

1 **Interplay of hydrology, community ecology and pollutant**
2 **attenuation in the hyporheic zone**

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17 **Summary**

18 1) We describe the hierarchical interplay of hydrology, hyporheic ecology and
19 transformation of nutrients and pollutants in the hyporheic zone (HZ). The exchange of water
20 between the surface-subsurface generates the hyporheic exchange flow: the engine that drives
21 the ecological functioning of the HZ. The magnitude and direction of hydrological fluxes in
22 the HZ follow complex spatial patterns, strongly influenced by the temporal dynamics of
23 surface flow in rivers.

24 2) The direction and magnitude of hydrological fluxes also shapes the structure of
25 hyporheic communities (hyporheos). During surface disturbances such as flooding or
26 drought, benthic organisms may also use the HZ as a refuge, although the importance of this
27 role is debated.

28 3) Streambed organisms differ in their ability to colonise the HZ depending on the
29 biological traits they possess. The reduction in oxygen concentration and pore size with
30 increasing sediment depth imposes a limit on the distribution of macroinvertebrates, which
31 are replaced by a suite of smaller organisms (meiofauna and protists) at deeper sediment
32 layers. Therefore, a concomitant reduction in net biomass and productivity might be expected
33 through depth. However, only a few studies have assessed the contribution of the hyporheos
34 to whole system production, and they have focused only on the fraction of relatively large
35 organisms.

36 4) The bioreactor ability of the HZ to transform nutrients and pollutants is an
37 important ecosystem service sustained by the life activities of hyporheos. Biofilms have the
38 key role in this process due to their capacity to metabolize a wide range of dissolved

39 compounds, including emerging pollutants. However, the residence time of water in pore
40 sediments (resulting from hyporheic exchange flow) and the rest of the community
41 (constantly reworking the sediments and grazing biofilms) are indirectly involved.

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43 **Key words:** macroinvertebrates, meiofauna, biofilms, micropollutants, bioremediation,
44 ecosystem services.

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53 **Introduction**

54 In most lotic systems, the surface water of the open channel is connected to groundwater
55 systems via the riverbed sediments. As a result, there is a bi-directional exchange between

56 the groundwater and the surface water along the continuum of stream and rivers (Bencala
57 1993). The volume of the sediments in which stream water mixes with groundwater is known
58 as the hyporheic zone (HZ). HZ functioning in the context of the whole–river ecosystem has
59 been studied by researchers belonging to many different disciplines and as a result selecting a
60 single inclusive definition for the HZ is difficult (Bencala 2000). Traditionally its definition
61 has depended on the discipline–specific interest in hyporheic processes (Tonina &
62 Buffington, 2009; Table 1). For example, in Geochemistry, the HZ is defined as the volume
63 of sediment containing a specified percentage of surface water, while in Biology it is
64 described as the volume of sediments housing a characteristic hyporheic community (Tonina
65 and Buffington 2009). These differences in definition and extent have important implications.
66 However, the fundamental concept behind all definitions is that water exchanges between the
67 open channel and the groundwater systems.

68 Recently, Ward (2016) proposed a more flexible and cross–disciplinary definition
69 (Table 1). A key idea from this definition of the HZ is the importance of the temporal scale
70 relevant to the processes of interest. In fact, flow paths and the rates of water exchange
71 through the HZ are strongly influenced by the temporal dynamics of surface flow in rivers.
72 This is especially evident on a seasonal scale. Despite the dynamics of rivers, seasonality may
73 result in a set of drastic changes in water flow conditions (Gasith & Resh 1999) and
74 determine the location and extent of the HZ (Wondzell 1993). Nonetheless, the HZ buffers
75 the amplitude of this variation, acting as a potential refuge of riverbed biota during adverse
76 conditions (Maazouzi et al 2017). This has important implications for variations in the
77 composition and abundance of organisms throughout the year (Stubington et al. 2009). The
78 HZ harbours diverse and productive communities whose distribution and composition is

79 strongly correlated with the direction and magnitude of hydrologic fluxes (Stanley and
80 Boulton 1993, Olsen and Townsend 2003). These hyporheic communities or hyporheos (*hypo*
81 = under, *rheos* = river) are composed of microbial biofilms (bacteria and fungi existing in an
82 exocellular matrix, Singer et al. 2006), protists (mainly ciliates, flagellates and amoebae) and
83 invertebrates. These groups differ notably in their biological traits and ability to colonize the
84 riverbed, shaping the budget of biomass and secondary production in the HZ.

85 The HZ is a mechanical filter mediated by the pore space of sediments and water
86 flows and a biogeochemical filter controlled by biological and chemical processes (Boulton et
87 al. 2010). As a result, the HZ provides an important ecosystem service by acting as a
88 bioreactor (hyporheic bioreactor, Table 1) with an impressive self-purification capacity, and
89 a barrier against contamination of aquifers, which is essential in the supply of water for
90 human consumption (Lewandowski et al. 2011). Thus the HZ of streams and rivers has a
91 critical role in the flows of biomass and energy, cycling of nutrients and pollution attenuation
92 (McClain et al. 2003, Smith et al. 2009, Robertson & Wood 2010, Boulton et al. 2010). A
93 large body of literature describes the nitrogen, phosphorus and organic carbon attenuation in
94 the HZ of streams and rivers (i.e. Harvey et al. 2013, Aubeneau et al. 2015, Stegen et al.
95 2016, Liu et al. 2017). However, there is little literature describing the fate and removal rates
96 of the emerging micropollutants (Table 1) in lotic systems (Lewandowski et al. 2011, Köhler
97 & Triebkorn, 2013), making understanding of the processing of these compounds by the
98 bioreactor a major remaining challenge in ecology.

