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Drivers of functional diversity in the hyporheic zone of a large river

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38 **Highlights: (3 to 5 point with 85 characters max)**

39 -Hydrogeology, current and past fluvial forms control large-scale distribution of the hyporheos

40 -Hydrology controls hyporheos composition at the station scale

41 -Visible characteristics of surface sediment had poor influence

42 -Upwelling zones are hotspot of groundwater biodiversity

43 -Downwelling zones harboured high abundances of temporary hyporheos

44

45 **Abstract**

46 The effects of regional (hydrogeology and geomorphology) and local (sediment and hydrology)
47 characteristics on hyporheic assemblages were studied along a 40-km reach of a large gravel-bed river.
48 Hyporheic water and fauna were sampled at the upstream and downstream positions of 15 large gravel
49 bars. The resulting 30 stations varied in their sediment grain size, stability and direction of river-aquifer
50 exchanges. The study concludes that at the 40-km (sector) scale, the longitudinal distribution of
51 hyporheic fauna was controlled by 1) the hydrogeology of the valley (i.e. gaining vs losing sectors)
52 that modifies abundance and taxonomic richness of stygobites 2) current channel morphometry of the
53 river (i.e. shape and location of meanders), and 3) historical changes (i.e. river incision) which modify
54 abundance and richness of assemblages. At the local scale, we found that surface grain size and
55 stability of the sediment evaluated by visual observation were poor predictors of hyporheos
56 composition. In contrast, the local hydrology (i.e. downwellings, upwellings, low vertical exchanges)
57 explained a large part of the abundance, taxonomic richness and composition of the hyporheic
58 assemblages. Stations with low vertical exchanges were found poorly colonized, while the upwelling
59 zones were rich in stygobites and downwelling areas harbor abundant and species-rich temporary
60 hyporheos. It was also observed that functional diversity was controlled by the same parameters, with
61 high relative abundances of stygobites in upwelling zones and POM feeders in downwelling zones. The
62 heterogeneity of hydrological patterns, with alternation of upwellings and downwellings may
63 represent the optimal spatial structure for hyporheic biodiversity conservation and resilience in rivers.

64

65

66

67 **Key words:** Hyporheos, stygobites, hydrogeology, geomorphology, river incision, dams

68 (Six max)

69

70 1. Introduction

71

72 Porous sediment deposits in rivers constitute the hyporheic habitat (Orghidan, 1959), where river-
73 aquifer exchanges occur together with a large number of ecological processes important for stream
74 metabolism (Findlay, 1995; Boulton et al., 1998). Hyporheic processes contribute to the recycling of
75 nutrients (e.g., nitrification / denitrification, Triska et al., 1993; Jones et al., 1995, Storey et al., 2004)
76 and of organic matter (e.g. biodegradation of leaf litter, Cornut et al., 2010). Additionally, the hyporheic
77 habitat contributes to the biodiversity of the river by combining colonization of both obligate
78 groundwater fauna (i.e. stygobites) and benthic organisms (Dole-Olivier and Marmonier 1992; Dole-
79 Olivier et al., 1997; Brunke and Gonzer, 1999). Hyporheic organisms can that modify the structure of
80 the sediment (through bioturbation, Mermillod-Blondin and Rosenberg, 2006; Shrivastava et al.,
81 2021a, 2021b), the dynamics of organic matter (through subsurface biodegradation, Crenshaw et al.,
82 2002; Marmonier et al., 2010) and the concentration of dissolved nutrients (Mermillod-Blondin et al.,
83 2000; Nogaro and Burgin, 2014). Thus, their biodiversity is of crucial importance for river integrity and
84 functioning.

85

86 The hydrological, geomorphological, physicochemical, and/or biological processes control major
87 ecological features of streams (Petts & Amoros, 1996). The local hydrology, with the occurrence of
88 water exchanges between the river and the adjacent groundwater is the major driver for the control
89 of hyporheic processes and biodiversity (Dole-Olivier and Marmonier 1992; Valett et al., 1993; Brunke
90 and Gonzer, 1999). Many studies have demonstrated the influence of river-aquifer exchanges on the
91 physicochemical characteristics, nutrient recycling and invertebrate biodiversity of streams (e.g., Olsen
92 and Townsend, 2003; Navel et al., 2011; Mathers et al., 2017). The intensity and direction of these
93 exchanges are crucial; in downwelling zones, the river water infiltrates into the riverbed sediments
94 carrying fresh organic matter and dissolved oxygen, while in upwelling zones, the flow of groundwater
95 towards the surface stabilizes stream temperature and water level (Claret et al., 1998; Franken et al.,
96 2001; Peralta-Maraver et al., 2018). Additionally, the direction and magnitude of hydrological
97 exchanges through the hyporheic zone also control hyporheic biodiversity (Dole-Olivier & Marmonier,
98 1992; Lin et al., 2020).

99

100 These river-aquifer interactions are generally controlled by the geology of the valley and the
101 geomorphology of the streambed at different scales (Williams, 1993; Poole, 2002, 2010; Capderrey et
102 al., 2013). The geology of the valley controls the characteristics of the aquifer (e.g. volume, general
103 slope) and the relative elevation of the piezometric level compared to the river level (Graillet et al.,
104 2014; Marmonier et al., 2019). Similarly, the geomorphology of the streambed controls the water

105 exchanges between the river and the hyporheic zone (Buffington and Tonina, 2009): at a regional scale,
106 the shape of the channel influences the direction of water exchanges (Cardenas et al., 2004). For
107 example, large gravel bars induce infiltration of surface water at their heads and exfiltration of
108 groundwater at their tails (Dole-Olivier and Marmonier, 1992). Furthermore, the geomorphology
109 controls the local characteristics of sediments like their grain sizes and their stability (e.g. Singer, 2008;
110 Snelder et al., 2011). For example, the occurrence of fine sediment patches may modify the nature and
111 intensity of most hyporheic microbial processes, due to reductions in vertical water exchanges,
112 availability of dissolved oxygen and organic matter (Lefebvre et al., 2005; Nelson et al., 2019). Likewise,
113 sediment grain size may control hyporheic assemblages, for both taxonomic richness and species
114 composition (Descloux et al., 2013; O'Sullivan et al., 2019) and sediment mobility maintain hyporheic
115 permeability and vertical migration of the hyporheic fauna (Dole-Olivier et al., 1997). The effects of
116 these large-scale characteristics (i.e. hydrogeology and geomorphology) are not limited to hyporheic
117 processes, but also control the global functioning of the river and the dynamics of most organisms that
118 live inside and in close relation to the river, supporting the importance to consider the river in its valley
119 (Hynes, 1975) as a global hydrosystem (Petts & Amoros, 1996).

120

121 Hyporheic assemblages (hyporheos) are characterized by a variety of taxa with very different ecological
122 requirements. Some species use the hyporheic zone for only part of their life cycle (i.e. temporary
123 hyporheos), while other species can spend their entire life cycle within the sediment (i.e. permanent
124 hyporheos). In addition, taxa such as stygobites are obligated to live in groundwater habitats (Williams,
125 1984; Marmonier et al., 1993). The relative composition of these three groups of species may
126 determine zones of high diversity (hyporheic hotspots, Marmonier et al., 2020; Hutchins et al., 2020).
127 Furthermore, the hyporheic fauna is very diverse in food preferences ranging from fine sediment
128 feeders, to particulate organic matter-feeders (detritivores) and predators (Williams et al., 2010).
129 While taxonomic composition of river assemblages may be strongly influenced by biogeography,
130 evolutionary processes and dispersal (Eme et al., 2015), the functional diversity based on ecological
131 groups (Usseglio-Polatera et al., 2000) can help to elucidate the dynamics of hyporheic assemblages.

132

133 Functional diversity relates to the range and value of those species and organismal traits that influence
134 how an ecosystem functions (Lawton, 1994; Tilman et al., 1997). It may also be affected by large-scale
135 gradients in habitat characteristics (Poff, 1997; Brown et al., 2018) or by local disturbances (Schmera
136 et al., 2017; Graco-Rosa et al., 2021). For example, Parker et al. (2018) used a functional index based
137 on fish habitats and foraging traits to evaluate the effect of long-term improvements in water quality
138 in four reaches of the Illinois River. In the same way, Paillex et al. (2013) used the functional diversity
139 of macroinvertebrate assemblages based on 11 biological traits to estimate the effect of the

140 restoration of the connectivity across the Rhône River floodplain. Also, functional diversity of
141 invertebrates may be of interest to evaluate the effects of disturbances in the hyporheic zone
142 (Magliozzi et al., 2019; Di Lorenzo et al., 2021). Changes in direction and/or intensity of river-
143 groundwater exchanges can lead to shifts in hyporheic biodiversity, whether they are natural (e.g.
144 floods) or human-induced (e.g. dewatering, groundwater abstraction; Dole-Olivier et al., 1997; Datry,
145 2012). Similarly, changes in the local characteristics of the bottom sediment (e.g. erosion, sediment
146 clogging) can also modify the composition of hyporheic assemblages (Strayer et al., 1997; Marmonier
147 et al., 2010b), but with poor changes in taxonomic richness due to species replacement (Descloux et
148 al., 2013). Functional diversity -that include ecological requirements of species-may be efficient to
149 evaluate the effect of changes in river-groundwater exchanges or in sediment characteristics on
150 hyporheic species composition (Marmonier et al., 2012; Descloux et al., 2014; Lewandowski et al.,
151 2019).

152

153 The present work aims to determine the combined effects of regional patterns (i.e. hydrogeological
154 and geomorphological characteristics of a 40-km long reach) and local characteristics (i.e. sediment
155 grain size, sediment stability and river-hyporheic water exchanges) on the composition and the
156 functional diversity of hyporheic assemblages. The local hydrology was described using water
157 chemistry, while the sediment characteristics was assessed by image analyses. The functional diversity
158 was characterized using two functional traits based on the affinity of the species for groundwater
159 habitats and their food-type preferences. For this purpose, 30 stations were selected along 15 gravel
160 bars of a large gravel-bed river (the Ain River, France), with varying river-groundwater exchanges,
161 sediment grain size and stability. Specifically, the following three hypotheses were proposed.

- 162 - First, hydrogeology and geomorphology control hyporheic assemblages at the scale of the 40-
163 km reach (**H1**, Poole, 2010). More precisely, both river-groundwater exchanges (obtained from
164 hydrogeology modelling) and channel morphology (e.g., location of incision zones, meanders)
165 explain the longitudinal variations of hyporheic abundance, its taxonomic richness, and the
166 variation of ecological groups along the studied reach.
- 167 - Second, surface sediment characteristics (visual estimation of the grain size composition and
168 stability) have an effect on the composition of the hyporheic assemblages at the local scale
169 (**H2**, Descloux et al., 2013). Taxonomic richness and abundances are high in coarse and mobile
170 sediment, which favor the vertical migration of benthic organisms (temporary hyporheos) and
171 organisms that feed on POM infiltrating from the surface. Whereas organisms that feed on
172 fine sediment and algae are more abundant in sandy and stable areas covered by abundant
173 biofilms. Finally, the stygobites that live in deep groundwater and are sensible to
174 environmental instability are more abundant in stable shallow sediments.

175 - Third, the vertical hydrological exchanges at a local scale (i.e. upwelling, downwelling, low
176 exchange zones) would control the abundance, taxonomic richness and functional diversity
177 (**H3**, Dole-Olivier & Marmonier, 1992). Unfavorable conditions for the hyporheic fauna exist in
178 low exchange zones (for both benthic and groundwater fauna); upwelling conditions lead to
179 enhanced taxonomic richness of obligate groundwater-dwellers (stygobites), while
180 downwelling conditions lead to increase the abundance and species richness of both
181 temporary (benthic) and permanent hyporheos.

182

183 **2. Study area**

184

185 The study was conducted in the lowest 40-km reach of the Ain River, a tributary of the Rhône River,
186 France (Dole-Olivier et al., 2019, Fig. 1). In this sector, the Ain River flows in a large alluvial plain
187 composed of recent alluvia covering 5 to 30 m thick fluvio-glacial deposits, overlying Miocene molasses
188 that represent the impermeable substratum. The alluvial plain width is reduced in its central part by
189 two morainic hills between which the river flows (dotted lines in Fig. 1). In this sector, the mean annual
190 discharge is $120 \text{ m}^3 \text{ s}^{-1}$, with low flow conditions during the summer period (mean July-August,
191 discharge: $54 \text{ m}^3 \text{ s}^{-1}$). The river hydrology is also strongly influenced by five dams located upstream of
192 the studied reach that produce daily discharge fluctuations and a significant sediment deficit (Rollet et
193 al., 2014).

194

195 The lower Ain River is relatively wide with a wetted channel width of 51.5 m (± 15.9 m) and an active
196 strip of 89.5 m wide (± 31.2 m, Dole-Olivier et al., 2019). The sediment deficit due to the dams induced
197 a degradation of the riverbed of 2 to 4 cm per year from 1976 to 1999 (Rollet et al., 2014) on the first
198 10 km of the upstream part of the studied sector, around the four upstream gravel bars (noted by a
199 pointed dark line on the Fig. 1). This riverbed degradation resulted in the erosion of fine particles and
200 the local pavement of the riverbed (Rollet et al., 2014; Słowik et al., 2021). From 2005 on, a restoration
201 program progressively took place in this sector of the Ain River, with injection of medium-to-fine
202 sediment, just upstream of gravel bar 5, to reduce the riverbed degradation (Lejot, 2008). A total of
203 89,000 m^3 were injected in 6 years (Syndicat de la Rivière d'Ain Aval et de ses Affluents). Presently, the
204 introduced fine sediment covers the river bottom and hides the local pavement (except at gravel bar
205 7).

206

207 **3. Material and methods**

208

209 **3.1. Sampling design**

210 Distribution of the hyporheic biodiversity along the Ain River was analysed in 2015 from June 29th
211 to July 3rd when the stream was at its lowest discharge (i.e. between 12.5 and 16 m³ s⁻¹) for at least 10
212 days. The sampling stations were located on gravel bars as they are relevant geomorphological units
213 to predict local downwellings and upwellings (at upstream and downstream positions along the bar
214 respectively, Dole-Olivier and Marmonier, 1992). Of the 40 gravel bars studied in 2014, a total of 15
215 gravel bars were selected from Pont d'Ain to Saint Maurice de Gourdans (coded from 5 to 33, similarly
216 to Dole-Olivier et al., 2019; Fig. 1). The gravel bars were sampled at two stations (upstream and
217 downstream of the bar), in three repeated points per position (hereafter called triplicates) randomly
218 chosen 1.5 m apart from one another (Boulton et al., 2003) and at three depths for each point, i.e. -
219 20, -40 and -60 cm below the sediment surface (i.e. 15 x 2 x 3 x 3 = 270 samples). The codes used for
220 stations were labelled by a number for the gravel bar (from 5 to 33) and a letter for the position (U for
221 upstream and D for downstream). These stations were selected to cover most of the studied sector
222 and to include all types of hydrological characteristics: 26 with downwelling or upwelling conditions
223 and 4 with low hyporheic exchanges, in a similar proportion to those selected in the study conducted
224 in 2014 by Dole-Olivier et al. (2019) (i.e. 84% of stations with vertical exchanges and 16% with low or
225 no exchanges).

226

227 **3.2. Hydrological characteristics of the study sector**

228 The hydrogeological characteristics of the Ain alluvial plain and resulting river-aquifer interactions
229 were studied using geomatics analysis (Graillet et al., 2014; Paran and Augeard, 2017; Marmonier et
230 al., 2019). The direction of groundwater/river hydraulic exchanges was inferred from point
231 observations of water levels (or piezometric maps) for both the aquifer and the river. In unconfined
232 alluvial aquifers, where the hydraulic connection with the river is proven, the water flow between the
233 groundwater and the river is proportional to the slope of the free surface of the groundwater near the
234 bank and the permeability of the alluvium following Darcy's law). We consider that the exchanges are
235 made through a vertical plane whose area is defined by the bank length and the water height.

