

1 **Does land use impact on groundwater invertebrate diversity and functionality in**
2 **alluvial wetlands?**

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26

27 **Abstract**

28 Land use change, especially the transformation of riparian forest to agricultural fields,
29 plays an important role in groundwater quality. Yet little is known about the effects of
30 land use change on groundwater invertebrate assemblages and diversity. This study
31 assesses for the first time the effect of land use (agricultural vs. forest use) on the
32 groundwater invertebrate community of four river floodplains representative of variable
33 conditions across SW Europe (the Garonne River in France, and the Bidasoa, Ebro and
34 Tajo Rivers in Spain). Groundwater invertebrate and water samples were collected
35 quarterly in 8 to 12 piezometers located in each floodplain over a year. Taxonomic and
36 functional diversity indices and ecosystem services (i.e. biogeochemical filtration and
37 particulate organic matter breakdown) were calculated. The taxonomic and functional
38 diversity of groundwater invertebrate communities increased linearly with the
39 increasing surface occupied by riparian forests and decreased under intensified
40 agriculture use. Moreover, the provision of key ecosystem services related to the
41 biogeochemical filtration and particulate organic matter breakdown also increased 2-
42 fold under more natural forest land. According to regression models, this is likely due to
43 the pressure of agricultural practices on groundwater quality, with high concentrations
44 of nitrates and sulphates exerting a negative influence over groundwater invertebrate
45 diversity and their associated ecosystem services. The results of this study have
46 important management implications, and suggest that the presence of large riparian
47 corridors enhances groundwater invertebrate diversity and functionality in floodplains
48 deeply disturbed by agricultural practices.

49

50 **Highlights**

- 51 • We study the effect of land use on groundwater invertebrate community.
- 52 • Groundwater invertebrate community change between land use types.
- 53 • Invertebrate diversity and ecosystem services increases with forest occupancy.
- 54 • Agriculture has a negative influence on groundwater invertebrate communities.
- 55 • Water quality controls groundwater invertebrate diversity and ecosystem
- 56 services.

57

58 **Keywords**

59 Biogeochemical filtration; biological traits; ecosystem services; particulate organic

60 matter breakdown; floodplain; stygofauna.

61

62 **1. Introduction**

63 Subterranean habitats have been traditionally considered as a biological desert, only
64 interesting as a major source of water resources (Gibert et al., 1994). However, recent
65 studies have demonstrated that groundwater systems are home to a great variety of
66 cryptic and endemic organisms (Avramov, 2014; Deharveng et al., 2009; Sket, 1999),
67 which provide important ecosystem services ranging from water purification and
68 bioremediation to water infiltration and transport (Boulton et al., 2008). Unfortunately,
69 the spread of groundwater habitats has also favoured their degradation, mainly because
70 of intensification of agriculture, forest clearance, industrial activities, groundwater
71 extraction, river flow regulation, and waste water discharge (Boulton et al., 2010;
72 Danielopol et al., 2003; Griebler et al., 2010; Tockner et al., 2008). These pressures
73 reduce the hydrological connection between ground and surface waters, and favour the
74 accumulation of pollutants that impair the characteristic biological structure and
75 functionality of groundwaters (Danielopol et al., 2003; Korbel et al., 2013).

76 Since the early 2000s, the importance of groundwater as a living ecosystem has
77 increased. Scientists and environmental managers are working to develop guidelines
78 and strategies for the conservation of groundwater ecosystems, taking into account their
79 biota and functionality (Gibert and Culver, 2009; Gibert et al., 2009; Gibert and
80 Deharveng, 2002). The majority of studies focus on describing groundwater
81 invertebrate (also called stygofauna) distribution patterns, such as the recent European
82 PASCALIS project (<http://pascalis.univ-lyon1.fr/>; Castellarini et al., 2007; Gibert et al.,
83 2009). Results from this project have identified temperate areas as hot-spots of
84 groundwater biodiversity, and have suggested that the full potential of groundwater
85 ecosystems as sources of biodiversity and ecosystem services is still to be fully

86 comprehended (Deharveng et al., 2009). For this reason, researchers have proposed the
87 use of groundwater invertebrates as bioindicators of ecological groundwater health in
88 the same way that they are used in surface waters (Boulton et al., 2008; Iepure et al.,
89 2013). The distribution of groundwater organisms indeed responds to hydrogeological
90 and environmental factors, such as floods, dissolved organic carbon, and the
91 concentration of nutrients and pollutants (Castellarini et al., 2007; Danielopol et al.,
92 1994; Di Lorenzo et al., 2014; Iepure et al., 2014, 2013;), which makes them suitable
93 candidates to reflect the ecological status of subterranean habitats. Furthermore,
94 groundwater invertebrates contribute to the functioning of groundwater ecosystems, and
95 consequently to the provision of groundwater ecosystem services used by humankind.
96 For example, amphipods and isopods are keystone taxa groups that enhance the
97 decomposition of organic matter, which in turn stimulates bacterial growth and activity,
98 supporting the biodegradation of contaminants (Tomlinson and Boulton, 2010; Ward et
99 al., 1998). These crustaceans also recycle nutrients through the excretion of faecal
100 pellets, and increase the flux of oxygenated water through burrowing (Hakenkamp and
101 Palmer, 2000). However, such functional processes have been poorly studied,
102 preventing the development of consistent ecological criteria for assessing groundwater
103 ecological status. More research is thus needed to ascertain the response of subterranean
104 biodiversity patterns and functionality to both natural and human-induced changes
105 (Culver and Sket, 2000; Deharveng et al., 2009; Hancock et al., 2005; Korbel et al.,
106 2013).

107 One of the most interesting subterranean habitats is the hyporheic zone, which is the
108 area of mixing between surface and subsurface water (White, 1993; Wondzell, 2011).
109 As surface water contains rich oxygen and organic matter and groundwater contains

110 abundant nutrient elements, the water mix between those two systems has a
111 significant impact on water quality, ecosystems and biogeochemistry cycling (Boulton
112 et al., 1998; Brunke and Gonser, 1997; Krause et al., 2013; Marmonier et al., 2012;
113 Sanchez-Pérez et al., 2003; Sánchez-Pérez and Trémolières, 2003; Vervier et al., 2009).
114 These ecosystems support important agricultural activities, and, consequently,
115 groundwater in alluvial plains often suffer from nitrate pollution (Arrate et al., 1997;
116 Almasri and Kaluarachchi, 2007; Liu et al., 2005; Sánchez-Pérez et al., 2003a). Several
117 studies show that the hyporheic zone (i.e. surface-groundwater interface) contributes to
118 nitrogen retention and/or transformation of the land-surface water continuum (Sabater et
119 al., 2003; Weng et al., 2003). This zone supports the purification of water by its ability
120 to eliminate nitrates during their infiltration through the vegetation-soil system to
121 groundwater, but also through diffusion from groundwater to surface water (Sanchez-
122 Perez et al., 1991a, 1991b; Takatert et al., 1999). However, little is known about the
123 groundwater biota and its role in these purification processes, and, definitely in the
124 general ecosystem functioning.

125 To this end, this study evaluates for the first time the effect of land use on the
126 groundwater invertebrate community of the hyporheic zone in four river floodplains
127 located across SW Europe. First, we hypothesized that forest areas would show higher
128 groundwater invertebrate diversity than agricultural areas, since forests provide high
129 heterogeneity and quality in terms of resources (Brunke and Gonser, 1997; Datry et al.,
130 2005). Moreover, we expected the groundwater invertebrate community to change
131 along a land use gradient, showing varying characteristic taxa and functions. For
132 example, the vegetation of forest areas adds debris to the system, increasing the organic
133 matter content in soils and groundwater. Consequently, the presence of detritivores and

134 omnivores, which are essential for organic matter decomposition and nutrient cycling in
135 groundwater ecosystems, is favoured (Avramov, 2014; Boulton et al., 2008; Vannote et
136 al., 1980). In contrast, pollution-tolerant taxa are likely to dominate agricultural areas
137 because of the high levels of fertilizers and manures employed, as was observed by
138 Dumas and Lescher-Moutoué (2001). Thus, our study allows us to evaluate the overall
139 role of riparian corridors as natural biofilters of pollutants from agricultural areas.
140 Ultimately, this study is essential for understanding the local variation in groundwater
141 assemblages and their response to human pressures, contributing to the development of
142 suitable conservation and management policies.

143

144 **2. Material and methods**

145 *2.1 Study area*

146 For this study, we selected four river floodplains located in South-western Europe:
147 Garonne, Ebro, Bidasoa and Tajo. Floodplains were selected in areas characterized by a
148 combination of agricultural occupation and patches of natural riparian forest that might
149 favour the potential degradation of agricultural pollutants. The study covers a wide
150 gradient of climatic and environmental conditions, from floodplains with little forest use
151 to others with a high forest surface. To our knowledge, no studies on groundwater
152 invertebrates have been performed in any of the four selected floodplains.

153 The Garonne study area was located 30 km north of Toulouse in South-West France
154 (UTM 355758/4861267; Figure 1, Table 1). This floodplain site is dominated by
155 agriculture, mainly corn, sunflower and poplar plantations (Table 1). Its riparian forest
156 is dominated by poplar (*Populus* spp.), ash (*Fraxinus* spp.) and oak (*Quercus* spp.). The
157 river flow at the study site is very variable, peaking in winter and spring ($2880 \text{ m}^3 \cdot \text{s}^{-1}$,

158 during the study period that corresponds to a 10 years return period) and declining in
159 summer ($47 \text{ m}^3 \cdot \text{s}^{-1}$).

