Drivers of Functional Diversity in the Hyporheic Zone of a Large River

Marie-José Dole-Olivier, Michel Creuzé Des Châtelliers, Diana M.P. Galassi, Michel Lafont, Florian Mermillod-Blondin, Frédéric Paran, Didier Graillot, Shishir Gaur, Pierre Marmonier

1
2
3
4
5 **Drivers of functional diversity in the hyporheic zone of a large river**
6
7

8 Marie-José Dole-Olivier[a], Michel Creuzé des Châtelliers[a], Diana M. P. Galassi[b], Michel Lafont[a], Florian
9 Mermillod-Blondin[a], Frederic Paran[c], Didier Graillot[c], Shishir Gaur[d] and Pierre Marmonier[a] (✉)
10
11
12 a - Univ. Lyon, University Claude Bernard Lyon 1, CNRS, ENTPE, UMR5023 LEHNA, 43 Boulevard du 11
13 Novembre 1918, F-69622, Villeurbanne, France
14
15 b - University of L'Aquila, Department of Life, Health and Environmental Sciences, Via Vetoio, Coppito,
16 67100 L'Aquila, Italy.
17
18 c – Ecole Nationale des Mines de Saint Etienne, CNRS, UMR5600 PEG, F-42023 Saint Etienne, France
19
20 d – Department of Civil Engineering, Indian Institute of Technology (BHU), 221005 Varanasi, India.
21
22 *(✉) Corresponding author:*
23 *e-mail: pierre.marmonier@univ-lyon1.fr*
24
25
26

36
37

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

Abstract

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

1. Introduction

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

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

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

4

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

5

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 m^3 s^{-1}, with low flow conditions during the summer period (mean July-August,
191 discharge: 54 m^3 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 m^3 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 (Graillot 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 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 (° C), electric conductivity (μS cm^{-1}) and dissolved oxygen (mg L^{-1}). Similar measurements

265   were collected concomitantly in the surface water. The vertical hydraulic gradient (VGH) 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 μS cm^{-1}, Fig. 2A); (ii) stations located in a

273   downwelling zone with negative VHG, low electric conductivity (generally below 400 μS 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 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:

9

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, thee images of 0.25 m^2 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

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

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

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

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

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

3.6. Data analysis

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

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

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

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

4. Results

12

4.1. Hyporheic water chemistry and hydrogeology of the sector

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

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

The hyporheic water characteristics also varied at the station scale, with variations according to the 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 µS cm^{-1} at -60 cm depth) and the stations 25U (fed by surface water,

434   20.3°C and 385 µS 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., *Potamothrix*

487   *moldaviensis*, the Epheremoptera *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*

15

489   *octoculata, 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   epigean 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.

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

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

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

4.6. Functional diversity

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

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

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

5. Discussion

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

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

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

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

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

5.3. Local hydrology, the major driver for hyporheic biodiversity

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

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

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

22

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

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

768

6. Conclusions

769

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. Graillot: 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

804   interests: Electricité de France (EDF) who built and manage the upstream dams funded the research

805   program, however the LTSER Rhône River Basin and the Rhône River Water Agency were included in

806   the following committee and guaranty the objectivity of our conclusions.

807

808

809 **References**.

810 Baxter, C., Hauer, F. R., Woessner, W. W. 2003. Measuring groundwater–stream water exchange: new

811   techniques for installing minipiezometers and estimating hydraulic conductivity. Transactions of

812   the American Fisheries Society. 132(3), 493-502. https://doi.org/10.1577/1548-

813   8659(2003)132<0493:MGWENT>2.0.CO;2

814 Benzécri, J. P. 1973. L'Analyse des Données. Volume II. L'Analyse des Correspondances. Dunod Ed.,

815   Paris.

816 Boano, F., Camporeale, C., Revelli, R., Ridolfi, L. 2006. Sinuosity-driven hyporheic exchange in

817   meandering rivers. Geophysical Research Letters. 33(18), 1-4. https://doi.org/10.1029/

818   2006GL027630

819 Boon, P. J., Willby, N., Gilvear, D., Pryce, D. 2016. The regional hyporheic fauna of gravel-bed rivers and

820   environmental controls on its distribution. Fundamental and Applied Limnology, 187(3), 223-239.

821   https://doi.org/10.1127/fal/2016/0705

822 Bornette, G., Foulquier, A., Graillot, D., Marmonier, P., Mimoun, D., Montuelle, B. 2014. Biodiversité

823   et fonctions des zones humides en réponse aux étiages sévères induits par le changement global.

824   Université Lyon 1, Mines Saint-Etienne, Cemagref Lyon, Projet ANR-CEP09_446043 Wetchange,

825   Final Report.

826 Bou, C., Rouch, R., 1967. Un nouveau champ de recherches sur la faune aquatique souterraine.

827   Compte-Rendu Académie des Sciences, 265, 369-370.

828 Boulton, A. J., Findlay, S., Marmonier, P., Stanley, E. H., Valett, H. M. 1998. The functional significance

829   of the hyporheic zone in streams and rivers. Annual review of Ecology and systematics, 29(1), 59-

830   81. https://doi.org/10.1146/annurev.ecolsys.29.1.59

831 Boulton, A. J. 2020. Conservation of groundwaters and their dependent ecosystems: Integrating

832   molecular taxonomy, systematic reserve planning and cultural values. Aquatic Conservation:

833   Marine and Freshwater Ecosystems, 30(1), 1-7. https://doi.org/10.1002/aqc.3268

834 Brown, L. E., Khamis, K., Wilkes, M., Blaen, P., Brittain, J. E., Carrivick, J. L., Fel, S., Friberg, N., Fureder,

835   L., Gislason, G.M., Hainie, S., Hannah, D.M., James W.H.M., Lencioni, V., Olafson, J.S., Robinson,

836   C.T., Saltveit, S.J., Thomlpson, C., Milner, A. M. 2018. Functional diversity and community assembly

837   of river invertebrates show globally consistent responses to decreasing glacier cover. Nature

838   Ecology & Evolution, 2(2), 325-333. https://doi.org/10.1038/s41559-017-0426-x

839 Brunke, M. 1999. Colmation and depth filtration within streambeds: retention of particles in hyporheic

840   interstices. International Review of Hydrobiology, 84(2), 99-117. https://doi.org

841   /10.1002/iroh.199900014

842 Brunke, M., Gonser, T. 1999. Hyporheic invertebrates: the clinal nature of interstitial communities

843 structured by hydrological exchange and environmental gradients. Journal of the North American

844 Benthological Society, *18*(3), 344-362. https://doi.org/10.2307/1468448

845 Buffington, J. M., Montgomery, D. R. 1997. A systematic analysis of eight decades of incipient motion

846 studies, with special reference to gravel-bedded rivers. Water Resources Research, 33(8), 1993-

847 2029. https://doi.org/10.1029/96WR03190

848 Buffington, J. M., Tonina, D. 2009. Hyporheic exchange in mountain rivers II: Effects of channel

849 morphology on mechanics, scales, and rates of exchange. Geography Compass, *3*(3), 1038-1062.

850 https://doi.org/10.1111/j.1749-8198.2009.00225.x

851 Capderrey, C., Datry, T., Foulquier, A., Claret, C., Malard, F. 2013. Invertebrate distribution across

852 nested geomorphic features in braided-river landscapes. Freshwater Science, 32(4), 1188-1204.

853 https://doi.org/10.1899/12-188.1

854 Cardenas, M. B., Zlotnik, V. A. 2003. Three-dimensional model of modern channel bend deposits.

855 Water Resources Research, 39(6), 1-12. https://doi.org/10.1029/2002WR001383

856 Cardenas, M. B., Wilson, J. L., Zlotnik, V. A. 2004. Impact of heterogeneity, bed forms, and stream

857 curvature on subchannel hyporheic exchange. Water Resources Research, 40(8), 1-13.

858 https://doi.org/10.1029/2004WR003008

859 Caschetto, M., Barbieri, M., Galassi, D.M.P., Mastrollio, L., Rusi, S., Stoch, F., Di Cioccio A., Petita, M.

860 2014. Human alteration of groundwater–surface water interactions (Sagittario River, Central Italy):

861 implication for flow regime, contaminant fate and invertebrate response. Environmental Earth

862 Sciences, 71, 1791–1807. https://doi.org/10.1007/s12665-013-2584-8

863 Caschetto M., Galassi D.M.P., Petitta M., Aravena R. 2017. Evaluation of the sources of nitrogen

864 compounds and their influence on the biological communities in the hyporheic zone of the

865 Sagittario River, Italy: an isotopic and biological approach. *Italian Journal of Geosciences* 136(2),

866 145-156. https://doi.org/10.3301/IJG.2016.07

867 Claret, C., Fontvieille, D. 1997. Characteristics of biofilm assemblages in two contrasted hydrodynamic

868 and trophic contexts. Microbial Ecology, 34(1), 49-57. https://doi.org/10.1007/s002489900033

869 Claret, C., Marmonier, P., Bravard, J. P. 1998. Seasonal dynamics of nutrient and biofilm in interstitial

870 habitats of two contrasting riffles in a regulated large river. Aquatic Sciences, 60(1), 33-55.

871 https://doi.org/10.1007/s000270050025

872 Cook, S., Price, O., King, A., Finnegan, C., van Egmond, R., Schäfer, H., Pearson, J.M., Abolfathi, S.,

873 Bending, G. D. 2020. Bedform characteristics and biofilm community development interact to

874 modify hyporheic exchange. Science of the Total Environment, 749, 141397.

875 https://doi.org/10.1016/j.scitotenv.2020.141397

876 Cornut, J., Elger, A., Lambrigot, D., Marmonier, P., Chauvet, E. 2010. Early stages of leaf decomposition
877   are mediated by aquatic fungi in the hyporheic zone of woodland streams. Freshwater Biology,
878   55(12), 2541-2556. https://doi.org/10.1111/j.1365-2427.2010.02483.x

879 Costaz, I. 2005-2006. Modélisation de la nappe alluviale de la basse plaine de l'Ain et de ses milieux
880   annexes. Burgeap, Conseil Général de l'Ain, Rapports phases 1 (27p.), 2 (23p.) et 3 (21p.).

881 Crenshaw, C. L., Valett, H. M., Tank, J. L. 2002. Effects of coarse particulate organic matter on fungal
882   biomass and invertebrate density in the subsurface of a headwater stream. Journal of the North
883   American Benthological Society, 21(1), 28-42. https://doi.org/10.2307/1468297

884 Curry, R. A., Noakes, D. L. 1995. Groundwater and the selection of spawning sites by brook trout
885   (*Salvelinus fontinalis*). Canadian Journal of Fisheries and Aquatic Sciences, 52(8), 1733-1740.
886   https://doi.org/10.1139/f95-765

887 Dahm, C. N., Trotter, E. H., Sedell, J. R. 1987. Role of anaerobic zones and processes in stream
888   ecosystem productivity, in Averett R.C., McKnight D.M. (Eds.), Chemical Quality of Water and the
889   Hydrologic Cycle, Lewis Publishers, Chelsea, pp 157-178.

890 Datry, T. 2012. Benthic and hyporheic invertebrate assemblages along a flow intermittence gradient:
891   effects   of   duration   of   dry   events.   Freshwater   Biology,   57(3),   563-574.
892   https://doi.org/10.1111/j.1365-2427.2011.02725.x

893 Dehedin, A. 2012. Changements globaux et assèchement des zones humides fluviales : conséquences
894   sur les processus biogéochimiques et les communautés d'invertébrés. PhD Thesis, University of
895   Lyon 1, 254p.

