

1 **Community structure and functioning below the streambed across contrasting**
2 **geologies**

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13 **ABSTRACT**

14 There is little understanding of the variability in the structure and function of metazoan
15 hyporheic communities across streams draining geologies that weather to produce
16 different pore sizes and, by extension, different hydrological conditions. In this study
17 we selected two catchments in each of three geologies that had differing values of
18 hydraulic conductivity and porosity, and sampled four riffles in each catchment at
19 high and low water levels and at two depths. We found clear differences in the
20 physical template of streams draining different geologies and in the composition and
21 abundance of communities inhabiting the hyporheic zones of streams draining chalk/
22 sandstone and limestone geologies. However, we did not detect any significant
23 differences in body size, biomass or functional measures (diversity, richness,
24 redundancy) between the geologies. Our findings imply that ecosystem functioning in
25 streams draining geologies that produce fine grain sediments may be similar to those
26 draining geologies that produce coarse grained sediments irrespective of differences
27 in the physical template and community structure.

28 **Keywords: Hyporheic zone, hyporheos, functional diversity, biomass,**
29 **streambed biotope, functional redundancy**

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32 1. INTRODUCTION

33 The central aim of ecological science is to understand the relationship between
34 organisms and their environment, a relationship driven by the habitat template on
35 which evolution forges characteristic life history strategies (Southwood 1977, 1988).
36 Determining the nature of the physical habitat template in the benthic compartment of
37 river systems has a long history (see Townsend & Hildrew 1994). Here we focus on
38 another connected compartment of the same system, the interface between rivers and
39 aquifers, the 'hyporheic zone' (HZ), that hosts a continuous exchange of water,
40 nutrients and materials between the surface stream and the aquifer (e.g., Knapp et al.
41 2017). The complex interactions of these two sources of water produce a unique and
42 dynamic set of physicochemical conditions where there are strong gradients of pH and
43 oxygen (e.g., Krause et al. 2009, Williams et al. 2010, Bardini et al. 2012). These
44 gradients encourage the biogeochemical cycling of nutrients and attenuation of
45 contaminants (e.g., Gandy et al. 2007, Lewandowski et al. 2011, Hester et al. 2013,
46 Stegen et al. 2016). This function is mediated by microbial biofilms (e.g., Brunke &
47 Gonser 1997) that form part of a hyporheic community that also includes small
48 invertebrates (<1 mm in size) such as microcrustaceans, tardigrades, rotifers, small
49 oligochaetes, chironomids and nematodes as well as the early instars of
50 macroinvertebrates (Robertson et al. 2000, Peralta-Maraver et al. submitted). This
51 metazoan hyporheic community (the hyporheos; Orghidan 1959) is the focus of our
52 study.

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55 The composition, abundance and biomass of metazoan hyporheos, and perhaps
56 their ecological functioning, are strongly influenced by the physical and chemical
57 conditions experienced within the HZ (Robertson & Wood 2010). Sediment grain
58 size is an important factor because of its strong relationship to interstitial pore size,
59 porosity and permeability (Brunke and Gonser, 1997). In turn grain size is partly
60 dependent on the geology from which the sediments have been weathered although
61 other factors, such as topography and land use, also play a role. Easily weathered
62 geologies are associated with fine alluvial sediments whereas geologies resistant to
63 weathering are associated with coarse sediments (Morrice et al., 1997). Sediment
64 grain size determines the dimensions of the habitat available to the hyporheos and
65 they are often most abundant in coarse-grained sediment where habitat availability is
66 high (Strayer et al. 1997). In fine grained sediments, where pore spaces are
67 reduced, small sized organisms such as the meiofauna are disadvantaged because
68 there is little available habitat (Descloux et al 2014, Jones et al. 2015) whereas
69 larger bodied invertebrates (e.g., amphipods, isopods, coleopteran Elmidae larvae)
70 are still able to move through these sediments (Boulton et al. 2008). Thus, although
71 no previous publications have presented data on body size across geologies with
72 varying pore sizes, we might expect body size to be larger in rivers with small sized
73 sediments (i.e., those draining chalk and sandstone geologies) than in rivers with
74 larger sized sediments (draining limestone). Sediment grain size (and interstitial
75 velocity) is also closely coupled to the hyporheic exchange rate (the movement of
76 water between the HZ and the surface stream), which determines the supply of
77 organic matter and dissolved oxygen and thus hyporheos abundance and
78 distribution (Hakenkamp & Palmer 2000). Depth in the HZ is another important
79 factor. In deeper layers the reduction in oxygen availability and pore space due to

80 sediment agglomeration limits the vertical distribution of larger organisms which are
81 replaced by a suite of numerous but small-bodied taxa (Schmid–Araya 1994,
82 Maridet & Philippe 1995, Strayer et al. 1997, Stead et al. 2004, Peralta-Maraver et
83 al. 2018, Peralta-Maraver et al. submitted).