99 The role of the hyporheic bioreactor in the whole river system might be seen as the
100 ‘rivers liver’ (Fischer et al. 2005). The HZ has an important role in the production,
101 metabolism, exchange and transformation of dissolved compounds, and health of the whole

102 ecosystem. Here we describe the hierarchical relationship between hyporheic exchange flow,
103 community ecology, and pollutant attenuation of the HZ. These subjects have been mainly
104 assessed separately in discipline specific studies but they are intimately connected and
105 together drive the functioning of the hyporheic bioreactor.

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107 **Hyporheic hydrodynamics: the motor of hyporheic zone ecology**

108 The water exchange between the open river channel and a groundwater system
109 generates the hyporheic exchange flow and strongly influences the whole ecosystem by
110 determining the transport of solutes between compartments (Ward et al. 2012) and the
111 chemistry of stream water (Duff and Triska 1990, Bencala et al. 1993). Streambed sediments
112 are a porous medium through which exchange of water occurs. Thus, the hyporheic exchange
113 and the flow paths throughout sediments could be theoretically studied by applying Darcy's
114 Law, as the product of hydraulic gradient and hydraulic conductivity (Jones et al. 1996).
115 However, hyporheic exchange flow does not show a uniform pattern along the river. The
116 flow between surface and groundwater follows complex dynamics (Rutheford & Hynes
117 1987), in which upwelling (UW) and downwelling (DW) zones occur alternately (White
118 1990). Thus, as a result of this mosaic of physical features, sediment conditions may change
119 substantially even at centimetre scales (Boano et al. 2014). In addition, temporal dynamics
120 from daily to seasonal processes result in change of hyporheic flow through time (Wondzell
121 2011).

122 Streambed topography from sediment-scale (ripples or pebbles) to larger
123 geomorphologic features (i.e., riffle-pool sequence, steps), is the primary control of

124 hyporheic exchange (Maddock et al. 1995, Vallet et al. 1996, Dahm et al. 1998, Calver 2001,
125 Ward et al. 2012, Gomez–Velez et al. 2014). These features act as obstacles to the water flow
126 along the open channel, extending the HZ both vertically and laterally from the stream
127 (Harvey & Bencala, 1993), and generating sequences of DW and UW zones (Savant et al.
128 1987, Hendriks & White, 1991, Harvey & Bencala 1993). For example, within a single
129 stream riffle, surface water enters the HZ at the beginning of the riffle (DW zone) and returns
130 to the open channel at the end of it (UW zone) (Hendrik & White 1991, Hendriks 1993,
131 Evans & Petts 1997).

132 Hyporheic exchange flows are generally faster in headwater streams, which typically
133 have shallow and steep reaches with cobble– and gravel–bed sediments (i.e. they are quite
134 porous). Hyporheic exchange is progressively slower, deeper and more complex as riverbed
135 sediments become finer (less porous) in slower flow zones (Buffington & Tonina 2009).
136 Nevertheless, quantifying this exchange is complicated because streambed materials range
137 from relatively homogeneous, to cases where the range of sediment sizes (and therefore the
138 hydraulic conductivity) exceeds six orders of magnitude (Calver et al. 2001).

139 In recent years a wide range of available sampling techniques has been developed to
140 determine the heterogeneous interactions between groundwater and surface water (Kalbus et
141 al. 2006). These methods range from direct measurements of water flux across the
142 groundwater–surface water interface (i.e. seepage meter, Lee 1977), to indirect techniques
143 such as heat tracer methods, mass balance approaches or mathematical modelling (Kalbus et
144 al. 2006). Until recently, these were almost exclusively employed in studies of hydrology and
145 engineering (Boulton et al. 2010). However, understanding the importance of hyporheic
146 exchange flow as a controlling factor of hyporheic communities and biogeochemical

147 processes has led to increased implementation of hydrodynamic analysis in recent ecological
148 studies of the HZ (Boulton 1993, Hendricks 1993, Standley and Boulton 1993, Schmid–
149 Araya 1998, Kasahara et al. 2009, Miyake & Shigeru 2002, Malard et al. 2003, Davy–
150 Bowker et al. 2006, Robertson & Wood 2010). One interesting strategy to address these
151 issues, which has been widely used in hydrological studies, is to implement time–series
152 analysis of streambed profiling–thermal records (i.e. Hatch et al. 2006, Keery et al. 2007,
153 Irvine & Lautz 2015, Irvine et al. 2015). These methods are based on quantifying changes in
154 phase and amplitude of temperature variations between pairs of subsurface sensors through
155 depth (Hatch et al. 2006). Nevertheless, results exclusively from these methods are often
156 limited and contradictory (Shanafield et al. 2011, Briggs et al. 2014).

