). For main stem sites the PCA1 that explained 38.4 % of the variation
227 correlated significantly with velocity and oxygen %, while the PCA2, explaining 32.3 %, correlated

228 with conductivity. For tributary sites PCA1, explaining 42.7 % of the variation, correlated
229 significantly with width, depth, mean velocity, size WA and Pielou S, while PCA2 that explained
230 27.8 %, correlated with temperature range and oxygen %.

231

232 3.2 Macroinvertebrates – overall characteristics

233 In total, 44,982 specimens and 138 taxa representing eleven groups (classes, orders) were recorded
234 from the studied sites (SM3). Taxon richness was especially high among the dipterans
235 Chironomidae (33 taxa), Trichoptera (28 taxa), Plecoptera (16 taxa) and Ephemeroptera (12 taxa).
236 These groups also contributed substantially to the abundance that overall ranged between 1216-
237 6038 individuals m⁻², while taxon richness varied from 50 to 75 between sites (SM 3). We found no
238 significant correlation between abundance and taxon richness ($r = 0.31$, $p > 0.05$). There were no
239 differences between main stem and tributary sites in mean values of abundance per sample (1723
240 and 1736 ind, respectively), taxon richness (67 and 58, respectively) or Fisher's α (14.22 and 11.93,
241 respectively) (t -test, $p > 0.05$).

242 The nMDS based on Bray-Curtis similarities between sites in the macroinvertebrate
243 composition had a stress value of 0.07 showing that the 2-dimensional presentation of the data was
244 highly reliable (Fig 2). The ANOSIM showed that there was no significant separation of main stem
245 and tributary sites (Global R = -0.004, $p = 0.42$).

246

247 3.3 Macroinvertebrates – longitudinal patterns

248 Analysing main stem and tributaries separately, taxon richness and Fisher's alpha increased only
249 slightly going downstream, i.e. neither correlated ($p > 0.05$) with altitude and distance from source
250 (Fig 3). In contrast, the community composition (DCA1) was much better explained by altitude and
251 distance from source (Fig. 3, and DCA shown in SM4). Overall, relations were the same for main

252 stem and tributary sites, except that the relationship between DCA1 and distance was linear for
253 tributary sites, but exponential for main stem sites.

254 The ANOSIM based on the Bray-Curtis similarities from the nMDS found that upper sites
255 (M1-M3 and T1-T4 located ≥ 880 m a.s.l.) and lower sites (M4-M6 and T5-T7 located ≤ 680 m a.s.l.)
256 formed two highly significantly different groups, (Global $R = 0.66$, $p = 0.001$). According to the
257 SIMPER analysis, upper sites were primarily characterised by 11 taxa contributing about 50% to
258 the taxon composition (SM5). Among these were the triclade *Crenobia alpina*, stoneflies
259 *Dictyogenus alpinus*, *Leuctra rosinae*, *Isoperla lugens*, *I. rivulorum*, mayflies *Rhithrogena* spp.,
260 *Haproleptoides confusa*, and the chironomid dipteran *Heleniella serratosioi*. Lower sites were
261 primarily characterised by 24 taxa similarly contributing about 50% to taxon composition (SM5).
262 Among these were the oligochaete *Stylogrilus heringianus*, the crustacean *Gammarus fossarum*,
263 mayflies *Ephemerella mucronata* and *Ecdyonurus venosus*, caddisflies *Glossosoma boltoni*, *G.*
264 *conformis*, *Micrasema minimum* and *Drusus monticola*, chironomids *Orthocladius frigidus*,
265 *Rheocricotopus fuscipes* and *Synorthocladius semivirens*, and the crane fly *Anthoca vitripennis*.

266

267 3.4 Macroinvertebrates – beta diversity

268 A supplementary DCA performed separately on main stem and tributary sites showed that tributary
269 sites had 19 and 41% longer gradients on axes 1 and 2, respectively compared to main stem sites
270 (SM4). However, a difference in overall beta diversity between the two groups was not confirmed
271 by the qualitative Sørensen dissimilarity index (mean 0.38 in both groups), or by the quantitative
272 Bray-Curtis dissimilarity index; mean 0.51 for main stem and mean 0.50 for tributaries (t -test, $p >$
273 0.05). Total Sørensen dissimilarity β_{SOR} between sites was more driven by replacement β_{REPL} than
274 by the richness component β_{RICH} , and replacement contributed equally in main stem (78.2%) and
275 tributary sites (77.0%) (t -test, $p > 0.05$).