84

85 Increased fine sediment loading (colmation), often resulting from agricultural
86 practices, alters the streambed physical habitat template and is a major and well
87 recognised global threat to stream ecological integrity, leading to reduced hyporheos
88 diversity and modified community structure and functions (e.g., Jones et al. 2015,
89 Descloux et al. 2014, Mathers et al 2017). In contrast, there is very limited information
90 on the underlying variability in the structure and function of metazoan HZ communities
91 (hyporheos) across streams draining different geologies, despite the centrality of such
92 data to understanding how fine sediment ingress will affect hyporheos.

93

94 The range of species' functional traits (functional diversity) likely underpins a key
95 mechanistic link between species richness and ecosystem functioning and a growing
96 body of evidence suggests that community functional diversity predicts ecosystem
97 functioning better than species based indices (e.g. Gagic et al. 2015, Griffin et al.
98 2009). Functional diversity can be decomposed into functional richness and
99 functional redundancy (Carmona et al 2016); communities with high functional
100 redundancy, where two ecological units have the same trait values and therefore
101 occupy the same functional space, are thought to be more resilient to disturbances
102 (i.e., they can lose species without a great loss of function; Carmona et al 2016).
103 Thus understanding how functional diversity, richness and redundancy vary across
104 the physical habitat template in the hyporheic zone is an important goal given the

105 likely role of the hyporheic community in riverine ecosystem functioning. However,
106 very few studies have used a trait-based approach for hyporheic communities and of
107 those that have, to our knowledge, none have included measures of functional
108 diversity, richness and redundancy (see Claret et al. 1999, Robertson & Wood 2010,
109 Descloux et al. 2014, Bona et al. 2016, Doretto et al. 2107, Mathers et al. 2017).

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111 The objective of this study was to assess differences in hyporheos composition and
112 function across streams draining differing geologies. We investigated eight UK rivers
113 of three distinct geologies with differing values of hydraulic conductivity and porosity.
114 We sampled multiple locations incorporating upwelling and downwelling sites and two
115 depths on two occasions. We expected that the community composition, abundance,
116 body size, biomass, and functional measures of the hyporheos in the rivers would differ
117 in response to the variation in these features of the physical habitat template.

118 We hypothesised that:

- 119 1. Hyporheos structure (in terms of composition and abundance) will vary
120 between geologies resulting from the varying habitat template. We expect that
121 hyporheos will be more abundant and characterised by meiofauna in geologies
122 that weather to produce coarse-grained sediments.
- 123 2. Taxonomic richness, body size, biomass and diversity will also differ between
124 geologies and with depth. We expect that body size will be greatest where
125 pore size is small and the other measures will be highest where sediment size/
126 pore space is greatest and decline with increasing depth.

127 3. Functional diversity, richness and redundancy will differ between geologies in
128 response to differences in the physical habitat template. We also expect that
129 these measures will decline with increasing depth.

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131 2. METHODS

132 2.1 Survey design

133 Three geologies of differing hydraulic conductivity (k) and porosity (n) were studied
134 (i) cretaceous chalk $k = 10^{-8}$ - 10^{-5} m/s, $n = 0.15 - 0.45$; (ii) permotriassic sandstone
135 $k=10^{-10}$ - 10^{-5} m/s, $n=0.05$ - 0.35 ; (iii) carboniferous limestone $k=10^{-6}$ - 10^0 m/s, $n = 0.05$ -
136 0.5 . (Hiscock 2007). Two catchments each were selected in chalk, sandstone,
137 limestone (unglaciaded) and limestone (glaciaded) geologies (Table 1). In each
138 catchment four sites (permanently flowing riffles) were selected on the main stem
139 and tributaries (Table 1). We ensured that sites differed as little as possible apart
140 from geology; all sites had similar water quality, discharge, catchment area and
141 elevation (Table 1). The land use in all catchments was predominantly pasture. All
142 sites were located on 3rd to 4th order streams (stream order derived from Ordnance
143 Survey maps of the local area following Strahler 1964). Stream bank widths were
144 between 3- 5 metres and streams were at least 5cm deep.

145 2.2. Sediment Grain Size

146 The assessment of sediment grain size at each site was undertaken using a variety
147 of methods, i) broad scale site selection determined by the local geology and
148 associated hydraulic properties of the aquifer (see above), ii) measurement of
149 vertical hydraulic gradient (VHG) from both the head and tail of each riffle following

150 Baxter et al. (2003). Briefly a mini-piezometer was inserted to a depth of 30cm,
151 purged and left to settle for 2 hours before measurement. $VHG = (h_s - h_p)/L$. h_s is
152 the distance the water in the mini-piezometer rises above the stream water surface.
153 h_p is the distance from the top of the mini-piezometer to the water level in the mini-
154 piezometer and L is the depth of the mini-piezometer in the sediment, iii) collection
155 of sediment samples from the head of each riffle during late autumn when
156 groundwater levels and river flow velocities were reduced. Sediment was collected
157 using a spade (depth penetration 20cm, surface area 20cm²), emptied into a 100 µm
158 mesh net held just downstream and stored in a plastic bag. Sediment samples were
159 air dried then weighed to the nearest gram to obtain the total sediment mass and fed
160 into a sediment shaker following the approach of Gordon et al. (2004). Sediment
161 fractions were expressed as a percentage of the total sediment mass. iv) at 50cm
162 depth, granulometric conditions were assessed by recording the time taken to pump
163 5 litres of water because there is a strong positive relationship between hydraulic
164 conductivity and sediment grain size (Sharp 1988; Descloux et al. 2010).
165 The combined measures gave a weight of evidence approach to quantification of the
166 sediment proportions and associated properties for each catchment across the three
167 geologies.