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158 **Seasonality in flow exchange and its effect on riverbed communities**

159 Stream and rivers are dynamic ecosystems par excellence. The incessant open channel flow
160 produces continuous movements of substrata, changes streambed topography and reorganizes
161 the morphology of the channel boundaries. In this manner, the temporal dynamics of surface
162 flow in a lotic ecosystem is closely related to the spatial heterogeneity of flow paths and rate
163 of exchange through the HZ at all scales. The multiple temporal and spatial scales and the
164 rate of exchange collectively define the hyporheic residence time of water (Buffington &
165 Tonina 2009). The residence time is an important property of the HZ, because most of the
166 biogeochemical processes that occur in sediments depend on the rate of water flow through
167 them (Mulholland & De Angelis 2000, Duff & Triska 2000). Furthermore, the temporal
168 dynamic that affects the hydrological exchange also produces fluctuations of HZ boundaries

169 (Gibert et al. 1990) and this variation in the size of the HZ determines its influence on both
170 the open channel and the underlying groundwater (Vervier et al. 1992).

171 Seasonality in rivers is an extreme example of the temporal variation of the discharge
172 of the open channel (e.g. due to snowmelt or the alternation between dry and rainy seasons).
173 Accordingly, these changes alter the flow exchange patterns in the HZ (Kalbus et al. 2006)
174 and may act as disturbance events for sediment organisms (Robertson et al. 1995, Townsend
175 et al. 1997, Robertson 2000). These potential disturbances may be reduced in the HZ due to
176 its ability to maintain humidity after surface drying and remain stable during floods
177 (Robertson & Wood 2010). Thus the HZ might serve as a refuge for the local biota during
178 disturbances events enabling recolonization of the surface once the disturbance ends
179 (Williams & Hynes 1974, Dole–Olivier 2011). The HZ can also act a refuge for the early
180 instars of some macroinvertebrates due to the more stable environmental conditions and
181 reduced predator pressure (Williams 1984). Nevertheless, the importance of the HZ as a
182 refuge is debated (Robertson & Wood 2010), because some studies found no evidence of HZ
183 refuge use by aquatic invertebrate fauna (Boulton et al. 2002, Olsen & Towsned 2003, James
184 et al. 2008). In contrast, the importance of the HZ as a refuge might be more evident in
185 seasonal intermittent streams. These systems are common worldwide and support diverse
186 communities of aquatic organisms including many taxa that survive in dry riverbeds and/or
187 rapidly recolonize when water returns (Stubbington & Datry 2013, Datry et al. 2014). Indeed,
188 the influence of drought may be even more intense in streams that lack a marked seasonally
189 (unpredictable intermittent streams, López–Rodríguez et al. 2012). This is the case for some
190 Mediterranean streams with supra–seasonal drought, where many organisms that survived in
191 the HZ during the dry season recolonized the stream during the first month of the wet season

192 (López-Rodríguez et al. 2012). Use of the HZ as a refuge is not exclusive to large biota, it
193 occurs across a wide range of organism size. Febria et al. (2012) observed that biofilms also
194 use hyporheic sediments as a refuge from desiccation, mainly transported by hydrological
195 pathways through the sediments. During periods of drought, the HZ supports bacteria
196 associated with the infiltration of water and the creation of microhabitats in the sediment;
197 when interstitial pore spaces become filled with water during flood events, HZ and the
198 surface become connected allowing bacteria recolonization (Febria et al. 2012).

199 Another factor that markedly affects the hydrology of streams and rivers is the
200 seasonal change of in-stream vegetation cover. In-stream macrophytes are typically
201 abundant in many lotic ecosystems during spring and summer; altering river flow and
202 trapping sediments (Champion & Tanner 2000, Dodds & Biggs 2002). In-stream vegetation
203 may reach from 0% to over 70% of spatial coverage between winter and summer in European
204 rivers (Cotton et al. 2006). This increase in macrophytes is coupled with a drastic reduction
205 of the open channel flow velocity and the deposition of fine sediments (Cotton et al. 2006).
206 Nevertheless, to our knowledge, there is no published research that assesses the effect of in-
207 stream macrophyte dynamics on hyporheic flow and hyporheic communities. In addition,
208 daily fluctuations in stream flow may also be caused by evapotranspiration of vegetation
209 (including riparian vegetation) and it has been hypothesized that this transpiration enlarges
210 hyporheic flow paths during the day and decreases them at night (Wondzell et al. 2010).

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212 **Hyporheic zone as a budget of biomass and production in streams and** 213 **rivers**

214 Defining system boundaries is an important aspect of the study of ecological processes
215 (Smock et al. 1992). Streams and rivers have been viewed traditionally as having three
216 interactive spatial compartments: firstly the open channel and benthic zone (BZ), secondly
217 the HZ and thirdly the riparian zone (Cummins et al. 1983, Ward et al. 1989) and each
218 compartment could play a different role depending on the ecological process under study.
219 Secondary production is a useful measure of the energy flux (as biomass) produced by
220 heterotrophic organisms over time and space (Benke & Huryn, 2007). However, there are
221 only a few studies, which have determined the relative contribution of the HZ compartment
222 to whole system production (i.e. Smock et al. 1992, Collier et al. 2004, Wright–Stow et al.
223 2006, Reynolds & Benke 2012). Most previous studies have defined the top 0 to 5–10 cm of
224 sediments as the benthic zone (BZ), and lower depths as HZ (i.e. Smock et al. 1992, Collier et
225 al. 2004, Wright–Stow et al. 2006, Reynolds & Benke 2012) despite its proposed biological
226 definition (Table 1). In order to accurately define the limits of the HZ compartment, a small-
227 scale approach across a depth gradient is needed (i.e. variation in assemblage structure at
228 centimetre scale, determination of distributional limits of characteristic taxa from both
229 compartments).