896 Dehedin, A., Piscart, C., Marmonier, P. 2013. Seasonal variations of the effect of temperature on lethal
897   and sublethal toxicities of ammonia for three common freshwater shredders. Chemosphere, 90(3),
898   1016-1022. https://doi.org/10.1016/j.chemosphere.2012.07.055

899 Descloux, S., Datry, T., Philippe, M., & Marmonier, P. 2010. Comparison of different techniques to
900   assess surface and subsurface streambed colmation with fine sediments. International Review of
901   Hydrobiology, 95(6), 520-540. https://doi.org/10.1002/iroh.201011250

902 Descloux, S., Datry, T., Marmonier, P. 2013. Benthic and hyporheic invertebrate assemblages along a
903   gradient of increasing streambed colmation by fine sediment. Aquatic Sciences, 75(4), 493-507.
904   https://doi.org/10.1007/s00027-013-0295-6

905 Descloux, S., Datry, T., Usseglio-Polatera, P. 2014. Trait-based structure of invertebrates along a
906   gradient of sediment colmation: Benthos versus hyporheos responses. Science of the Total
907   Environment, 466, 265-276. https://doi.org/10.1016/j.scitotenv.2013.06.082

908 Di Lorenzo, T., Stoch, F., Galassi, D.M.P. 2013. Incorporating the hyporheic zone within the river
909   discontinuum: longitudinal patterns of subsurface copepod assemblages in an Alpine stream.
910   Limnologica, 43(4), 288-296. https://doi.org/10.1016/j.limno.2012.12.003

911 Di Lorenzo, T., Fiasca, B., Di Cicco, M., Cifoni, M., Galassi, D.M.P. 2021. Taxonomic and functional trait
912   variation along a gradient of ammonium contamination in the hyporheic zone of a Mediterranean
913   stream, *Ecological Indicators*, 132, 108268, https://doi.org/10.1016/j.ecolind.2021.108268.

914 Dole, M. J., Chessel, D. 1986. Stabilité physique et biologique des milieux interstitiels. Cas de deux
915   stations du Haut-Rhône. Annales de limnologie – International Journal of Limnology, 22(1), 69-81.
916   https://doi.org/10.1051/limn/1986008

917 Dole-Olivier, M. J., Marmonier, P. 1992. Patch distribution of interstitial communities: prevailing
918   factors. Freshwater Biology, 27(2), 177-191. https://doi.org/10.1111/j.1365-2427.1992.tb00532.x

919 Dole-Olivier, M. J., Marmonier, P., Beffy, J. L. 1997. Response of invertebrates to lotic disturbance: is
920   the hyporheic zone a patchy refugium? Freshwater Biology, 37(2), 257-276.
921   https://doi.org/10.1046/j.1365-2427.1997.00140.x

922 Dole-Olivier, M. J., Galassi, D. M. P., Marmonier, P., Creuzé des Châtelliers, M. 2000. The biology and
923   ecology of lotic microcrustaceans. Freshwater biology, 44(1), 63-91.
924   https://doi.org/10.1046/j.1365-2427.2000.00590.x

925 Dole-Olivier, M. J., Malard, F., Martin, D., Lefebure, T., Gibert, J. 2009. Relationships between
926   environmental variables and groundwater biodiversity at the regional scale. Freshwater biology,
927   54(4), 797-813. https://doi.org/10.1111/j.1365-2427.2009.02184.x

928 Dole-Olivier, M. J. 2011. The hyporheic refuge hypothesis reconsidered: a review of hydrological
929   aspects. Marine and Freshwater Research, 62(11), 1281-1302. https://doi.org/10.1071/MF11084

930 Dole-Olivier, M. J., Galassi, D. M., Fiers, F., Malard, F., Martin, P., Martin, D., Marmonier, P. 2015.
931   Biodiversity in mountain groundwater: the Mercantour National Park (France) as a European
932   hotspot. Zoosystema, *37*(4), 529-550. https://doi.org/10.5252/z2015n4a1

933 Dole-Olivier, M. J., Wawzyniak, V., Des Châtelliers, M. C., Marmonier, P. 2019. Do thermal infrared (TIR)
934   remote sensing and direct hyporheic measurements (DHM) similarly detect river-groundwater
935   exchanges? Study along a 40 km-section of the Ain River (France). Science of the Total Environment,
936   646, 1097-1110. https://doi.org/10.1016/j.scitotenv.2018.07.294

937 Dudunake, T., Tonina, D., Reeder, W. J., Monsalve, A. 2020. Local and reach-scale hyporheic flow
938   response from boulder-induced geomorphic changes. Water Resources Research, 56(10), 1-15.
939   https://doi.org/10.1029/2020WR027719

940 Dwivedi, D., Steefel, C. I., Arora, B., Newcomer, M., Moulton, J. D., Dafflon, B., Faybishenko, B., Fox, P.,
941   Nico, P., Spycher, N., Carol, R., Williams, K. H. 2018. Geochemical exports to river from the
942   intrameander hyporheic zone under transient hydrologic conditions: East River Mountainous
943   Watershed, Colorado. Water Resources Research, 54(10), 8456-8477.
944   https://doi.org/10.1029/2018WR023377

945 Eme, D., Zagmajster, M., Fišer, C., Galassi, D., Marmonier, P., Stoch, F., Cornu, J.F., Oberdorf, T., Malard,
946   F. 2015. Multi-causality and spatial non-stationarity in the determinants of groundwater crustacean
947   diversity in Europe. Ecography, 38(5), 531-540. https://doi.org/10.1111/ecog.01092

948 Ercoli, F., Lefebvre, F., Delangle, M., Gode, N., Caillon, M., Raimond, R., Souty-Grosset, C. 2019.
949   Differing trophic niches of three French stygobionts and their implications for conservation of
950   endemic stygofauna. Aquatic Conservation: Marine and Freshwater Ecosystems, 29: 2193– 2203.
951   https://doi.org/10.1002/aqc.3227

952 Fattorini, S., Fiasca, B., Di Lorenzo, T., Di Cicco, M., Galassi, D. M. P., 2020. A new protocol for assessing
953   the conservation priority of groundwater-dependent ecosystems. Aquatic Conservation: Marine
954   and Freshwater Ecosystems, 30: 1483– 1504. https://doi.org/10.1002/aqc.3411

955 Faulkner, B. R., Brooks, J. R., Forshay, K. J., Cline, S. P. 2012. Hyporheic flow patterns in relation to large
956   river floodplain attributes. Journal of hydrology, *448*, 161-173.
957   https://doi.org/10.1016/j.jhydrol.2012.04.039

958 Findlay, S. 1995. Importance of surface-subsurface exchange in stream ecosystems: The hyporheic
959   zone. Limnology and oceanography, 40(1), 159-164. https://doi-org.docelec.univ-
960   lyon1.fr/10.4319/lo.1995.40.1.0159

961 Franken, R. J., Storey, R. G., Williams, D. D. 2001. Biological, chemical and physical characteristics of
962   downwelling and upwelling zones in the hyporheic zone of a north-temperate stream.
963   Hydrobiologia, 444(1), 183-195. https://doi-org.docelec.univ-lyon1.fr/10.1023/A:1017598005228

964 Gallardo, B., Gascón, S., Cabezas, Á., González-Sanchis, M., García-Antón, M., Comín, F. A. 2009.
965   Relationship between invertebrate traits and lateral environmental gradients in a Mediterranean
966   river-floodplain. Fundamental and Applied Limnology, 173(4), 281-292. https://doi-org/
967   10.1127/1863-9135/2009/0173-0281

968 Gayraud, S., Philippe, M. 2001. Does subsurface interstitial space influence general features and
969   morphological traits of the benthic macroinvertebrate community in streams? Archiv für
970   Hydrobiologie, 151(4), 667-686. https://doi-org/10.1127/archiv-hydrobiol/151/2001/667

971 Gibbins, C., Vericat, D., Batalla, R. J. 2007. When is stream invertebrate drift catastrophic? The role of
972   hydraulics and sediment transport in initiating drift during flood events. Freshwater Biology, 52(12),
973   2369-2384. https://doi-org.docelec.univ-lyon1.fr/10.1111/j.1365-2427.2007.01858.x

974 Gomez, B. 1984. Typology of segregated (armoured/paved) surfaces: some comments. Earth Surface
975   Processes and Landforms, 9(1), 19-24. https://doi-org.docelec.univ-
976   lyon1.fr/10.1002/esp.3290090103

977 Gomez-Velez, J. D., Wilson, J. L., Cardenas, M. B., Harvey, J. W. 2017. Flow and residence times of
978   dynamic river bank storage and sinuosity-driven hyporheic exchange. Water Resources Research,
979   53(10), 8572-8595. https://doi-org.docelec.univ-lyon1.fr/10.1002/2017WR021362

980 Graco-Roza, C., Soininen, J., Corrêa, G., Pacheco, F. S., Miranda, M., Domingos, P., Marinho, M. M.
981   2021. Functional rather than taxonomic diversity reveals changes in the phytoplankton community
982   of a large dammed river. Ecological Indicators, 121, 107048.
983   https://doi.org/10.1016/j.ecolind.2020.107048

984 Graillot, D., Paran, F., Bornette, G., Marmonier, P., Piscart, C., Cadilhac, L., 2014. Coupling groundwater
985   modelling and biological indicators for identifying river/aquifer exchanges. Springerplus 3, 68.
986   https://doi.org/10.1186/2193-1801-3-68

987 Harbaugh, A.W., Banta, E.R., Hill, M.C., McDonald, M.G., 2000. Modflow-2000, the U.S. Geological
988   survey modular Ground-Water model – user guide to modularization concepts and the ground-
989   water flow process. U.S. Geological Survey, Open-File Report 00-92, 1-121, 26p.

990 Harvey, J. W., Böhlke, J. K., Voytek, M. A., Scott, D., Tobias, C. R. 2013. Hyporheic zone denitrification:
991   Controls on effective reaction depth and contribution to whole-stream mass balance. Water
992   Resources Research, *49*(10), 6298-6316. https://doi-org.docelec.univ-lyon1.fr/10.1002/wrcr.20492

993 Hunt, W. G., Stanley, E. H. 2003. Environmental factors influencing the composition and distribution of
994   the hyporheic fauna in Oklahoma streams: Variation across ecoregions. Archiv fur Hydrobiologie,
995   158(1), 1-23. https://doi-org /10.1127/0003-9136/2003/0158-0001

996 Hutchins, B. T., Swink, A. P., Diaz, P. H., Schwartz, B. F. 2020. Environmental influences on invertebrate
997   diversity and community composition in the hyporheic zone ecotone in Texas, USA: contrasts
998   between co-occurring epigean taxa and stygobionts. Hydrobiologia, 847(19), 3967-3982.
999   https://doi-org.docelec.univ-lyon1.fr/10.1007/s10750-020-04379-8

1000 Hynes, H. B. N. 1975. The stream and its valley. Internationale Vereinigung für theoretische und
1001   angewandte Limnologie: Verhandlungen, 19(1), 1-15. https://doi-org.docelec.univ-
1002   lyon1.fr/10.1080/03680770.1974.11896033

1003 Jain, S. C. 1990. Armor or pavement. Journal of Hydraulic Engineering, 116(3), 436-440.
1004   https://doi.org/10.1061/(ASCE)0733-9429(1990)116:3(436)

1005 Jones Jr, J. B., Fisher, S. G., Grimm, N. B. 1995. Nitrification in the hyporheic zone of a desert stream
1006   ecosystem. Journal of the North American Benthological Society, 14(2), 249-258.
1007   https://doi.org/10.2307/1467777

1008 Juget, J., Lafont, M. 1994. Theoretical habitat templets, species traits, and species richness: aquatic
1009   oligochaetes in the Upper Rhône River and its floodplain. Freshwater Biology, 31(3), 327-340.
1010   https://doi-org.docelec.univ-lyon1.fr/10.1111/j.1365-2427.1994.tb01744.x

1011 Lawton, J. H. 1994. What do species do in ecosystems? Oikos, 71(3), 367-374. https://doi-
1012   org.docelec.univ-lyon1.fr/10.2307/3545824

Lefebvre, S., Marmonier, P., Pinay, G., Bour, O., Aquilina, L., Baudry, J. 2005. Nutrient dynamics in interstitial habitats of low-order rural streams with different bedrock geology. Archiv fur Hydrobiologie, 164(2), 169-191. https://doi.org/10.1127/0003-9136/2005/0164-0169

Leigh, C., Bonada, N., Boulton, A. J., Hugueny, B., Larned, S. T., Vander Vorste, R., Datry, T. 2016. Invertebrate assemblage responses and the dual roles of resistance and resilience to drying in intermittent rivers. Aquatic Sciences, 78(2), 291-301. https://doi-org.docelec.univ-lyon1.fr/10.1007/s00027-015-0427-2

Lejot, J. 2008. Suivi des formes fluviales par télédétection à très haute résolution. Application aux programmes de restauration de la basse vallée de l'Ain et du haut-Rhône (Chautagne). PhD thesis, University of Lyon 2, 257p.