160 The Ebro study site was located 12 km downstream of Zaragoza, Spain (UTM
161 686179/4607010; Figure 1, Table 1). It is mostly subjected to agricultural land use;
162 mainly corn and irrigated cereals, but it shows an extensive riparian corridor dominated
163 by white poplar (*Populus alba*), European black poplar (*Populus nigra*), white willow
164 (*Salix alba*) and salt cedar (*Tamarix* spp.) (González et al., 2010). River discharge
165 varied from 205 to $1450 \text{ m}^3 \cdot \text{s}^{-1}$ during the study period.

166 The Bidasoa study area was located 7 km upstream of Irun, Spain (UTM
167 602190/4797250, Figure 1). Agriculture (mainly corn and pasture) and livestock
168 practically cover the entire floodplain surface, leaving a tight riparian corridor along the
169 river shore (Table 1). White willow (*Salix alba*), alder (*Alnus glutinosa*) and ash
170 (*Fraxinus excelsior*) dominate this riparian corridor, and the invasive species American
171 pokeweed (*Phytolacca Americana*) is also abundant. River discharge varied between 9
172 and $630 \text{ m}^3 \cdot \text{s}^{-1}$ during the studied period.

173 The Tajo study site was located 30 km downstream of Toledo, Spain (UTM
174 380482/4410115; Figure 1, Table 1). This floodplain site is under extensive agriculture;
175 mainly irrigated cereals, corn and pasture (Table 1). Only some isolated patches of
176 riparian forest remain, which are dominated by white poplar (*Populus alba*) and salt
177 cedar (*Tamarix* spp.). The river is highly regulated by dams and water derivations, with
178 a discharge that hardly varied during the study period (10 to $12 \text{ m}^3 \cdot \text{s}^{-1}$).

179 The piezometric network in each floodplain was designed considering the floodplain
180 surface and land use. For this study, we sampled 11 piezometers in the Garonne River,
181 12 piezometers in the Ebro River, 7 piezometers in the Bidasoa River, and 10

182 piezometers in the Tajo River, covering different percentages of land use surface (see
183 Figure 1). More detailed information about the piezometric network design,
184 environmental conditions, and hydrodynamics of each studied area can be found in
185 Antiguada et al. (to be submitted in the same special issue) and Bernard-Jannin et al.
186 (to be submitted in the same special issue).

187 We calculated the surface occupied by each type of land use within a buffer of 50 m
188 around each piezometer using QGIS v2.6.1. For the buffer selection, we took into
189 account the groundwater velocity in the alluvial aquifers (i.e. hydraulic conductivity).
190 For the hydraulic conductivity range (10^{-3} to 10^{-5} $\text{m}^3 \cdot \text{s}^{-1}$) in our studied areas, we
191 estimated that a particle (e.g. nutrients, pollutants) would move between 1 and 100
192 metres per day. It is for this reason that we selected an average surface of influence (i.e.
193 buffer) of 50 metres. Each piezometer was classified as agriculture-dominated when this
194 land use represented $\geq 40\%$ of the buffer surface and otherwise as forest-dominated.

195 *2.2 Taxonomic and functional composition of the invertebrate community*

196 One groundwater invertebrate sample was collected at each piezometer every three
197 months for one year: (i.e. April/May 2013, July/August 2013, October/November 2013
198 and January/February 2014). This meant that 90-100 L of groundwater were extracted
199 at each piezometer with a manual pump (based on Malard et al., 2002) and filtered
200 through a net with a mesh of 65 μm . Samples were preserved in situ in 70% ethanol.
201 Groundwater invertebrate samples were sorted and identified in the laboratory at least to
202 order level (Table A1, Appendix).

203 At the taxonomic level, three metrics were calculated: the total abundance of
204 individuals, the total richness of taxa, and the Shannon-Wiener diversity index. To
205 characterize the functional composition of the macroinvertebrate community, we used

206 feeding habits, the biogeochemical filtration capacity, and the particulate organic matter
207 (POM) breakdown capacity. In terms of feeding habits, we used affinity scores from
208 zero to five for each of the following habits: absorber, deposit feeder, shredder, scraper,
209 filter-feeder, piercer, predator, and parasite. We used a fuzzy coding approach based on
210 scores published in Tachet et al. (2002). For those taxa not included in this reference
211 guide, we classified organisms using information from the scientific literature as well as
212 expert consultation (Table A2, Appendix). A score of zero indicated no affinity, and a
213 score of five indicated the highest affinity of the taxon to a particular feeding habit. For
214 taxa identified at higher taxonomic levels than genera, the most frequent score across all
215 taxa belonging to a particular taxonomic group was selected. This may have resulted in
216 a certain underestimation of functional diversity, although according to Dolédec et al.
217 (2000) the overall functional structure of the invertebrate communities is conserved.
218 The matrix containing sites per taxa abundance was multiplied by the taxa per feeding
219 habit matrix to calculate three functional diversity metrics: abundance of individuals
220 showing affinity for each feeding habitat (i.e. functional abundance), total richness of
221 feeding habits (i.e. functional richness), and the Shannon-Wiener diversity of feeding
222 habits (i.e. functional Shannon diversity index). To assess the biogeochemical filtration
223 and POM breakdown capacities of the community, we used the functional scores
224 defined by Boulton et al. (2008). Thus, for each taxon, a biogeochemical filtration and
225 POM breakdown efficiency score was assigned: 0: no or unknown direct role, 1: minor
226 role, 2: moderate role, and 3: major role (Table A2, Appendix). We expressed the
227 biogeochemical filtration capacity of the groundwater community as the product of the
228 absolute abundance of each taxon by its efficiency score.

229 Taxonomic and functional diversity metrics were computed with the ‘vegan’ (Oksanen
230 et al., 2013) and ‘ade4’ (Thioulouse et al., 1997) packages of R software, 2.15.3 (R
231 Core Team, 2013).

232 *2.3 Environmental variables*

233 Water samples were collected at each piezometer with a submersible pump. To ensure
234 that the water sample corresponded to the aquifer and not stagnant water accumulated in
235 the piezometer, we previously extracted groundwater until conductivity and oxygen
236 values were constant (Griebler et al., 2010; Sánchez-Pérez, 1992). Water samples were
237 transferred into a 1.5 L PVC bottle previously washed in acid (CIH 0.1 N), and placed
238 on ice for transportation to the laboratory (see total number of samples in Table 1).

239 The alkalinity of unfiltered water samples was estimated within 4 h of collection by
240 automatic titration with H₂SO₄ 0.04 N (APHA, 1989). Total suspended solids, total
241 dissolved solids and organic matter content were determined by the gravimetric method,
242 i.e. filtering of samples through pre-combusted (450°C, 4 h) Whatman GF/F glass-fibre
243 filters following standard protocols (APHA, 1989). Filtered water aliquots were stored
244 at -20 °C and used within one month for the following analyses. Ion chromatography
245 (Metrohm 861 Advanced Compact IC; APHA, 1989) was applied to determine
246 ammonia (NH₄⁺), nitrate (NO₃⁻) and sulphate (SO₄²⁻) concentration. Modified Berthelot
247 reaction using salicylate and dichloroisocyanurate was used to determine the amount of
248 ammonia (NH₄⁺) by the colorimetric method (Krom, 1980) for Bidasoa River samples.
249 Soluble reactive phosphorus (SRP) was measured by the ascorbic acid method and
250 determined by the colorimetric method (APHA, 1989). Total dissolved phosphorus
251 (TDP) was also estimated by the ascorbic acid method, but potassium persulphate
252 digestion was performed beforehand (90 min, 115 °C) (APHA, 1989). Finally, water

253 temperature, pH, conductivity, dissolved oxygen, and oxidation-reduction potential
254 (ORP) were recorded in situ with portable probes. The depth of the water table was also
255 measured with a sound piezometric probe.

256 *2.4 Statistical analysis*

257 All statistical analyses were based on log-transformer data (with the exception of water
258 pH, left untransformed) to normalize distributions and linearize relationships. However,
259 water physicochemical parameters and diversity metrics still showed a non-normal
260 distribution (Kolmogorov-Smirnoff test, $P < 0.05$). Thus, non-parametric Mann-
261 Whitney U (for two samples) and Kruskal-Wallis (for k samples) tests were applied to
262 identify significant differences in diversity metrics between rivers, land use types within
263 each river, and seasons.

264 The response of diversity indices (both taxonomic and functional) and ecosystem
265 services (biogeochemical filtration and POM breakdown capacities) to increasing forest
266 surface was plotted and evaluated by simple linear regression after checking that no
267 increase in fitness was achieved when quadratic or logistic functions were used.

268 To evaluate changes in the composition of groundwater invertebrate assemblages in
269 response to land use types, a similarity test was used. This test allows detection of the
270 most characteristic taxa and functions associated to each land use, as well as those that
271 better explain the dissimilarity between land use types. Similarity tests were performed
272 for each floodplain separately by means of two-way SIMPER analysis with squared-
273 root transformed abundances, Bray-Curtis similarity distance, and a cut-off for low
274 contributions set at 90%. Taxa and feeding traits that contributed more to dissimilarity
275 (i.e. with values higher than one of the quotient between average dissimilarity and

276 standard deviation [Av. Dissimilarity/SD > 1]), were considered significant in terms of
277 discriminating land use types (Clarke and Warwick, 2001; Ruhí et al., 2012).