230 Some studies have shown that invertebrate assemblages comprising a suite of
231 relatively few and large individuals near the surface are replaced by numerous but small-
232 bodied organisms with increasing depth (Schmid–Araya 1994, Stead et al. 2004). This is
233 because the taxa in the hyporheos differ in their ability to penetrate the HZ depending on their
234 biological attributes (Nogaro et al. 2009, Robertson & Wood 2010, Descloux et al. 2014).
235 The reduction in oxygen concentration and pore size due to sediment agglomeration along the
236 depth gradient (Fig 1) limits the distribution of large macroinvertebrates with higher

237 metabolic rates (Maridet & Philippe 1995, Strayer et al. 1997). As a result, the density of
238 meiofauna (microscopically small metazoans) and protists should increase with depth (Fig 1).
239 In fact, the reduction in density of large organisms through depth has been broadly reported
240 as a general pattern in studies of riverbed communities (i.e. Dole–Olivier et al. 1994,
241 Marchant, 1995, Maridet & Philippe 1995, Davy–Bowker et al. 2006, Pacioglu & Robertson
242 2017). Accordingly, it might be hypothesized that the depth gradient, as a set of different
243 physicochemical factors, is also a key variable causing the decline of biomass and secondary
244 production of riverbed systems. Our metadata analysis of invertebrate communities from
245 different river systems (using data from Smock et al 1992, Reynolds and Benke 2012)
246 corroborates this prediction, showing a negative and significant effect of depth both on
247 biomass and secondary production (Fig 2; an explanation of these analyses is available in
248 appendix 1). However, despite its significance, the regression model explains only a small
249 part of the observed variation (marginal $R^2 = 11\%$, and conditional $R^2 = 54\%$, see appendix 1)
250 and so other variables must be important. This meta-analysis is limited to macroinvertebrate
251 communities because studies of changes in biomass and secondary production along the
252 depth gradient have focused exclusively on large size organisms ignoring most meiofauna
253 and the Protozoa.

254 Remembering that taxa comprising the hyporheos differ in their ability to penetrate
255 into the HZ depending on their size, we expect a significant interaction between depth and
256 size group (flagellates, ciliates, meiofauna and macroinvertebrates). Including these groups
257 and their interaction with depth, hydrology and sediment characteristics in future research
258 studies would notably improve predictive modelling and compartment comparisons. This is
259 of particular interest because it has been proposed that the bioreactor ability of the HZ is

260 sustained and maintained by diverse and active hyporheic communities (Krause et al. 2009).
261 Accordingly, it might be predicted that hot spots of nutrient and pollutant transformation may
262 coincide with areas containing higher biomass and secondary production rates. It could also
263 be expected that the role of different organisms in the bioreactor would vary following the
264 depth gradient. For example, bioturbation and bioirrigation resulting from life activities of
265 relatively large burrowers (such as Chironomidae larvae, Ephemera nymphs or
266 Oligochaeta), would be more important in the benthic zone and upper layers of the HZ than
267 in deeper layers. These processes promote sediment permeability, respiration of freshwater
268 sediments and bacterial activity (Betrics and Ziebis 2009, Hölker et al. 2015, Baranov et al.
269 2016), and so have a great impact on water biogeochemistry (Morad et al. 2010).

270

271 **The hyporheic bioreactor**

272 Flow exchange and pore water chemistry of the HZ can be also affected by anthropogenic
273 activity, typically with negative effects on ecosystem health. A common alteration that occurs
274 in rivers across the world is the artificial water input from wastewater treatment plants
275 (WWTPs) (Carey & Migliaccio 2009) due to which many rivers receive permanent or pulsed
276 inputs of nutrients (i.e. organic carbon, nitrate, phosphate) and other pollutants (i.e.
277 pesticides) as a result of human activities (Boyer et al. 2006, Mulholland et al. 2008).
278 However, once these compounds penetrate into the sediments as a consequence of the
279 hydrological patterns, they may be transformed into oxidized or reduced substances by
280 metabolic reactions, mediated by active and productive hyporheic communities (Krause et al.
281 2009, Bardini et al. 2012, Sánchez-Perez et al. 2013).

282 Accordingly, the HZ acts as a true water-purifying bioreactor in which microbial
283 biofilms play an important role. Hyporheic biofilms are dominated by highly diverse bacteria
284 and archaea communities embedded in the same matrix of polysaccharides (Battin et al.
285 2016). This results in the coexistence of a great range of operational taxonomic units (Zeglin
286 2015), diverse metabolic capabilities (Singer et al. 2010, Battin et al. 2016) and sites of high
287 enzymatic activity (Romaní et al. 2008). Thus, in-stream biofilms are important components
288 of the global biogeochemical fluxes of carbon, nitrogen and phosphorous (Mulholland et al.
289 2008, Battin et al. 2008, Boano et al. 2014). The supply of nutrients is assumed to be a
290 limiting factor in determining the biomass, activity, and physiology of subsurface microbial
291 communities (Bengtsson 1989). Thus bacterial biomass and metabolic activity should be
292 significantly greater under situations of higher input of DOC (Foulquier et al. 2011). In
293 addition, water is pumped in and out of the HZ and riparian zone on a daily cycle because
294 water stage variation, generates large hydraulic gradients and enhanced mixing in highly
295 regulated rivers (Gerecht et al. 2011). These daily fluctuations of the river stage stimulate
296 bacterial respiration and organic carbon turnover (Stegen et al. 2016).