236

237 The exchange evaluation was based on a groundwater model by Burgeap (an environmental
238 engineering group, Costaz, 2005-2006) with the modular three-dimensional finite-difference
239 groundwater flow model Modflow (USGS, Harbaugh et al., 2000). In a second step, the model was
240 improved by D. Mimoun, S. Gaur and F. Paran (Bornette et al., 2014) using river and groundwater
241 levels, volume of water extraction, and hydraulic conductivity obtained from the Syndicat de la Basse
242 Vallée de l'Ain (SBVA), the Centre d'Étude Technique de l'Équipement (CETE Lyon), the Diren Rhône-
243 Alpes and the French geological survey (BRGM). The resulting regional groundwater flow model (grid
244 size 60 m; 249,000 grid cells) for the Ain basin area (360 km²) was constructed and calibrated for

245 transient-state conditions. The regional piezometric surface was based mainly on water hydrographs
246 for 280 wells in the alluvial plain, while the mean thickness of the surface soil layer and vadose zone is
247 about 3 m. The median values of hydraulic conductivity range from 0.0018 m s^{-1} (for the old fluvio-
248 glacial deposits) to 0.003 m s^{-1} (for the recent layers). The horizontal hydraulic conductivity has been
249 estimated from well tests and published data (Costaz, 2005, 2006) and found to vary between 3.10^{-5}
250 to $4.10^{-2} \text{ m.s}^{-1}$, with a strong decrease in the central part of the studied sector (between the two
251 morainic hills, downstream of the gravel bars 10 to 12). Specific yield (Ss) values for alluvial deposits
252 ranged from 1 to 17% (mean 5%).

253 The geomatic analysis was based on the model calibration expressed as a water table contour maps of
254 the alluvial aquifer during a dry period (July 2006). A TIN mesh (Triangulated Irregular Network) was
255 used to build the 3D model of the groundwater surface, using the slope of the water table (in %) and
256 the direction of the flow (in degrees). The permeability of the aquifer (K) was estimated using model
257 calibrated values. The calculation of flow exchanges between the alluvial groundwater and the Ain
258 River was carried out using the ArcGIS Desktop 10.6 software. Because of uncertainties inherent in the
259 geomatic analysis only the direction of exchanges was considered for this study.

260

261 **3.3. Local scale hydrological patterns**

262 A mobile standpipe was pushed inside the sediment at 20, 40 and 60 cm depth, and 1 L of hyporheic
263 water was pumped using a peristaltic pump. A HACH thermo-conducti-oxymeter was used to measure
264 temperature ($^{\circ} \text{C}$), electric conductivity ($\mu\text{S cm}^{-1}$) and dissolved oxygen (mg L^{-1}). Similar measurements
265 were collected concomitantly in the surface water. The vertical hydraulic gradient (VHG) was obtained
266 by measuring the difference between the groundwater level in the mobile piezometer and the surface
267 water level reported for the sampling depth (Baxter et al., 2003).

268

269 Using conductivity-temperature biplots (Dole-Olivier et al., 2019; Fig. 2A to 2C), the stations were
270 separated into three groups according to the direction of the hydrological exchanges: (i) stations
271 located in an upwelling zone with a positive VHG, a low hyporheic temperature (generally below 17°C)
272 and high electric conductivity (generally above $400 \mu\text{S cm}^{-1}$, Fig. 2A); (ii) stations located in a
273 downwelling zone with negative VHG, low electric conductivity (generally below $400 \mu\text{S cm}^{-1}$) and high
274 temperature (generally above 17°C , Fig. 2C) and (iii) stations with weak or no exchange between river
275 and aquifer with null values of VHG combined with low oxygen concentration (average O_2
276 concentration $< 2.5 \text{ mg L}^{-1}$, Fig. 2B). Accordingly, 9 stations were located in “upwelling zones” (Fig. 2A),
277 4 stations in “low exchange sites” (Fig. 2B) and 17 stations in “downwelling zones” (Fig. 2C, full data in
278 supplementary material Fig. S1). No upstream-downstream segregation along the study reach was
279 observed in these 3 groups, but a segregation of the stations was found with river geomorphology:

280 most of the downwelling stations (10 out of 17) were located upstream of gravel bars (noted U),
281 whereas most of the upwelling stations (6 out of 9) were located downstream of gravel bars (noted D
282 in the Fig. 2). The low-exchange stations were evenly distributed at the upstream and downstream
283 ends of three different gravel bars.

284

285 **3.4. Sediment characteristics**

286 Several methods were proposed to estimate the sediment characteristics (Descloux et al., 2010). Yet,
287 most of them only consider the surface layer, despite potential vertical heterogeneity of the river
288 sediment due to variability in fine particle penetration (e.g. Brunke 1999, Gayraud & Philippe, 2001).
289 To evaluate the relevance of these methods based on surficial sediment assessment for hyporheic
290 biodiversity, we used a visual estimation of sediment characteristics based photograph analysis. At
291 each station, three images of 0.25 m² of the surface sediments were randomly taken with a digital
292 camera to define 3 types of substrate, scored as follows (Fig. 2D to 2E): substrate type dominated by
293 sand, gravel and small pebbles (score 1), substrate type defined by a mixture of gravel and large
294 pebbles (score 2), substrate type dominated by large pebbles (score 3). The mean score values
295 (calculated with the three replicate photographs) identified three groups of station (Fig. 2D to 2E): 13
296 stations were characterized by fine sediments, 10 by mixed sediments and 7 by coarse sediments,
297 without upstream-downstream segregation or any relation with the last century eroded zone (stations
298 5 to 9).

299

300 The sediment stability was estimated using the same images considering biofilm cover (Fig. 2G and
301 2H). We used differences in biofilm development to evaluate sediment stability because the river
302 discharge was low for only 10 days. The mobile and instable areas were devoid of biofilm (score 0),
303 while the stable sediment was covered by a filamentous biofilm (score 1). The mean score values
304 identified two homogeneous groups: 13 stations were devoid of biofilm, while 17 were covered by
305 filamentous algae. This stability index did not show any upstream-downstream segregation or relation
306 with the last century eroded zone.

307

308 Coarse Particulate Organic Matter (CPOM) content per mass of dried sediment was measured by loss
309 on ignition (550°C for 4.5 hours) in the laboratory for each 5L faunal sample (see below) after all
310 invertebrate removal (CPOM expressed in mg L⁻¹).

311

312 **3.5. Hyporheic invertebrates**

313 The hyporheic fauna was collected using Bou-Rouch sampling equipment (Bou & Rouch, 1967,
314 Stubbington et al., 2016) including a steel standpipe and a piston pump. Each sample consisted of 5 L

315 mixture of water, sediment, particulate organic matter, and organisms. This procedure was applied at
316 each point and depth (-20, -40 and -60 cm). Samples were sieved through a 200 µm mesh after
317 elutriation to retain invertebrates and CPOM, and preserved with 96% alcohol. In the laboratory
318 sorting and identification of invertebrates were performed with the aid of a stereomicroscope
319 Olympus ZSX16 and a light microscope Olympus BX40, when needed. Clitellata, Copepoda, Ostracoda,
320 Cladocera, Amphipoda, and Isopoda were identified to the species level. Flatworms, leeches, molluscs
321 and most insect instars were identified to the genus or species levels (when possible). Diptera were
322 identified to the family or the tribe levels. Finally, Acari Hydrachnidia Hydrachnidae and Nematoda
323 were not identified further.

324

325 To study spatial patterns of functional diversity, the invertebrates were separated into two functional
326 groups:

327 -Affinity to the groundwater habitat: (i) temporary hyporheos: organisms found inside the
328 sediment that have an obligate aerial stage (i.e. benthic insects, excluding some Coleoptera that have
329 aquatic adults); (ii) permanent hyporheos: organisms without specialization to groundwater life but
330 that can complete their biological cycle without aerial stages (mainly flatworms, leeches, some
331 oligochaetes, molluscs, some micro and macro-crustaceans, and Coleoptera with aquatic adults); and
332 (iii) stygobites: obligate groundwater species that cannot survive in surface water (some oligochaetes,
333 molluscs, and micro and macro-crustaceans in the Ain River).

334 -Food types used: (i) organisms feeding on biofilm covering fine sediments, hereafter
335 sediment-feeders; (ii) feeding on particulate organic matter, hereafter POM-feeders; (iii) feeding on
336 algae; and (iv) predators. Abundances in each group correspond to the sum of the abundances of all
337 taxa of this group, corrected by a percentage of affinity when a species is known to belong to more
338 than one trophic group. Food type uses were assessed using Tachet et al. (2000) for most benthic
339 organisms, Juget and Lafont (1994) for Clitellata, and Marmonier et al. (1994) and Dole-Olivier et al.
340 (2000) for micro-crustaceans.

341 The functional group diversity was calculated separately on the two types of grouping using
342 the Simpson Index ($D = 1 / \sum p_i^2$) calculated on abundance-weighted functional groups (Gallardo et al.,
343 2009; Schmera et al., 2017).

344

345 **3.6. Data analysis**

346 The spatial variation in physicochemical characteristics of the hyporheic water (n = 270 samples) was
347 analysed using Principal Component Analysis (PCA) for the 5 parameters (i.e. VHG, electric
348 conductivity, dissolved oxygen (concentration and saturation), and temperature). Concentrations of
349 dissolved oxygen and CPOM were compared among groups of stations defined by sediment grain sizes

350 (fine sediment, mixed, coarse), sediment stability (stable, unstable) or hydrology (upwellings, low
351 exchanges, downwellings) using one-way ANOVAs after log-transformation, with mean value per
352 station as replicates.

353

354 At the scale of the study reach, spatial autocorrelation among stations has been estimated for each
355 variable using the acf function in R 4.1.2 software (R Core Team, 2021). As these preliminary analyses
356 did not reveal any significant spatial autocorrelation, the longitudinal distribution of the hyporheic
357 fauna was analysed using linear correlation, with the distance downstream of the last dam (expressed
358 in km) as independent variable, while dependent variables were taxonomic richness and abundances
359 of the total assemblages, of the 3 ecological groups and the 4 trophic groups. Taxonomic richness of
360 trophic groups was not used because most taxa belongs to more than one trophic group. The
361 composition of hyporheic assemblages was studied using a Correspondence Analysis (CA, Benzécri,
362 1973) to highlight spatial trends along the study reach and for different groups of stations based on
363 sediment grain size, sediment stability and hydrology. PCA and CA were both performed using
364 ExcelStat 2014. Total taxonomic richness was compared among the three groups based on hydrology.
365 Due to the uneven distribution of samples among downwelling, upwelling and no-exchange stations
366 (153, 81, and 36 samples each), rarefaction curves were generated based on sample-based incidence
367 data to allow comparison of taxa richness at the same level of sampling effort using EstimateS, Version
368 9 (R. K. Colwell, <http://purl.oclc.org/estimates>).

369

370 At the local scale, statistical differences among taxonomic richness and abundances of all functional
371 groups were investigated for groups of stations based on grain size categories (fine, mixed and coarse),
372 sediment stability categories (stable vs unstable) or hydrology (upwelling, downwelling and low
373 exchange) by one-way ANOVAs with stations as replicates (mean values for all depths and replicates
374 and at the -20cm depth alone). In a last step, abundances and taxonomic richness of the total
375 assemblages, of the ecological groups, and the abundances of the functional groups were compared
376 among depths (-20, -40 and -60 cm depths) and hydrological patterns (downwelling, upwelling, no
377 exchange) using two-way ANOVAs with stations as replicates, after log (x+1) transformation when
378 necessary, followed by a Tukey's test. For all variables, the normality and the homoscedasticity of the
379 residues were verified using the Shapiro-Wilk's test and the Levene's test, respectively. Significance
380 level for statistical tests was set at $\alpha < 0.05$. All ANOVAs were performed using Statistica (Statsoft Inc.,
381 USA).

382

383 **4. Results**

384

385 **4.1. Hyporheic water chemistry and hydrogeology of the sector**

386 The dissolved oxygen concentrations in the hyporheic water increased from upstream to downstream
387 ($r^2=0.21$, $p = 0.006$; Supplementary material Fig. S3), with low oxygen values upstream (stations 5 to 9)
388 and higher values downstream (stations 22 to 33). In contrast, no longitudinal trends for temperature,
389 electric conductivity, VGH and CPOM were detected (Supplementary material Fig. S3). Any longitudinal
390 variability for these parameters was better explained by river-groundwater exchanges evaluated by
391 geomatic modelling (Fig. 3A). On one hand, the groundwater flow in the Ain alluvial plain was north-
392 east to south-west directed, which resulted in local groundwater inflow along the river (e.g. near the
393 stations 7, 22, 31-33 for the left bank). On the other hand, when the geomatic model was combined
394 with the river forms (here meanders), the hyporheic water chemistry showed repeated inversions of
395 the river-aquifer exchanges between upstream and downstream ends of the meanders (e.g. at gravel
396 bars 12, 15 and at the large meander surrounded by the morainic hills from gravel bars 25 to 29). The
397 geomatic analysis thus supported the majority of the diagnostics based on hyporheic water chemistry
398 (20 of 30 stations, Fig. 3A and B). The stations classified “low exchange” type were poorly predicted by
399 the geomatic analysis (3 of 4 stations were incorreced classified as “groundwater inflow” type by the
400 geomatic model). In contrast, most stations classified in the “upwelling zone” type by the hyporheic
401 water chemistry (8 out of 9) were consistent with the geomatic analysis. The only exception was the
402 upwelling observed at station 31U, which occurred in a section where low exchange was predicted by
403 the geomatic analysis (Fig. 3B). In the same way, most of the stations classified as “downwelling zones”
404 by the hyporheic water chemistry (11 of 17) concurred with the geomatic model. The six cases of
405 discrepancy between the geomatic model and the water chemistry were linked to narrow gravel bars
406 located in straight sections of the river (i.e. not associated to meanders). In all cases, the downwelling
407 zones observed at the upstream end of these small gravel bars (i.e. in stations 22U and 29U) or at both
408 ends (i.e. stations 9U-9D and 17U-17D) were not predicted by the geomatic model. In contrast, when
409 the gravel bars were associated with meanders, the prediction based on the geomatic analysis and the
410 hyporheic water chemistry agreed quite well, with a reversion of exchange direction between
411 upstream and downstream positions (i.e. for stations 12U-12D and 15U-15D) or a massive
412 groundwater influence all along the downstream part of the large central meander (i.e. stations 26D,
413 28U-28D, 29D).

414

415 **4.2. Hyporheic water chemistry and sediment characteristics at the local scale.**

416

417 The hyporheic water characteristics also varied at the station scale, with variations according to the
418 position, the sediment characteristics and the sampling depth. The PCA on the 270 samples (i.e. 15

419 stations, 2 positions, 3 depths and 3 replicates, Fig. 4) showed a gradient of high electric conductivity
420 and positive VHG to warmer and well oxygenated water on the 1st axis.