278 Linear mixed-effect models (LME, Laird and Ware, 1982) were used to identify the
279 environmental characteristics that controlled taxonomic and functional diversity. This
280 statistical technique was used to avoid the co-dependence effect introduced by repeated
281 measurements over time and across the four riparian areas (Demidenko, 2004).
282 Physicochemical parameters (non-correlated, Spearman rank test $P < 0.6$) and forest
283 surface percentage were included as fixed effects in LME models. Sampling season and
284 floodplain identity were included as random factors. Model selection followed an
285 automatic stepwise forward regression selection of predictors, based on the lowest
286 Akaike Information Criteria (AIC) that quantify the goodness of fit of multiple
287 alternative models.

288 Non-parametric analyses of variance (Mann-Whitney U test and Kruskal-Wallis test)
289 and linear regressions were performed with SPSS version 18.0 (©SPSS, Inc., Chicago,
290 IL). SIMPER tests were performed with PRIMER v. 6.0 for Windows. LME models
291 were computed with the 'nlme' (Pinheiro et al., 2013), 'MuMIn' (Barton, 2013),
292 'MASS' (Venables and Ripley, 2002), 'lme4' (Bates et al. 2013), and 'effects' (Fox,
293 2003) packages in R version 2.15.3 (R Core Team, 2013).

294

295 **3. Results**

296 *3.1 Groundwater invertebrate community and associated ecosystem services*

297 Diversity indices and the associated ecosystem services (i.e. biogeochemical filtration
298 and POM breakdown) showed a linear positive response with increasing forest land use.
299 Moreover, the percentage of forest land use was able to explain between 14 and 52% of

300 the diversity indices and associated ecosystem services of groundwater communities
301 (Figures 2, 3, and A1, Appendix).

302 In general, the taxonomic diversity and composition of groundwater invertebrate
303 communities showed differences between river and land use types (Table 1, Figures 4
304 and A2, Appendix). Particularly, more than 30% of the community in forest areas was
305 composed of crustacea (mainly Copepoda and Amphipoda), and Oligochaeta was one of
306 the most abundant taxa in agricultural areas. Moreover, SIMPER analysis showed that
307 Amphipoda was one of the taxa that contributed most to the dissimilarity between land
308 use types in three out of the four studied floodplains (Garonne, Ebro, and Bidasoa). The
309 abundance of Ostracoda was particularly important in the dissimilarity between land
310 uses in the Tajo River floodplain (Table 3, and A3, Appendix). At the functional level,
311 the most abundant (ca. 50%) functional feeding group corresponded to deposit feeders
312 (Figure 5), which also contributed most to the dissimilarity between land use types in all
313 of the studied floodplains (SIMPER analysis, Table 3 and A4, Appendix). The
314 proportion of shredders, scrapers, and filter-feeders was generally higher in forest than
315 in agricultural areas (Figure 5). Diversity indices and the associated ecosystem services
316 remained practically unchanged between sampling campaigns (i.e. seasons, Figure A3,
317 Appendix).

318

319 *3.2 Environmental factors controlling diversity indices and ecosystem services*

320 In general, concentration values of dissolved oxygen and nitrate in piezometers located
321 in agricultural areas were almost double those in forest areas, whereas conductivity and
322 DOC increased under forest land use (Table 2).

323 According to LME, the percentage of forest area and water quality (mainly, nitrate,
324 sulphate, phosphate, and DOC concentrations) explained between 51 and 71% of the
325 response of diversity indices and the associated ecosystem services (Table 4; Figure A4,
326 Appendix). Diversity indices and ecosystem services had a positive response to the
327 increase in the forest use surface and the groundwater content of nitrates, sulphates, and
328 DOC. Likewise, phosphate concentration had a negative influence on diversity indices
329 (Table 4; Figure A4, Appendix).

330

331 **4. Discussion**

332 In this study we demonstrate that the diversity and functionality of groundwater
333 invertebrates increase with the area occupied by riparian corridors in floodplains
334 affected by intensive agricultural development. This is probably because riparian forests
335 provide greater availability of quality resources (such as organic matter) to groundwater
336 organisms and unpolluted groundwater. More importantly, riparian corridors supported
337 organisms that provide important ecosystem services, such as biogeochemical filtration
338 and POM breakdown. Similar results were observed in other studies (Boulton et al.,
339 2008; Korbil and Hose, 2011), where changes in the composition, abundance, and
340 richness of the groundwater fauna were directly related to ecosystem functions. Our
341 study therefore highlights the need to conserve or even increase the forest area in
342 floodplains affected by intense agricultural use as a means to create buffer areas that
343 favour groundwater biodiversity and, consequently, the biogeochemical degradation of
344 pollutants coming from agricultural areas.

345

346 *4.1 How does land use affect groundwater biodiversity and functionality?*

347 Groundwater invertebrate assemblages showed notable changes between land use types,
348 at both taxonomic and functional levels. For instance, Amphipoda and Copepoda were
349 the most abundant taxa in the forest areas, whereas Oligochaeta and Ostracoda were
350 more frequent in agricultural areas. In the Bidasoa floodplain, where agricultural and
351 livestock activities are intensively developed, we mainly found species associated with
352 poor water quality, such as Oligochaeta, Diptera and Nematoda (see Figure 4). In
353 contrast, Ostracoda, often associated with low groundwater level fluctuations (Malard et
354 al., 1996), was the most abundant taxon in the Tajo River, which is consistent with the
355 low river discharge fluctuations registered during the study period (10 to $12 \text{ m}^3 \cdot \text{s}^{-1}$).
356 Similar results were found by Danielopol (et al., 2003), who suggested that river
357 regulation combined with the negative effect of organic pollution strongly alters
358 groundwater habitats. This is partly explained through river regulation which reduces
359 water energy, thereby increasing the transport large amounts of fine sediments (e.g. silt
360 and clay) that are accumulated in the aquifer, favouring its siltation. This siltation
361 reduces the porosity of alluvial aquifers and, consequently, reduces the space to host
362 fauna and reduces water and energy exchange between rivers and alluvial aquifers.
363 Moreover, the high content of organic pollution together with the characteristic low
364 oxygen concentration of alluvial aquifers favours the creation of chemical-reducing
365 conditions that also eliminate groundwater fauna. Consequently, free-moving
366 crustaceans such as stygobiotic copepods and isopods are replaced by epigeal animals
367 (i.e. surface animals), mostly nematodes and oligochaetes, which are able to live under
368 these conditions (Danielopol et al., 2003).

369 Unlike agricultural use, forest use does not add pollutants to groundwater, favouring the
370 provision of better-quality habitats and resources. Forest areas can thus act as local hot-

371 spots of food resources for groundwater invertebrates, thanks mainly to the organic
372 matter provided by the vegetation (Boulton and Foster, 1998). More particularly,
373 organic matter is degraded by surface fauna, increasing dissolved organic carbon
374 content in soils and infiltrated water. Consequently, dissolved organic carbon content
375 increases in groundwater under forest areas and is used as a food resource for many
376 taxa, such as Amphipoda and Isopoda. The increase of this kind of taxon also favours
377 better ecosystem functioning. For example, shredder crustaceans are keystone species
378 for nutrient cycling in groundwater ecosystems (Avramov, 2014; Boulton et al., 2008)
379 because of their role in bioturbation and the compaction of fine sediments into faecal
380 pellets (Boulton et al., 2008). Such biological processes boost the substrate availability
381 for bacteria, which in turn enhance denitrification processes and the transformation of
382 micropollutants in groundwaters, thereby contributing to healthy waters (Gibert and
383 Deharveng, 2002; Tomlinson and Boulton 2010; Ward et al., 1998; Yao et al.,
384 submitted). Consequently, floodplains dominated by forest land use, such as the Ebro
385 and Garonne, showed the greatest biogeochemical filtration and POM breakdown
386 capacities. It thus follows that the loss of groundwater invertebrates in agricultural
387 areas, particularly of keystone species such as amphipods and isopods, can have serious
388 repercussions for the functioning of groundwater ecosystems (Boulton et al., 2008;
389 Korbelt et al., 2013) and the associated supporting and provisioning ecosystem services
390 (e.g. purification of water, sustainability of surface ecosystems, provision of fit/clean
391 water for agricultural use) (Danielopol et al., 2003).