297

298 **Micropollutants, the new challenge for the hyporheic bioreactor**

299 Recent research has shown that nitrogen, phosphorus and organic carbon are
300 important pollutants in the HZ and aquatic ecosystems generally (i.e. Lewandowski &
301 Nützmann, 2010, Bardini et al. 2012, Harvey et al. 2013, Maazouzi et al. 2013). However,
302 surface water systems and their interactions with groundwater systems are increasingly under
303 pressure from a new group of chemicals; the micropollutants (Langenhoff et al. 2013). The

304 occurrence of micropollutants (such as pharmaceutical and personal care products; i.e.
305 ibuprofen or antibiotics) due to WWTPs inputs has greatly increased in stream and rivers.
306 The concern about their presence is mainly related to potential adverse effects on
307 environmental systems (i.e. bioaccumulation) and to human toxicology (i.e. aquifer
308 contamination) (Hernández Leal et al. 2010, Langenhoff et al. 2013). Furthermore, the
309 chronic low–level antibiotic exposures detected in aquatic systems acts as a selective process
310 on bacteria communities (Hirsch et al. 1999, Yang & Carlson 2003). Thus, differential
311 antibiotic tolerance of a bacterial community may produce a shift in the biofilm structure
312 (composition, richness, density), affect the spatial distribution of members of the community
313 (Roose–Amsaleg & Laverman 2016) and change the ability of biofilms to conduct ecosystem
314 services (e.g. reducing denitrification processes due to deleterious impacts on denitrifying
315 bacteria, Costanzo et al. 2005). However, in some cases, micropollutants can be efficiently
316 attenuated along flow paths in the HZ (Lewandowski et al. 2011). Indeed, some of these
317 compounds (i.e. diclofenac, bezafibrate, ibuprofen, and naproxen) are more efficiently
318 transformed in river sediments than in WWTPs by biofilms (i.e. Schulz et al. 2008, Radke et
319 al. 2009). This is mainly related to the higher diversity of microbial communities in
320 environmental systems. In addition, water residence times in the HZ are longer than in the
321 open channel and surface sediments (and WWTP), allowing more efficient biodegradation
322 processes (Lewandowski et al. 2011).

323 Notwithstanding the role of the biofilm is recognized in pollutant attenuation, more
324 complex questions behind this ecological process remained unanswered (i.e. the role of the
325 rest of the community). Furthermore, it is also important to consider the hierarchical
326 interaction between hydrological patterns and biogeochemical processes in the study of the

327 nutrient and pollutant breakdown in the HZ. Hydrodynamics throughout the sediments may
328 induce two opposite effects on solute reactions. Higher inward water fluxes lead to a larger
329 input of substances into the HZ enhancing reaction rates, but also the hyporheic microbiota
330 has less time to perform biogeochemical reactions due to the lower residence times of the
331 compounds in the sediments (Fig 3a) (Bardini et al. 2012). These mechanisms will become
332 even more complex when we recall that hydraulic conductivity may also be affected by the
333 action of the hyporheos. Growth of biofilm matrices in the sediment pores reduces
334 permeability and increases residence times of water in the HZ (Findlay & Sobczac, 2000,
335 Battin et al. 2003). In addition, biofilm theory holds that uptake of solutes is diffusion–
336 limited by the thickness of the biofilm polysaccharide matrix (Gantzer 1988). Before
337 assimilation, solutes must pass first from the pore water to the biofilm surface (external mass
338 transfer) and then through the biofilm matrix to the cells (internal mass transfer) (Battin et al.
339 2003). However, sediments are constantly being reworked, increasing the sediments
340 permeability locally (Boulton, 2000). Life activities of macroinvertebrates, meiofauna and
341 protists (e.g. ciliates and flagellates) in the HZ (digging, removing the sediments and grazing
342 on biofilms) result in preferential flow paths, increasing biofilm surface and boosting
343 bacterial densities (Danielopol 1976, Boulton 2000, Mermillod–Blondin et al. 2003, Battin et
344 al. 2003), acting as ecosystem engineers (Fig 3c, d). Thus, the net effect on breakdown rates
345 depends on the balance between all these opposing factors (Arnon et al. 2007, Cardenas et al.
346 2008, Bardini et al. 2012). In this manner, mechanistic understanding of biofilms function
347 can be acquired only through carefully designed experiments under well–defined conditions
348 and appropriate cultivation techniques (Singer et al. 2006). Accordingly, controlled
349 experiments are needed to explore the underlying causal mechanisms that generate the

350 patterns seen in reach-scale descriptive surveys (Boulton et al. 1998, Olsen and Townsend
351 2003).