276 There were no differences in mean values and ranges of the explanatory variables
277 environmental PCA distance, watercourse distance, overland distance and altitudinal differences
278 between main stem and tributary sites (t -test, $p > 0.05$). Relationships between Sørensen
279 dissimilarities and these four explanatory factors differed for main stem and tributary sites (Fig 4).
280 Mantel tests showed that main stem dissimilarities were significantly correlated to all four
281 explanatory factors, with altitude difference being the most significant. In contrast, tributary site
282 similarities were not correlated to any of the four factors, but almost so to environmental differences
283 measured as Euclidian PCA distances. However, several of these explanatory factors were pair-wise
284 significantly correlated; for both main stem and tributary sites altitudinal difference was correlated
285 with watercourse and overland distance, and so were watercourse and overland distance. Further, in
286 main stem sites altitudinal difference was correlated with PCA environmental distance. Therefore
287 significance and power of the four explanatory variables were also analysed simultaneously in
288 GLMs. These showed that dissimilarity among main stem sites was explained only by altitude
289 difference ($p = 0.012$), while no factors were significant among tributary sites, even though nearly
290 so for PCA distance ($p = 0.064$) (Table 1).

291

292 **4. Discussion**

293 4.1 Longitudinal patterns

294 Our main hypothesis that taxonomic richness would increase and assemblage composition change
295 along an altitudinal gradient, from high towards low altitude, in the main stem and among
296 tributaries, and that these patterns would be strongest for main stem sites, was only partly confirmed
297 by the results. Richness did not change significantly, while composition did, and these patterns were
298 largely similar along the main stream and among tributaries.

299 Surprisingly, assemblages in small downstream tributaries were more similar to those in
300 downstream main stem sites than to those in small headwater sites. We identified several
301 macroinvertebrate species that could be used to distinguish upper from lower sites and these species
302 were present in both the main stem and in tributaries. The majority of the taxa from upper sites (*C.*
303 *alpina*, *D. alpinus*, *L. rosinae*, *I. rivulorum* and *H. seratosioi*) are known to prefer cold water (i.e.
304 inhabiting springs or groundwater fed streams) (e.g. Silveri, 2008; Bitušik and Trnková, 2016;
305 Monbertrand et al., 2019). Likewise for several of the species characterizing downstream sites such
306 as *G. fossarum*, confined to low mountain sites (Janetzky, 1994), *E. mucronata*, *G. boltoni*, *G.*
307 *conformis* (Hanetseder, 2015) and *A. vitripennis* (Živić et al., 2006).

308 Temperature seems to have the overall highest explicative power of altitudinal community
309 patterns across ecosystems (Peters et al., 2016), and together with dissolved oxygen, water
310 temperature also seems to be a key driver of macroinvertebrate assemblages in mountain streams
311 (Jacobsen et al., 1997; Jacobsen, 2008). Therefore, even though measurements of mean temperature
312 (8.4 -11.9 °C) did not differ greatly among our study sites, probably due to the limited altitudinal
313 range and dominant forest cover, and were not at all correlated to stream size, altitude, longitudinal
314 position, or to any of the faunal metrics, we suggest that a variable ubiquitously related to altitude,
315 such as temperature, is responsible for the high similarity between downstream assemblages in
316 tributary and main stem sites, overruling the effects of stream size, but not strong enough to drive
317 patterns in taxon richness. We only measured temperature over a 24 h period at each site, on a warm
318 and sunny summer day, and these temperature measurements may not be representative of
319 differences during other seasons or on a yearly basis. In support of the importance of temperature,
320 Urbanic and Toman (2007) found that maximum temperature and range were the most important
321 factors structuring Trichoptera assemblages at 94 Slovenian stream sites within an altitude gradient
322 of 10-835 m a.s.l. and stream orders 1-7. Likewise, in a study of 39 Polish headwater streams

323 located within an altitude gradient of 500-1700 m a.s.l. it was found that temperature, pH,
324 conductivity, stream gradient, habitat quality and altitude were the most important drivers of
325 macroinvertebrate distribution and metrics (Lewin et al., 2015).

326 Studies like the present, aiming at detecting changes in macroinvertebrate richness and
327 composition along river systems, covering gradients in altitude and stream order, are few (Lujan et
328 al., 2013). Our results are in accordance with the study by Lujan et al. (2013) of the Arazá-
329 Inambari-Madre de Dios watershed in Peru, covering an exceptionally wide altitudinal gradient of
330 4100 m and a longitudinal distance of hundreds of kilometers, that found similar patterns in richness
331 and composition of macroinvertebrate assemblages along the main channel and its tributaries,
332 indicating a prominent effect of altitude over that of longitudinal placement in the riverine network,
333 or stream size as such.

334

335 4.2 Beta diversity

336 Even though the DCAs showed that tributary sites had longer gradients compared to main stem
337 sites, there were no differences in mean Sørensen or Bray-Curtis dissimilarities between the two
338 groups. This finding is not surprising considering the comparable ranges and mean values of
339 potential explanatory environmental and spatial factors, and makes this system ideal to compare
340 relationships between main stem and tributary sites. Indeed, published studies on beta diversity
341 patterns and the relative importance of environmental versus spatial factors in main stem compared
342 to headwaters (or tributaries) report contradictory conclusions, probably because results are
343 dependent on the specific properties of the catchment (Schmera et al., 2018; Tonkin et al., 2018).
344 Another reason, which is rarely addressed in the literature, lies in the concept of environmental
345 distance. While geographical distances are exactly defined and precisely measurable, quantifying
346 overall environmental distance between two sites is rather fuzzy, and depends on the underlying

347 variables that have been measured. However, one finding seems to dominate among studies: There
348 is often no clear difference between the explicative power of often highly correlated overland and
349 watercourse distances (Grönroos et al., 2013; Kärnä et al., 2015; Tonkin et al., 2018). Our study
350 contributes to that.