392 On the other hand, increase of nitrates, phosphates, and sulphates as a consequence of
393 agricultural fertilization and pesticides application influenced the groundwater
394 invertebrate community (Tables 2 and 4). These compounds can inhibit the

395 development of key taxa (mainly amphipods and isopods) at high concentrations
396 (Fakher et al., 1998), and favour the presence of other pollution-tolerant taxa such as
397 Oligochaeta. Other studies (Di Lorenzo et al., 2014; Iepure et al., 2013; Yao et al.,
398 submitted) also observed a negative influence of human use (e.g. agricultural, urban or
399 industrial) on groundwater invertebrate communities that was mainly associated with
400 the deterioration of water quality and ecosystem functioning. In contrast, other authors
401 (Boulton et al., 1998; Castellarini et al., 2007; Di Lorenzo et al., 2005; Dole-Olivier et
402 al., 2009; Dumas and Lescher-Moutoué, 2001) found little evidence that land use and
403 the associated changes in water chemistry affect the composition of stygobiotic
404 assemblages. These authors emphasized the role of hydrological (e.g. hydraulic
405 conductivity) and geomorphological (e.g. pore size) factors instead. This discordant
406 result can be explained by the strong agricultural pressure in our studied areas,
407 characterized by long periods of low discharges that usually coincide with fertilization
408 and irrigation periods. Diffuse pollution from agricultural areas may thus override the
409 effects of other hydrological and geomorphological determinants. However, the role of
410 hydrological factors on groundwater invertebrate communities should not be
411 disregarded, especially given its importance for surface waters (Español et al., 2015;
412 Gallardo et al., 2008, 2009).

413

414 *4.2 Conclusions*

415 The results of this study demonstrate that land use impacts on groundwater biota and
416 their associated ecosystem services. We observed a negative influence of agricultural
417 land use on invertebrate diversity and associated ecosystem services, which is attributed
418 to a high concentration of nitrates and sulphates. In contrast, riparian corridors provide

419 food resources (dissolved organic matter) and better water quality (low nitrate and
420 sulphate concentration) that enhance biota development and, consequently, the
421 associated ecosystem services (e.g. POM breakdown and biogeochemical filtration
422 capacities). Therefore, management measures to protect groundwater ecosystems should
423 include the conservation, creation, and eventually expansion of riparian forest corridors
424 in floodplains occupied by intensive agriculture. In addition, the rationalization of
425 agricultural fertilization activities and pesticides application are needed to reduce
426 diffuse contamination of subterranean habitats.

427 In terms of biomonitoring, our results suggest that groundwater invertebrate abundance,
428 taxonomic richness, POM breakdown capacity, and biogeochemical filtration capacity
429 are appropriate indicators for comparing the composition and functionality of alluvial
430 aquifers and assessing the effect of human pressures. However, more detailed regional
431 long-term studies are needed for better understanding of the structure and function of
432 groundwater ecosystems, which remain poorly investigated. Such basic studies are
433 essential for developing the ecological criteria proposed by the European standard (EU-
434 GWD, 2006) for assessing groundwater ecosystem status at the same level as surface
435 aquatic ecosystems.

436

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446

447 **6. References**

- 448 Almasri, M.N., Kaluarachchi, J.J., 2007. Modeling nitrate contamination of
449 groundwater in agricultural watersheds. *Journal of Hydrology* 343: 211–229 DOI:
450 10.1016/j.jhydrol.2007.06.016
- 451 Antiguëdad, I., Zabaleta, A., Martínez-Santos, M., Ruiz, E., Uriarte, J., Morales, T.,
452 Comin, F.A., Carranza, F., Español, C., Navarro, E., Bodoque, J.M., Ladera, J.,
453 Bernard-Jannin, L., Sun, X., Teissier, S., Sauvage, S., Sanchez-Perez, J.M.,
454 2015submitted. From hydrochemical observation to hydrological conceptualization: a
455 multi-criteria assessment in four different riparian zones. *Ecological Engineering*
456 (submitted).
- 457 APHA, 1989. Standard methods for the examination of water and wastewater,
458 seventeenth ed. American Public Health Association, Washington, DC, USA.
- 459 Arrate, I., Sanchez-Perez, J.M., Antiguëdad, I., Vallecillo, M.A., Iribar, V., Ruiz, M.,
460 1997. Groundwater pollution in Quaternary aquifer of Vitoria: Gasteiz (Basque
461 Country, Spain): Influence of agricultural activities and water-resource management.
462 *Environmental geology* 30: 257–265 DOI: 10.1007/s002540050155
- 463 Avramov, M., 2014. Sensitivity and Stress of Groundwater Invertebrates to Toxic
464 Pollution and Changes in Temperature. München, Technische Universität München,
465 Diss.
- 466 Barton, K., 2013. MuMIn: Multi-model inference. R package version 1.9.13.
467 <http://CRAN.R-project.org/package=MuMIn>.
- 468 Bates, D., Maechler, M., Bolker, B., Walker, S., 2013. lme4: Linear mixed-effects
469 models using Eigen and S4. R package version 1.0-4. [http://CRAN.R-](http://CRAN.R-project.org/package=lme4)
470 [project.org/package=lme4](http://CRAN.R-project.org/package=lme4)

471 Boulton, A.J., Findlay, S., Marmonier, P., Stanley, E.H., Valett, H.M., 1998. The
472 Functional Significance of the Hyporheic Zone in Streams and Rivers. *Annual Review*
473 *of Ecology and Systematics* 29: 59–81 DOI: 10.1146/annurev.ecolsys.29.1.59

474 Boulton, A.J., Datry, T., Kasahara, T., Mutz, M., Stanford, J.A., 2010. Ecology and
475 management of the hyporheic zone: stream–groundwater interactions of running waters
476 and their floodplains. *Journal of the North American Benthological Society* 29, 26–40.
477 doi:10.1899/08-017.1

478 Boulton, A.J., Fenwick, G.D., Hancock, P.J., Harvey, M.S., 2008. Biodiversity,
479 functional roles and ecosystem services of groundwater invertebrates. *Invertebrate*
480 *Systematics* 22, 103-116. doi:10.1071/IS07024

481 Boulton, A.J., Foster, J.G., 1998. Effects of buried leaf litter and vertical hydrologic
482 exchange on hyporheic water chemistry and fauna in a gravel-bed river in northern New
483 South Wales, Australia. *Freshwater Biology* 40, 229–243.

484 Brunke, M., Gonser, T., 1997. The ecological significance of exchange processes
485 between rivers and groundwater. *Freshwater Biology* 37: 1–33 DOI: 10.1046/j.1365-
486 2427.1997.00143.x

487 Castellarini, F., Malard, F., Dole-Olivier, M.-J., Gibert, J., 2007. Modelling the
488 distribution of stygobionts in the Jura Mountains (eastern France). Implications for the
489 protection of ground waters: Stygobiotic distribution. *Diversity and Distributions* 13,
490 213–224. doi:10.1111/j.1472-4642.2006.00317.x

491 Clarke, K.R., Warwick, R.M., 2001. *Change in marine communities: an approach to*
492 *statistical analysis and interpretation*, second ed. PRIMER-E, Plymouth.

493 Culver, D.C., Sket, B., 2000. Hotspots of subterranean biodiversity in caves and wells.
494 *Journal of Cave and Karst Studies* 62, 11–17.

495 Danielopol, D.L., Creuzé des Châtelliers, M., Moeszlacher, F., Pospisil, P., Popa, R.,
496 1994. Adaptations of crustacea to interstitial habitats: a practical agenda for ecosystem
497 studies, in: Gibert, J., Danielopol, D.L., Stanford, J.A. (Eds), *Groundwater Ecology*,
498 Academic Press, San Diego, pp 217–243.

499 Danielopol, D.L., Griebler, C., Gunatilaka, A., Notenboom, J., 2003. Present state and
500 future prospects for groundwater ecosystems. *Environmental Conservation* 30, 104–
501 130. doi:10.1017/S0376892903000109.

502 Datry, T., Malard, F., Gibert, J., 2005. Response of invertebrate assemblages to
503 increased groundwater recharge rates in a phreatic aquifer. *Journal of the North
504 American Benthological Society* 24, 461–477.

505 Deharveng, L., Stoch, F., Gibert, J., Bedos, A., Galassi, D., Zagnajster, M., Brancelj,
506 A., Camacho, A., Fiers, F., Martin, P., Giani, N., Magniez, G., Marmonier, P., 2009.
507 Groundwater biodiversity in Europe. *Freshwater Biology* 54, 709–726.
508 doi:10.1111/j.1365-2427.2008.01972.x

509 Demidenko, E., 2004. *Mixed models: theory and applications*. Wiley-Interscience,
510 Malden.

511 Di Lorenzo, T., Di Marzio, W.D., Sáenz, M.E., Baratti, M., Dedonno, A.A., Iannucci,
512 A., Cannicci, S., Messana, G., Galassi, D.M.P., 2014. Sensitivity of hypogean and
513 epigean freshwater copepods to agricultural pollutants. *Environmental Science and
514 Pollution Research* 21, 4643–4655. doi:10.1007/s11356-013-2390-6

515 Di Lorenzo, T., Stoch, F., Fiasca, B., Gattone, E., De Laurentiis, P., Ranalli, F., Galassi,
516 D.M.P., 2005. Environmental quality of deep groundwater in the Lessinian massif
517 (Italy): signposts for sustainability. *Symposium on world subterranean biodiversity* (ed.

518 by J. Gibert). HBES — Université Claude Bernard Lyon 1 — UMR-CNRS 5023.
519 Villeurbanne, France.

520 Dolédec, S., Olivier, J.M., Statzner, B., 2000. Accurate description of the abundance of
521 taxa and their biological traits in stream invertebrate communities: effect of taxonomic
522 and spatial resolution. *Archiv für Hydrobiologie* 14, 25–43

523 Dole-Olivier, M.-J., Castellarini, F., Coineau, N., Galassi, D.M.P., Martin, P., Mori, N.,
524 Valdecasas, A., Gibert, J., 2009. Towards an optimal sampling strategy to assess
525 groundwater biodiversity: comparison across six European regions. *Freshwater Biology*
526 54, 777–796. doi:10.1111/j.1365-2427.2008.02133.x

527 Dumas, P., Lescher-Moutoué, F., 2001. Cyclopid distribution in an agriculturally-
528 impacted alluvial aquifer. *Archiv für Hydrobiologie* 150, 511–528.