421

422 The consistence of the three groups of stations based on the hydrology (using the biplots of
423 temperature and electric conductivity) could be verified with their location in the PCA (Fig. 4B). The
424 stations classified in upwelling zones (blue dots) had negative coordinates on the 1st axis, stations
425 classified as downwelling zones (red dots) had mostly positive coordinates on the same axis, and
426 stations classified as low exchange (black dots) were all closely grouped on the negative side on the
427 two principal components (Fig. 4B). The distribution of these groups along the 2nd axis was due to a
428 significant gradient in dissolved oxygen concentration that decreased from downwelling to upwelling
429 zones and to low exchange areas (Fig. 4E; $F_{(2, 27)} = 11.45$, p-value = 0.0002). Nevertheless, the three
430 groups of stations were not strictly separated by the PCA: some sampling points pertaining to
431 upwelling and downwelling zones were close to or overlapped with low exchange stations (Fig. 4B).
432 This mixing was due to varying hydrological exchange intensities. For example, the station 12D (fed by
433 groundwater, 16.8°C and 481 $\mu\text{S cm}^{-1}$ at -60 cm depth) and the stations 25U (fed by surface water,
434 20.3°C and 385 $\mu\text{S cm}^{-1}$ at -60 cm depth) were mixed with “low exchange” stations because of zero
435 VGH. More than three clearly separated groups, the PCA highlighted a gradient of stations with
436 different exchange intensities.

437

438 In contrast, stations characterized by contrasting sediment grain sizes (Fig. 4C) or sediment stability
439 (Fig. 4D) were poorly or not separated at all by the two first axes of the PCA. The only chemical
440 parameter that significantly changed with grain size was the dissolved oxygen concentration (Fig. 4F;
441 $F_{(2, 27)}=5.21$, p-value = 0.012), with higher mean values below “mixed sediments” than under sediments
442 visually estimated as fine or coarse. Finally, the CPOM concentrations were not significantly different
443 between the groups of stations, regardless of the criteria used (hydrology, grain size or stability)
444 because of high intragroup variability (p-value > 0.05).

445

446 **4.3. The hyporheic fauna distribution at the sector scale**

447

448 A total of 110,106 individuals belonging to 156 taxa were collected in the 270 samples (see dominant
449 species in the frequency histograms, Supplementary material, Fig. S3). At the scale of the study reach
450 (Fig. 5), both abundances and taxonomic richness of hyporheic fauna increased from upstream to
451 downstream, with very low values measured from gravel bars 5 to 9 and higher values obtained
452 downstream (e.g. gravel bars 30 and 33), resulting in significant correlations with the distance from
453 the last dam located upstream of the studied reach (p-values < 0.01; Fig. 5).

454

455 One of the ecological groups, the permanent hyporheos, followed a similar increase with distance
456 downstream of the dams (p -values < 0.01 ; Fig. 5), with very low abundances measured from gravel
457 bars 5 to 9. In contrast, the temporary hyporheos (aquatic insect instars with flying adults) did not
458 show any significant longitudinal trend (p -value > 0.05). High abundances were measured in all
459 stations along the studied sector. The stygobites showed a heterogeneous longitudinal distribution,
460 with high abundances measured in stations located downstream of meanders (i.e. stations 12D and
461 15D), or close to the morainic hills (i.e. gravel bars from 26 to 29) and in two gravel bars downstream
462 of the sector (i.e. 30 and 33). The link between stygobite abundance and the presence of the meanders
463 and morainic hills was associated with stygobite crustaceans, such as the amphipods *Niphargus* spp.
464 that reached their highest abundances in the stations 12D, 15D, 26D and 28U-D (Fig. 5), four stations
465 located in areas where the hydrological model predicted groundwater inflows (Fig. 3).

466

467 All trophic functional groups followed a significant increase with the distance downstream of dams (p -
468 values < 0.01 ; Fig. 5). The upstream area harbored low abundances of all feeding groups, which
469 increased downstream. When the proportions (in %) were calculated, the sediment feeders were
470 dominant in the upstream part of the sector (more than 50% of the individuals) and decreased
471 downstream (negative correlation with distance $r^2 = 0.270$, p -value = 0.0016), while the POM feeders
472 were poorly represented in upstream areas (around 5% of the assemblage) and increased with the
473 distance downstream ($r^2 = 0.274$, p -value = 0.0015). No significant trend was observed in the
474 percentages of algae feeders and predators at the scale of the study reach.

475

476 **4.4. Effect of sediment characteristics and local hydrology on hyporheic fauna at the station scale**

477

478 The Correspondance Analysis (Fig. 6) highlighted a gradient in the composition of the assemblages, but
479 with a rather low percentage of variance explained by the two first axes (11.3%). The 1st axis
480 represented a gradient of taxa with different affinities to groundwater. Most stygobite species were
481 located on the negative side of this axis: oligochaetes *Haber turquini*, *Trichodrilus* spp., *Rhyacodrilus*
482 *balmensis*, *Spiridion phreaticola*, the mollusc *Spiralix* sp., *Bythiospeum* cf *diaphanum*, *Islamia* cf *minuta*,
483 the Crustacea *Microcharon reginae*, *Parabathynella* cf *stygia*, *Salentinella angelieri*, *Niphargus* spp.,
484 *Proasellus* sp., *Parastenocaris* cf *glareola*, *Phreatalona phreatica*, *Cryptocandona kieferi* and
485 *Marmocandona zschokkei*. On the positive side of this axis, most species were burrowers in fine
486 sediments (e.g. the Clitellata *Lophochaeta ignota*, *Tubifex tubifex*, *Tubifex* spp., *Potamothenis*
487 *moldaviensis*, the Ephemeroptera *Ephemera* sp., the Diptera Chironomini and Tanytarsini) together
488 with species living at the surface in moderate to slow flowing zones of rivers (e.g. *Erpobdella*

489 *octocolata*, *Glossiphonia* sp., *Asellus aquaticus*, *Cypria ophtalmica*). Finally, a group of taxa were
490 located close to the origin of the 1st axis, but with negative values on the 2nd axis, largely consisting of
491 insects (e.g. *Potamanthus luteus*, *Micronecta* sp., *Stenelmis* sp., *Leuctra cf nigra*, *Corinoneura* sp.) or
492 epigeal crustaceans (e.g. *Alona rectangula*, *Chydorus sphaericus*, *Cypridopsis vidua*) that mostly live in
493 the benthic layer of rivers. As some stygobite species have positive coordinates on the 2nd axis (e.g.
494 *Spiralix* sp., *Microcharon cf reginae* or *Parabathynella cf stygia*) there is a light redundancy between
495 the 1st and the 2nd axis that induced an arc distribution of the samples (Fig. 6D to F).

496 Considering the distribution of samples along the two first axes (Fig. 6D to F), sediment characteristics
497 (grain size (Fig. 6E) and stability (Fig. 6F)) had poor influences on the hyporheic fauna: samples of the
498 different groups strongly overlapped. Similarly, no differences were observed for the abundance and
499 taxonomic richness of the whole assemblage, for the ecological groups and for the trophic functional
500 groups under coarse, mixed, and fine sediment or under stable or unstable sediment patches (Table 1,
501 Supplementary material Fig. S4, in all cases p-values < 0.05). This lack of difference was also observed
502 when the -20 cm depth was analysed alone (p-values < 0.05 for abundances and richness of all taxa
503 groups).

504
505 In contrast, the local hydrology influenced the composition of the hyporheic assemblages (Fig. 6D). The
506 samples from upwelling zones were all located on the negative side of the 1st axis, closely associated
507 with the stygobite fauna. A part of the samples from the downwelling and most of those from low
508 exchange zones were mixed together on the positive side of the 1st axis, closely associated with species
509 living in slow flowing water and fine sediment. Finally, a large set of samples from downwelling zones
510 was located close to the origin of the 1st axis and on the negative side of the 2nd axis, closely associated
511 to benthic species. These differences in assemblage composition among hydrology groups were also
512 linked to higher abundances and taxonomic richness in areas with vertical exchanges (i.e. upwelling
513 and downwelling zones) than in low exchange areas. The total abundance, the abundance of the
514 temporary hyporheos and the abundances of organisms feeding on fine sediment, POM and algae
515 were significantly higher in the stations located in downwelling zones than in the two other contexts
516 (Table 1, Supplementary material Fig. S4). The stygobites followed a rather different trend with higher
517 abundance and richness found in stations located in upwelling zones (but only significant for taxonomic
518 richness, Table 1, see stygobite abundance in the Supplementary material Fig. S4).

519

520 **4.5. Local hydrology, taxonomic richness and functional groups.**

521 The local hydrology (downwelling, upwelling and low exchange) had a strong effect on the taxonomic
522 richness of the hyporheic assemblages. The taxonomic richness estimated by rarefaction curves (i.e.

523 for a similar sampling effort of 36 samples, Fig. 7) was consistently higher in stations located in the
524 downwelling and upwelling zones than in stations from low exchange zones.

525
526 In addition to local hydrology, the sampling depths had major effects on the hyporheic assemblages
527 (Fig. 8): the abundances and the taxonomic richness of 1) all fauna, 2) the temporary and permanent
528 hyporheos and 3) the abundances of all food type groups decreased from the downwelling zones to
529 the low exchange areas and with depths (from -20 to -60 cm depths). These changes linked to
530 hydrology were similar at all depths (no significant interaction between hydrology and depth was
531 detected, see Supplementary material Table S2). A very different pattern was observed for stygobites
532 (Fig. 8). These organisms adapted to groundwater habitats reached their highest species richness and
533 abundances in the upwelling zones and did not show any significant change with depth.

534 In addition, the total taxonomic richness and total abundances did not uniquely differ with the
535 direction of water exchanges, but also changed with the intensity of these exchanges. This was
536 particularly clear at -60 cm depth in the downwelling zones, where the taxonomic richness and the
537 abundance increased with the water temperature ($r^2=0.159^{**}$ and 0.155^{**} for taxonomic richness and
538 total abundance, respectively) and with the concentration in dissolved oxygen ($r^2=0.257^{***}$ and
539 0.158^{**} for taxonomic richness and total abundance, respectively, Supplementary material, Fig. S5) is
540 highlighting a more abundant and diversified hyporheic fauna in areas where the infiltration of surface
541 water was most intense.

542

543 **4.6. Functional diversity**

544 Different trends were observed for the two types of functional groups (Fig. 9). The functional diversity
545 based on food type groups significantly increased with the distance downstream of the last dam
546 ($r^2=0.31$, p-value = 0.0006). In contrast, the functional diversity based on the ecological groups (i.e.
547 affinities with groundwater) did not increase as predicted from upstream to downstream. Functional
548 diversity reached maximum values in the central part of the studied sector (resulting in a correlation
549 with a bell-shaped trend, $r^2=0.21$, p-value = 0.0052), but with high values in two downstream stations
550 (29D and 31U), located in upwelling zones.

551

552 The functional diversity based on ecological groups was higher in the upwelling zones compared to low
553 exchange areas (without vertical trend; Fig. 9), while the functional diversity based on food type groups
554 changed with the combination of local hydrology and depth. The later was slightly higher in the two
555 groups of stations with vertical exchanges compared to low exchange zones, and decreased
556 significantly with depth (at least in downwelling and upwelling zones).

557

558 The diversity based on affinity to groundwater was mainly controlled by the relative abundance of
559 stygobites in the assemblage. The diversity index was significantly correlated to the percentage of
560 stygobites for the longitudinal trend ($r^2=0.13$, p-value = 0.022) and for the local hydrology ($r^2= 0.59$, p-
561 value = 0.03). The diversity based on food type groups increased with the decreasing relative
562 abundance of sediment feeders (negative correlation $r^2=0.95$, p-value = 4.10^{-20}), the increasing relative
563 abundances of predators ($r^2=0.45$, p-value = 2.10^{-5}) and POM feeders ($r^2=0.37$, p-value = 0.0002), two
564 groups that followed the same upstream-downstream increase in the hyporheos. A similar relationship
565 with the relative abundances of sediment feeders (negative correlation $r^2=0.86$, p-value = 0.003) was
566 observed when the depth and the hydrology were combined.

567

568 **5. Discussion**

569

570 **5.1. Distribution of the hyporheic fauna at the scale of the study reach.**

571 The hypothesis of a combined control of hyporheic fauna distribution by hydrogeology of the valley
572 and geomorphology of the river channel (**H1**) was clearly verified when considering the distribution of
573 the hyporheic fauna along the studied 40-km reach. Several studies highlight the role of large scale
574 hydrogeological heterogeneity and local scale geomorphology in the spatial patterns of river-
575 groundwater exchanges (e.g. Faulkner et al., 2012, Gomez-Velez et al., 2017), especially the location
576 of large-scale draining sectors (i.e. sections of several kilometers where local upwelling zones are fed
577 by deep groundwater, Marmonier et al., 2020).

578

579 In the Ain River, these large-scale groundwater inflows were mostly located in the central part of the
580 studied sector, controlled by the dominant direction of the groundwater flow (illustrated by the
581 geomatic model) and by the presence of the two morainic hills. At this regional scale, the groundwater
582 inflows had a weak influence on the richness and abundances of the hyporheic assemblages, of the
583 permanent and the temporary hyporheos and on the abundance of the trophic functional groups.
584 However, we found a significant effect on the stygobite abundance and taxonomic richness. The
585 stygobites can establish permanent and sometime abundant populations at shallow depths, in areas
586 where the environmental characteristics (e.g. temperature, solute contents, flow velocity) exhibit low
587 temporal variability (Dole-Olivier and Marmonier, 1992; Malard et al., 2003). For example, several
588 *Niphargus* species (here *N. casparyi*, *N. rhenorhodanensis* and *N. fontanus*) are known to live in the
589 hyporheic zone of other tributaries of the Rhône River, especially in upwelling areas of gaining sectors
590 (Marmonier et al., 2019, 2020). In the Ain River, they were mostly sampled downstream of meanders
591 located in the central part of the sector (station 12D and 15D) or close to the morainic hills (stations
592 26D, 28U-D). In addition to these very abundant amphipods, some other rare stygobite crustaceans

593 (e.g. *Parabathynella cf stygia* and *Microcharon reginae* according to Dole and Chessel, 1986; Dole-
594 Olivier et al., 2009) were sampled here, representing shallow populations of species living deeper in
595 the sediments. These species are rare (in abundance and distribution) and represent a high heritage
596 value for the region. Thus, they must be considered in the management plan for the protection of
597 river-groundwater exchanges and global river biodiversity (Michel et al, 2009; Boulton, 2020). The
598 proportion of stygobites in the assemblages control the level of functional diversity (based on the
599 ecological groups). In addition to their importance in the total taxonomic richness (e.g. Dole-Olivier et
600 al., 2015; Marmonier et al., 2020), their role in ecological functions must be considered with attention
601 (Ercoli et al., 2019; Fattorini et al., 2020; Di Lorenzo et al., 2021).

602

603 This study also highlights the importance of the geomorphology of the Ain River. The shape and
604 location of the channel within its floodplain, and the occurrence of meanders are essential for river-
605 groundwater exchanges (Magliozzi et al., 2018), because these channel forms induce intra-meander
606 hyporheic flows (Boano et al., 2006). The hydrological role of meanders has been documented by
607 several authors, both for water exchanges between the river and the hyporheic zone (Stonedahl et al.,
608 2013; Nelson et al., 2019) and for biogeochemical processes (e.g. for nitrogen dynamics, Dwivedi et
609 al., 2018). In the present study, all gravel bars located along meanders showed clear downwelling-
610 upwelling successions (e.g. stations 12U-D, 15U-D, 25U to 28D). In contrast, gravel bars in straight
611 sections were generally narrow and did not follow the expectation of the geomatic modelling. For
612 example, stations 22U and 29U, predicted to be in two upwelling zones by the geomatic model, were
613 found in downwelling conditions (based on water chemistry). These narrow and straight lateral bars
614 were not taken into account by the large-scale geomatic model, even if they were large enough to
615 support downwelling conditions at their head and upwelling at their tail (Dole-Olivier & Marmonier,
616 1992). Thus large-scale hydrogeological modelling has some limits for integrating small gravel bars and
617 local heterogeneities in the prediction of hydrological exchanges (Stonedahl et al., 2013).