Lewandowski, J., Arnon, S., Banks, E., Batelaan, O., Betterle, A., Broecker, T., et al. 2019. Is the hyporheic zone relevant beyond the scientific community? Water, 11(11), 2230. https://doi-org/0.3390/w11112230

Lin, Q., Song, J., Gualtieri, C., Cheng, D., Su, P., Wang, X., Fu, J., Peng, J. 2020. Effect of Hyporheic Exchange on Macroinvertebrate Community in the Weihe River Basin, China. Water, 12(2), 457. https://doi-org/10.3390/w12020457

Magliozzi, C., Grabowski, R. C., Packman, A. I., Krause, S. 2018. Toward a conceptual framework of hyporheic exchange across spatial scales. Hydrology and Earth System Sciences, 22(12), 6163-6185. https://doi.org/10.5194/hess-22-6163-2018

Magliozzi, C, Usseglio-Polatera, P, Meyer, A, Grabowski, RC. 2019. Functional traits of hyporheic and benthic invertebrates reveal importance of wood-driven geomorphological processes in rivers. Functional Ecology, 33: 1758– 1770. https://doi.org/10.1111/1365-2435.13381

Malard, F., Galassi, D., Lafont, M., Doledec, S., Ward, J. V. 2003. Longitudinal patterns of invertebrates in the hyporheic zone of a glacial river. Freshwater Biology, 48(10), 1709-1725. https://doi-org.docelec.univ-lyon1.fr/10.1046/j.1365-2427.2003.01118.x

Malcolm, I. A., Soulsby, C., Youngson, A. F., Hannah, D. M. 2005. Catchment-scale controls on groundwater–surface water interactions in the hyporheic zone: implications for salmon embryo survival. River Research and Applications, 21(9), 977-989. https://doi-org.docelec.univ-lyon1.fr/10.1002/rra.861

Mao, L., Surian, N. 2010. Observations on sediment mobility in a large gravel-bed river. Geomorphology, 114(3), 326-337. https://doi.org/10.1016/j.geomorph.2009.07.015

Marion, A., Packman, A. I., Zaramella, M., Bottacin-Busolin, A. 2008. Hyporheic flows in stratified beds. Water Resources Research, 44(9), 1-10. https://doi-org.docelec.univ-lyon1.fr/10.1029/2007WR006079

1047   Marmonier, P., Vervier, P., Giber, J., Dole-Olivier, M. J. 1993. Biodiversity in ground waters. Trends in
1048   Ecology & Evolution, 8(11), 392-395. https://doi.org/10.1016/0169-5347(93)90039-R

1049   Marmonier, P., Bodergat, A. M., Doledec, S. 1994. Theoretical habitat templets, species traits, and
1050   species richness: ostracods (Crustacea) in the Upper Rhône River and its floodplain. Freshwater
1051   biology,   31(3),   341-355.   https://doi-org.docelec.univ-lyon1.fr/10.1111/j.1365-
1052   2427.1994.tb01745.x

1053   Marmonier, P., Piscart, C., Sarriquet, P. E., Azam, D., Chauvet, E. 2010a. Relevance of large litter bag
1054   burial for the study of leaf breakdown in the hyporheic zone. Hydrobiologia, 641(1), 203-214.
1055   https://doi-org.docelec.univ-lyon1.fr/10.1007/s10750-009-0082-5

1056   Marmonier, P., Luczyszyn, H., Des Chatelliers, M. C., Landon, N., Claret, C., Dole-Olivier, M. J. 2010b.
1057   Hyporheic flowpaths and interstitial invertebrates associated with stable and eroded river sections:
1058   interactions between micro-and meso-scales. Fundamental and Applied Limnology, 176(4), 303-
1059   317. https://doi.org/10.1127/1863-9135/2010/0176-0303

1060   Marmonier, P., Archambaud, G., Belaidi, N., Bougon, N., Breil, P., Chauvet, E., Claret, C., Cornut, J.,
1061   Datry, T. , Dole-Olivier, M.-J., Dumont, B., Flipo, N., Foulquier, A., Gérino, M., Guilpart, A., Julien, F.,
1062   Maazouzi, C., Martin, D. , Mermillod-Blondin, F., Montuelle, B., Namour, Ph., Navel, S., Ombredane,
1063   D., Pelte, T., Piscart, C., Pusch, M., Stroffek, S., Robertson, A., Sanchez-Pérez, J.-M., Sauvage, S.,
1064   Taleb, A., Wantzen, M., Vervier, P. 2012. The role of organisms in hyporheic processes: gaps in
1065   current knowledge, needs for future research and applications.   Annales de Limnologie-
1066   International Journal of Limnology, 48(3), 253-266. https://doi.org/10.1051/limn/2012009

1067   Marmonier, P., Olivier, M.J., Creuzé des Châtelliers, M., Paran, F., Graillot, D., Winiarski, T., Konecny-
1068   Dupré, L., Navel, S., Cadilhac, L., 2019. Does spatial heterogeneity of hyporheic fauna vary similarly
1069   with natural and artificial changes in braided river width? Science of the Total Environment, 689,
1070   57–69. https://doi.org/10.1016/j.scitotenv.2019.06.352

1071   Marmonier, P., des Châtelliers, M. C., Dole-Olivier, M. J., Radakovitch, O., Mayer, A., Chapuis, H.,
1072   Graillot, D., Re-Bahuaud, J., Johannet, A., Cadilhac, L. 2020. Are surface water characteristics
1073   efficient to locate hyporheic biodiversity hotspots? Science of the Total Environment, 738, 139930.
1074   https://doi.org/10.1016/j.scitotenv.2020.139930

1075   Marzadri, A., Tonina, D., Bellin, A. 2012. Morphodynamic controls on redox conditions and on nitrogen
1076   dynamics within the hyporheic zone: Application to gravel bed rivers with alternate-bar
1077   morphology. Journal of Geophysical Research: Biogeosciences, 117(G3), 1-14. https://doi-
1078   org.docelec.univ-lyon1.fr/10.1029/2012JG001966

1079   Mathers, K. L., Hill, M. J., Wood, P. J. 2017. Benthic and hyporheic macroinvertebrate distribution
1080   within the heads and tails of riffles during baseflow conditions. Hydrobiologia, 794(1), 17-30.
1081   https://doi-org.docelec.univ-lyon1.fr/10.1007/s10750-017-3092-8

1082 Mermillod-Blondin, F., Creuzé des Châtelliers, M., Gerino, M., Gaudet, J. P. 2000. Testing the effect of

1083 *Limnodrilus* sp. (Oligochaeta, Tubificidae) on organic matter and nutrient processing in the

1084 hyporheic zone: a microcosm method. Archiv für Hydrobiologie, 149(3), 467-487.

1085 https://doi.org/10.1127/archiv-hydrobiol/149/2000/467

1086 Mermillod-Blondin, F., Rosenberg, R. 2006. Ecosystem engineering: the impact of bioturbation on

1087 biogeochemical processes in marine and freshwater benthic habitats. Aquatic sciences, 68(4), 434-

1088 442. https://doi-org.docelec.univ-lyon1.fr/10.1007/s00027-006-0858-x

1089 Mermillod-Blondin, F., Winiarski, T., Foulquier, A., Perrissin, A., Marmonier, P. 2015. Links between

1090 sediment structures and ecological processes in the hyporheic zone: ground-penetrating radar as a

1091 non-invasive tool to detect subsurface biologically active zones. Ecohydrology, 8(4), 626-641.

1092 https://doi-org.docelec.univ-lyon1.fr/10.1002/eco.1530

1093 Michel, G., Malard, F., Deharveng, L., Di Lorenzo, T., Sket, B., De Broyer, C. 2009. Reserve selection for

1094 conserving groundwater biodiversity. Freshwater Biology, 54(4), 861-876. https://doi-

1095 org.docelec.univ-lyon1.fr/10.1111/j.1365-2427.2009.02192.x

1096 Navel, S., Mermillod-Blondin, F., Montuelle, B., Chauvet, E., Simon, L., Piscart, C., Marmonier, P. 2010.

1097 Interactions between fauna and sediment control the breakdown of plant matter in river

1098 sediments. Freshwater Biology, 55(4), 753-766. https://doi-org.docelec.univ-

1099 lyon1.fr/10.1111/j.1365-2427.2009.02315.x

1100 Navel, S., Mermillod-Blondin, F., Montuelle, B., Chauvet, E., Simon, L., Marmonier, P. 2011. Water–

1101 sediment exchanges control microbial processes associated with leaf litter degradation in the

1102 hyporheic zone: a microcosm study. Microbial ecology, 61(4), 968-979. https://doi-

1103 org.docelec.univ-lyon1.fr/10.1007/s00248-010-9774-7

1104 Nelson, A. R., Sawyer, A. H., Gabor, R. S., Saup, C. M., Bryant, S. R., Harris, K. D., Briggs M.A., Williams

1105 K.H., Wilkins, M. J. 2019. Heterogeneity in hyporheic flow, pore water chemistry, and microbial

1106 community composition in an alpine streambed. Journal of Geophysical Research: Biogeosciences,

1107 124(11), 3465-3478. https://doi-org.docelec.univ-lyon1.fr/10.1029/2019JG005226

1108 Nogaro, G., Datry, T., Mermillod-Blondin, F., Descloux, S., Montuelle, B. 2010. Influence of streambed

1109 sediment clogging on microbial processes in the hyporheic zone. Freshwater biology, 55(6), 1288-

1110 1302. https://doi-org.docelec.univ-lyon1.fr/10.1111/j.1365-2427.2009.02352.x

1111 Nogaro, G., Burgin, A. J. 2014. Influence of bioturbation on denitrification and dissimilatory nitrate

1112 reduction to ammonium (DNRA) in freshwater sediments. Biogeochemistry, 120(1), 279-294.

1113 https://doi-org.docelec.univ-lyon1.fr/10.1007/s10533-014-9995-9

1114 Olsen, D. A., Townsend, C. R. 2003. Hyporheic community composition in a gravel-bed stream:

1115 influence of vertical hydrological exchange, sediment structure and physicochemistry. Freshwater

Biology, 48(8), 1363-1378. https://doi-org.docelec.univ-lyon1.fr/10.1046/j.1365-2427.2003.01097.x

Orghidan, T. (1959). Ein neuer Lebensraum des unterirdischen Wassers: der hyporheische Biotop. Archiv für Hydrobiologie, 55(3), 392-414.

O'Sullivan, M., Huallachain, D. O., Antunes, P. O., Jennings, E., Kelly-Quinn, M. 2019. The impacts of cattle access to headwater streams on hyporheic zones. Biology and Environment: Proceedings of the Royal Irish Academy, 119(1), 13-27. https://doi.org/10.3348/BIOE.2019.02

Paillex, A., Dolédec, S., Castella, E., Mérigoux, S., Aldridge, D. C. 2013. Functional diversity in a large river floodplain: anticipating the response of native and alien macroinvertebrates to the restoration of hydrological connectivity. Journal of Applied Ecology, 50(1), 97-106. https://doi-org.docelec.univ-lyon1.fr/10.1111/1365-2664.12018

Palmer, M. A., Bely, A. E., Berg, K. E. 1992. Response of invertebrates to lotic disturbance: a test of the hyporheic refuge hypothesis. Oecologia, 89(2), 182-194. https://doi-org.docelec.univ-lyon1.fr/10.1007/BF00317217

Paran, F., Augeard, B., 2017. Guide technique Interactions nappe/rivière: des outils pour comprendre et mesurer les échanges. Guides et protocoles. Agence française pour la biodiversité, pp. 1–102.