529 Español, C., Gallardo, B., Comín, F.A., Pino, M.R., 2015. Constructed wetlands
530 increase the taxonomic and functional diversity of a degraded floodplain. *Aquatic*
531 *Sciences* 77, 27–44. doi:10.1007/s00027-014-0375-2

532 EU-GWD, 2006. Directive 2006/118 of the European Parliament and the Council of the
533 12 December 2006. *Official Journal of the European Communities* L372, 371–319.

534 Fakher, E.A., Oulbaz, Z., Yacoubi-Khebiza, M., Coineau, N., Boutin, C., 1998. Etude
535 expérimentale de la sensibilité comparée de trois crustacés stygobies vis-à-vis de
536 diverses substances toxiques pouvant se rencontrer dans les eaux souterraines.
537 *Mémoires de Biospéologie* 25, 167–181.

538 Fox, J., 2003. Effect displays in R for Generalised Linear Models. *Journal of Statistical*
539 *Software*, 8(15), 1–27. URL <http://www.jstatsoft.org/v08/i15/>

540 Gallardo, B., García, M., Cabezas, Á., González, E., González, M., Ciancarelli, C.,
541 Comín, F.A., 2008. Macroinvertebrate patterns along environmental gradients and

542 hydrological connectivity within a regulated river-floodplain. *Aquatic Sciences* 70,
543 248–258. doi:10.1007/s00027-008-8024-2

544 Gallardo, B., Gascón, S., Cabezas, Á., Gonzalez, M., García, M., Comín, F.A., 2009.
545 Relationship between invertebrate traits and lateral environmental gradients in a
546 Mediterranean river-floodplain. *Fundamental and Applied Limnology/Archiv für*
547 *Hydrobiologie* 173, 281–292.

548 Gibert, J., Danielopol, D.L., Stanford, J.A., 1994. *Groundwater Ecology*. Academic
549 Press, New York.

550 Gibert, J., Culver, D.C., 2009. Assessing and conserving groundwater biodiversity: an
551 introduction. *Freshwater Biology* 54, 639–648. doi:10.1111/j.1365-2427.2009.02202.x

552 Gibert, J., Culver, D.C., Dole-Olivier, M.-J., Malard, F., Christman, M.C., Deharveng,
553 L., 2009. Assessing and conserving groundwater biodiversity: synthesis and
554 perspectives. *Freshwater Biology* 54, 930–941. doi:10.1111/j.1365-2427.2009.02201.x

555 Gibert, J., Deharveng, L., 2002. Subterranean Ecosystems: A Truncated Functional
556 Biodiversity. *BioScience* 52, 473–481.

557 González, E., González-Sanchis, M., Cabezas, Á., Comín, F.A., Muller, E., 2010.
558 Recent Changes in the riparian forest of a large regulated Mediterranean river:
559 Implications for management. *Environmental Management* 45, 669–681.
560 doi:10.1007/s00267-010-9441-2

561 Griebler, C., Stein, H., Kellermann, C., Berkhoff, S., Brielmann, H., Schmidt, S., Selesi,
562 D., Steube, C., Fuchs, A., Hahn, H.J., 2010. Ecological assessment of groundwater
563 ecosystems – Vision or illusion?. *Ecological Engineering* 36, 1174–1190.
564 doi:10.1016/j.ecoleng.2010.01.010

565 Hakenkamp, C.C., Palmer, M.A., 2000. The ecology of hyporheic meiofauna, In: Jones,
566 J.B., Mulholland, P.J. (Eds.), *Streams and ground waters*. Academic Press, San Diego,
567 pp. 307–336.

568 Hancock, P.J., Boulton, A.J., Humphreys, W.F., 2005. Aquifers and hyporheic zones:
569 Towards an ecological understanding of groundwater. *Hydrogeology Journal* 13, 98–
570 111. doi:10.1007/s10040-004-0421-6

571 Iepure, S., Martinez-Hernandez, V., Herrera, S., Rasines-Ladero, R., de Bustamante, I.,
572 2013. Response of microcrustacean communities from the surface—groundwater
573 interface to water contamination in urban river system of the Jarama basin (central
574 Spain). *Environmental Science and Pollution Research* 20, 5813–5826.
575 doi:10.1007/s11356-013-1529-9

576 Iepure, S., Meffe, R., Carreño, F., Rasines, R.L., de Bustamante, I., 2014. Geochemical,
577 geological and hydrological influence on ostracod assemblages distribution in the
578 hyporheic zone of two Mediterranean rivers in central Spain. *International Review of*
579 *Hydrobiology* 99, 435–449. doi:10.1002/iroh.201301727

580 Korbek, K.L., Hose, G.C., 2011. A tiered framework for assessing groundwater
581 ecosystem health. *Hydrobiologia* 661, 329–349. doi:10.1007/s10750-010-0541-z

582 Korbek, K.L., Lim, R.P., Hose, G.C., 2013. An inter-catchment comparison of
583 groundwater biota in the cotton-growing region of north-western New South Wales.
584 *Crop and Pasture Science* 64, 1195-1208. doi:10.1071/CP13176.

585 Krause, S., Tecklenburg, C., Munz, M., Naden, E., 2013. Streambed nitrogen cycling
586 beyond the hyporheic zone: Flow controls on horizontal patterns and depth distribution
587 of nitrate and dissolved oxygen in the upwelling groundwater of a lowland river.

588 Journal of Geophysical Research: Biogeosciences 118: 54–67 DOI:
589 10.1029/2012JG002122

590 Krom, M.D., 1980. Spectrophotometric determination of ammonia: A study of a
591 modified Berthelot reaction using salicylate and dichloroisocyanurate. *The Analyst* 105,
592 305–316.

593 Laird, N.M., Ware, J.H., 1982. Random-effects models for longitudinal data. *Biometrics*
594 38, 963–974.

595 Liu, A., Ming, J., Ankumah, R.O., 2005. Nitrate contamination in private wells in rural
596 Alabama, United States. *Science of The Total Environment* 346, 112–120.
597 doi:10.1016/j.scitotenv.2004.11.019

598 Malard, F., Dole-Olivier, M.-J., Mathieu, J., Stoch, F., 2002. Sampling manual for the
599 assessment of regional groundwater biodiversity. Sampling manual published within the
600 framework of the EU Project PASCALIS (Protocols for the ASsessment and
601 Conservation of Aquatic Life In the Subsurface). <http://www.pascalis-project.com>.

602 Malard, F., Mathieu, J., Reygrobellet, J.-L., Lafont, M., 1996. Biomonitoring groundwater
603 contamination: Application to a karst area in Southern France. *Aquatic Sciences* 58,
604 158–187.

605 Marmonier, P., Archambaud, G., Belaidi, N., Bougon, N., Breil, P., Chauvet, E., Claret,
606 C., Cornut, J., Datry, T., Dole-Olivier, M.-J., Dumont, B., Flipo, N., Foulquier, A.,
607 Gérino, M., Guilpart, A., Julien, F., Maazouzi, C., Martin, D., Mermillod-Blondin, F.,
608 Montuelle, B., Namour, P., Navel, S., Ombredane, D., Pelte, T., Piscart, C., Pusch, M.,
609 Stroffek, S., Robertson, A., Sanchez-Pérez, J.-M., Sauvage, S., Taleb, A., Wantzen, M.,
610 Vervier, P., 2012. The role of organisms in hyporheic processes: gaps in current

611 knowledge, needs for future research and applications. *Annales de Limnologie -*
612 *International Journal of Limnology* 48, 253–266. doi:10.1051/limn/2012009

613 Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O’Hara, B.,
614 Simpson, G.L., Solymos, P., Stevens, M.H.H., Wagner, H., 2013. *Vegan: community*
615 *ecology package*. R package version 2.0-10.

616 Pinheiro, P., Bates, D., DebRoy, S., Sarkar, D., R Development Core Team, 2013.
617 *nlme: Linear and Nonlinear Mixed Effects Models*. R package version 3.1-108.

618 R Core Team, 2013. *R: A language and environment for statistical computing*. R
619 Foundation for Statistical Computing, Vienna, Austria. <http://www.R-project.org/>.

620 Ruhí, A., Herrmann, J., Gascón, S., Sala, J., Boix, D., 2012. How do early successional
621 patterns in man-made wetlands differ between cold temperate and Mediterranean
622 regions? *Limnologica - Ecology and Management of Inland Waters* 42, 328–339.
623 [doi:10.1016/j.limno.2012.07.005](https://doi.org/10.1016/j.limno.2012.07.005).