618

619 Finally, the history of river incision was surprisingly important for most of the characteristics of the
620 hyporheic fauna at the 40-km scale. Abundances and taxonomic richness of the total assemblages, and
621 of the permanent hyporheos and the abundances of all food type groups increased with the distance
622 downstream, because of very low values in the four upstream gravel bars (from 5 to 9). These stations
623 were located in an area where an intense incision occurred during the XXth century, after the
624 construction of dams and the reduction of sediment transit (Rollet et al., 2014). This was particularly
625 surprising because the riverbed erosion is not yet visible. The recent restoration program with
626 sediment reinjection in the main channel (Lejot, 2008) resulted in fine sediment deposits at the surface
627 (e.g. at gravel bars 5, 8 and 9). Nevertheless, the historical erosion, while completely hidden, is still

628 detectable in the composition of the hyporheic fauna. During river incision, the erosion of sand and
629 gravel generated a re-arrangement of coarse pebbles resulting in a pavement of the river bottom and
630 an accumulation of fine sediment between these large particles (Gomez, 1984; Jain, 1990). In these
631 conditions, the habitability of the interstices progressively decreased and apparently still persisted 10
632 years after the beginning of the injection of gravel and sand in the main channel. The effect of the
633 historical pavement of the upstream sector was also visible in the low concentrations of dissolved
634 oxygen (i.e. positive correlation with distance to the dams), generally associated with low vertical
635 exchanges (Lefebvre et al., 2005) and the reduction of the abundances and richness of the hyporheic
636 fauna (Strayer et al., 1997). None of the ecological groups were affected in a similar way: the very
637 mobile temporary hyporheos (i.e. the aquatic insect instars with flying adults) did not exhibit its lowest
638 richness and abundances in the upstream stations. While the less mobile permanent hyporheos (i.e.
639 Clitellata, Mollusca and Crustacea) showed lower abundance and lower richness in the four upstream
640 gravel bars than downstream. In addition, the hyporheic assemblages sampled in these stations
641 showed specific feeding traits, with a dominance of the fine sediment feeders, certainly favored here
642 by the storage of fine particles. This dominance resulted in lower functional diversity, which increased
643 downstream together with the proportion of other trophic groups, such as POM feeders. Some of
644 these POM feeders, being attracted by porous and mobile sediment (e.g. the *Gammarus* species,
645 Vadher et al., 2015; Navel et al., 2010), were more abundant downstream. The functional diversity
646 based on trophic traits appeared thus to be sensitive to the physical characteristics of the hyporheic
647 zone, even if differences are not visually obvious.

648

649 **5.2. Weak influence of surface grain size and sediment stability**

650 Surprisingly, our results did not support the influence of heterogeneity in surface sediment grain size
651 and sediment stability on the hyporheic fauna (**H2** rejected). The importance of sediment grain size
652 and heterogeneity on hyporheic processes has been widely documented (Richards and Bacon, 1994;
653 Wood and Armitage, 1997; Tonina et al., 2016; Nelson et al., 2019). For example, decreases in dissolved
654 oxygen concentrations and nitrification processes were generally observed in fine sediment, while
655 ammonium concentrations and denitrification process increased (Dahm et al., 1987; Lefebvre et al.,
656 2005). This shift from oxic to hypoxic or anoxic processes was related to fine sediment being the
657 substrate of the biofilm, which stimulated its growth and respiration (Claret and Fontvieille, 1997) and
658 decreased vertical hydrologic exchanges and oxygen supply in the hyporheic zone (Tonina and
659 Buffington, 2009; Cook et al., 2020). A relationship between surface sediment grain size and hyporheic
660 processes was not always detected (Descloux et al., 2010; Nogaro et al., 2010), but appeared strong
661 enough to justify methods of visual assessment of surface sediment characteristics for river bed
662 clogging (Peck et al., 2000; Sennatt et al., 2006). In the present study, very few changes were observed

663 in the habitat characteristics and in the fauna composition between areas differing in surface sediment
664 grain size. First, the hyporheic water chemistry was poorly controlled by the surface sediment grain
665 size. Most chemical parameters did not change at all, and only a minor increase in dissolved oxygen
666 concentrations was measured at stations with mixed sediment size and lower values in the fine and
667 coarse sediment contexts. This increase in oxygen content in mixed sediment was probably a side
668 effect of the local hydrology: stations 22U, 30D and 33D were all located in strong downwelling zones,
669 with more than 11 mg L⁻¹ of dissolved oxygen at 60 cm depth inside sediments. Second, the expected
670 decrease in the hyporheos abundance and taxonomic richness with decreasing sediment size was not
671 observed in this study: the surface characteristics of the bed sediment did not explain the composition
672 and the functional diversity of the hyporheic assemblages. This was likely due to a strong vertical
673 stratification of bed sediment (as observed with a geological radar by Mermillod-Blondin et al., 2015
674 or with field measures and modelling by Cardenas and Zlotnik, 2003) that generally controls hyporheic
675 water fluxes. The vertical stratification of river sediment is linked to the history of the sediment origin,
676 transit and deposition (Marion et al., 2008). The visual estimate of surface sediment grain size may be
677 efficient for small streams with a thin layer of bottom sediment, but it is less informative for a
678 consistent diagnostic of hyporheic habitat quality in large gravel-bed rivers (Nogaro et al., 2010;
679 Descloux et al., 2010).

680
681 The stability or mobility of surface sediment has also been evaluated as an explanation for changes in
682 hyporheic processes (e.g. Peterson et al., 2008; Zheng et al., 2019; Dudunake et al., 2020). Sediment
683 mobility may have different effect on hyporheic biodiversity because of direct (invertebrate migration)
684 and indirect consequences (sediment modifications). At a small time-scale sediment mobility may
685 induce an increase in invertebrate drift (Gibbins et al., 2007) and in the vertical migration of the
686 hyporheic fauna (Dole-Olivier et al., 1997, Schmid-Araya, 2000). At a long time-scale, a low mobility of
687 bed sediment may reduce the resuspension of fine particles, resulting in local clogging of the
688 interstices, and the formation of anoxic microzones with local denitrification (Roy Chowdhury et al.,
689 2020). In the studied sector, the development of a filamentous biofilm at the surface of the particles
690 was not a consistent predictor of the hyporheic habitat suitability and hyporheos composition. Even
691 the expected increase in algae-feeder densities below the thick biofilm was not supported, probably
692 because of very different porosity under similar level of biofilm development. Hence, the use of surface
693 biofilm development to evaluate sediment stability seems poorly informative. In the future studies,
694 the sediment stability must be considered using combined methods, such as hydraulic modelling (e.g.
695 Buffington and Montgomery, 1997) or direct measures of sediment movement (e.g. spray-painted or
696 magnetically tagged stones, Mao and Surian, 2010) to evaluate gravel mobility.

697

698 **5.3. Local hydrology, the major driver for hyporheic biodiversity**

699 The present study clearly demonstrated that local hydrology (i.e. downwelling, upwelling *versus* low
700 exchange areas) had a major role in the distribution and composition of hyporheic fauna (**H3** verified).
701 Several significant differences were observed in the abundance, the taxonomic richness and the
702 functional diversity according to the hydrological connections, with a similar trend at all depths.

703

704 First, specific environmental and faunal characteristics were observed in low exchange areas. Here the
705 zero VHG suggested nearly immobile hyporheic water with poor exchanges with both surface water
706 and groundwater. These hydrological conditions resulted in very low oxygen concentrations (i.e. less
707 than 2 mg.L⁻¹ at -60 cm depth). Similar low values in dissolved oxygen were measured in poorly
708 connected riverbeds and were generally associated with denitrification and ammonium accumulation
709 (Dahm et al., 1987; Lefebvre et al., 2005; Marzadri et al., 2012; Harvey et al., 2013). In this study, the
710 low water exchanges induced low hyporheic abundances and low taxonomic richness (for total
711 hyporheos, most ecological groups and the food type groups) compared to the well-connected stations
712 located in downwelling or upwelling zones. The negative impact of a reduction in the water exchange
713 on the hyporheic fauna has been well documented (Brunke and Gonser, 1999; Hunt and Stanley, 2003;
714 O'Sullivan et al., 2019). The hyporheos abundance was generally correlated positively with dissolved
715 oxygen concentrations (Strayer et al., 1997; Boon et al., 2016) and negatively with the accumulation
716 of potentially toxic ammonia inside sediment (Dahm et al., 1987; Dehedin et al., 2013; Caschetto et al.,
717 2014, 2017; Di Lorenzo et al., 2021). In contrast, the consequence of a reduced hydrological vertical
718 exchange on the functional diversity is less understood. In this study, the functional diversity evaluated
719 by the ecological groups (i.e. the affinity of organisms with groundwater) followed a similar pattern as
720 taxonomic richness with lower values in the low exchange area than in the other hydrological contexts.
721 While the low taxonomic richness was due to the reduction in the number of temporary hyporheic
722 species (i.e. the benthic insects), the low values in functional diversity were linked to a lower
723 proportion of stygobites. The limitation of vertical exchanges reduced the hyporheic assemblages to a
724 set of permanent hyporheic species (mostly oligochaetes and micro-crustaceans) as already observed
725 in sandy and lowland rivers (e.g. Palmer et al., 1992).

726

727 In areas fed by groundwater (i.e. upwelling zones), the environmental conditions were less stressful
728 for the hyporheic fauna because of rather large interstices (fine sediment washout) and regular water
729 renewal. These conditions resulted in intermediate dissolved oxygen concentrations (at least in the
730 studied sector where groundwater-fed springs contains between 5.2 and 8.7 mg.L⁻¹ of oxygen,
731 Dehédin, 2012). Here, the total hyporheic abundance was intermediate between the low exchange
732 zones and the downwelling zones. In contrast, the taxonomic richness was high in upwelling zones,

733 because of the large number of stygobite species. Such increases in stygobite richness and abundance
734 in upwellings were frequently observed in a wide range of rivers (Dole-Olivier and Marmonier, 1992;
735 Malard et al., 2003; Di Lorenzo et al., 2013). The upwellings did not represent a real storage zone for
736 river biomass (Dole-Olivier, 2011), but they appeared to be hotspots for biodiversity, especially for rare
737 stygobite species. The high relative abundance of stygobites in the upwelling zones induced an increase
738 in functional diversity based on ecological groups, which did not decrease with increasing depth,
739 because of the ability of most of the ambient stygobites to live in deep river sediment (Dole and
740 Chessel, 1986). In contrast, the functional diversity based on food types (that poorly changed with the
741 local hydrology) decreased with depth in the upwelling zones. This vertical pattern was probably due
742 to a reduction of food diversity with depth, selecting preferentially sediment feeders, which was the
743 trophic group feeding on the most available resource: fine sediments and associated biofilm.

744

745 Finally, in the downwelling zones, the abundance and taxonomic richness were the highest, especially
746 for the temporary hyporheos (i.e. benthic insects with flying adults). This high density may be explained
747 by the inflow of surface water into the sediment, which brings dissolved oxygen and organic matter
748 (Brunke & Gonser, 1999; Franken et al., 2001). This study demonstrates that downwelling zones
749 represent essential storage zones for the benthic fauna, contributing to river resilience through
750 recolonization process of the benthic layer (Dole-Olivier, 2011, Vander Vorste et al., 2016). Although
751 not all downwelling zones were similarly populated: the highest densities of the temporary hyporheos
752 were measured in strong downwellings. This influence of infiltration intensity was illustrated by the
753 positive correlation between the abundance and taxonomic richness with temperature and dissolved
754 oxygen at -60 cm depth. Intense downwellings represented attractive areas for faunal vertical
755 migration (Dole-Olivier, 2011). This richness in benthic species was probably due to the higher diversity
756 of food types in the downwelling zones than in other zones (upwelling and no exchange zones). These
757 downwelling areas had high dissolved oxygen concentration and fresh organic matter, which was
758 reflected in a higher proportion of POM feeders, algae feeders and predators in comparison with other
759 zones with no direct supply of fresh organic matter from surface waters. Therefore, the downwellings
760 were hotspots for biomass and taxonomic richness in the hyporheic zone, but with a different
761 assemblage of species compared to upwelling zones. The importance of upwelling zones is often
762 emphasized by river managers, particularly because of their role in fish reproduction and survival
763 during low water periods (e.g. Curry and Noakes, 1995; Malcolm et al., 2005). In contrast, the
764 importance of downwelling zones is rarely considered. This study supports the idea that the
765 heterogeneity of hydrological patterns, with alternation of upwellings and downwellings, may
766 represent the optimal spatial structure for biodiversity conservation in rivers, with both contributing
767 to the diversity and resilience of the river (Leigh et al., 2016).

768

769 **6. Conclusions**

770

771 At the local scale, the composition of the hyporheos was controlled by vertical exchanges of water
772 between the river, the hyporheic zone and the deep groundwater (i.e. downwelling, upwelling or low
773 exchange areas). The upwellings were hotspot of taxonomic richness, but also for functional diversity
774 based on ecological groups. Here, the functional diversity was mostly related to the relative abundance
775 of stygobites and the reduction of dominance of the permanent hyporheos. In contrast, the
776 downwelling zones were hotspots of abundances, especially for the temporary hyporheos (mostly
777 benthic insects). These organisms, living temporarily inside sediment, contribute to the river biomass
778 and resilience. In downwellings, the food-type diversity was mostly controlled by the proportion of
779 POM feeders and the reduction in dominance of sediment feeders. Low exchange zones appeared to
780 be less populated areas with low functional diversity.

781

782 Compared to hydrology, the surface characteristics of the riverbed sediment (i.e. visual estimation of
783 the grain size and the stability) was not informative for the hyporheos composition of this large gravel-
784 bed river. The visual estimation of surface sediment characteristics neglected the vertical stratification
785 of the sediment, which is a consequence of the history of sediment transit and deposition.

786

787 These links between vertical water exchanges and hyporheic assemblages must be combined with
788 large-scale patterns of hyporheic distribution. In the 40-km long reach assessed in this study, the
789 hyporheic fauna was controlled by a combination of two factors: the hydrogeology of the alluvial plain
790 (i.e. large-scale groundwater movement) and the geomorphology of the river channel (i.e. location of
791 meanders and history of the river incision). In consequence, river managers must carefully consider
792 the ongoing activities that modify the dynamics of the river-groundwater exchanges (e.g. river
793 regulation or groundwater extraction), but also the long-term history of sediment fluxes due to past
794 regulations.

795

796 **CRedit authorship contribution statement.**

797 M.J. Dole-Olivier: Conceptualization, Investigation, Writing – original draft. M. Creuzé des Châtelliers:
798 Conceptualization, Investigation. D. Galassi: Investigation. M. Lafont: Investigation. F. Mermillod-
799 Blondin: Investigation. F. Paran: Investigation. D. Graillet: Investigation. S. Gaur: Investigation. P.
800 Marmonier: Conceptualization, Investigation, Writing – original draft.

801

802 **Declaration of competing interest**

803 The authors declare the following financial interests, which may be considered as potential competing
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807

808

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Table 1: Comparison of the effects of local hydrology, sediment grain size and sediment stability on the functional traits of the hyporheic assemblages. One way ANOVAs were performed with stations as replicates (mean values for all depths and replicates, after log transformation; n.s. above the significant level, - no test possible for food type groups because a species may belong to more than one trophic group, see histogram in Supplementary material Fig. S4).