168 *2.3. Hyporheic community sampling:*

169 Each riffle was sampled on two occasions 1) at high groundwater levels (summer
170 2008) and 2) at low groundwater levels (autumn 2008). In summer 2008, three
171 replicate hyporheos samples were taken from the deep (50 cm) HZ at both the head
172 (downwelling) and tail (upwelling) of each riffle (192 samples in total). In autumn
173 2008, the same survey design was followed except samples were additionally taken

174 from the shallow (20 cm) HZ (384 samples in total) because low numbers of
175 individuals were collected at 50 cm depth.

176 Samples were collected using the Bou-Rouch method (Bou and Rouch 1967; Bou
177 1974). Piezometers were installed at least 1 metre apart and allowed to settle for at
178 least one hour before pumping 5 litres of water (after Kibichi et al 2009). This was
179 filtered through a 63 µm sieve and preserved in 70% IMS. Preserved samples were
180 sorted under a stereomicroscope and photographed at calibrated magnifications.
181 Subsequently the length and width of all organisms (except Oligochaeta, Nematoda
182 and Acari) were measured to the nearest micrometre using these images and an
183 image analyser. If large groups of similar sized instars were found (>50 µm, primarily
184 *Bryocamptus* sp. and Chironomidae larvae) all individuals were counted, then 20%
185 of the group was measured to obtain a mean value which was then applied to the
186 whole group. The prolate ellipsoid volume of each group was calculated following
187 Omesova et al. (2008).

$$188 \quad V = 4/3\pi l w^2$$

189 $w = \text{width}, l = \text{length}, V = \text{volume (mm}^3\text{)}$

190 Invertebrates were identified to species level where possible using the following
191 keys: Copepoda (Einsle 1993, Janetzky et al. 1996); Cladocera (Scourfield 1994);
192 Macroinvertebrates (Holland 1972, Elliott and Humpesch 1983, Hynes 1993, Wallace
193 et al. 2003, Edington and Hildrew 2005) and Groundwater Crustacea (Gledhill 1993).
194 Oligochaeta, Diptera, Ostracoda and Nematoda were not identified further due to the
195 poor preservation of Oligochaeta and Nematoda and the predominance of early
196 instar Chironomidae larvae.

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198 *2.4. Water chemistry*

199 Measurements were taken directly from each piezometer. Conductivity, dissolved
200 oxygen (DO), temperature, and pH were determined using portable meters (pH -
201 Hanna HI-9025; electrical conductivity - Hanna HI-9635; dissolved oxygen -
202 temperature - Hanna HI-9145). Then, 0.1 litre of water was withdrawn from the
203 piezometer and frozen within 12 hours. Subsequently, on the day of analysis, water
204 samples were defrosted and passed through a 0.7 µm filter to remove particulate
205 matter. Cations and anions were analysed using high performance anion-exchange
206 chromatography (DIONEX ED40 electrochemical detector (minimum reporting value
207 =1.0 mg/L; Thermo Fisher Scientific, Waltham, Massachusetts).

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209 *2.5. Functional traits*

210 Traits were characterised for 37 taxa using trait tables from Descloux et al. (2014) for
211 the permanent meiofauna and Tachet et al. (2010) for temporary meiofauna. Trait
212 profiles were described mainly at genus level except for some Diptera, Oligochaeta
213 (described as trait profiles of potential families in the corresponding biogeographic
214 area) and Copepoda (described as Harpacticoida or Cyclopoida). Nine traits with 55
215 modalities incorporating a range of life history, mobility, morphological, and
216 ecological traits were included following Descloux et al. (2014) but excluding the trait
217 'fecundity' due to lack of data (see Supplementary Information Table S25). A score
218 was assigned to each taxon describing its affinity for that modality (Descloux et al.
219 2014; Tachet et al. 2010). The mean trait profile of each riffle assemblage was
220 obtained by weighting the individual trait profiles of taxa by their total abundance in
221 the sample (replicate and upwelling/ downwelling samples were pooled to give a
222 single value of abundance per riffle and water level because we did not detect

223 significant differences in abundance depending on the direction of vertical flow).
224 Then, the sums of the weighted scores (one per trait category) were rescaled to sum
225 to one for each trait and riffle and depth, following Descloux et al. (2014).