351 For main stem sites overall beta diversity (Sørensen dissimilarity) was significantly related
352 to both environment (PCA distances) and spatial factors (watercourse, overland and altitudinal
353 distance), and this basically supports our *a priori* hypothesis. This is to be expected from a system
354 covering an altitudinal gradient, where both environmental and spatial variables are inevitably inter-
355 correlated (Wang et al., 2012; Tonkin et al., 2017), and thus their effect hard to disentangle. Yet,
356 when their power was compared directly in the GLM, altitudinal difference was clearly the most
357 influential along the main stem. It has been suggested that spatial processes seem to dominate in
358 downstream regions because high dispersal rates between flow-connected main stem sites may
359 override local environmental species sorting through mass effects and species spill-over into
360 suboptimal habitats (Brown and Swan, 2010; Göthe et al., 2013). Our hypothesis that beta diversity
361 in tributary sites would correlate mostly to environmental factors was also confirmed. Intermediate
362 levels of connectivity and dispersal, as assumed among tributaries (compared to high levels along
363 the main stem) allow species to be best sorted according to their environmental preferences
364 (Astorga et al., 2012; Heino et al., 2015; Tonkin et al., 2018).

365 For this same reason we also hypothesized that the richness (and nestedness) component
366 would be somewhat more important among main stem sites. However, even though in riverine
367 networks replacement indeed appears to be more pronounced among headwaters than downstream
368 sites (Tonkin et al., 2018), the replacement component was equally dominant (77 – 78 %) in main
369 stem and tributaries. This confirms the general notion that in most freshwater and riverine systems

370 replacement (or turnover) seems to be the dominant component of beta diversity (Soininen et al.,
371 2018; Parityadar et al., 2020).

372 Our study illustrates how local taxon richness (alpha diversity) may be rather constant or
373 homogenous along investigated gradients while species composition indeed responds to a gradient,
374 reflecting the qualities of a heterogeneous and diverse landscape, and it thus underlines the
375 importance of measuring beta diversity along ecological gradients. As an example, future climate
376 warming may displace the faunal composition in River Kokra upwards and result in an unchanged
377 local diversity despite a potentially altered species-turnover (e.g. Friberg et al., 2013).

378

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381 trip to Slovenia, proposed and initiated the present study. Peter was a brilliant and creative
382 freshwater scientist, inspiring both students and colleagues. He was also among the founders of the
383 “Old Danish Organ Builder Syndicate”, a social and freshwater zoological society to which all the
384 authors belong, existing since the year 1991. It is therefore a natural decision to include Peter as an
385 author and we especially dedicate the present paper to him.

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585 **Tables**

Table 1. Results of General Linear Models (full factorial, Type III sum of squares) with Sørensen dissimilarity between all pair-wise combinations of sites as dependent variable and including four covariates: euclidian distance from a PCA on environmental parameters, altitudinal difference, flow distance and geographic euclidian distance between sites.

Main stem sites	SS	df	F	<i>p</i>	Power
Corrected Model	1363.93	4	9.23	0.002	0.98
PCA distance	0.24	1	0.01	0.937	0.05
Altitude difference	349.70	1	9.47	0.012	0.79
Flow distance	0.29	1	0.01	0.931	0.05
Geographic distance	10.82	1	0.29	0.600	0.08
Total	23775.00	15			

R² = 0.787 (Adjusted R² = 0.702)

Tributary sites	SS	df	F	<i>p</i>	Power
Corrected Model	432.70	4	3.20	0.041	0.70
PCA distance	133.70	1	3.96	0.064	0.46
Altitude difference	41.77	1	1.24	0.283	0.18
Flow distance	12.46	1	0.37	0.552	0.09
Geographic distance	23.64	1	0.70	0.415	0.12
Total	30542.00	21			

R² = 0.445 (Adjusted R² = 0.306)

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606 **Figure legends**

607 Fig. 1. Position of sites in the River Kokra system and sampling sites.

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609 Fig. 2. Nonmetric Multidimensional scaling (nMDS) of Bray-Curtis similarities of
610 macroinvertebrate assemblages from thirteen sites of the River Kokra system. M = main stem sites;
611 T = tributary sites. Numbering is according to distance from source. Stress value: 0.07.

612

613 Fig. 3. Relationships between selected macroinvertebrate metrics (taxon richness, Fisher's alpha,
614 DCA 1 scores) and altitude/distance from source of the Kokra River system. Filled circles – main
615 stem sites; open circles – tributary sites. Statistics from Pearson correlations.

616

617 Fig 4. Regressions of pair-wise Sørensen dissimilarities in main stem (filled circles, continuous
618 line) and tributary sites (open circles, dashed line) in the Kokra River system as a function of four
619 distance measures. Results from Mantel tests are provided.