	Local hydrology ⁽¹⁾				Grain size ⁽²⁾		Stability ⁽³⁾	
	Abundances		Richness		Abd	Rich	Abd	Rich
	F _(2,27)	p	F _(2,27)	p	F _(2,27)	F _(2,27)	F _(1,28)	F _(1,28)
Total fauna	4.97	0.014	4.96	0.014	n.s.	n.s.	n.s.	n.s.
Ecological groups								
Temp. hyporheos	12.05	0.0002	8.94	0.001	n.s.	n.s.	n.s.	n.s.
Perm. hyporheos		n.s.	3.51	0.04	n.s.	n.s.	n.s.	n.s.
Stygobites		n.s.	6.83	0.004	n.s.	n.s.	n.s.	n.s.
Food type groups								
Sediment feeders	5.40	0.011	-	-	n.s.	-	n.s.	-
POM feeders	4.69	0.017	-	-	n.s.	-	n.s.	-
Algae feeders	3.47	0.04	-	-	n.s.	-	n.s.	-
Preadators		n.s.	-	-	n.s.	-	n.s.	-

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⁽¹⁾ Local hydrology are downwelling (n=16), low exchange (n=4) and upwelling contexts (n=10).

⁽²⁾ Sediment grain size classes are fine (n=13), mixed (n=10) and coarse surface sediment (n=7).

⁽³⁾ Sediment stability are with (n=17) or without biofilm development (n=13).

1268 **Figure legends:**

1269 Figure 1: Study area (A), the Lower Ain River from Pont d'Ain to the confluence with the Rhône River
1270 (background elevation map from Geographic National Institute, Géoportail). Blue lines: Ain and Rhône
1271 river channels; pointed dark line: upstream eroded sector before 2005; dotted lines: limits of the
1272 morainic hills. Location of the 15 sampled gravel bars (A), upstream and downstream stations at each
1273 gravel bar (B) and sampling design at each station (C).

1274
1275 Figure 2. Chemical characteristics of hyporheic water, sediment grain size and sediment stability.
1276 Examples showing the classification of the stations into the three groups of stream-groundwater
1277 exchanges according to electric conductivity and temperature of the surface and hyporheic waters: A-
1278 Upwellings (UPW), B- Low exchanges (LOW EX), C- Downwelling (DNW). Examples of sediment
1279 characteristics: D- fine sediment, E- mixed, F- coarse. Example of biofilm development on unstable (G)
1280 or stable (H) benthic layer. List of the stations in each group with U for upstream and D for downstream
1281 positions along the gravel bars (see Fig. 1).

1282
1283 Figure 3: Hydrogeological characteristics of the studied sector based on geomatic analysis of the study
1284 reach (A) and details of the eight stations with upwelling zones as derived by the hyporheic water
1285 chemistry (B).

1286
1287 Figure 4. Principal Component Analysis on hyporheic water characteristics. Correlation circle of
1288 physico-chemical parameters (A) Cond-Electric conductivity, VGH-Vertical Hydraulic Gradient, O₂-
1289 Dissolved oxygen, T°C-Temperature. Location of the sampling points on the Axis 1 x Axis 2 biplots,
1290 according to the local hydrology (B), the grain size (C) and the sediment stability (D). Oxygen
1291 concentrations by groups and depths (means and standard errors) for local hydrology groups (E), grain
1292 size groups (F) and stability groups (G) with results of ANOVA tests between groups.

1293
1294 Figure 5: Longitudinal distribution of hyporheic assemblages. Means (and standard errors) for the 15
1295 gravel bars and positions (i.e. 30 stations) for total abundance and taxonomic richness (left panels), for
1296 ecological groups based on affinities to groundwater (center panels) and for trophic functional groups
1297 (right panels).

1298 Figure 6. Correspondance analysis on hyporheic assemblages (the 1st axis represented 6% and the 2nd
1299 axis 5.3% of the total inertia). Location of the taxa in the 1st and 2nd axes is separated in three major
1300 taxonomic groups: (A) oligochaetes, molluscs, leeches, (B) crustaceans and (C) insects, with stygobite
1301 species in blue and other hyporheic species in black. The location of all samples between the 1st and

1302 2nd axes also separated by hydrological exchange groups (D), sediment grain size groups (E) and
1303 sediment stability groups (F).

1304 Figure 7: Rarefaction curves of the taxonomic richness in different hydrological contexts (Dwn:
1305 downwelling zones, Upw: upwelling zones and LowEX: low exchange zones). Shaded areas represented
1306 confidence intervals.

1307

1308 Figure 8: Taxonomic richness (left panels), abundances of the total fauna and of the 3 functional groups
1309 based on affinities with groundwater (center panels), and abundances of the food type groups (right
1310 panels) in different hydrological contexts and depths (means and standard errors). Differences
1311 between hydrological contexts (H) and depths (D) tested by a two-ways ANOVA with *<5%, **<1%,
1312 ***<0.1%, n.s. non significant.

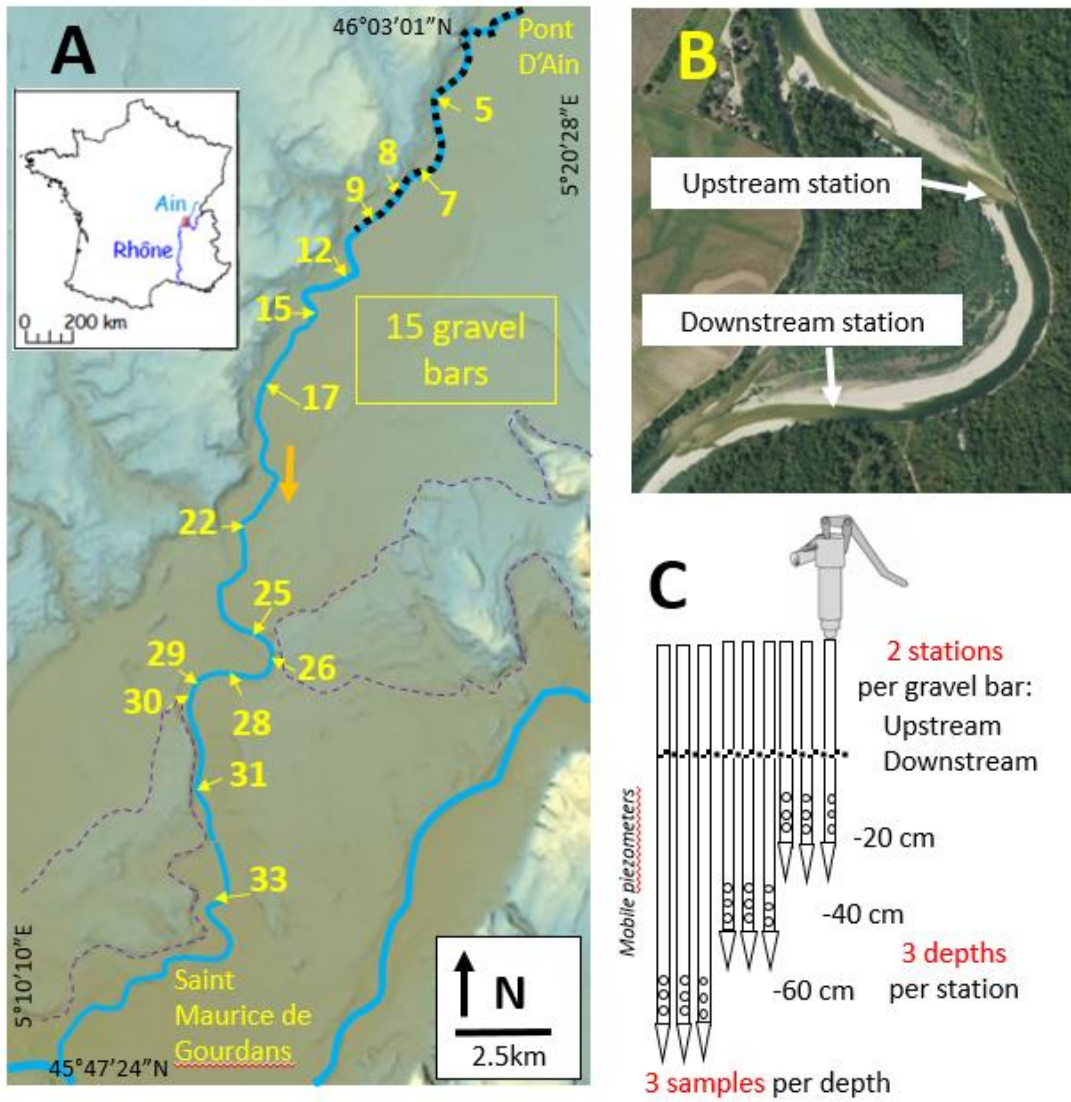
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1314 Figure 9. Functional diversity for ecological groups (A) and food type groups (B) for the longitudinal
1315 trend (left) and the combination of depth and hydrological contexts (right, means and standard errors).
1316 The ecological group diversity significantly correlated with the distance (in km) with a polynomial
1317 regression curve ($y = 0.0021 x^2 + 0.075 x + 1.3$), while the food type group diversity significantly fitted
1318 with a linear regression curve ($y = 0.023 x + 2.396$). Differences between hydrological contexts (H) and
1319 depths (D) tested by a two-ways ANOVA with *<5%, **<1%, ***<0.1%, n.s. not significant.

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1322 Fig. 1

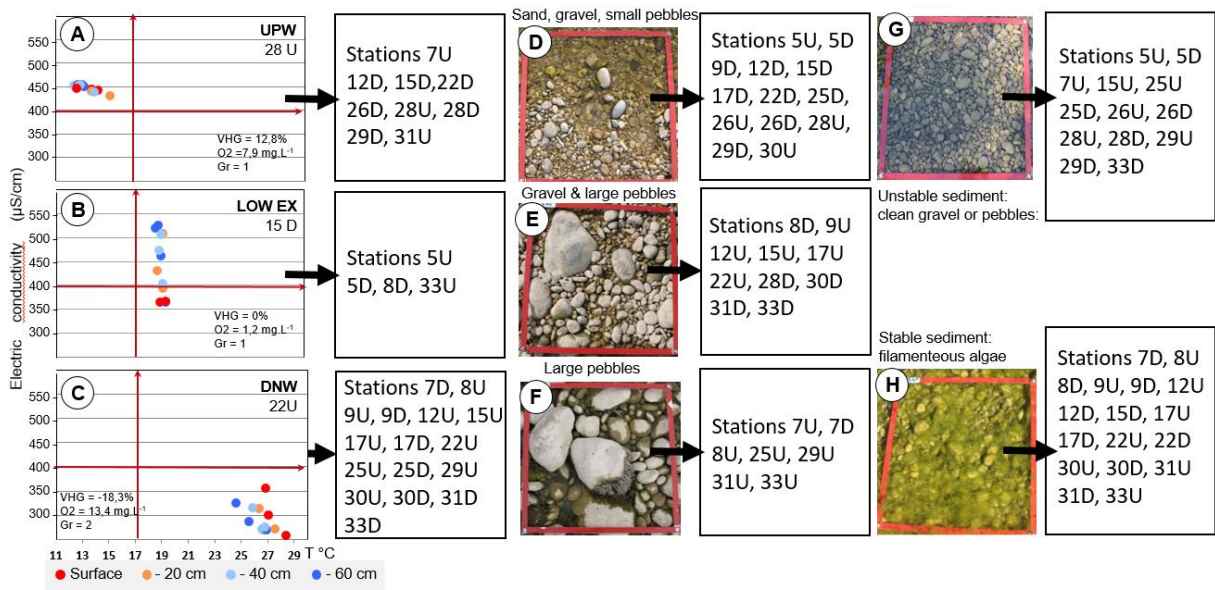


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1326 Fig. 2



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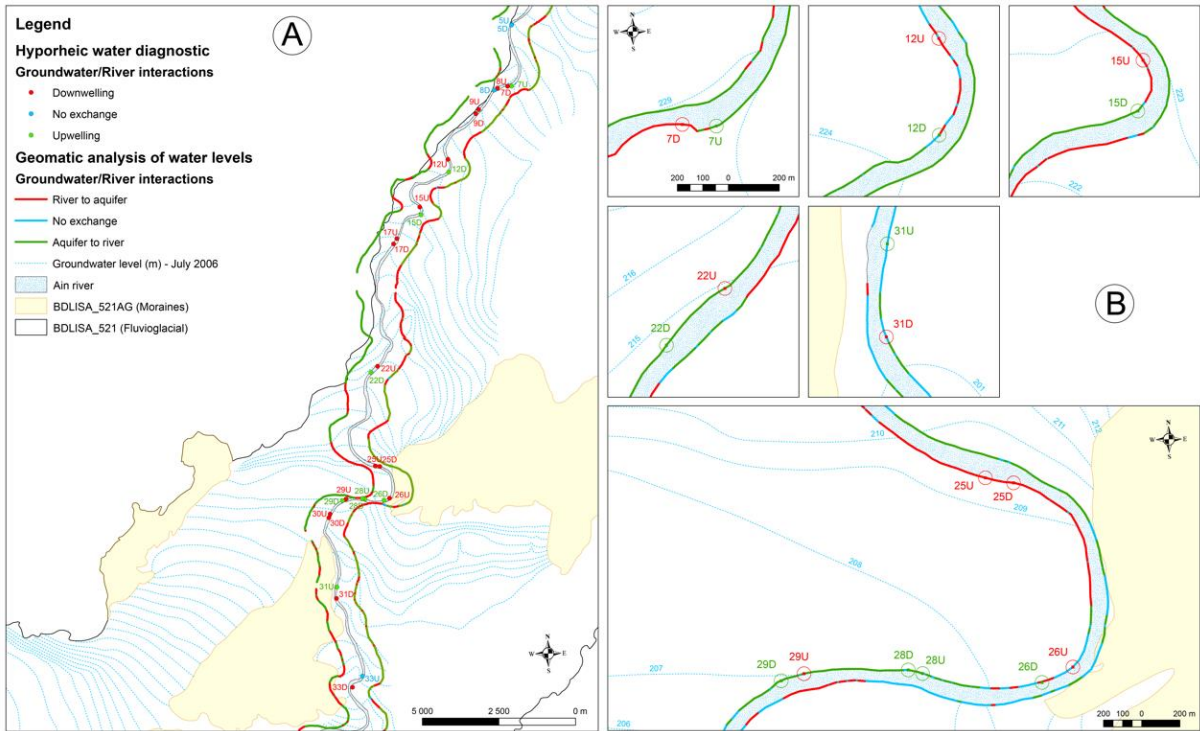
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1331 Fig. 3

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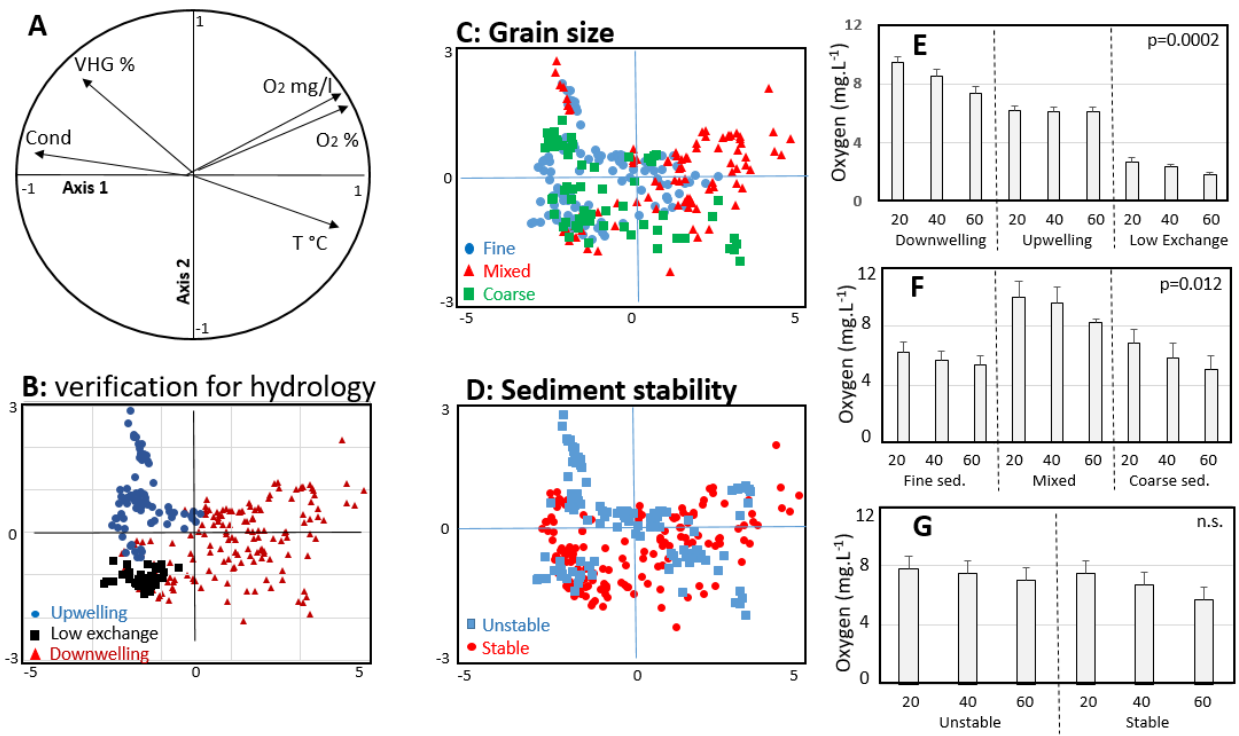