226

227 *2.6. Data analysis*

228 *2.6.1 Community structure* - Composition and abundance of all identified taxa during
229 the study period was averaged by site and depth in order to merge all the recovered
230 information of the community structure per geology treatment. Then, a NMDS
231 analysis (Non-metric Multi-Dimensional Scaling) was performed to describe the
232 dissimilarity among communities based on their structure (taxonomic composition
233 and abundance). This is an effective method for the ordinations of ecological data
234 that works with rank orders dissimilarity matrixes (rather than absolute values) and
235 can handle nonlinear responses of the biological attributes of any shape and
236 effectively and robustly find the underlying gradients (Oksanen, 2015; Quinn &
237 Keough, 2002). In order to handle excessively large differences between abundance
238 values, these were transformed using Wisconsin double standardization (Bray &
239 Curtis, 1957). Dissimilarity matrices between communities used in the ordination
240 were calculated with the quantitative Bray-Curtis Index. An iterative process was
241 carried out to find the ordination with the best goodness of fit (Oksanen, 2015).

242 Subsequently, analysis of similarity (ANOSIM) was performed to determine
243 whether there was a significant effect of the geology on the structure of the
244 communities (*p-value* < 0.05) and the reliability of the test (ANOSIM R value). This
245 analysis also uses a rank order dissimilarity matrix to determine whether there is a
246 significant difference between two or more sample groups based on whether there are
247 greater differences between these groups compared to within groups (Oksanen,

248 2015). In this manner, we compared the magnitude of differences intra-treatments
249 (ANOSIM within- and between-group rank dissimilarities are available as
250 Supplementary information: Fig S2). Finally, post-hoc comparisons between geologies
251 were conducted with PerMANOVA pairwise contrasts to identify those treatments that
252 differed significantly. All analyses were performed with the Vegan package (Oksanen
253 et al., 2013) within the R software platform (R Core Team 2014).

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255 *2.6.2 Taxonomic richness, body size, biomass, community diversity and functional*
256 *measures (diversity, richness, redundancy).* Taxonomic richness, body size,
257 biomass, Shannon-Wiener diversity, functional diversity, functional richness, and
258 functional redundancy were compared between geologies (factor with four levels:
259 chalk, sandstone, unglaciated limestone and glaciated limestone) and depth
260 compartment (factor with two levels: 20 and 50 cm) using low water level data and
261 applying 2-way ANOVA tests. Then, taxonomic richness, body size, biomass,
262 Shannon-Wiener diversity, functional diversity, functional richness and functional
263 redundancy were also compared between geologies during the whole study period
264 by using only data from 50 cm depth with a nested 1-way ANOVA test, correcting by
265 water level. In order to solve heterogeneity in the residuals, body size was log
266 transformed, but this was not necessary for the rest of responses. No differences
267 were found in the studied responses between downwelling and up-welling conditions
268 within riffles during data exploration. Accordingly, metrics were pooled by riffle (riffle
269 as a replicate per site). Dependency structure of the residuals with the study site
270 (*Site*) was incorporated in the previous ANOVA tests in the design of the study
271 (random factor). In this manner, we accounted for repeated measures (non-
272 dependence of residuals with study site in the ANOVA tests). In order to fit the

273 random factor to the ANOVA tests we used the *lmer* function of the R package lme4
274 (Bates et al. 2017, R Core Team 2016). Validation of underlying assumptions of
275 normality and homocedasticity of tests residuals was applied following Zuur et al.
276 (2009) (supplementary material: Fig S1). Finally, Wald chi-square tests were
277 performed in order to assess the significance of the analysed factors on the
278 responses using the *Anova* function from the *car* R-package (Fox & Weisberg 2011)
279 and, subsequently, post-hoc Tukey tests were applied to compare which specific
280 treatments differ significantly using the *diffsmeans* function from the R-package
281 *lmerTest* (Kuznetsova et al. 2015).

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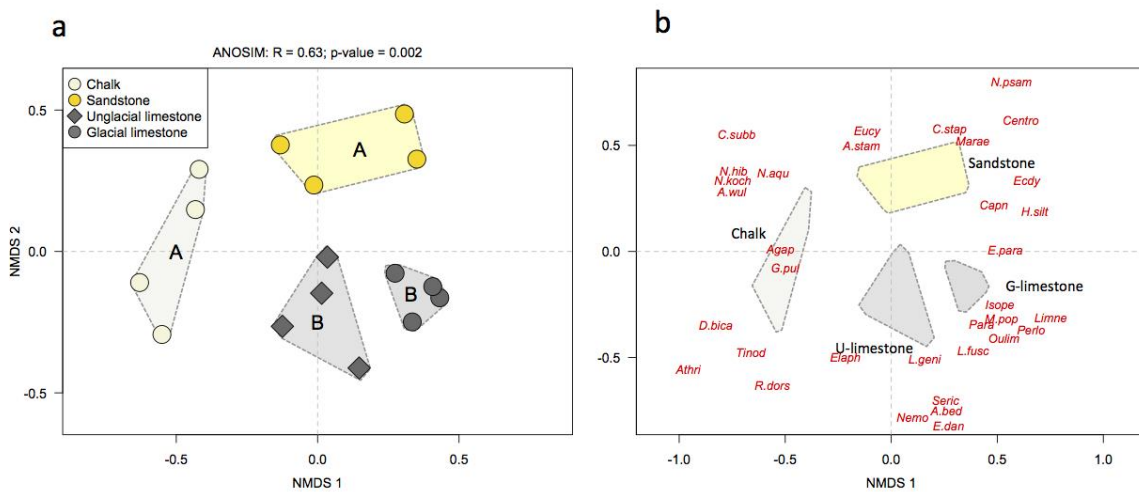
283 **3. RESULTS**