Parker, J., Cao, Y., Sass, G. G., Epifanio, J. 2018. Large river fish functional diversity responses to improved water quality over a 28 year period. Ecological indicators, 88, 322-331. https://doi.org/10.1016/j.ecolind.2018.01.035

Peck, D. V., Lazorchak, J. M. Klemm, D. J. 2000. Environmental monitoring and assessment program-surface water: Western pilot study field operations manual for wadeable streams. - US Environmental protection Agency, Washington D.C.

Peralta-Maraver, I., Reiss, J., Robertson, A. L. 2018. Interplay of hydrology, community ecology and pollutant attenuation in the hyporheic zone. Science of the Total Environment, 610-611, 267-275. https://doi.org/10.1016/j.scitotenv.2017.08.036

Peterson, E. W., Sickbert, T. B., Moore, S. L. 2008. High frequency stream bed mobility of a low-gradient agricultural stream with implications on the hyporheic zone. Hydrological Processes, 22(21), 4239-4248. https://doi-org.docelec.univ-lyon1.fr/10.1002/hyp.7031

Petts, G. E., Amoros, C. 1996. The fluvial hydrosystem, Springer, Dordrecht. https://doi-org.docelec.univ-lyon1.fr/10.1007/978-94-009-1491-9_1

Poff LeRoy, N. L. 1997. Landscape filters and species traits: towards mechanistic understanding and prediction in stream ecology. Journal of the North American Benthological Society, 16(2), 391-409. https://doi.org/10.2307/1468026

Poole, G. C. 2002. Fluvial landscape ecology: addressing uniqueness within the river discontinuum. Freshwater Biology, 47(4), 641-660. ttps://doi-org.docelec.univ-lyon1.fr/10.1046/j.1365-2427.2002.00922.x

Poole, G. C. 2010. Stream hydrogeomorphology as a physical science basis for advances in stream ecology. Journal of the North American Benthological Society, 29(1), 12-25. https://doi.org/10.1899/08-070.1

R Core Team, 2021. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Richards, C., Bacon, K. L. 1994. Influence of fine sediment on macroinvertebrate colonization of surface and hyporheic stream substrates. The Great Basin Naturalist, 54(2), 106-113. http://www.jstor.org/stable/41712819

Rollet, A. J., Piégay, H., Dufour, S., Bornette, G., Persat, H. 2014. Assessment of consequences of sediment deficit on a gravel river bed downstream of dams in restoration perspectives: application of a multicriteria, hierarchical and spatially explicit diagnosis. River Research and Applications, 30(8), 939-953. https://doi-org.docelec.univ-lyon1.fr/10.1002/rra.2689

Roy Chowdhury, S., Zarnetske, J. P., Phanikumar, M. S., Briggs, M. A., Day-Lewis, F. D., Singha, K. 2020. Formation criteria for hyporheic anoxic microzones: Assessing interactions of hydraulics, nutrients, and biofilms. Water Resources Research, 56(3), 1-15. https://doi-org.docelec.univ-lyon1.fr/10.1029/2019WR025971

Schmera, D., Heino, J., Podani, J., Erős, T., Dolédec, S. 2017. Functional diversity: a review of methodology and current knowledge in freshwater macroinvertebrate research. Hydrobiologia, 787(1), 27-44. https://doi-org.docelec.univ-lyon1.fr/10.1007/s10750-016-2974-5

Schmid-Araya, J. M. 2000. Invertebrate recolonization patterns in the hyporheic zone of a gravel stream. Limnology and Oceanography, 45(4), 1000-1005. https://doi-org.docelec.univ-lyon1.fr/10.4319/lo.2000.45.4.1000

Sennatt, K. M., Salant, N. L., Renshaw, C. E., Magilligan, F. J. 2006. Assessment of Methods for Measuring Embeddedness: Application to Sedimentation in Flow Regulated STREAMS1. Journal of the American Water Resources Association, 42(6), 1671-1682. https://doi-org.docelec.univ-lyon1.fr/10.1111/j.1752-1688.2006.tb06028.x

Shrivastava, S., Stewardson, M. J., Arora, M. 2021a. Influence of bioturbation on hyporheic exchange in streams: Conceptual model and insights from laboratory experiments. Water Resources Research, 57, e2020WR028468. https://doi.org/10.1029/2020WR028468

Shrivastava, S., Stewardson, M. J., Arora, M. 2021b. Sediment reworking in streambeds with fine sediment deposits and its influence on hyporheic flow regime. Water Resources Research, 57, e2021WR030360. https://doi.org/10.1029/2021WR030360

Singer, M. B. 2008. Downstream patterns of bed material grain size in a large, lowland alluvial river subject to low sediment supply. Water Resources Research, 44(12). https://doi-org.docelec.univ-lyon1.fr/10.1029/2008WR007183

Słowik, M., Kiss, K., Czigány, S., Gradwohl-Valkay, A., Dezso, J., Halmai, A., Marciniak, A., Tritt R., Pirkhoffer, E. 2021. The influence of changes in flow regime caused by dam closure on channel planform evolution: insights from flume experiments. Environmental Earth Sciences, 80, 165. https://doi.org/10.1007/s12665-021-09437-5

Snelder, T. H., Lamouroux, N., Pella, H. 2011. Empirical modelling of large scale patterns in river bed surface grain size. Geomorphology, 127(3-4), 189-197. https://doi.org/10.1016/j.geomorph.2010.12.015

Stonedahl, S. H., Harvey, J. W., Packman, A. I. 2013. Interactions between hyporheic flow produced by stream meanders, bars, and dunes. Water Resources Research, *49*(9), 5450-5461. https://doi-org.docelec.univ-lyon1.fr/10.1002/wrcr.20400

Storey, R. G., Williams, D. D., Fulthorpe, R. R. 2004. Nitrogen processing in the hyporheic zone of a pastoral stream. Biogeochemistry, 69(3), 285-313. https://doi-org.docelec.univ-lyon1.fr/10.1023/B:BIOG.0000031049.95805.ec

Strayer, D. L., May, S. E., Nielsen, P., Wollheim, W., Hausam, S. 1997. Oxygen, organic matter, and sediment granulometry as controls on hyporheic animal communities. Archiv für Hydrobiologie, 140(1), 131-144.

Stubbington, R., Dole-Olivier, M. J., Galassi, D. M., Hogan, J. P., Wood, P. J. 2016. Characterization of macroinvertebrate communities in the hyporheic zone of river ecosystems reflects the pump-sampling technique used. PloS one, 11(10), e0164372. https://doi.org/10.1371/journal.pone.0164372

Tachet, H., Richoux, P. H., Bournaud, M., Usseglio-Polatera, P. 2000. Invertébrés d'eau douce. Systématique, biologie, écologie. CNRS éditions, Paris.

Tilman, D., Knops, J., Wedin, D., Reich, P., Ritchie, M., Siemann, E. 1997. The influence of functional diversity and composition on ecosystem processes. Science, 277(5330), 1300-1302. https://doi.org/10.1126/science.277.5330.1300

Tonina, D., Buffington, J. M. 2009. Hyporheic exchange in mountain rivers I: Mechanics and environmental effects. Geography Compass, 3(3), 1063-1086. https://doi-org.docelec.univ-lyon1.fr/10.1111/j.1749-8198.2009.00226.x

Tonina, D., de Barros, F. P., Marzadri, A., Bellin, A. 2016. Does streambed heterogeneity matter for hyporheic residence time distribution in sand-bedded streams? Advances in Water Resources, 96, 120-126. https://doi.org/10.1016/j.advwatres.2016.07.009

1218 Triska, F. J., Duff, J. H., Avanzino, R. J. 1993. The role of water exchange between a stream channel and
1219   its hyporheic zone in nitrogen cycling at the terrestrial—aquatic interface, in Hillbricht-Ilkowska, A.,
1220   Pieczynska, E. (Eds), Nutrient dynamics and retention in land/water ecotones of lowland, temperate
1221   lakes and rivers. Springer, Dordrecht, pp. 167-184. https://doi-org.docelec.univ-
1222   lyon1.fr/10.1007/978-94-011-1602-2_20

1223 Usseglio-Polatera, P., Bournaud, M., Richoux, P., Tachet, H. 2000. Biological and ecological traits of
1224   benthic freshwater macroinvertebrates: relationships and definition of groups with similar traits.
1225   Freshwater Biology, 43(2), 175-205. https://doi-org.docelec.univ-lyon1.fr/10.1046/j.1365-
1226   2427.2000.00535.x

1227 Vadher, A. N., Stubbington, R., Wood, P. J. 2015. Fine sediment reduces vertical migrations of
1228   *Gammarus pulex* (Crustacea: Amphipoda) in response to surface water loss. Hydrobiologia, 753(1),
1229   61-71. https://doi-org.docelec.univ-lyon1.fr/10.1007/s10750-015-2193-5

1230 Valett, H. M., Hakenkamp, C. C., Boulton, A. J. 1993. Perspectives on the hyporheic zone: integrating
1231   hydrology and biology. Introduction. Journal of the North American Benthological Society, 12(1),
1232   40-43. https://doi.org/10.2307/1467683

1233 Vander Vorste, R. V., Corti, R., Sagouis, A., Datry, T. 2016. Invertebrate communities in gravel-bed,
1234   braided rivers are highly resilient to flow intermittence. Freshwater Science, 35(1), 164-177.
1235   https://doi.org/10.1086/683274

1236 Williams, D. D. 1984. The hyporheic zone as an habitat for aquatic insects and associated arthropods,
1237   in Resh, V.H., Rosenberg, D.M. (Eds), The ecology of aquatic insects, Praeger Scientific, New York,
1238   pp.430-455.

1239 Williams, D. D. 1993. Nutrient and flow vector dynamics at the hyporheic/groundwater interface and
1240   their effects on the interstitial fauna, in Hillbricht-Ilkowska, A., Pieczynska, E. (Eds), Nutrient
1241   dynamics and retention in land/water ecotones of Lowland, Temperate Lakes and Rivers, Springer,
1242   Dordrecht, pp. 185-198. https://doi.org/10.1007/978-94-011-1602-2_21

1243 Williams, D. D., Febria, C. M., Wong, J. C. 2010. Ecotonal and other properties of the hyporheic zone.
1244   Fundamental and Applied Limnology, 176(4), 349-364. https://doi.org/10.1127/1863-
1245   9135/2010/0176-0349

1246 Wood, P. J., Armitage, P. D. 1997. Biological effects of fine sediment in the lotic environment.
1247   Environmental management, 21(2), 203-217. https://doi.org/10.1007/s002679900019

1248 Zheng, L., Cardenas, M. B., Wang, L., Mohrig, D. 2019. Ripple effects: Bed form morphodynamics
1249   cascading into hyporheic zone biogeochemistry. Water Resources Research, 55(8), 7320-7342.
1250   https://doi-org.docelec.univ-lyon1.fr/10.1029/2018WR023517

1251

1252

1253

1254

1255

1256 Table 1: Comparison of the effects of local hydrology, sediment grain size and sediment stability on the

1257 functional traits of the hyporheic assemblages. One way ANOVAs were performed with stations as

1258 replicates (mean values for all depths and replicates, after log transformation; n.s. above the significant

1259 level, - no test possible for food type groups because a species may belong to more than one trophic

1260 group, see histogram in Supplementary material Fig. S4).

1261

| | 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.	-

1262

1263 [1] Local hydrology are downwelling (n=16), low exchange (n=4) and upwelling contexts (n=10).