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1336 Fig. 4



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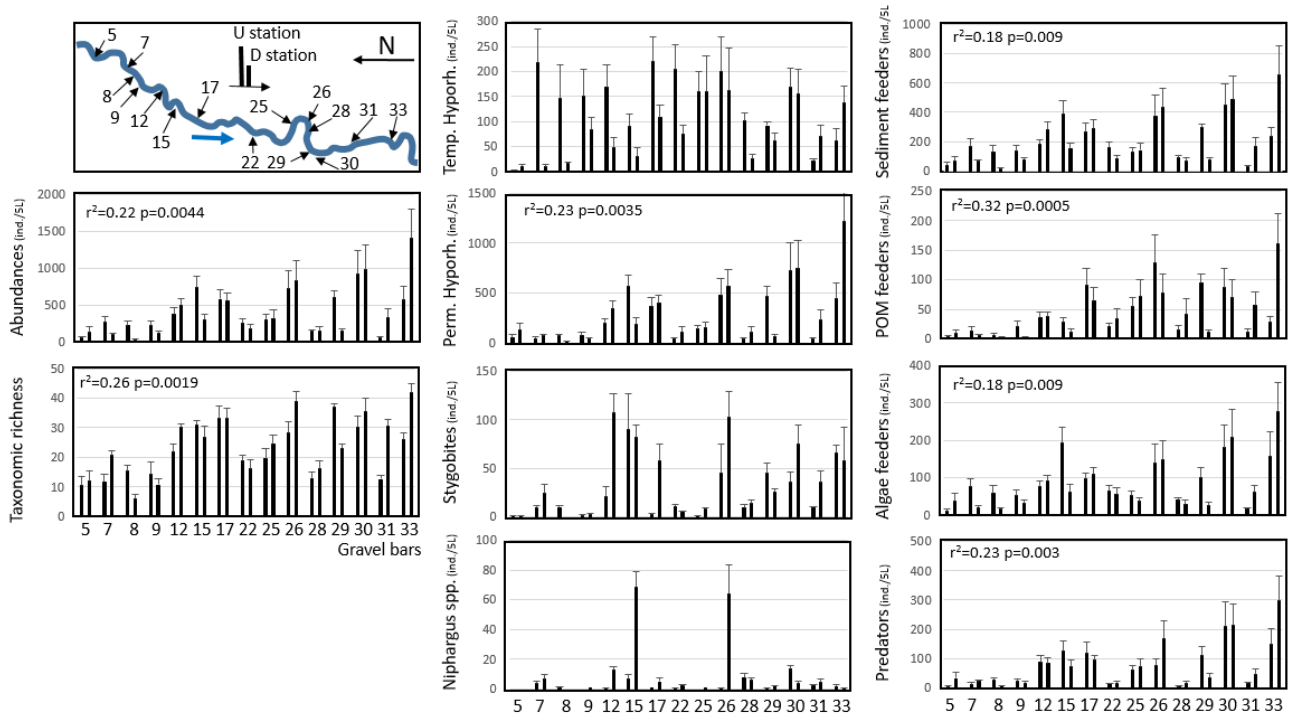
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1341 Fig. 5

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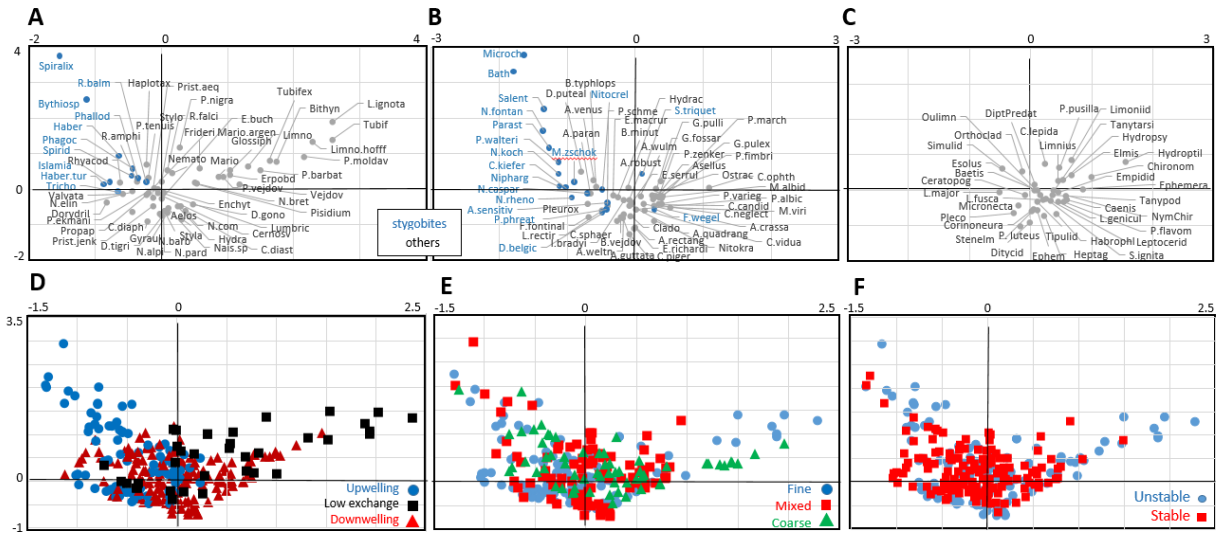
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1346 Fig. 6

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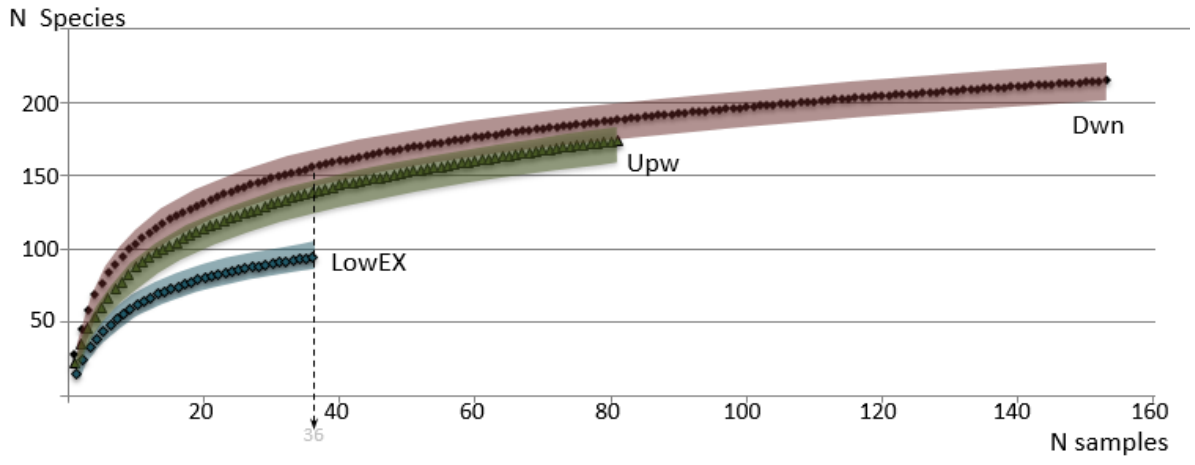
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1351 Fig. 7

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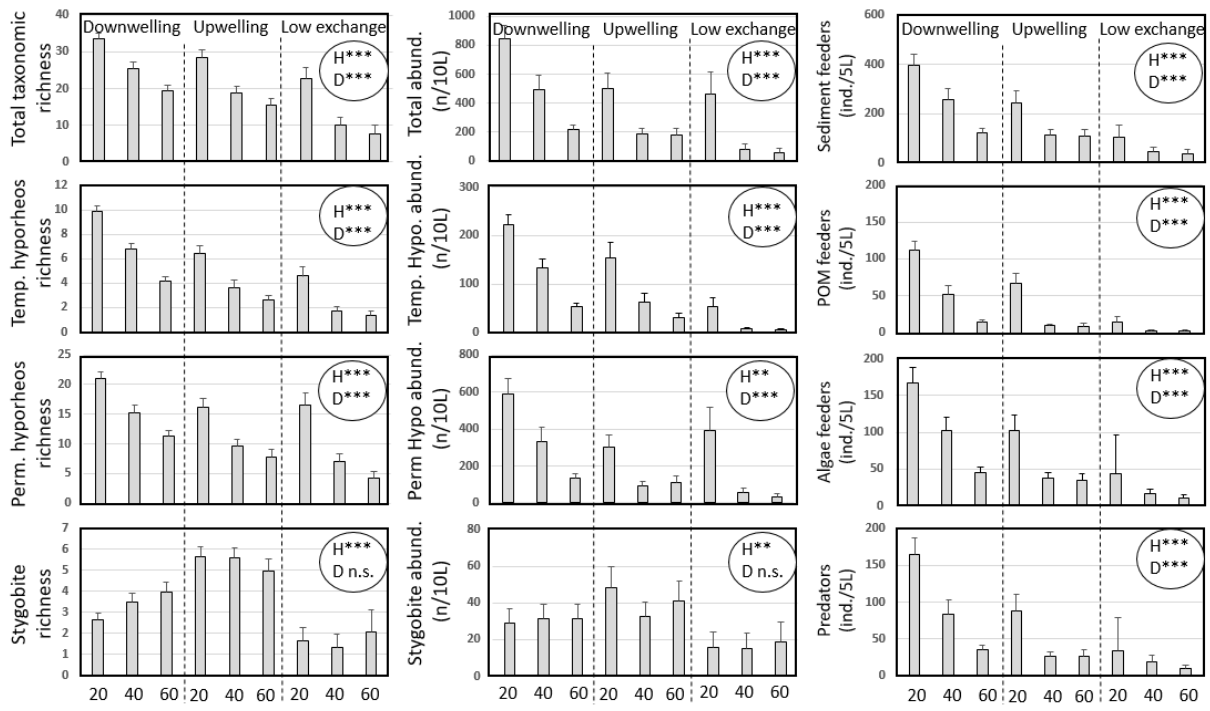


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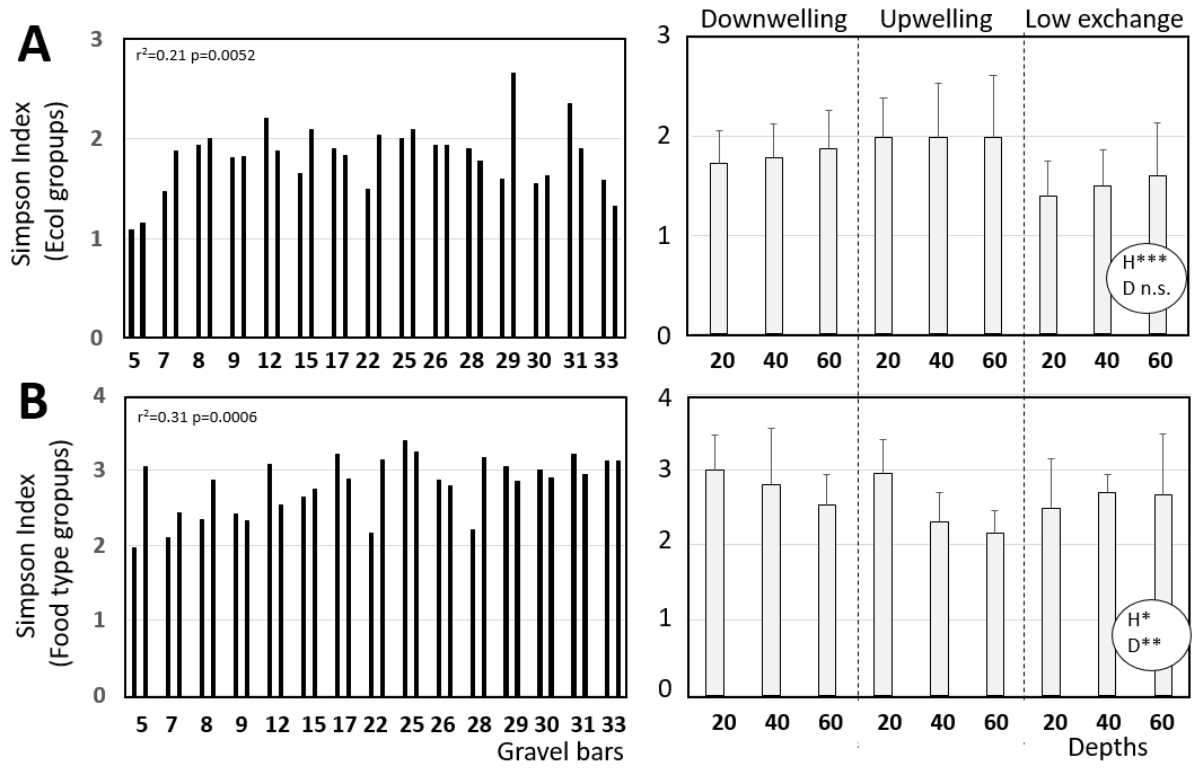
1358 Fig. 8



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1366 Supplementary material: Tables.