624 Sabater, S., Butturini, A., Clement, J-C, Burt, T., Dowrick, D., Hefting, M., Matre, V.,
625 Pinay, G., Postolache, C., Rzepecki, M., et al., 2003. Nitrogen Removal by Riparian
626 Buffers along a European Climatic Gradient: Patterns and Factors of Variation.
627 *Ecosystems* 6: 0020–0030. doi: 10.1007/s10021-002-0183-8

628 Sánchez-Pérez, J.M., Antigüedad, I., Arrate, I., García-Linares, C., Morell, I., 2003a.
629 The influence of nitrate leaching through unsaturated soil on groundwater pollution in
630 an agricultural area of the Basque country: a case study. *Science of The Total*
631 *Environment* 317: 173–187. doi: 10.1016/S0048-9697(03)00262-6

632 Sanchez-Perez, J.M., Tremolieres, M., Carbiener, R., 1991a. Une station d’épuration
633 naturelle des phosphates et nitrates apportés par les eaux de débordement du Rhin : la

634 forêt alluviale à frêne et orme. Comptes rendus de l'Académie des sciences. Série 3,
635 Sciences de la vie 312: 395–402.

636 Sanchez-Perez, J.M., Tremolieres, M., Schnitzler, A., Carbiener R., 1991b. Evolution
637 de la qualité physico-chimique des eaux de la frange superficielle de la nappe
638 phréatique en fonction du cycle saisonnier et des stades de succession des forêts
639 alluviales rhénanes (Querco-Ulmetum minoris Issl. 24). Acta oecologica : (1990) 12:
640 581–601.

641 Sánchez-Pérez, J.M., Trémolières, M., 2003. Change in groundwater chemistry as a
642 consequence of suppression of floods: the case of the Rhine floodplain. Journal of
643 Hydrology 270: 89–104 DOI: 10.1016/S0022-1694(02)00293-7

644 Sánchez-Pérez, J.M., Vervier, P., Garabétian, F., Sauvage, S., Loubet, M., Rols, J.L.,
645 Bariac, T., Weng, P., 2003. Nitrogen dynamics in the shallow groundwater of a riparian
646 wetland zone of the Garonne, SW France: nitrate inputs, bacterial densities, organic
647 matter supply and denitrification measurements. Hydrology and Earth System Sciences
648 Discussions 7: 97–107

649 Sánchez-Pérez, J.M. 1992. Fonctionnement hydrochimique d'un Ecosysteme forestier
650 inondable de la plaine du Rhin. La foret alluviale du secteur de l'île de Rhinau en
651 Alsace (France). PhD, Université Louis Pasteur, Strasbourg, 176 pp.

652 Sket, B., 1999. The nature of biodiversity in hypogean waters and how it is endangered.
653 Biodiversity & Conservation 8, 1319–1338.

654 Tachet, H., Richoux, P., Bournaud, M., Usseglio-Polatera, P., 2002. Invertébrés d'Eau
655 Douce, second corrected impression. CNRS Éditions, Paris.

656 Takatert, N., Sanchez-Pérez, J.M., Trémolières, M., 1999. Spatial and temporal
657 variations of nutrient concentration in the groundwater of a floodplain: effect of
658 hydrology, vegetation and substrate. *Hydrological Processes* 13: 1511–1526.

659 Thioulouse, J., Chessel, D., Dolédec, S., Olivier, J.M., 1997. ADE-4: a multivariate
660 analysis and graphical display software. *Statistics and Computing* 7, 75–83.

661 Tockner, K., Bunn, S.E., Gordon, C., Naiman, R.J., Quinn, G.P., Stanford, J.A., 2008.
662 Flood plains: critically threatened ecosystems, in: Polunin, N. (Ed.), *Aquatic
663 ecosystems: trends and global prospects*. Cambridge University Press, Cambridge,
664 pp.45–61.

665 Tomlinson, M., Boulton, A.J., 2010. Ecology and management of subsurface
666 groundwater dependent ecosystems in Australia - a review. *Marine and Freshwater
667 Research* 61, 936-949.

668 Vannote, R. L., Minshall, G. W., Cummins, K. W., Sedell, J. R., Cushing, C. E., 1980.
669 The river continuum concept. *Canadian Journal of Fisheries and Aquatic Sciences* 37,
670 130–137.

671 Venables, W.N., Ripley, B.D., 2002. *Modern Applied Statistics with S*, fourth ed.
672 Springer, New York.

673 Vervier, P., Bonvallet-Garay, S., Sauvage, S., Valett, H.M., Sanchez-Perez, J-M., 2009.
674 Influence of the hyporheic zone on the phosphorus dynamics of a large gravel-bed river,
675 Garonne River, France. *Hydrological Processes* 23: 1801–1812, DOI:
676 10.1002/hyp.7319.

677 Ward, J.V., Bretschko, G., Brunke, M., Danielopol, D., Gibert, J., Gonser, T., Hildrew,
678 A.G., 1998. The boundaries of river systems: the metazoan perspective. *Freshwater
679 Biology* 40, 531-569.

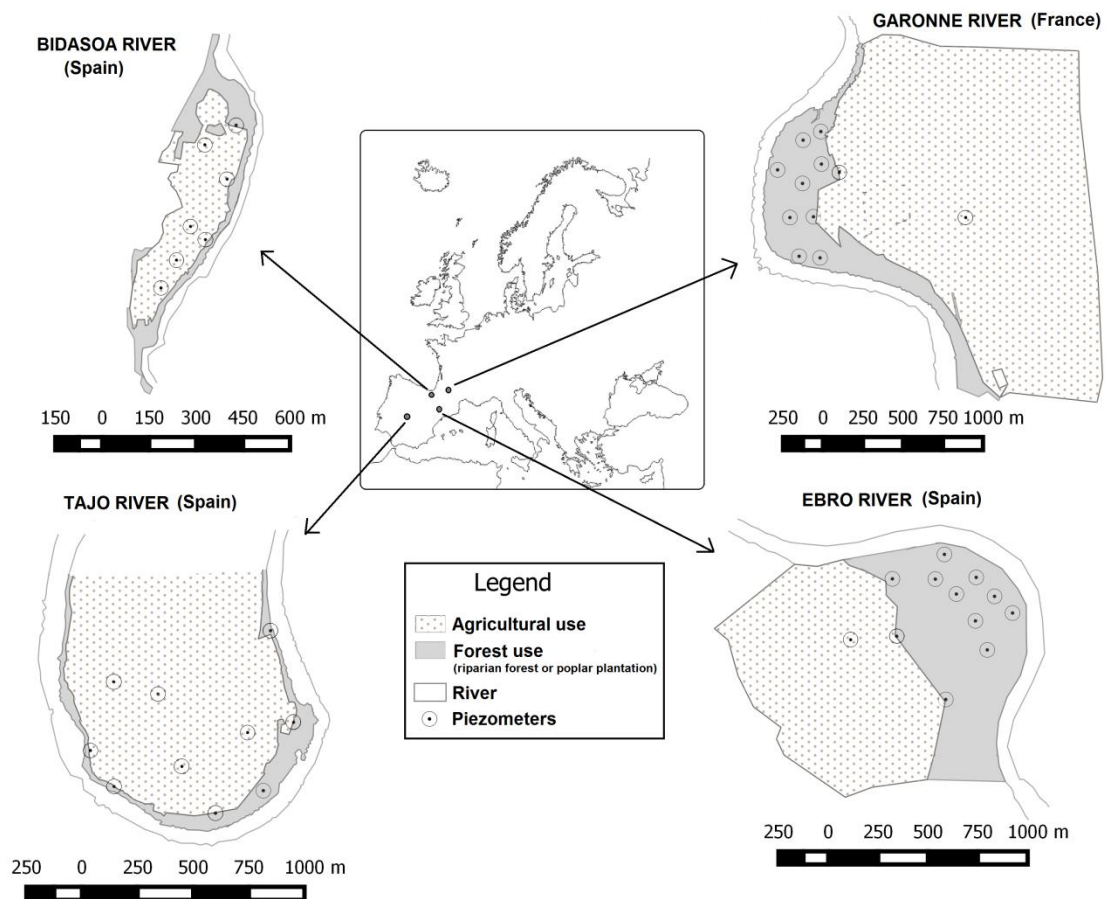
680 Weng, P., Sánchez-Pérez, J.M., Sauvage, S., Vervier, P., Giraud, F., 2003. Assessment
681 of the quantitative and qualitative buffer function of an alluvial wetland: hydrological
682 modelling of a large floodplain (Garonne River, France). *Hydrological Processes* 17:
683 2375–2392. doi: 10.1002/hyp.1248

684 White, D.S., 1993. Perspectives on Defining and Delineating Hyporheic Zones. *Journal*
685 *of the North American Benthological Society* 12: 61–69. doi: 10.2307/1467686

686 Wondzell, S.M., 2011. The role of the hyporheic zone across stream networks.
687 *Hydrological Processes* 25: 3525–3532. doi: 10.1002/hyp.8119