352

353 **Concluding remarks**

354 Ecological research in the HZ is still a challenge due to the great number of variables
355 involved in its operation. Furthermore, there is a notable hierarchy of these variables, many
356 of them being nested or correlated spatially and temporally. Its study is also becoming one of
357 the most developed areas in freshwater science in recent years, principally due to the
358 development and increasing accessibility of better technological approaches. Nevertheless,
359 studies on the role of the HZ in the functioning and ecosystem service delivery of the whole
360 river system are still maturing and remain a major research focus and challenge for future
361 freshwater researchers (Robertson and Wood 2010). Complex and interesting questions about
362 how pollutants and nutrients are transformed by the hyporheos during their travel through the
363 HZ, or how hyporheic exchange flow may determine energy fluxes of these communities are
364 still unanswered. Furthermore, future research should also consider and focus on a more
365 thorough understanding of impacts caused by rivers regulations on hyporheic exchange flow
366 (Gerecht et al. 2011). Accordingly, holistic and truly interdisciplinary approaches at the
367 interface of hydrology, geomorphology and ecology will be the only valid strategy to assess
368 all these issues (Pacioglu 2010, Boulton et al. 2010, Robertson and Wood 2010).

369

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375

376 **Appendix**

377 Biomass and secondary production data along the depth gradient were extracted from
378 reported values in Smock et al. (1992) and Reynolds and Benke (2012). A similar sampling
379 method (sediment corers) and spatial scale resolution were used in both studies. Smock et al.
380 (1992) reported biomass and secondary production of the whole community of
381 macroinvertebrates in a sandy-sediment river (Buzzards Branch). While, Reynolds and
382 Benke (2012) measured genus-specific biomass and secondary production of chironomid
383 larvae (Diptera) assemblage (composed of 26–31 genera) along a hyporheic gradient,
384 comparing gravel-cobble of a high-alkalinity stream (Hendrik Mill Brach) with sandy and
385 mud-silt habitats of a low-alkalinity stream (Payne Creek). The chironomid assemblages
386 were reasonably similar between studied streams. Furthermore, chironomid larvae are usually
387 the dominant and richest invertebrate group in freshwater benthic habitats (Ferrington et al.
388 2008). Hence, it could be assumed as a good model group to infer general production patterns
389 of invertebrates.

390 Two linear mixed effect models (LMMs) were applied to test the effect of, depth
391 (continuous covariant) on biomass and production (responses). Responses were Log_{10}
392 transformed to solve heterogeneity in the residuals. The Akaike Information Criterion (AIC)

393 was then used to find the most parsimonious model by combining the fixed term (depth) and
 394 potential random effects (i.e. studied site, differences between taxa). As a result, biomass and
 395 production models included depth (single covariate), study site (studied river as random
 396 intercept) and the interaction between depth and taxa (random slope) as effective parameters:

$$397 \quad \text{Log}_{10}(\hat{y}_{ij}) = \beta_0 + a_{site} + \beta_1 \times \text{Depth}_j + b_i \times \text{Depth}_j + \varepsilon_{ij}$$

$$\varepsilon_{ij} \sim \text{Norm}(0, \sigma^2)$$

$$a_{site} \sim \text{Norm}(0, \sigma_{site}^2)$$

$$b_i \sim \text{Norm}(0, \sigma_i^2)$$

398 where \hat{y}_{ij} is the biomass or production for each taxa i at depth j (0,10,...,50). Intercept of the
 399 model is given by $\beta_0 + a_{site}$ with changes randomly by a_{site} , and $b_i \times \text{Depth}_j$ represent the
 400 random variation of slope β_1 .

401 Model validation was applied following Zuur et al. (2009). Previous models were
 402 fitted using the restricted maximum likelihood estimation (REML) with functions *lmer* of the
 403 R package lme4 (Boulton et al. 2016, R Core Team 2016). Finally, 5000 values from the
 404 posterior joint distribution of the model parameters were simulated with the function *sim* of
 405 the R package arm (Gelman and Hill 2007). This function uses an analytical direct-
 406 simulation method with uninformative priors (Korner–Nievergelt 2015). Obtained means of
 407 the simulated values from the joint posterior distribution of model parameters were used as
 408 estimates, and the 2.5% and 97.5% quintiles as lower and upper limits of 95% credible
 409 intervals. Finally, the marginal and conditional R^2 (as a technique to describe the predictive
 410 capacity of mixed effect models; Nakagawa & Schielzeth 2013) was calculated to assess
 411 model fit.

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807 **Legend Figures and Tables**

808 **Table 1.** Glossary of terms.

809 **Fig 1.** BOX 1: Scheme of the streambed community distribution throughout the depth profile
810 in relation to pore size and redox potential. Arrows represent colonization depth of large
811 macroinvertebrates (a), temporary and permanent meiofauna (b) and Protozoa (c). Also
812 shown is the theoretical boundary between benthic zone (BZ) and hyporheic zone (HZ) as the
813 colonization limit between benthos and hyporheos. The grey/black scale in the sediment
814 profile indicates the redox potential with dark black as strongly anoxic conditions. BOX 2:
815 Body size and density distributions of different groups in the community structure.
816 Organisms are not drawn to scale.

817 **Fig 2.** Depth-related biomass and production of invertebrates based on reported values from
818 Smock et al. (1992) and Reynolds Jr. and Benke (2012). Predictions (black line) represent the
819 \log_{10} biomass and production values and are derived from the linear mixed models explained
820 in the Appendix, with 95% credible intervals (shaded grey). Open circles represent the \log_{10} -
821 transformed values per taxa. Note that even though the model detected the negative effect of
822 depth on the responses, the marginal R^2 is low. This explains the large dispersion in the
823 scatter plot and evidences the necessity of including more factors (i.e. taxonomic group,
824 hydrological conditions in future analyses).