1367 Table S1. Codes for the taxa used in the Factorial Analysis (Stygobite species in red).

Taxa names	Codes	Taxa names	Codes	Taxa names	Codes
Aeolosomatidae	Aelos	<i>Valvata</i> sp.	Valvata	<i>Cypridopsis vidua</i>	C.vidua
Lumbricidae	Lumbric	<i>Bithynia tentaculata</i>	Bithyn	<i>Potamocypris variegata</i>	P.variegata
<i>Dorydrilus michaelsoni</i>	Dorydril	<i>Gyraulus</i> sp.	Gyraul	<i>Gammarus</i> sp. (juveniles)	G.pulli
<i>Stylodrilus</i> sp.	Stylo	<i>Islamia cf minuta</i>	Islamia	<i>Gammarus pulex</i>	G.pulex
<i>Trichodrilus</i> sp.	Tricho	<i>Bythiospeum cf diaphanum</i>	Bythiosp	<i>Gammarus fossarum</i>	G.fossar
<i>Chaetogaster diaphanus</i>	C.diaph	<i>Spiralix cf vitrea</i>	Spiralix	<i>Niphargus</i> sp. (juveniles)	Niphargus
<i>Chaetogaster diastrophus</i>	C.diast	<i>Pisidium</i> sp.	Pisidium	<i>Niphargus rhenorhodanensis</i>	N.rheno
<i>Nais alpina</i>	N.alpi	Hydracarina	Hydrac	<i>Niphargus gr. kochianus</i>	N.koch
<i>Nais barbata</i>	N.barb	<i>Acanthocyclops venustus</i>	A.venustus	<i>Niphargus casparyi</i>	N.caspar
<i>Nais bretscheri</i>	N.bret	<i>Acanthocyclops robustus</i>	A.robustus	<i>Niphargus gr fontanus</i>	N.fontan
<i>Nais gr communis</i>	N.com	<i>Acanthocyclops sensitivus</i>	A.sensitivus	<i>Salentinella</i> sp	Salentinel
<i>Nais elinguis</i>	N.elin	<i>Macrocyclus albidus</i>	M.albidus	<i>Asellus</i> sp.	Asellus
<i>Nais pardalis</i>	N.pard	<i>Megacyclops viridis</i>	M.viridis	<i>Proasellus walteri</i>	P.walteri
<i>Nais</i> div sp	Nais.sp	<i>Paracyclops fimbriatus</i>	P.fimbri	<i>Microcharon cf reginae</i>	Microcharon
<i>Stylaria lacustris</i>	Styla	<i>Eucyclops serrulatus</i>	E.serrul	<i>Parabathynella cf stygia</i>	Bathynel
<i>Vejdovskiiella intermedia</i>	Vejdov	<i>Euyclops macruroides</i>	E.macrur	Plecoptera (juveniles)	Pleco
<i>Pristina aequisetata foreli</i>	Prist.aeq	<i>Diacyclops belgicus</i>	D.belgicus	<i>Leuctra major</i>	L.major
<i>Pristina jenkiniae</i>	Prist.jenk	<i>Diacyclops putealis</i>	D.putealis	<i>Leuctra cf fusca</i>	L.fusca
Tubificinae	Tubif	<i>Bryocamptus minutus</i>	B.minutus	<i>Euleuctra geniculata</i>	L.geniculata
<i>Haber</i> sp.	Haber	<i>Bryocamptus typhlops</i>	B.typhlops	Ephemeroptera (juveniles)	Ephem
<i>Haber turquini</i>	Haber.tur	<i>Bryocamptus vej dovskiyi</i>	B.vej dov	<i>Serratella ignita</i>	S.ignita
<i>Limnodrilus</i> sp.	Limnodri	<i>Attheyella crassa</i>	A.crassa	<i>Baetis</i> sp.	Baetis
<i>Limnodrilus hoffmeisteri</i>	Limno.hof	<i>Attheyella paranaphthalica</i>	A.paran	Heptageniidae (juveniles)	Heptagen
<i>Lophochaeta ignota</i>	L.ignota	<i>Attheyella wulmeri</i>	A.wulm	<i>Potamanthus luteus</i>	P. luteus
<i>Potamothele moldaviensis</i>	P.moldav	<i>Pesceus schmeili</i>	P.schme	<i>Habroleptoides</i> sp.	Habrolep
<i>Potamothele vej dovskiyi</i>	P.vej dov	<i>Nitocrella psammophila</i>	Nitocrella	<i>Ephemera</i> sp	Ephemera
<i>Psammoryctides barbatus</i>	P.barbat	<i>Nitokra hibernica</i>	Nitokra	<i>Caenis</i> sp.	Caenis
<i>Tubifex</i> sp.	Tubifex	<i>Epactophanes richardi</i>	E.richardi	<i>Hydropsyche</i> sp.	Hydropsy
<i>Rhyacodrilus</i> sp.	Rhyacod	<i>Fontinalicaris fontinalis</i>	F.fontinalis	<i>Cheumatopsyche lepida</i>	C.lepida
<i>Rhyacodrilus amphigenus</i>	R.amphi	<i>Parastenocaris cf. glareola</i>	Parasteno	<i>Polycentropus flavomaculatus</i> cf	P.flavom
<i>Rhyacodrilus falciformis</i>	R.falci	Cladocera (juveniles)	Clado	<i>Psychomyia pusilla</i>	P.pusilla
<i>Pararhyacodrilus ekmani</i>	P.ekmani	<i>Alona guttata</i>	A.guttata	Hydroptilidae	Hydroptil
<i>Phallodrilinae</i>	Phallod	<i>Alona quadrangularis</i>	A.quadrang	Leptoceridae	Leptocerid
<i>Rhyacodrilus balmensis</i>	R.balmensis	<i>Alona rectangula</i>	A.rectang	<i>Esolus</i> sp.	Esolus
<i>Spiridion phreaticola</i>	Spirid	<i>Alona weltneri</i>	A.weltn	<i>Limnius</i> sp.	Limnius
Enchytraeidae	Enchyt	<i>Chydorus piger</i>	C.piger	<i>Stenelmis</i> sp.	Stenelm
<i>Cernosvitoviella</i> sp	Cernosv	<i>Chydorus sphaericus</i>	C.sphaer	<i>Elmis</i> sp.	Elmis
<i>Enchytraeus buchholzi</i>	E.buch	<i>Lathonura rectirostris</i>	L.rectir	<i>Oulimnius</i> sp.	Oulimn
<i>Fridericia</i> sp.	Frideri	<i>Phreatalona phreatica</i>	P.phreatica	Dyticidae div. sp.	Ditycid
<i>Marionina</i> sp.	Marion	<i>Pleuroxus</i> sp.	Pleurox	<i>Micronecta</i> sp.	Micronecta
<i>Marionina argentea</i>	M.argent	Ostracoda (juveniles)	Ostrac	Ceratopogonidae	Ceratopog
<i>Propappus volki</i>	Propap	<i>Pseudocandona albicans</i>	P.albicans	Empididae	Empididae
<i>Haplotaxis gordioides</i>	Haplotax	<i>Pseudocandona marchica</i>	P.marchica	Limoniidae	Limoniid
<i>Hydra</i> sp.	Hydra	<i>Marmocandona zschokkei</i>	M.zschok	Simuliidae	Simuliidae
<i>Dugesia gonocephala</i>	D.gono	<i>Cryptocandona kieferi</i>	C.kieferi	Tipulidae	Tipulidae
<i>Dugesia tigrina</i>	D.tigri	<i>Schellencandona triquetra</i>	S.triquetra	Brachycera predators	DiptPredat
<i>Phagocata</i> sp.	Phagoc	<i>Fabaeformiscandona wegelini</i>	F.wegelini	Nymphe of Chironomidae	NymChir
<i>Polycelis nigra</i>	P.nigra	<i>Candona candida</i>	C.candida	Orthoclaadiinae	Orthoclad
<i>Polycelis tenuis</i>	P.tenuis	<i>Candona neglecta</i>	C.neglecta	<i>Corinoneura</i> sp.	Corinoneura
Nematoda	Nemato	<i>Cypria ophthalmica</i>	C.opth	Tanypodinae	Tanypodinae
<i>Erpobdella cf octoculata</i>	Erpobd	<i>Ilyocypris bradyi</i>	I.bradyi	Tanytarsini	Tanytarsini
<i>Glossiphonia</i> sp.	Glossiph	<i>Prionocypris zenkeri</i>	P.zenkeri	Chironomini	Chironomini

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1370 Table S2. Results of two-ways ANOVA between depths (-20, -40 and -60cm) and hydrological patterns
 1371 (downwelling, upwelling, no exchange) for abundances and richness of the total hyporheic fauna, the
 1372 temporary and the permanent hyporheos and the stygobites, after log(x+1) transformation.

1373 Total richness of the hyporheos

Effects	DF	F	p
Depths	2	15.61	0.000002
Hydrological patterns	2	12.96	0.000013
Hydro x depths	4	1.11	n.s.

1374

1375 Total abundances of the hyporheos

Effects	DF	F	p
Depths	2	20.88	< 10 ⁻⁵
Hydrological patterns	2	15.72	0.000002
Hydro x depths	4	0.79	n.s.

1376

1377 Richness of the temporary hyporheos

Effects	DF	F	p
Depths	2	19.17	< 10 ⁻⁵
Hydrological patterns	2	20.19	< 10 ⁻⁵
Hydro x depths	4	0.38	n.s.

1378

1379 Abundances of the temporary hyporheos

Effects	DF	F	p
Depths	2	23.17	< 10 ⁻⁵
Hydrological patterns	2	30.63	< 10 ⁻⁵
Hydro x depths	4	0.61	n.s.

1380

1381 Richness of the permanent hyporheos

Effects	DF	F	p
Depths	2	18.65	< 10 ⁻⁵
Hydrological patterns	2	8.61	0.0004
Hydro x depths	4	0.65	n.s.

1382

1383 Abundances of the permanent hyporheos

Effects	DF	F	p
Depths	2	15.41	0.000002
Hydrological patterns	2	7.26	0.001
Hydro x depths	4	0.34	n.s.

1384

1385 Richness of the stygobites

Effects	DF	F	p
Depths	2	0.028	n.s.
Hydrological patterns	2	16.34	0.000001
Hydro x depths	4	0.52	n.s.

1386

1387 Abundances of the stygobites

Effects	DF	F	p
Depths	2	0.056	n.s.

Hydrological patterns	2	6.64	0.002
Hydro x depths	4	0.38	n.s.

1388

1389 Abundances of the sediment feeders

Effects	DF	F	p
Depths	2	18.03	< 10 ⁻⁵
Hydrological patterns	2	15.79	0.000002
Hydro x depths	4	0.94	n.s.

1390

1391 Abundances of the POM feeders

Effects	DF	F	p
Depths	2	23.45	< 10 ⁻⁵
Hydrological patterns	2	11.07	0.00005
Hydro x depths	4	0.31	n.s.

1392

1393 Abundances of the Algae feeders

Effects	DF	F	p
Depths	2	19.31	< 10 ⁻⁵
Hydrological patterns	2	13.04	0.000012
Hydro x depths	4	0.70	n.s.

1394

1395 Abundances of the predators

Effects	DF	F	p
Depths	2	11.34	0.00004
Hydrological patterns	2	9.05	0.0003
Hydro x depths	4	0.11	n.s.

1396

1397 Ecological groups' diversity

Effects	DF	F	p
Depths	2	0.42	n.s.
Hydrological patterns	2	8.11	0.0006
Hydro x depths	4	0.19	n.s.

1398

1399 Food-type groups' diversity

Effects	DF	F	p
Depths	2	5.25	0.007
Hydrological patterns	2	4.15	0.019
Hydro x depths	4	1.23	n.s.

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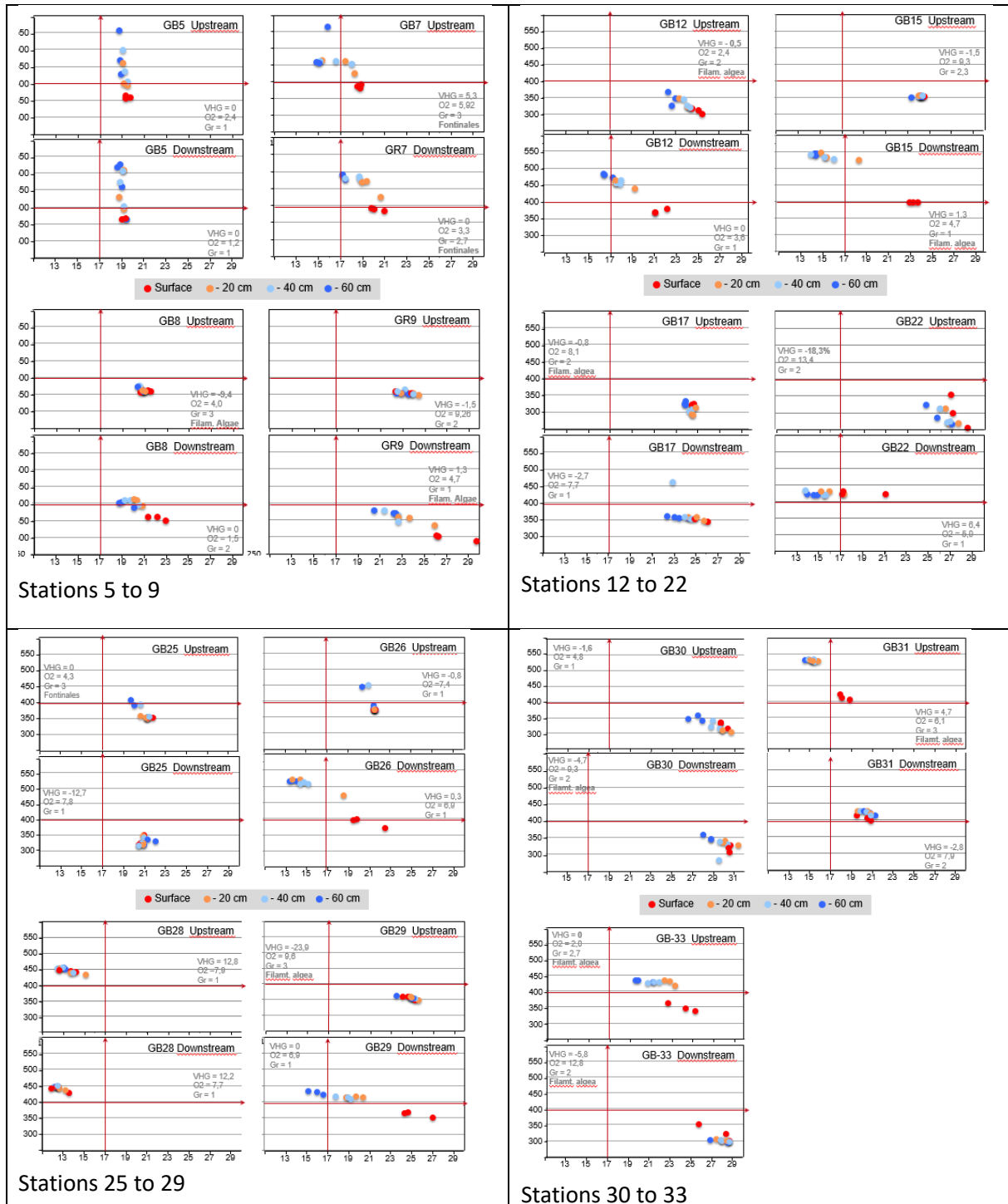
1405