284 The physical habitat template showed clear differences between geologies and
285 across water levels. Pump times were significantly higher in rivers draining chalk
286 than in other geologies ($F_{3,176}=21.9$, $p<0.001$, Table 2) and these findings were
287 supported by VHG measurements (Table 2). Chalk rivers also had the highest
288 percentage of sediments in the 0.5 – 2mm size category. Conductivity ($F_{3,563}=403.0$,
289 $p<0.001$), pH ($F_{3,563}=116.1$, $p<0.001$), dissolved oxygen ($F_{3,563}=7.52$, $p<0.001$),
290 temperature ($F_{3,563}=200.3$, $p<0.001$), and nitrate ($F_{3,563}=124.2$, $p<0.001$), also
291 differed significantly between geologies. Conductivity ($F_{1,563}=12.3$, $p<0.001$), pH
292 ($F_{1,563}=11.9$, $p<0.001$), dissolved oxygen ($F_{1,563}=86.0$, $p<0.001$), temperature
293 ($F_{1,563}=2097.9$, $p<0.001$), and nitrate ($F_{1,563}=4.4$, $p<0.001$), also differed significantly
294 between water levels. See Table 2 for means and standard errors of these
295 parameters across geologies and water levels.

296

297 The NMDS ordination model based on taxonomic composition and abundance was
298 run 20 times for the two-dimensional ordination with a very high goodness of fit
299 between the distances in the ordination against the original data (stress = 0.20,
300 linear fit $R^2 = 0.98$, nonmetric fit $R^2 = 0.86$). Accordingly, the Shepard plot of the final
301 model has small scatter around the fitted line (Supplementary Information Fig S1)
302 and the bi-dimensional ordination is representative of the original differences
303 between assemblages. The NMDS-plot clearly discriminated assemblages between
304 geologies (Fig 1a) and showed which taxa contributed most to the observed
305 dissimilarities (Fig 1b). ANOSIM analysis found a significant effect of geology on the
306 observed dissimilarities between assemblages ($R = 0.63$, p -value = 0.002),
307 supporting the NMDS plot. Thus our first hypothesis, community composition will
308 differ between geologies, was upheld. The post hoc PerMANOVA pairwise contrasts
309 detected significant differences between limestone assemblages (both glacial and
310 un-glacial) with sandstone and chalk sites respectively. However, these differences
311 were not significant between sandstone and chalk assemblages, or between glacial
312 and un-glacial limestone assemblages (Fig 1a, Table S25) i.e., differences in
313 assemblage structure (composition and abundance) between sandstone and chalk
314 sites and between both limestones were too small to be able to characterise them as
315 different communities. The hyporheos of Limestone catchments was characterised
316 by a suite of stoneflies and caddisflies (e.g., *Leuctra* spp., Perlodidae, *Limnephilus*
317 sp.) and microcrustacea (e.g., *Paracyclops* sp., *Moraria* sp., *Elaphoidella* sp.) (Fig.
318 1b). Chalk catchments were defined by large-bodied Crustacea (e.g., *Gammarus*
319 *pulex*, *Niphargus* spp., *Crangonyx* sp.) whereas indicative taxa of sandstone
320 catchments included harpacticoid (e.g., *Maraenobiotus* sp., *Canthocamptus* sp.) and
321 cyclopoid (*Eucyclops* sp.) copepods (Fig. 1b).

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Fig 1: (a) NMDS ordination model of different HZ assemblages by geology including ANOSIM statistic (R -value) and significance (p -value). Polygons group the assemblages by geology sites in the ordination. Assemblages whose structure is not significantly different after post-hoc PerMANOVA tests (P -value < 0.005) are indicated with the same letter. (b) Distribution of the most influential taxa on the ordination. In order to simplify interpretation of the plot, those taxa falling in the centre of the ordination (low influence in the ordination) have been removed. For explanations of species abbreviations see Supplementary Table 23.

338 Our second hypothesis; taxonomic richness, body size, biomass and Shannon-Wiener
339 diversity will differ across geologies and depths was partly supported. Only taxonomic
340 richness showed significant differences across some geologies with larger values in
341 rivers draining unglaciated limestone (Fig. 2b). In contrast, depth showed a strong
342 effect on all these responses. Taxonomic richness, body size, biomass and Shannon-
343 Wiener diversity declined with increasing depth for all geologies (Fig. 2a, c, e, g) and
344 in most cases these differences were significant.

345 3.4. Our third hypothesis; functional measures will differ with geology and decline
346 with increasing depth was only partially upheld. Functional measures did not differ
347 significantly across geologies either in the summer data or across the whole study
348 period (Fig. 2i – n). However, functional richness and redundancy declined
349 significantly with increasing depth for several geologies (Fig. 2 l,k). Numerical results
350 and p-values from the Wald chi-square and post-hoc Tukey tests are available as
351 Supplementary information (Tables S1 - S22).