825 **Fig 3.** The ability of the HZ to process dissolved solutes is mediated by a hierarchical
826 interaction between hydrological patterns and community ecology. (a) Daily and seasonal
827 fluctuations between the high river-stage (H-RS) and low river-stage (L-RS) cause variation
828 in the open channel discharge and the water table stage (dashed lines in the figure represent

829 high and low water table stage). **(b)** The increase in the surface flow promotes more input of
830 water (higher sub-surface flow) and input of dissolved solutes into the HZ, but also the
831 residence time of water in the sediments decreases. The grey/black scale indicates the redox
832 potential with dark black as strongly anoxic conditions. **(c)** Life activities of
833 macroinvertebrates and meiofauna result in bioturbation and biorrigation phenomena in the
834 streambed sediments, causing the occurrence of preferential flow paths and increasing
835 permeability locally. **(d)** Protists grazing on biofilms increase its absorption surface. As a
836 result, dissolved solutes diffusion gradient is higher in presence of grazers **(d1)** than in their
837 absence **(d2)**.

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TABLE 1

Term	Definition	Source
Hyporheic zone (Original definition)	The interstitial habitat beneath a stream, bordered by the surface water above and by the true groundwater below	Orghidan (1959)
Hyporheic zone (Geochemical definition)	The volume of sediment containing a specified percentage of surface water	Tonina and Buffington (2009)
Hyporheic zone (Hydrological definition)	The volume of sediment where water exchange between open channel and groundwater occur as a result of streambed pressure gradients and hydraulic conductivity	Tonina and Buffington (2009)
Hyporheic zone (Biological definition)	The volume of sediments housing a characteristic hyporheic community. This community can be defined as occasional users or permanent users	Tonina and Buffington (2009)
Hyporheic zone (Integrative definition)	Any location meeting four key criteria: [1] Saturate surface. [2] Existence of flow path that originate from and return to surface water. [3] Interaction with the stream occurs within a temporal scale relevant to the	Ward (2016).

	processes of interest. [4] Processes of interest occur continuously from the subsurface to the groundwater continuum.	
Hyporheos	The biota occupying saturated interstitial spaces below the stream surface (benthic zone)	Standley & Boulton (1993)
Upwelling (UW) zone	High-pressure areas in riverbed, where surface water comes out from HZ to the open channel	Franken et al. (2001)
Downwelling (DW) zone	Low-pressure areas in riverbed, where surface water enter in the HZ	Franken et al. (2001)
Hyporheic exchange flows	Strength and direction of the water mass through the sediment pore spaces in the HZ, resulting from the alternation of UW and DW zones	This article
Micropollutants	A vast and expanding array of emerging contaminants (including pharmaceuticals, personal care products, steroid hormones, industrial chemicals and pesticides) commonly present in waters at trace concentrations, ranging from a few	Luo et al. (2014)

	ng/L to several µg/L.	
Hyporheic bioreactor	Active biological system in which the transformation of chemical compounds occurs as result of the hyporheos life activities or the active substances they produce.	Lewandowski et al. (2011)
Residence time	Hydrodynamic retention time in the HZ during which biogeochemical processing of dissolved solutes occur	Buffington & Tonina (2009)

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FIG 1

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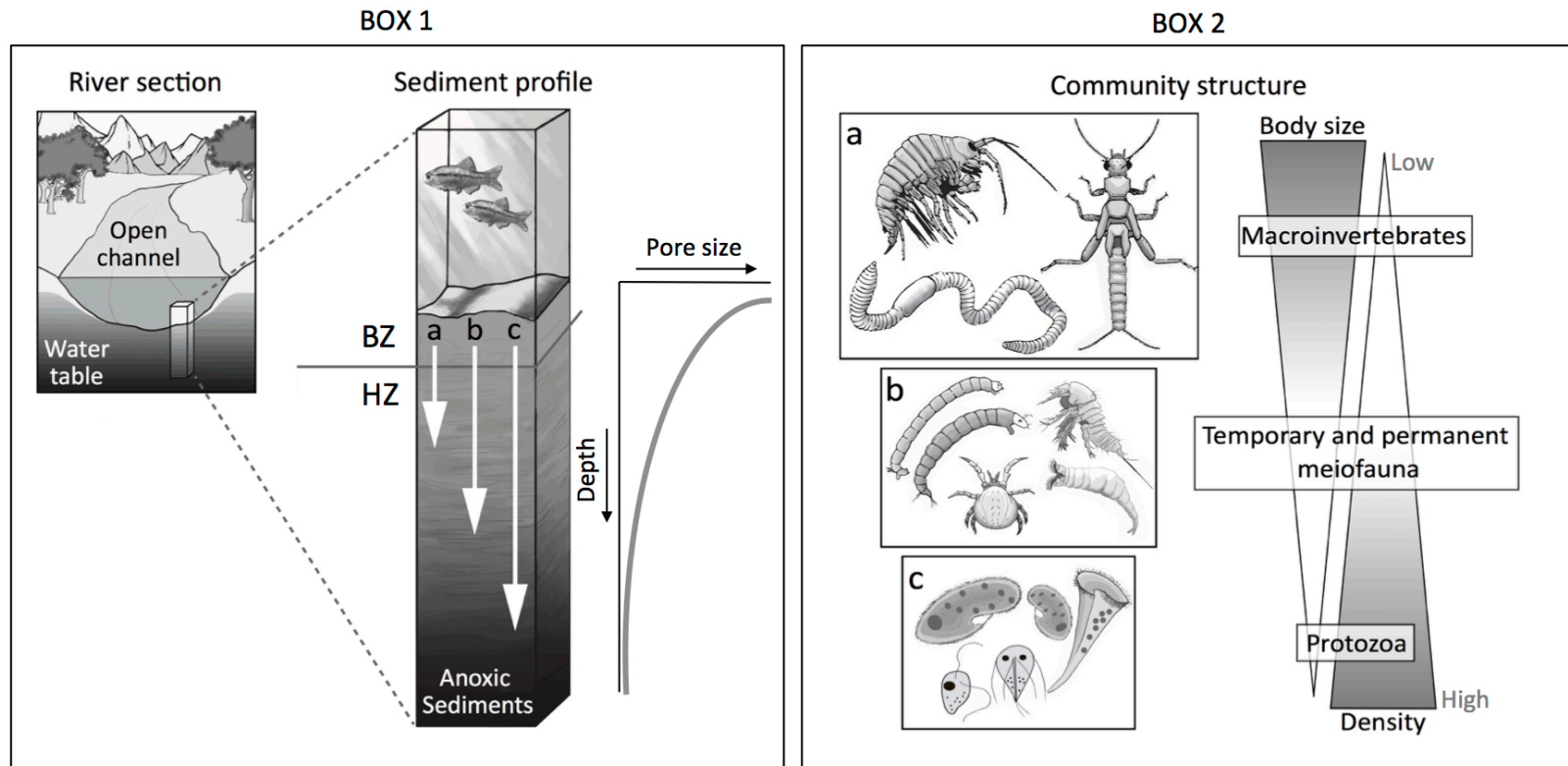