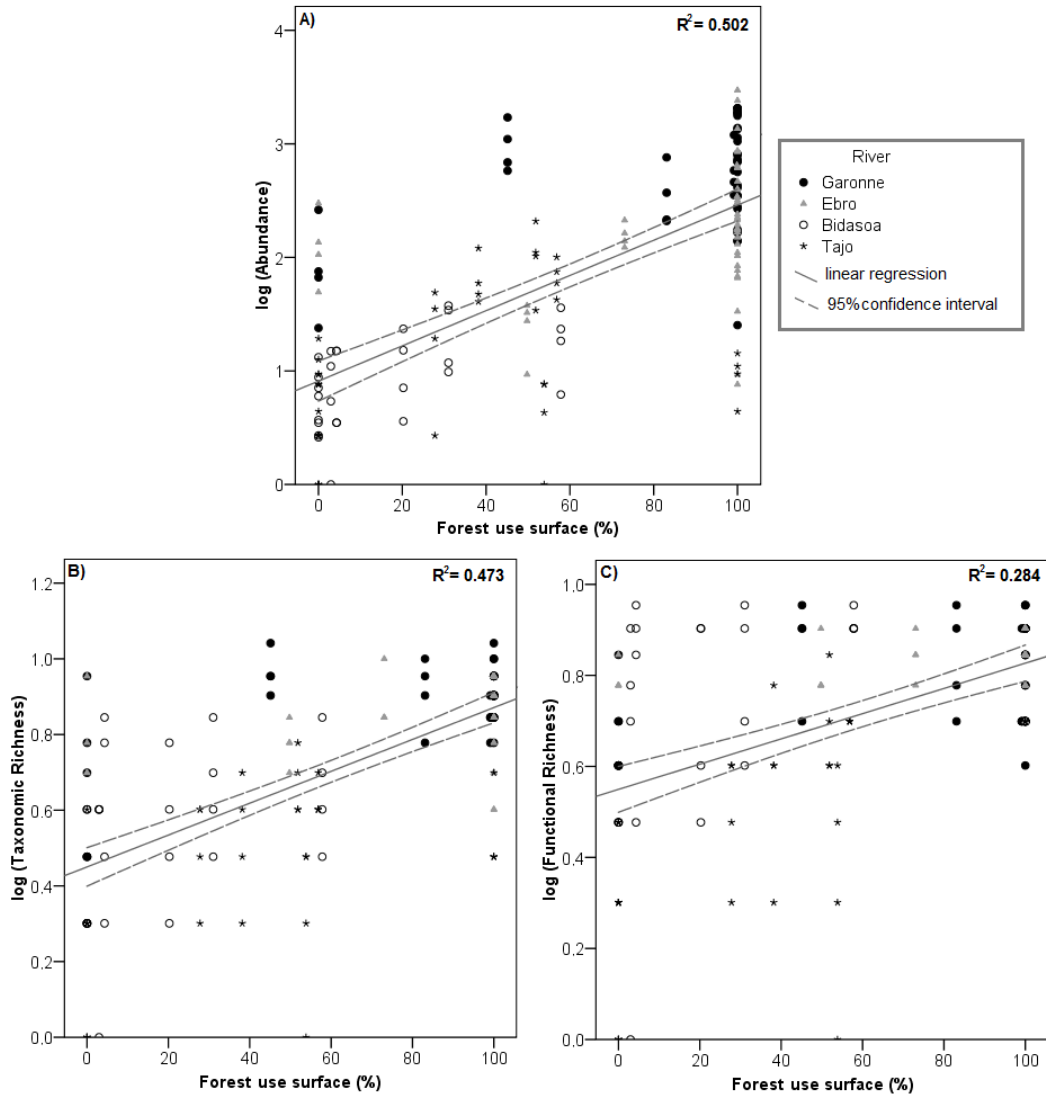
688 Yao, J.M., Sánchez-Pérez, J.M., Sauvage, S., Teissier, S., Attard, E., Lauga, B., Duran,
689 R., Julien, F., Bernard-Jannin, L., Ramburn, H., Gerino, M., 2015submitted.
690 Biodiversity, ecosystem purification service in alluvial wetlands. *Ecological*
691 *Engineering* (submitted).

- 1 Figure 1. Study area location and piezometric network of each studied river floodplain.
- 2 The study area is composed by four river floodplains located in South-western Europe
- 3 (Garonne, Ebro, Bidasoa and Tajo Rivers). Samples were collected in the piezometric
- 4 network (8 to 12 piezometers) of each floodplain.
- 5



6

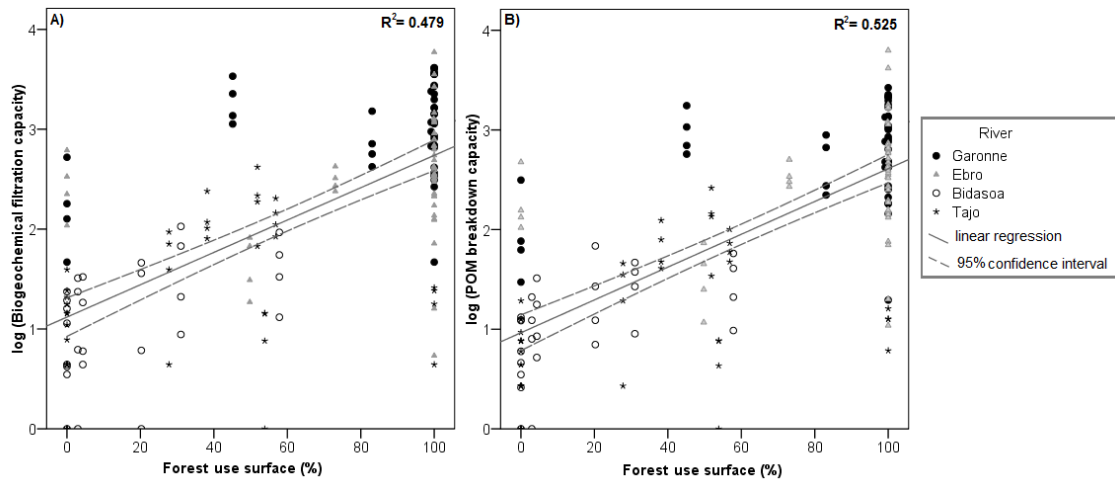
7 Figure 2. Relationship between taxonomic and functional diversity indices and the
 8 percentage of surface occupied by forest (buffer 50 m) in the floodplain of four rivers in
 9 South-western Europe.



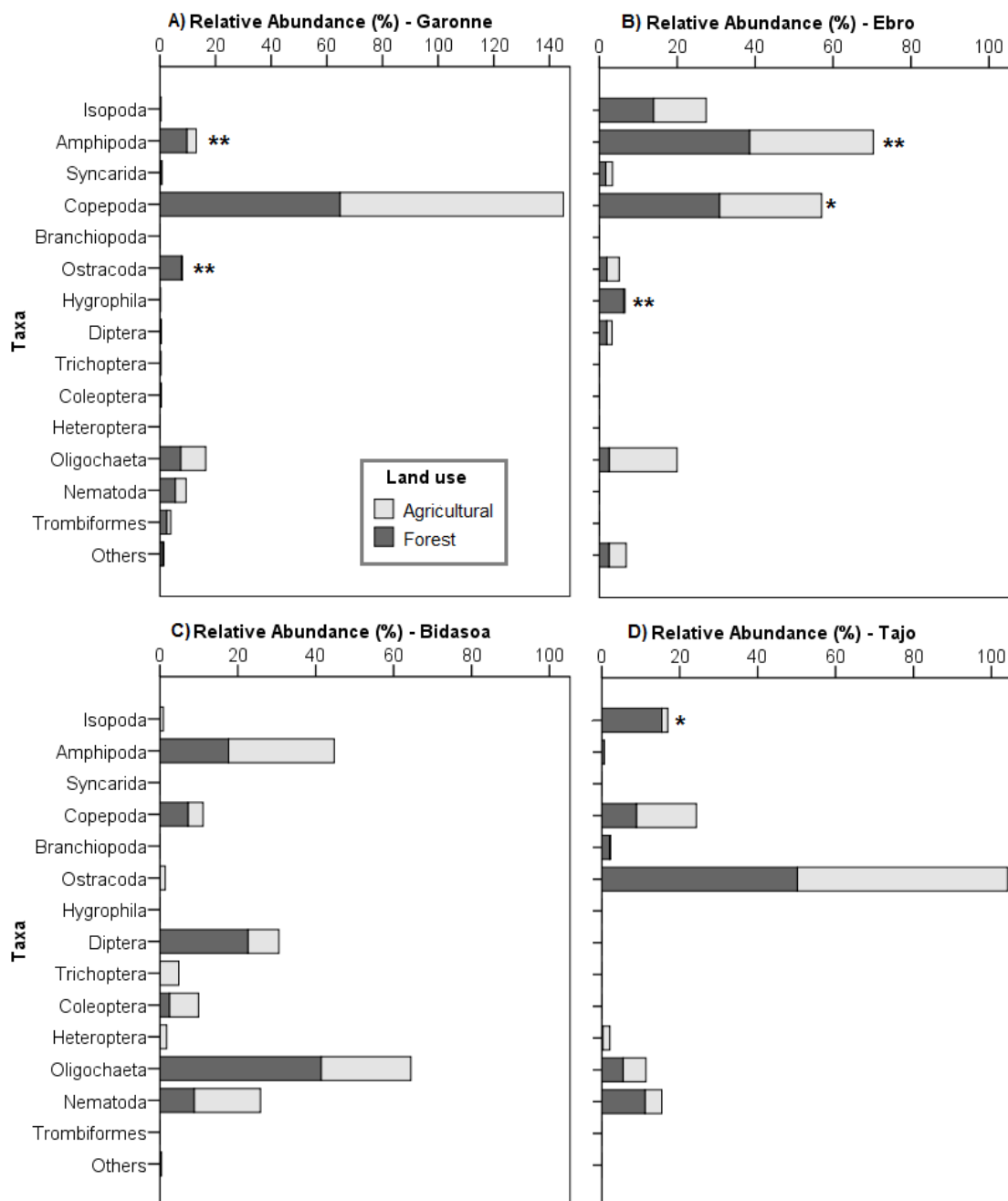
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12 Figure 3. Relationship between ecosystem services (i.e. biogeochemical filtration and
13 POM breakdown capacities) and the percentage of surface occupied by forest (buffer 50
14 m) in four river floodplains in South-western Europe.