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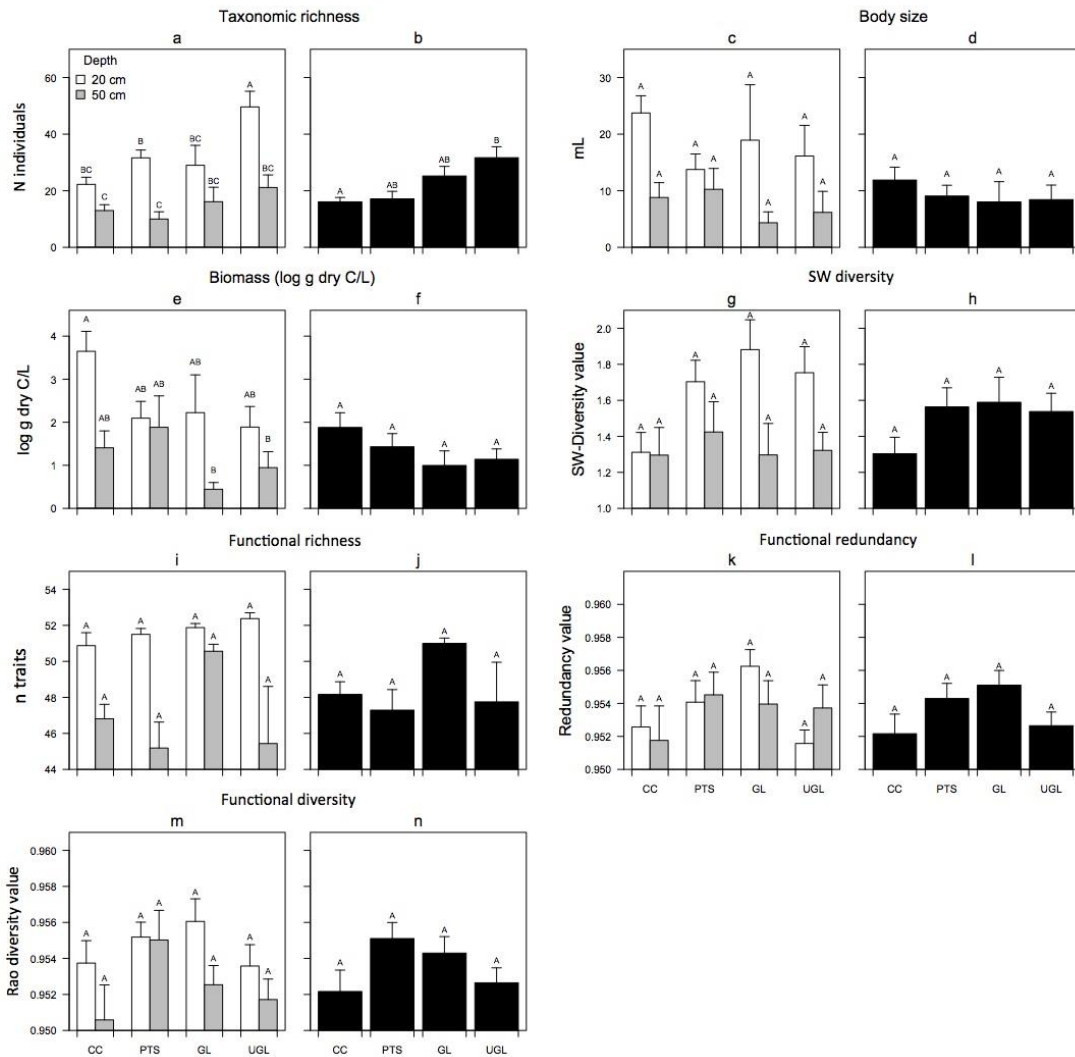
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363 Fig 2: Taxonomic richness, body size, biomass, Shannon-Wiener diversity and
 364 functional richness, redundancy and diversity (mean $\pm 1SE/5 L$). Summer data
 365 (a,c,e,g,i) are pooled across the four geologies (CC: Chalk, PTS: permo-triassic
 366 sandstone, UGL: unglaciated limestone, GL: glaciated limestone) and two depths
 367 (20cm and 50cm). While, data from 50 cm depth (b,d,f,h,j) of the whole study period
 368 are just pooled across geologies. Geologies where total responses are not significantly
 369 different are indicated with the same letter (95% CI).

370 **4. DISCUSSION**

371 Our study showed clear differences in the HZ physical habitat template in rivers
372 draining different geologies at the regional scale and corresponding variations in the
373 community composition of these HZ's. Geologies that weather easily to produce fine
374 grained alluvial sediments (chalk and sandstone) possessed hyporheos that differed
375 significantly from those that were present in rivers where the geology did not weather
376 so easily resulting in coarser grained sediments (carboniferous limestones). The
377 hyporheos in coarse-grained limestone rivers included an abundance of small-
378 bodied copepods, which were able to exploit the larger pore spaces available. In
379 contrast the hyporheos of chalk rivers was dominated by larger macroinvertebrates,
380 presumably because they were able to burrow through these sediments more easily
381 (Boulton et al. 2008). Although copepods did occur in these rivers, they were much
382 less abundant and diverse.