FIG 2

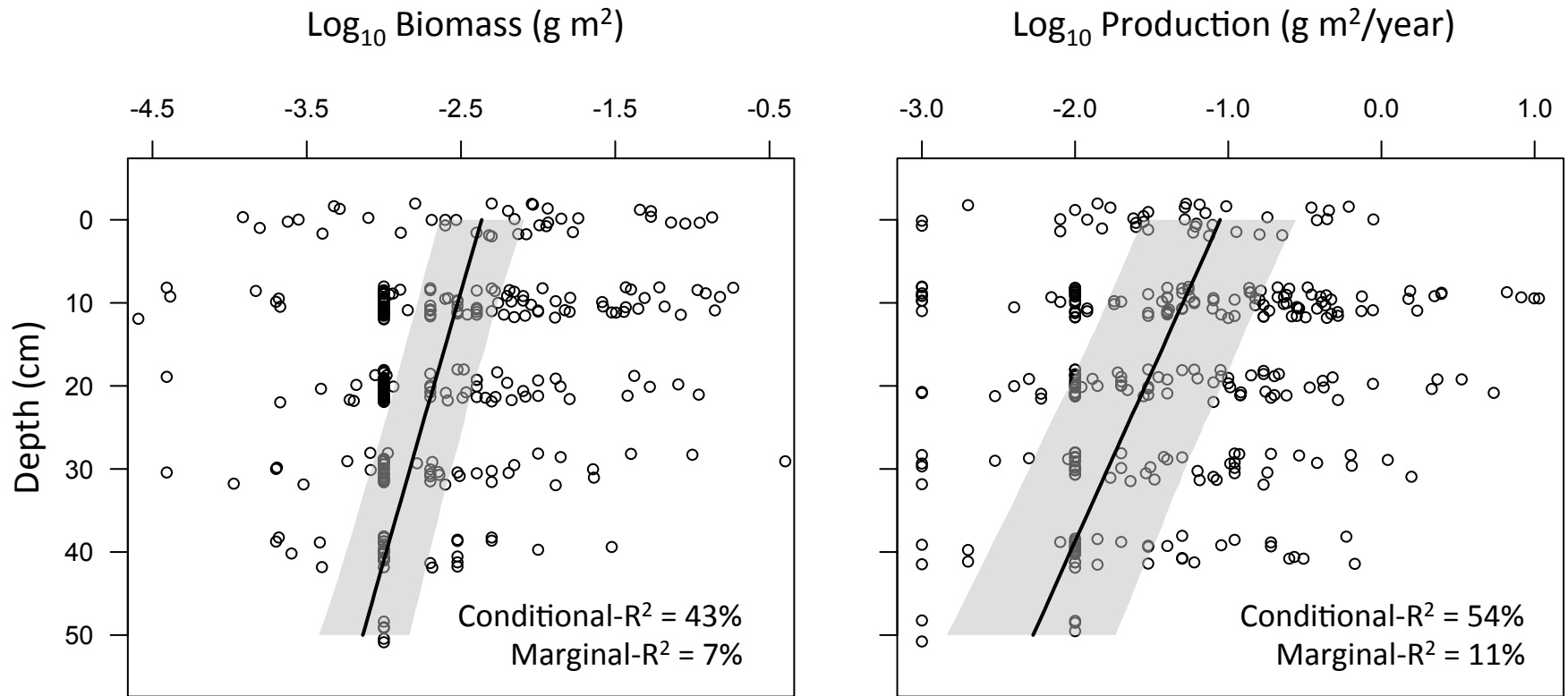


FIG 3