1264 [2] Sediment grain size classes are fine (n=13), mixed (n=10) and coarse surface sediment (n=7).

1265 [3] Sediment stability are with (n=17) or without biofilm development (n=13).

1266

1267

Figure legends:

Figure 1: Study area (A), the Lower Ain River from Pont d'Ain to the confluence with the Rhône River

(background elevation map from Geographic National Institute, Géoportail). Blue lines: Ain and Rhône

river channels; pointed dark line: upstream eroded sector before 2005; dotted lines: limits of the

morainic hills. Location of the 15 sampled gravel bars (A), upstream and downstream stations at each

gravel bar (B) and sampling design at each station (C).

Figure 2. Chemical characteristics of hyporheic water, sediment grain size and sediment stability.

Examples showing the classification of the stations into the three groups of stream-groundwater

exchanges according to electric conductivity and temperature of the surface and hyporheic waters: A-

Upwellings (UPW), B- Low exchanges (LOW EX), C- Downwelling (DNW). Examples of sediment

characteristics: D- fine sediment, E- mixed, F- coarse. Example of biofilm development on unstable (G)

or stable (H) benthic layer. List of the stations in each group with U for upstream and D for downstream

positions along the gravel bars (see Fig. 1).

Figure 3: Hydrogeological characteristics of the studied sector based on geomatic analysis of the study

reach (A) and details of the eight stations with upwelling zones as derived by the hyporheic water

chemistry (B).

Figure 4. Principal Component Analysis on hyporheic water characteristics. Correlation circle of

physico-chemical parameters (A) Cond-Electric conductivity, VGH-Vertical Hydraulic Gradient, O_2-

Dissolved oxygen, T°C-Temperature. Location of the sampling points on the Axis 1 x Axis 2 biplots,

according to the local hydrology (B), the grain size (C) and the sediment stability (D). Oxygen

concentrations by groups and depths (means and standard errors) for local hydrology groups (E), grain

size groups (F) and stability groups (G) with results of ANOVA tests between groups.

Figure 5: Longitudinal distribution of hyporheic assemblages. Means (and standard errors) for the 15

gravel bars and positions (i.e. 30 stations) for total abundance and taxonomic richness (left panels), for

ecological groups based on affinities to groundwater (center panels) and for trophic functional groups

(right panels).

Figure 6. Correspondance analysis on hyporheic assemblages (the 1st axis represented 6% and the 2nd

axis 5.3% of the total inertia). Location of the taxa in the 1st and 2nd axes is separated in three major

taxonomic groups: (A) oligochaetes, molluscs, leeches, (B) crustaceans and (C) insects, with stygobite

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.

1313

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.
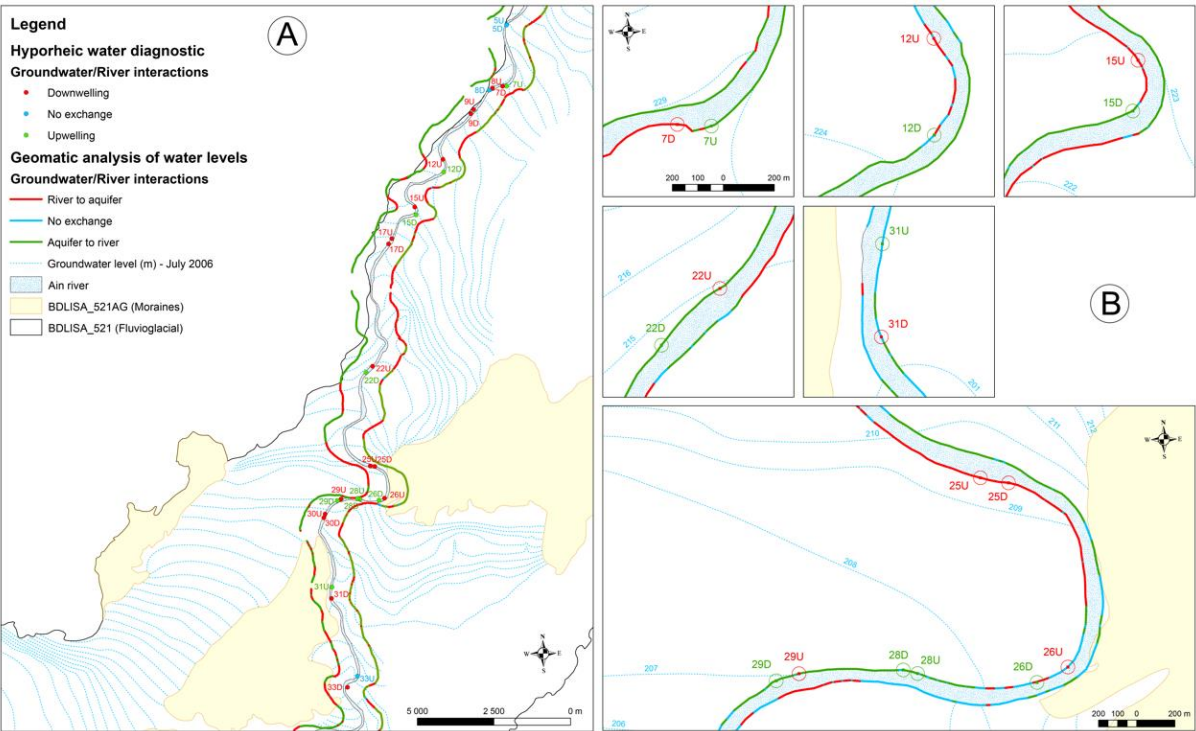
1320