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384 We detected clear differences in taxonomic richness between geologies in
385 agreement with previous studies (e.g. Strayer et al. 1997; Ward et al. 1998; Bo et al.
386 2007, Descloux et al. 2014) despite the lower % of fine sediments in our study.
387 These authors noted that decreasing sediment size and interstitial space exert a
388 negative effect on abundance and taxon richness (although we did not compare
389 abundance between geologies as the taxonomic composition of the hyporheos
390 varied so markedly). To our knowledge only one publication has compared the
391 biomass of metazoan hyporheos across sediment types in perennial streams
392 (Reynolds & Benke 2012). They found that the biomass of chironomid assemblages
393 was highest in the HZ's of gravel-cobble reaches, intermediate in sand reaches and
394 lowest in mud-silt reaches. Our study is the first to compare HZ biomass of the
395 whole metazoan community retained on a 63 μ m sieve across geologies with

396 different pore sizes. Our finding, that biomass was similar across geologies,
397 suggests that the HZ's of all these rivers support a similar quantity of hyporheos
398 although the structure and composition of these communities differ. Contrary to our
399 expectations, although rivers draining limestone included small bodied taxa and
400 chalk rivers were characterised by taxa with larger body sizes, this did not translate
401 into significant body size differences between the rivers, perhaps because
402 macroinvertebrate taxa were represented by very early instar individuals. Our
403 findings suggest that individuals of any body size are equally likely to penetrate bed
404 sediments of the three geologies and that the HZ's may therefore have equal
405 connectivity with the surface in terms of organism movement.

406

407 Our findings, that taxonomic richness declined with increasing depth and that body
408 size and biomass tended to decrease with depth, concur with those of other studies
409 (e.g., Strayer et al. 1997, Davy-Bowker et al. 2006, Pacioglu & Robertson 2017,
410 Peralta-Maraver et al. submitted). This likely results from a reduction in pore space
411 and oxygen with increasing depth (Maridet & Philippe 1995, Strayer et al. 1997,
412 Peralta-Maraver et al. 2018, Peralta-Maraver et al. submitted).

413

414 HZ's are increasingly recognised as a central component of the lotic ecosystem,
415 particularly regarding their functional role in the attenuation of nutrients and
416 pollutants (e.g., Lewandowski et al. 2011; Peralta-Maraver et al. 2018) and their
417 importance as a refuge from surface disturbances (e.g., Robertson & Wood 2010,
418 Maazouzi et al. 2017). Although biodiversity is understood to be a fundamental
419 driver of ecosystem functioning, there is a growing acceptance that the diversity of
420 species functional traits – functional diversity - within a community underpins and

421 better describes ecosystem functioning (Gagic et al. 2015). Our study is the first to
422 determine the functional richness, redundancy and diversity of hyporheos in
423 contrasting geologies. Despite differences in the physical habitat template, which
424 resulted in distinctive hyporheos per geology, none of our measures of ecosystem
425 function significantly differed between geologies implying equal functioning across
426 the HZ's. Several studies have demonstrated the impact of fine sediment deposition
427 (colmation) on individual functional traits of hyporheos (e.g., Descloux et al. 2014,
428 Bona et al. 2016, Doretto et al. 2017, Mathers et al. 2017). We therefore expected,
429 but did not find, that functional diversity would be lowest in the fine sediment rivers.
430 As anticipated, we found functional measures declined with increasing depth, as did
431 taxonomic richness suggesting that ecosystem functioning decreases with increasing
432 depth. Thus, our results imply that maximal functioning of the metazoan hyporheos
433 occurs in the shallow HZ in accordance with findings for microbial biofilms in the HZ
434 (Battin et al. 2003, O'Connor & Harvey 2008, Boano et al. 2014, Knapp et al. 2017).
435 Our findings suggest that, for these relatively unimpacted rivers draining three
436 different geologies, the HZ's all supported similar quantities of hyporheos, had
437 similar connectivity with the surface stream and had similar levels of functional
438 richness, redundancy and diversity, all of which implies relatively similar ecological
439 functioning irrespective of differences in the physical template and community
440 structure between geologies. However, our approach to characterising ecosystem
441 functioning within the HZ was quite broad brush; we do not yet know the extent to
442 which their capacity to undertake these functions is resilient to environmental
443 perturbations i.e., the degree of response diversity within the functionally redundant
444 elements of the community (Mori et al. 2013) and whether this differs across
445 geologies.

446

447 **5. CONCLUSION**

448 The effects of fine sediment accumulation in streams is a global concern yet there is
449 limited understanding of the underlying variation in hyporheos structure and function
450 across streams draining differing geologies despite their contribution to the delivery
451 of ecosystem services such as pollution attenuation. Such data is essential to
452 contextualise the effect of perturbations such as fine sediment ingress. We found
453 that despite clear differences in metazoan community structure between streams
454 with fine sediment and streams with coarse sediment, there were no significant
455 differences in biomass or functional measures between the geologies implying that
456 the HZ's in all geologies functioned equally.