1406 **Supplementary material: Figures**

1407

1408 Fig. S1 : Biplots for hydrological patterns.

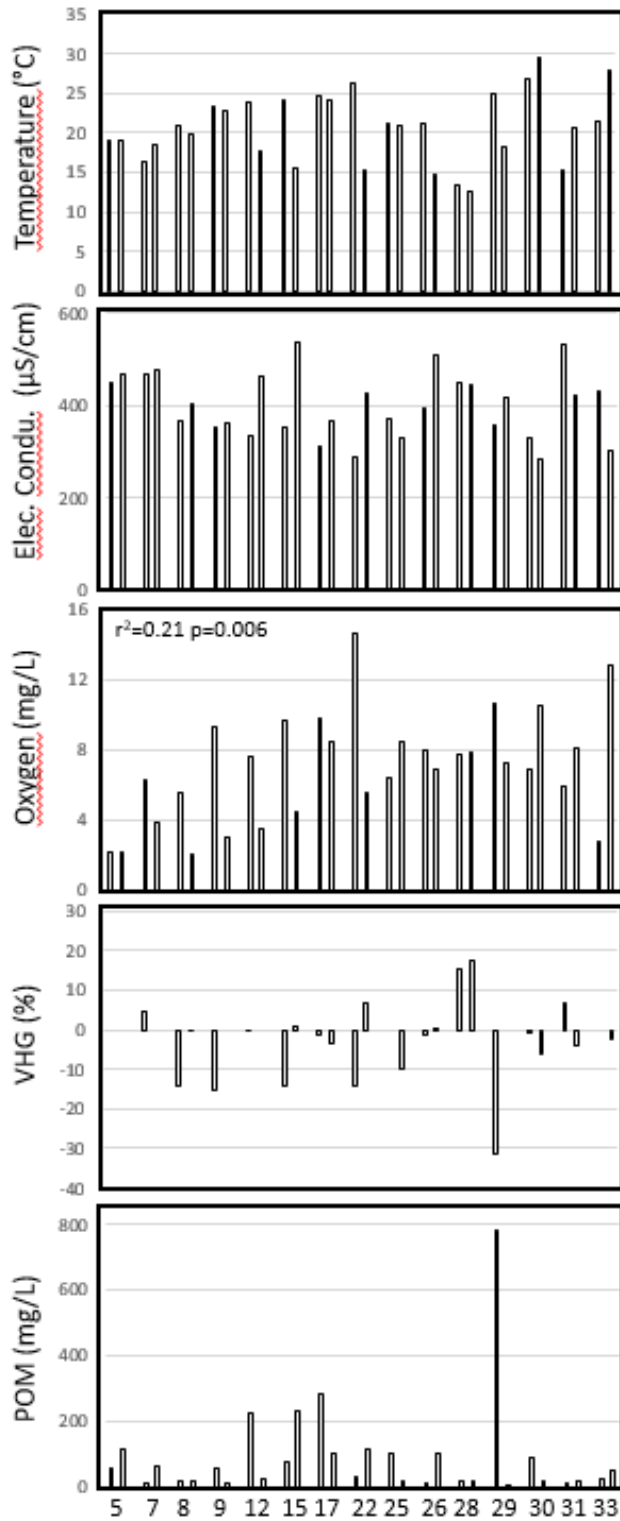
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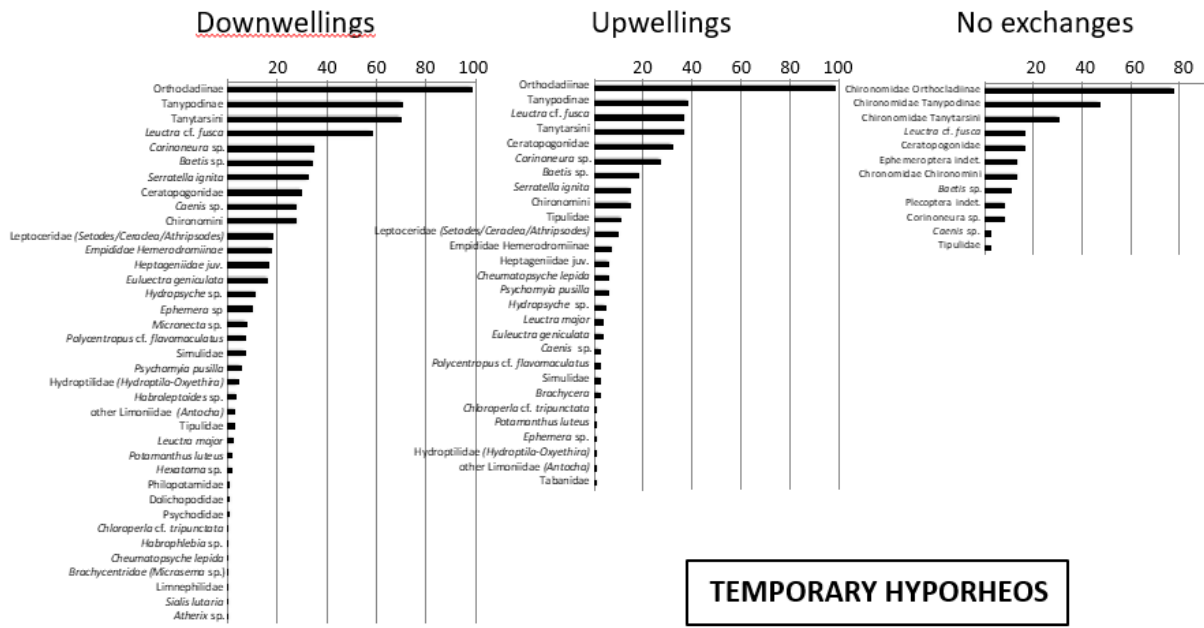
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1412 Fig. S2 : Longitudinal profiles of temperature, electric conductivity, dissolved oxygen, vertical hydraulic
1413 gradient and POM (mean values per stations, all depths and all replicated cumulated)



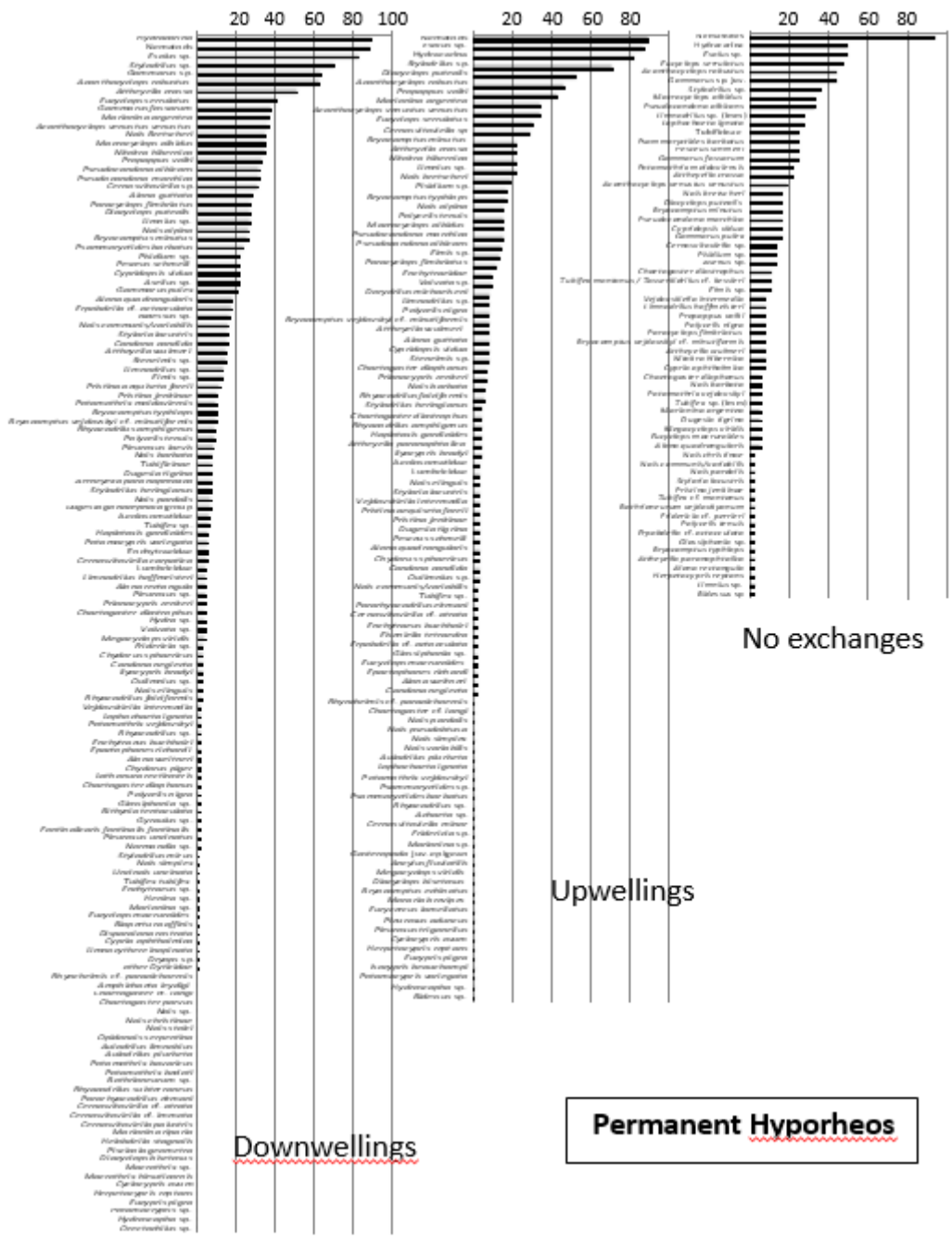
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1415 Fig. S3a: Frequences of temporary hyporheic taxa according to local hydrology
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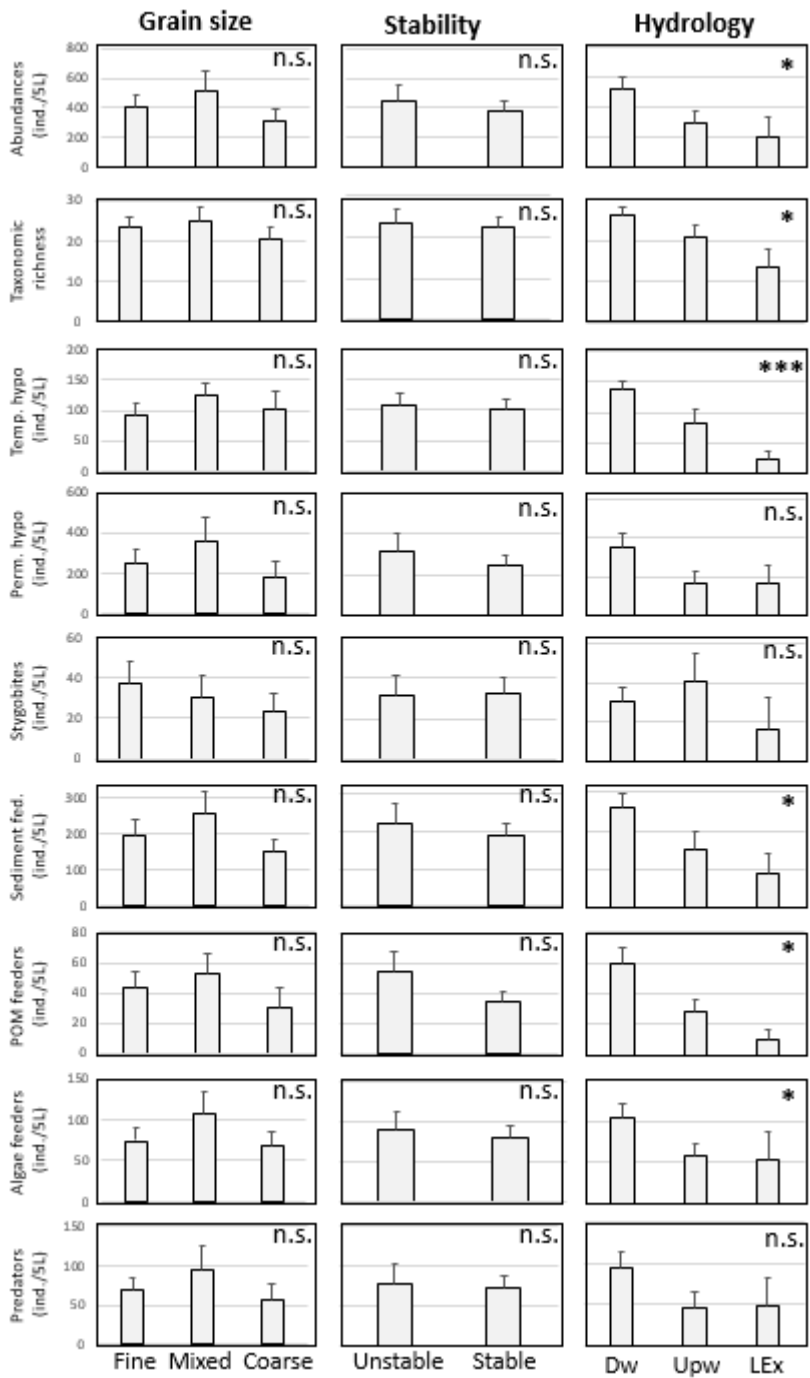
1421 Fig. S3b: Frequences of permanent hyporheic taxa according to local hydrology



1422

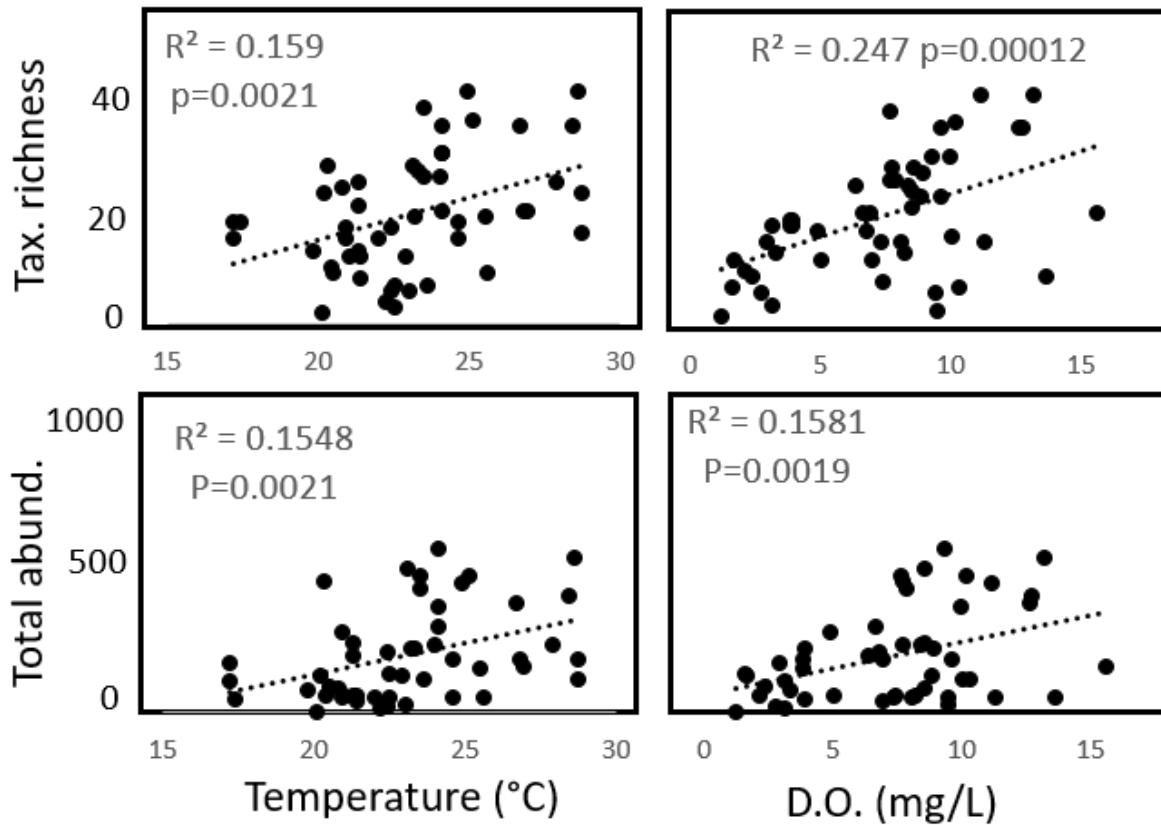
1423

1427 Fig. S4: Effects of sediment grain size (left column), sediment stability (central column) and hydrology (right column) on taxonomic richness, abundances and functional group abundances of the hyporheic
 1428 assemblages. Results of one-way ANOVAs on classes after log-transformation, using stations as
 1429 replicates. Mean values and error-types.
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1434 Fig. S5: Correlations between abundances and taxonomic richness and local characteristics
 1435 (Temperature and Dissolved Oxygen) at 60cm depth in downwelling zones.



1436
 1437

1438 When considering the relationship of the abundances (upper panels) and taxonomic richness (lower
 1439 panels) with temperature and Oxygen at -60 cm depth in downwelling zones, we observed similar
 1440 patterns with increasing number of species and individuals with increasing temperature (with
 1441 maximum values above 25°C) and with available Oxygen (with maximum values above 7 mg.L⁻¹). The
 1442 hyporheic fauna develop abundant and diversifies assemblages in strong downwelling zones (with
 1443 warm and well oxygenated water).

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