1321

1322   Fig. 1
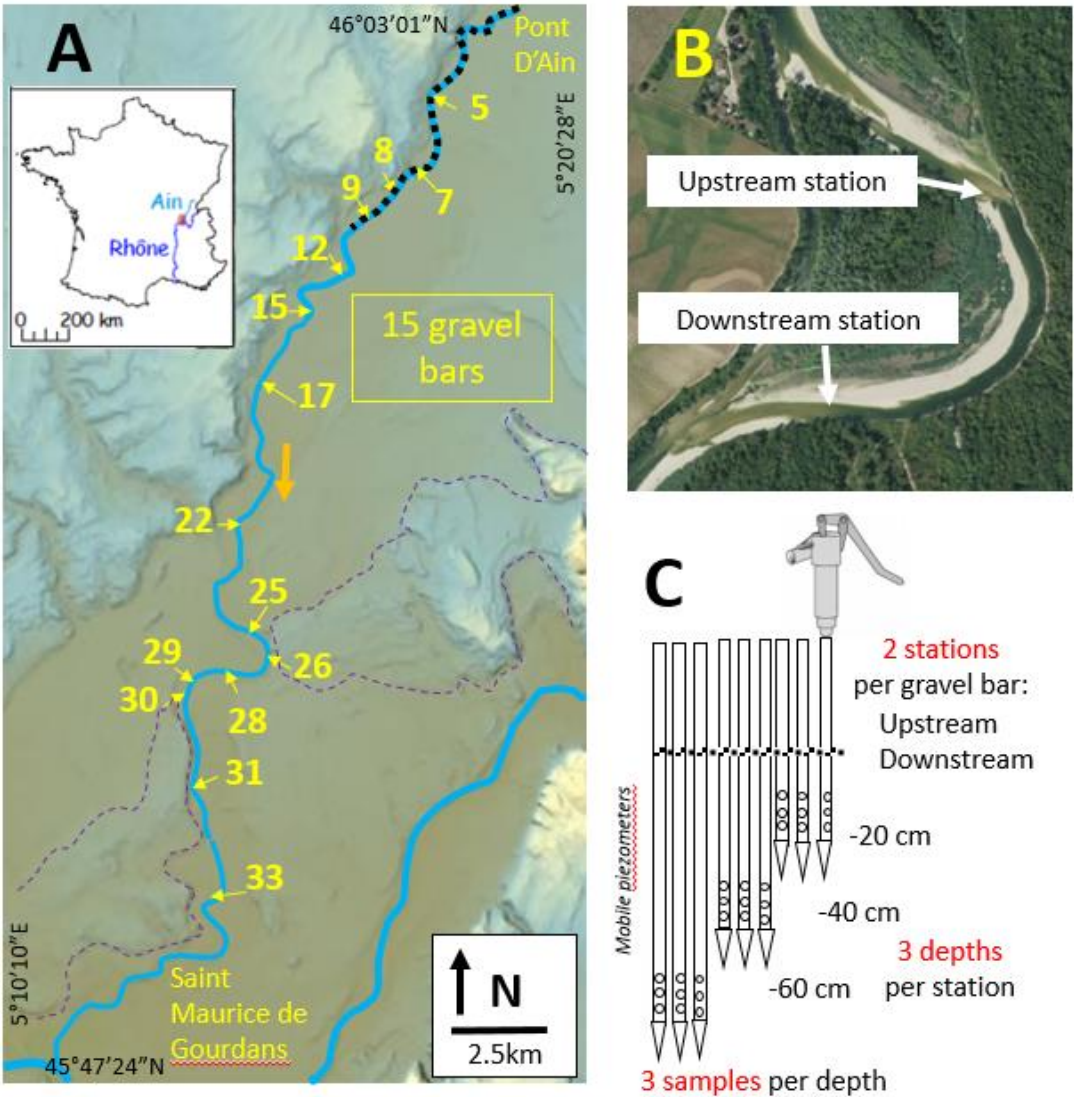
1323

1324

1325

1326   Fig. 2

1327

1328

1329

1330
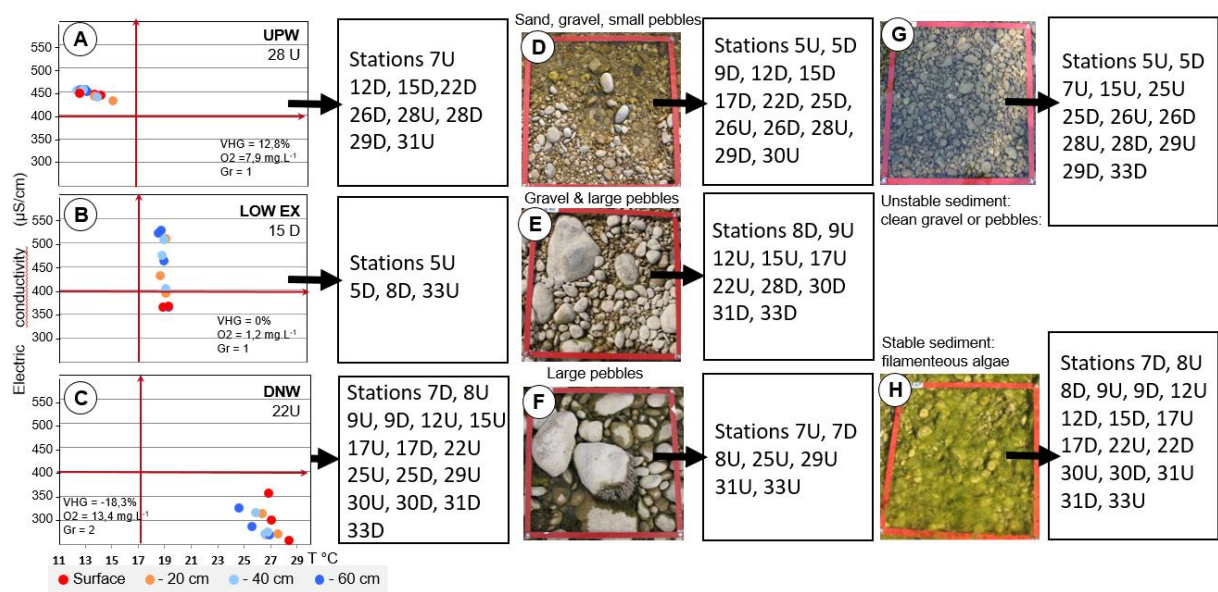
1331   Fig. 3

1332

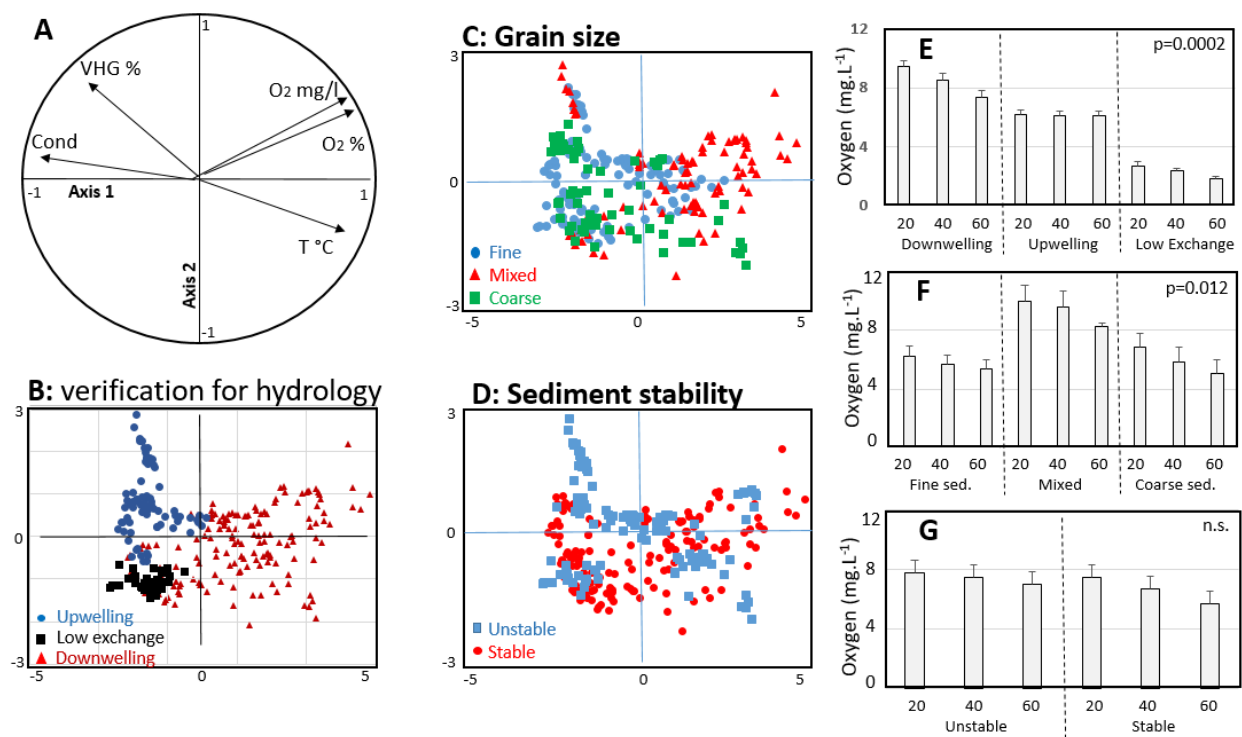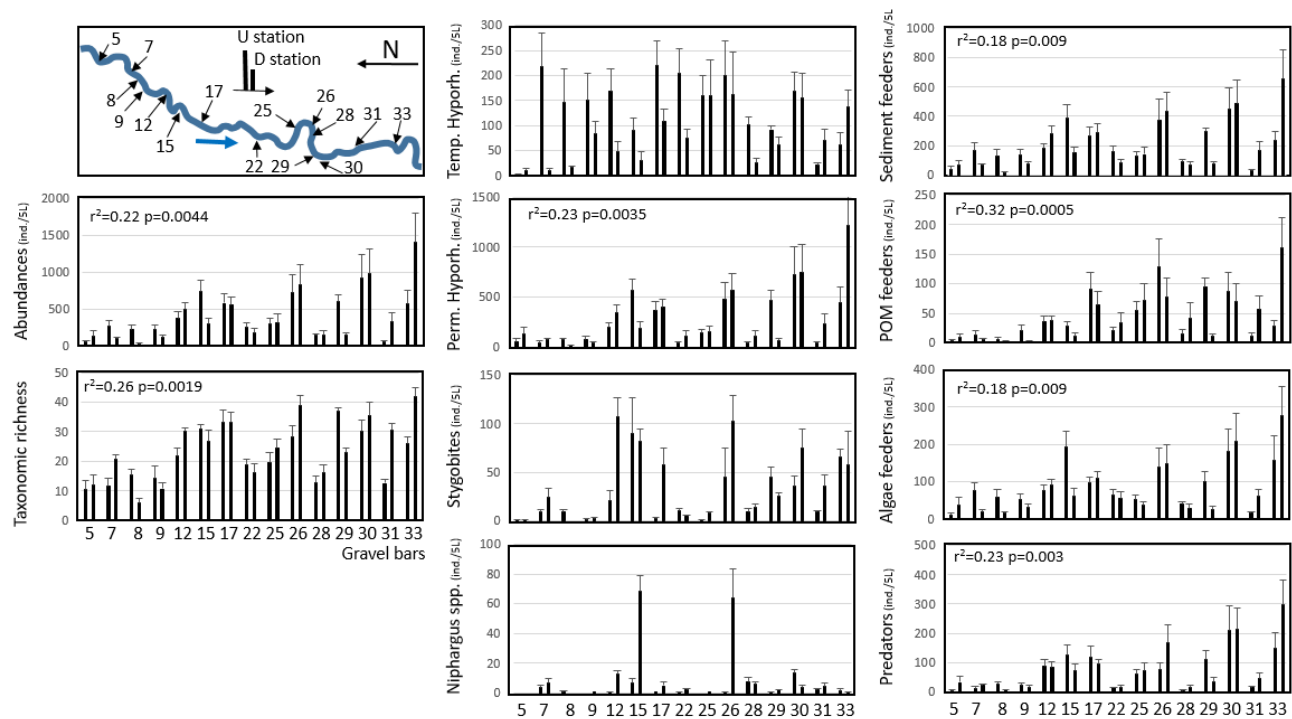

1333
1334
1335

1336   Fig. 4

1337

1338

1339

1340

1341   Fig. 5

1342

1343

1344

1345

1346   Fig. 6
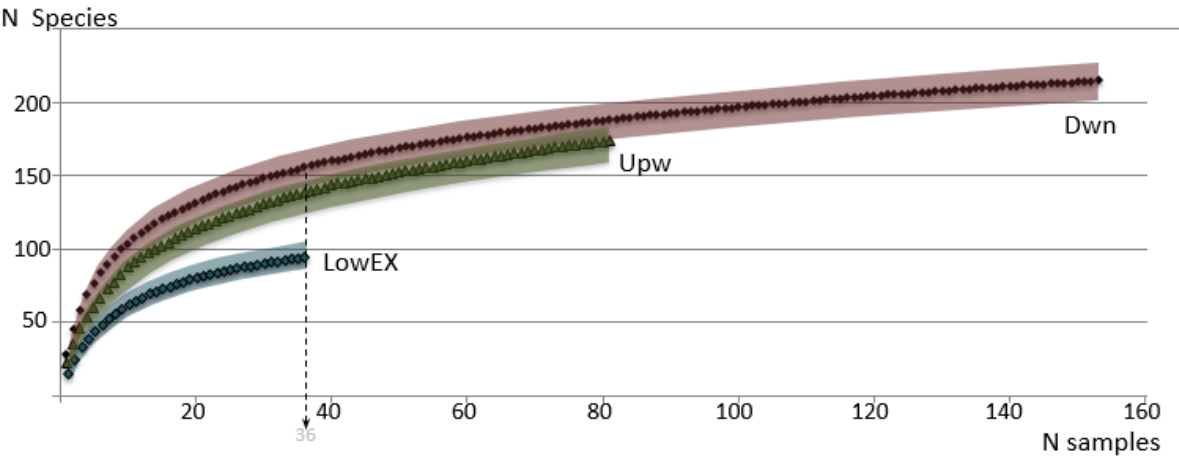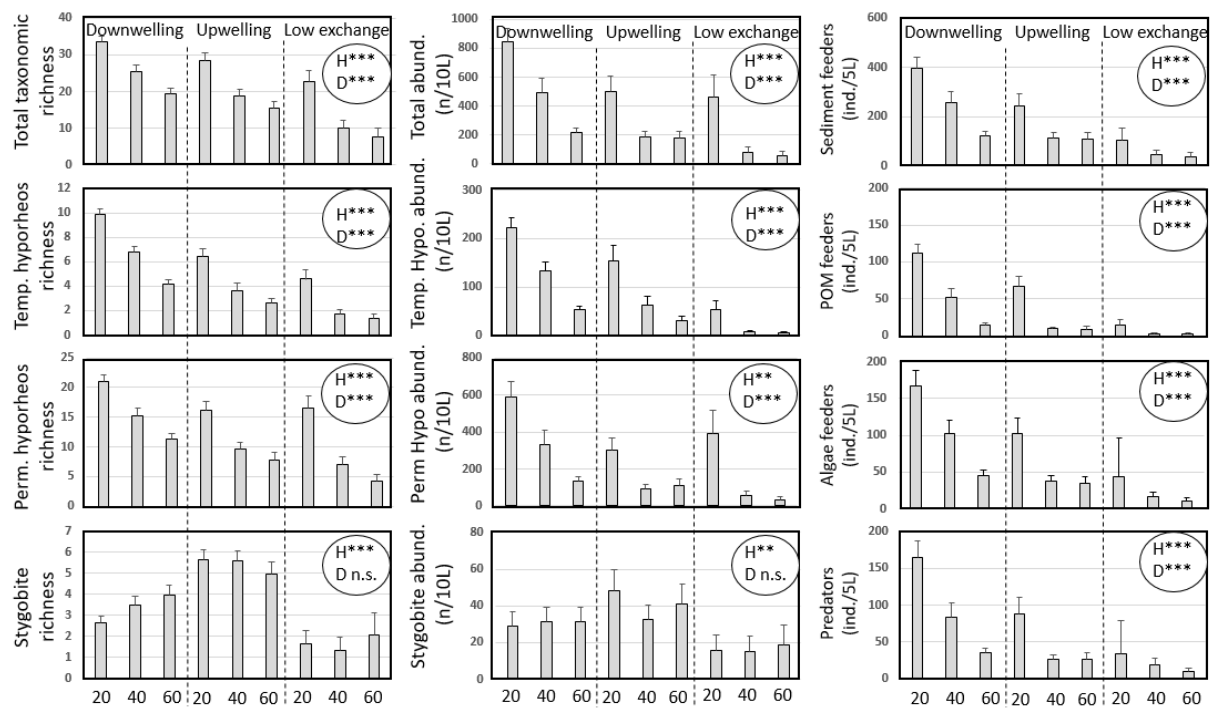
1347

1348

1349

1350

1351   Fig. 7

1352

1353

1354

1355

1356

1357

Fig. 8

1359

1360

1361
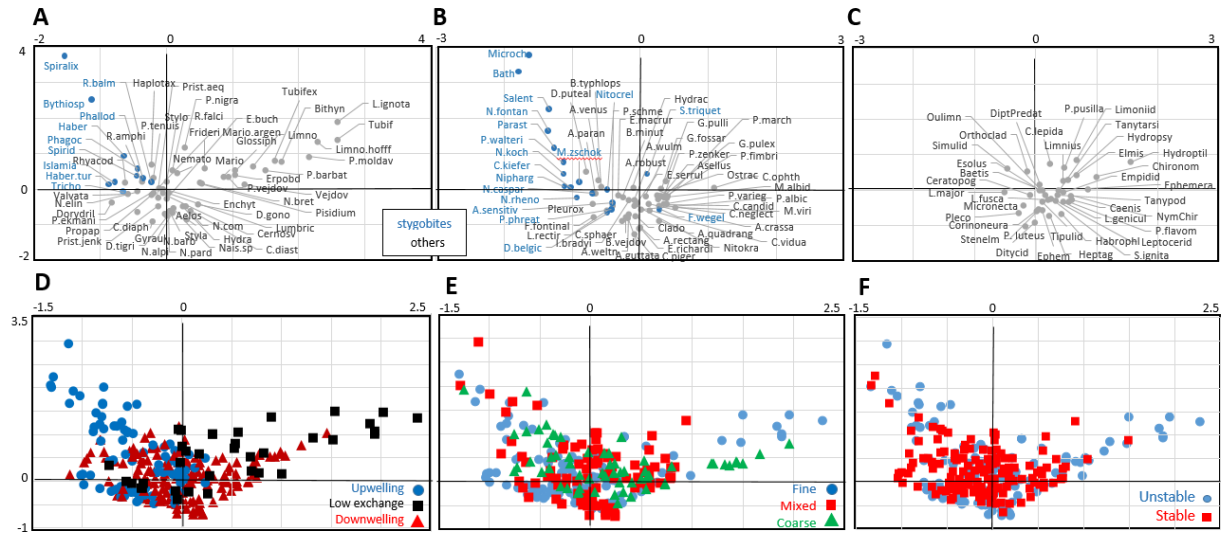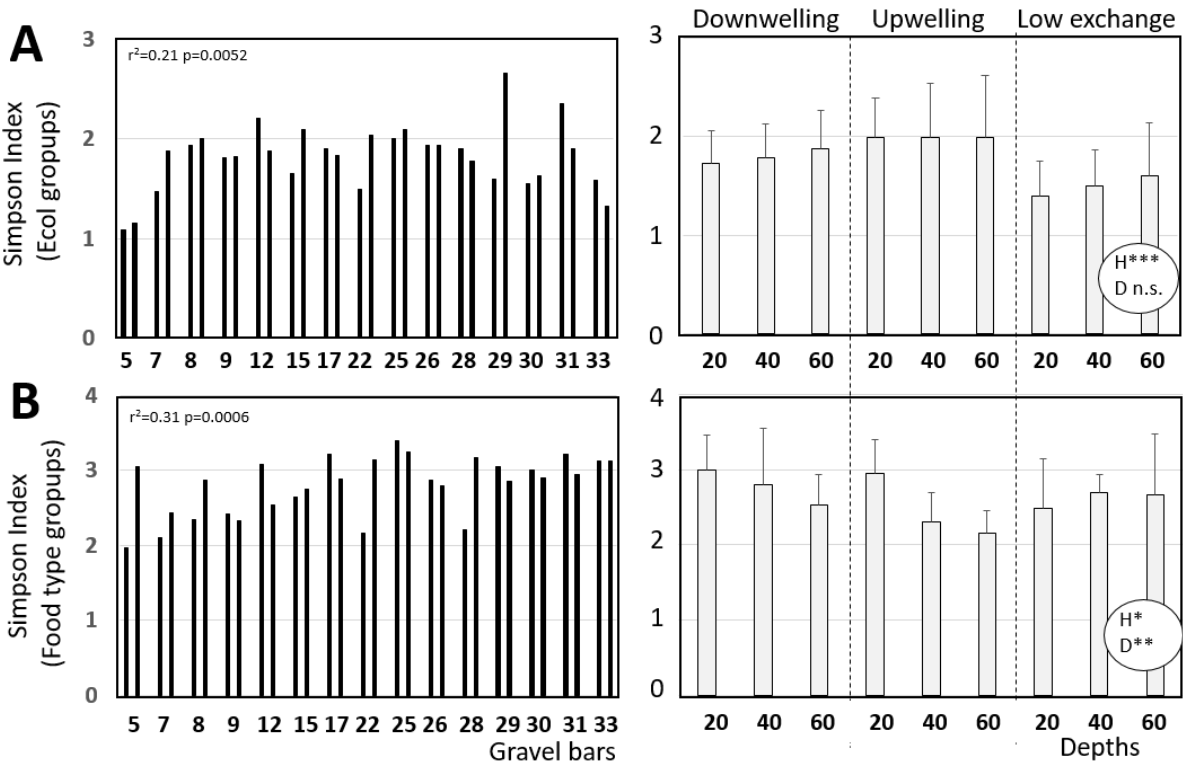
1366

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

1368

1369

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. |