457

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465

466 **7. COMPETING INTERESTS STATEMENT**

467 The authors have no competing interests to declare.

468

469 **8. REFERENCES**

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755 Table 1. Physical and water quality characteristics of the studied catchments

756

Geology	Site location	Catchment area (Km ²) ¹	Naturalized mean annual discharge (m ³ S ⁻¹)	Elevation (m)	RBMP C2 status MI ²
Chalk	RIVER FROME N50° 47' 23.0" W002° 34' 49.9" N50° 46' 36.3" W002° 28' 10.4" N50° 45' 07.3" W002° 30' 42.6" N50° 46' 35.4" W002° 34' 26.8"	30-40	0.31-1.25	78-95	High
	RIVER PIDDLE N50° 44' 20.7" W002° 15' 38.2" N50° 44' 42.5" W002° 19' 16.7" N50° 45' 22.3" W002° 13' 35" N50° 45' 17.4" W002° 21' 40.4"	48-70	0.31-0.62	44-60	High
Sandstone	RIVER TONE N51° 03' 12.6" W003° 14' 55.1" N51° 02' 11.4" W003° 16' 8.5" N50° 58' 24.7" W003° 18' 50.1" N50° 59' 14.1" W003° 15' 19.0"	42-65	0.31-1.25	16-62	Good- High
	RIVER EXE N50° 54' 49.4" W003° 27' 21.7" N50° 48' 35.2" W003° 38' 53.8" N50° 46' 40.8" W003° 40' 01.8" N50° 46' 41.0" W003° 39' 46.3"	42-65	0.62-1.25	35-83	High
Glaciated Limestone	RIVER URE N54° 16' 11.1" W002° 08' 22.3" N54° 17' 10.5" W001° 58' 38.1" N54° 17' 12.2" W001° 58' 1.9" N54° 13' 23.2" W001° 55' 55.1"	40	0.31-0.62	85-258	High
	RIVER WHARFE N54° 11' 26.01" W002° 05' 43.4" N54° 11' 26.0" W002° 05' 44.4" N54° 08' 51.5" W002° 07' 42.4" N54° 08' 23.8" W002° 06' 42.1"	23-33	0.62-1.25	209-225	Good- High
Unglaciated Limestone	RIVER DERWENT N53° 20' 45.1" W001° 46' 45.1" N53° 14' 58.0" W001° 51' 23.6" N53° 14' 23.2" W001° 44' 55.1" N53° 14' 17.5" W001° 44' 46.7"	20-70	0.31-2.5	128-255	Good- High
	RIVER DOVE N53° 05' 00.7" W001° 47' 30.0" N53° 03' 13.0" W001° 48' 01.9" N53° 03' 29.4" W001° 46' 37.7" N53° 03' 05.3" W001° 48' 30.1"	45	0.62-2.5	138-171	High

757

758 ¹ Data source <http://environment.data.gov.uk/catchment-planning/>

759 ²River Basin Management Plan Cycle 2 2016 <http://environment.data.gov.uk/catchment-planning/>

760

761 Table 2: Means (± 1 SE) of selected physico-chemical parameters for three geologies and two water levels. Pump time = time (in
 762 minutes) to pump 5 litres of water.

763

Geology	Vertical Hydraulic Gradient. H = riffle head, T = riffle tail	Water level	% sediment (0.5- 2mm)	% sediment (4-16mm)	Pump time	Conductivity (μ S)	pH	Dissolved oxygen (mg/L)	Temperature ($^{\circ}$ C)	NO ₃ (mg/L)
Chalk	0.008 \pm 0.003 (H)	High	30	48	2.9 \pm 0.2	542.2 \pm 7.39	7.7 \pm 0.03	5.8 \pm 0.17	18.2 \pm 0.15	17.2 \pm 0.75
	0.0006 \pm 0.002 (T)	Low				560.7 \pm 5.03	7.5 \pm 0.03	7.9 \pm 0.21	12.3 \pm 0.09	14.3 \pm 0.80
Sandstone	-0.02 \pm 0.005 (H)	High	17.5	62.5	1.6 \pm 0.1	404.2 \pm 10.7	7.1 \pm 0.03	5.2 \pm 0.18	15.3 \pm 0.1	15.2 \pm 1.14
	0.03 \pm 0.005 (T)	Low				317.2 \pm 11.5	7.3 \pm 0.04	7.2 \pm 0.13	11.8 \pm 0.08	11.5 \pm 1.31
Glaciated Limestone	-0.06 \pm 0.008 (H)	High	21	57	1.5 \pm 0.1	255.2 \pm 5.1	7.9 \pm 0.03	6.1 \pm 0.19	15.0 \pm 0.1	1.3 \pm 0.07
Unglaciated Limestone	0.08 \pm 0.011 (T)	Low	20	59.5		243.9 \pm 9.2	7.8 \pm 0.02	7.7 \pm 0.18	10.6 \pm 0.07	1.5 \pm 0.07
Unglaciated Limestone	-0.07 \pm 0.012 (H)	High			0.08 \pm 0.009 (T)	Low	1.5 \pm 0.1	444.9 \pm 12.01	7.7 \pm 0.03	7.4 \pm 0.32

764