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.

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.

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.

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.

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.

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.

Supplementary material: Figures

Fig. S1 : Biplots for hydrological patterns.

Stations 5 to 9

Stations 12 to 22

Stations 25 to 29

Stations 30 to 33
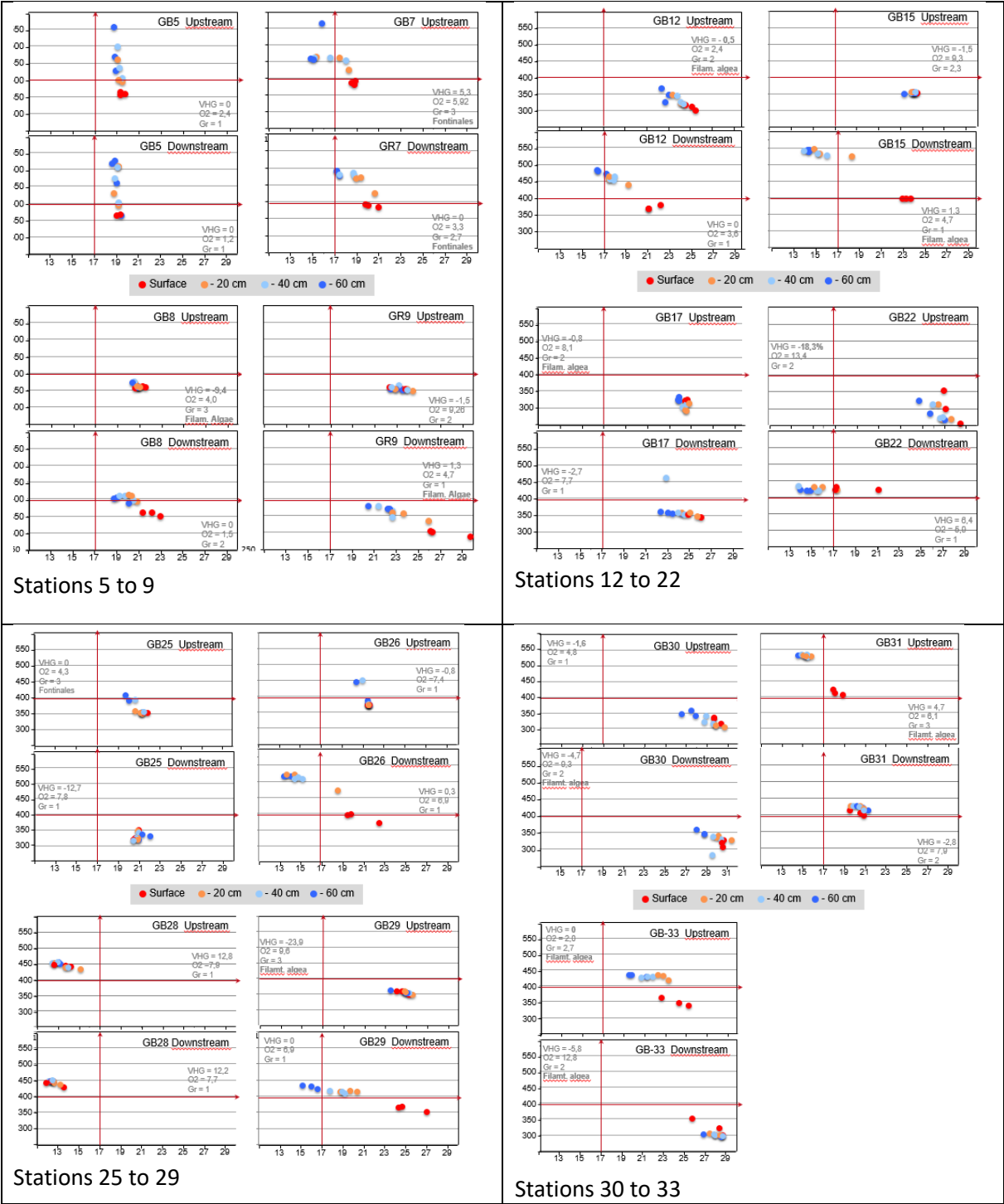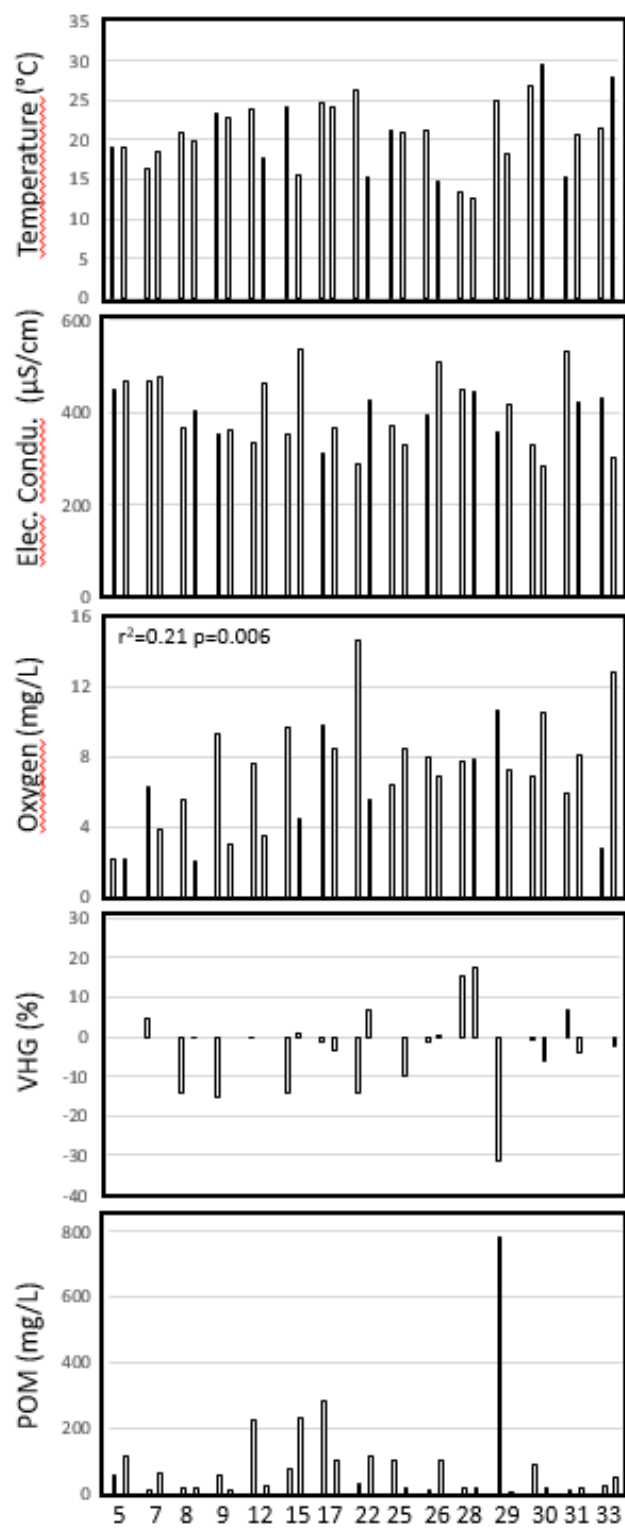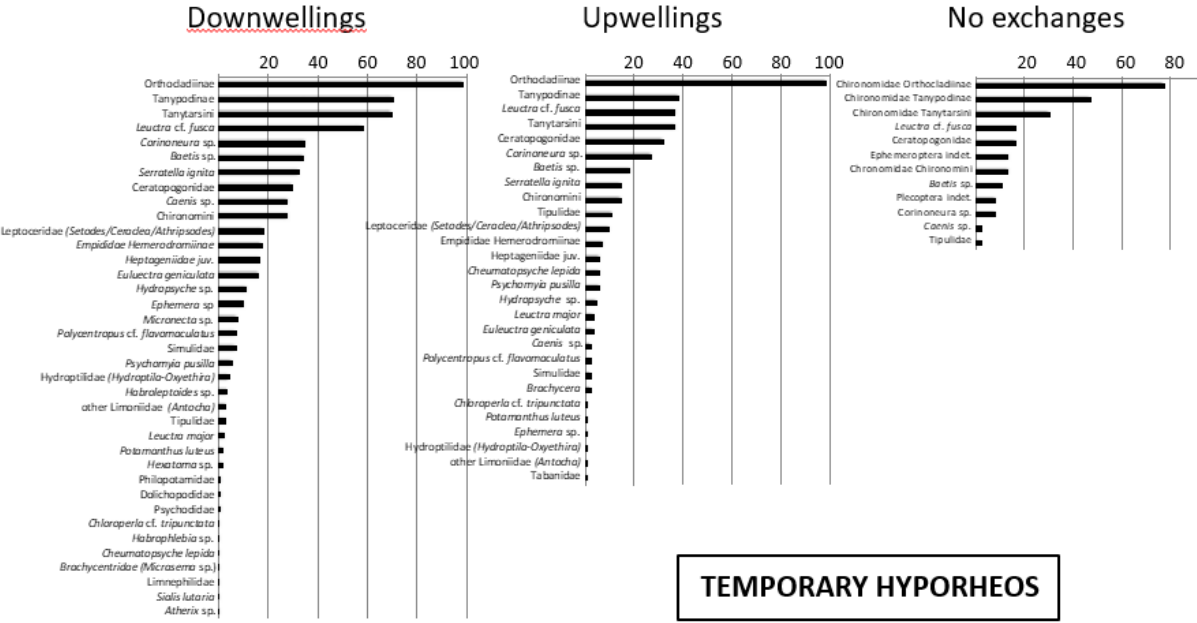
1412   Fig. S2 : Longitudinal profiles of temperature, electric conductivity, dissolved oxygen, vertical hydraulic
1413   gradient and POM (mean values per stations, all depths and all replicated cumulated)

1414

Fig. S3a: Frequences of temporary hyporheic taxa according to local hydrology
1416

1417
1418
1419

1420

Fig. S3b: Frequences of permanent hyporheic taxa according to local hydrology

Downwellings

Upwellings

No exchanges

Permanent Hyporheos

1422

1423

1424   Fig. S3c: Frequences of stygobites according to local hydrology
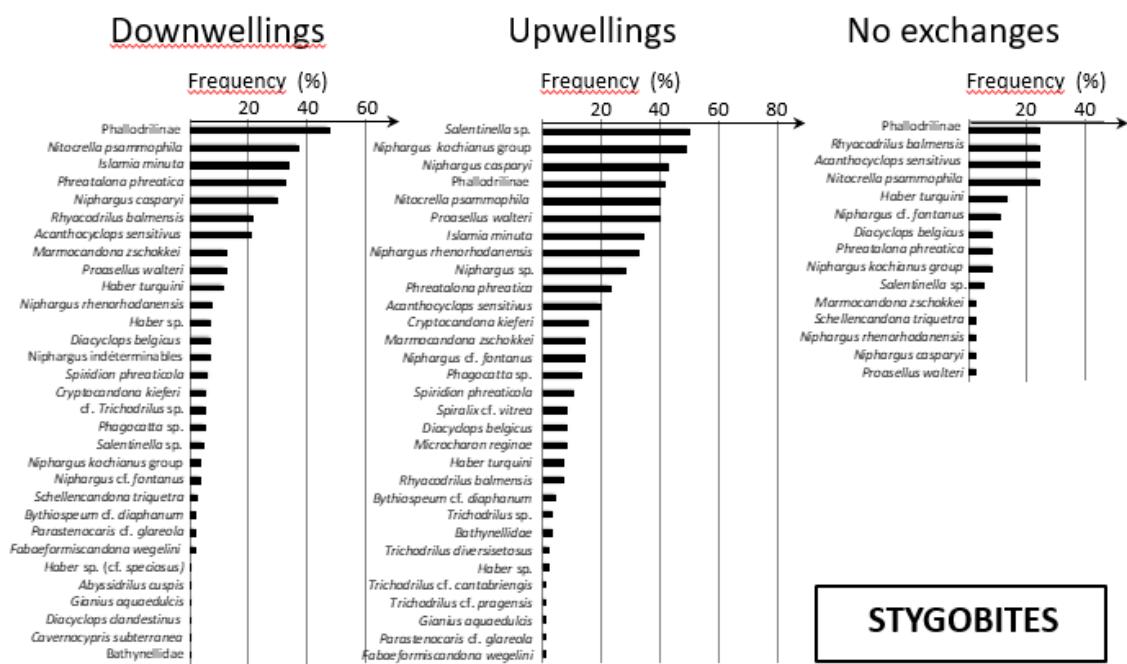
1425

1426

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
assemblages. Results of one-way ANOVAs on classes after log-transformation, using stations as
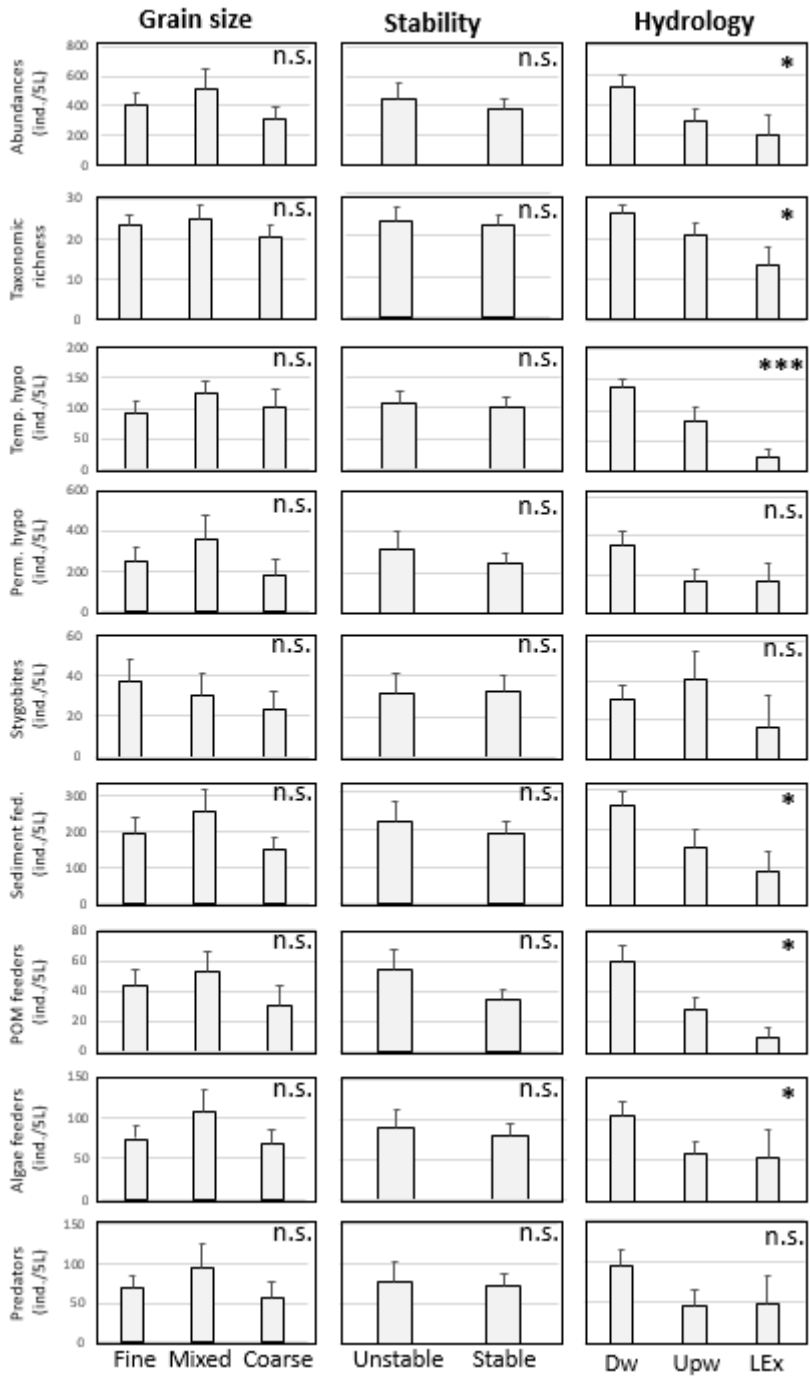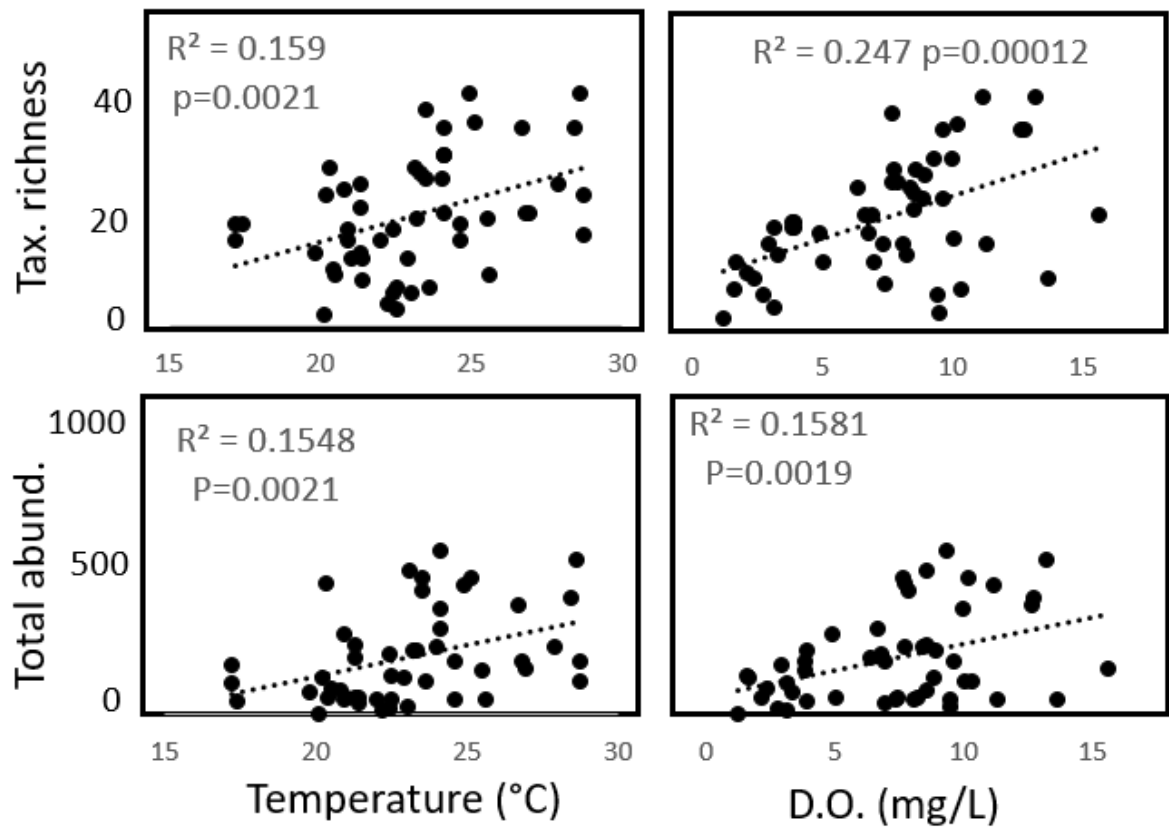replicates. Mean values and error-types.

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 pannels) and taxonomic richness (lower
1439 pannels) 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 developp abundant and diversifies assemblages in strong downwelling zones (with
1443 warm and well oxygenated water).
1444
1445

1446
1447
1448