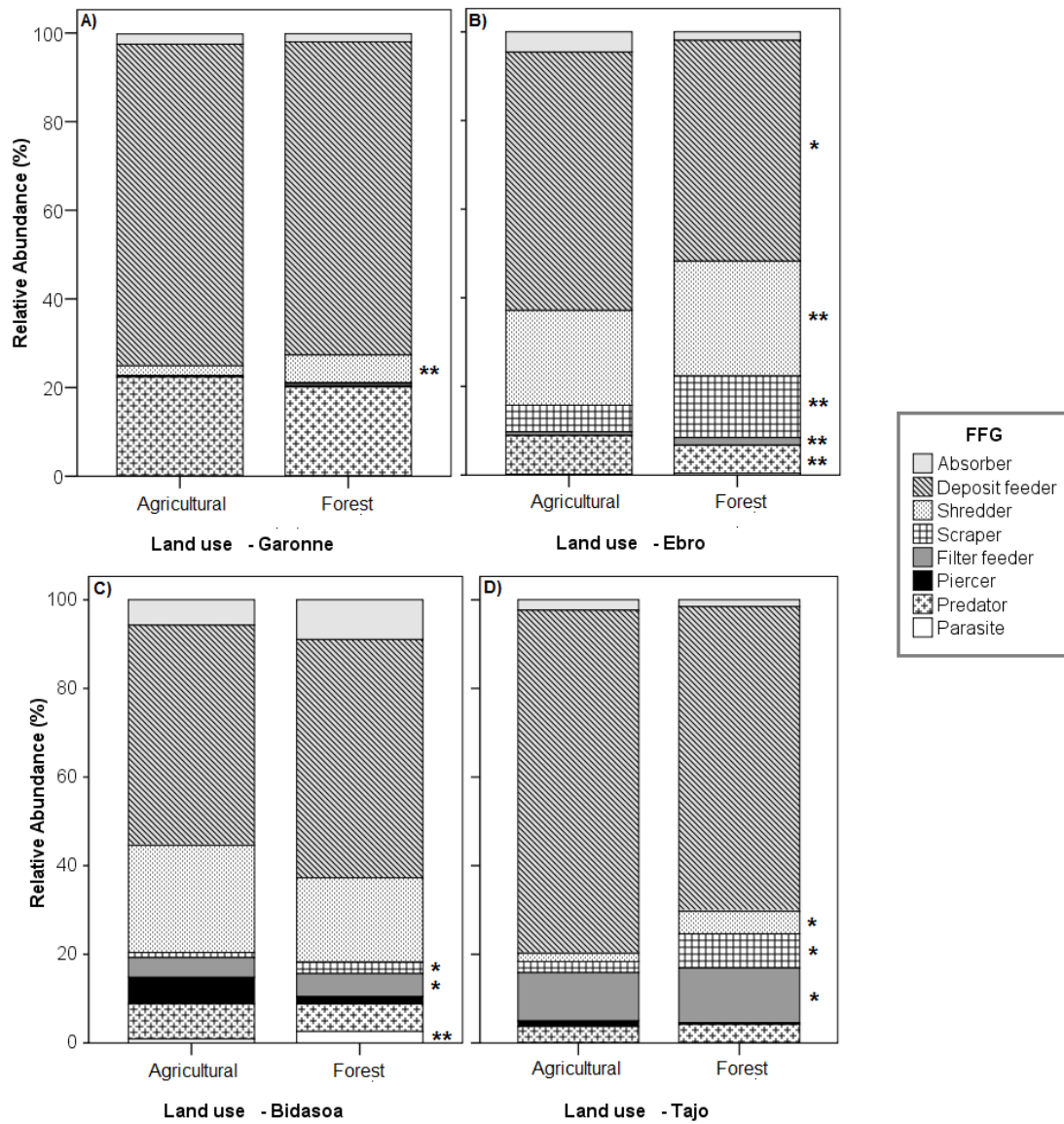
16 Figure 4. Taxonomic composition of the groundwater invertebrate community of four
 17 river floodplains according to their dominant land use type. Bars represent the relative
 18 abundance (average percentage) of each taxon in the community for each land use type.
 19 Significant differences between land use types are indicated by * ($P < 0.05$, Mann-
 20 Whitney U test) and ** ($P < 0.01$).



21

22

23 Figure 5. Functional feeding habits of groundwater invertebrates in four river
 24 floodplains according to their land use. Bars represent relative abundance (average
 25 percentage). Significant differences between land use types are indicated by * ($P < 0.05$,
 26 Mann-Whitney U test) and ** ($P < 0.01$).



27

28

1 Table 1. Study site characteristics, dominating land use type, diversity metrics (mean \pm
 2 SD), and provided ecosystem services (mean \pm SD) in four river floodplains (Garonne,
 3 Ebro, Bidasoa and Tajo Rivers). N = sample size. Significant differences between river
 4 floodplains are indicated by ** ($P < 0.05$, Kruskal-Wallis test).

5

	Units	Garonne (N = 44)	Ebro (N = 48)	Bidasoa (N = 28)	Tajo (N = 40)
Piezometers	n°	11	12	7	10
Total Floodplain Area	Ha	1200	80	17	62
<i>Habitat</i>					
Agricultural area	Ha	960	30	15	52
Forest area	Ha	240	50	2	8
<i>Hydrological conditions</i>					
Hydraulic conductivity (aquifer)	m/day	86	1 - 3	50 - 100	8-9
River discharge (average)	m ³ /s	195	250	25	11
Specific flow (average)	m ³ /s·km ²	1.4·10 ⁻²	5.3·10 ⁻³	3.7·10 ⁻²	4.3·10 ⁻⁴
<i>Diversity indices</i>					
Abundance *	n°indiv/100L	776 \pm 645	354 \pm 560	11 \pm 10	30 \pm 44
Taxonomic richness *	n° taxa	7 \pm 1	6 \pm 1	3 \pm 2	2 \pm 1
Taxonomic Shannon diversity index *		0.83 \pm 0.39	1.13 \pm 0.25	0.70 \pm 0.52	0.38 \pm 0.36
Functional richness *	n° categories	5 \pm 2	6 \pm 1	5 \pm 2	2 \pm 1
Functional Shannon diversity index *		0.77 \pm 0.16	1.24 \pm 0.10	1.07 \pm 0.40	0.52 \pm 0.41
<i>Ecosystem services</i>					
Biogeochemical filtration capacity *		1539 \pm 1272	639 \pm 1014	24 \pm 27	60 \pm 88
POM breakdown capacity *		873 \pm 717	635 \pm 1077	18 \pm 17	34 \pm 53

6

7

8 Table 2. Groundwater physicochemical features (mean \pm SD) of four floodplains
 9 located in the Garonne, Ebro, Bidasoa and Tajo Rivers under different land use types. N
 10 = sample size. Significant differences between land use types in each river floodplain
 11 are indicated by * ($P < 0.05$, Mann-Whitney U test).

12

Physicochemical features (Abbr.)	Units	Garonne		Ebro		Bidasoa		Tajo	
		Agricultural use (N = 8)	Forest use (N = 36)	Agricultural use (N = 12)	Forest use (N = 36)	Agricultural use (N = 24)	Forest use (N = 4)	Agricultural use (N = 24)	Forest use (N = 16)
Depth (Depth)	m	2.26 \pm 0.77 *	3.28 \pm 1.28 *	3.90 \pm 0.71*	2.49 \pm 0.45*	4.17 \pm 0.76	3.99 \pm 0.73	3.53 \pm 1.20*	2.13 \pm 0.73*
Temperature (Temp)	$^{\circ}$ C	14.2 \pm 1.7	13.79 \pm 1.23	16.3 \pm 1.4*	14.0 \pm 1.8*	15.1 \pm 2.0	14.1 \pm 1.3	17.5 \pm 1.3	17.6 \pm 2.4
pH (pH)		7.02 \pm 0.14	6.97 \pm 0.12	6.92 \pm 0.49*	7.36 \pm 0.28*	6.49 \pm 0.28	6.49 \pm 0.13	7.14 \pm 0.12	7.07 \pm 0.15
Dissolved oxygen (O2)	%	65.60 \pm 10.53 *	29.95 \pm 29.20 *	24.06 \pm 19.74	16.72 \pm 15.94	37.66 \pm 17.80	38.00 \pm 8.94	42.34 \pm 32.67*	26.10 \pm 29.96*
Conductivity (EC)	μ S/cm	945 \pm 115	901 \pm 225	3464 \pm 646*	1618 \pm 959*	367 \pm 101	397 \pm 31	2234 \pm 410*	2493 \pm 439*
Oxidation-reduction potential (ORP)	mV	252 \pm 35	195 \pm 91	104 \pm 21*	68 \pm 34*	98 \pm 66	163 \pm 50	118 \pm 36	116 \pm 39
Dissolved organic carbon (DOC)	mg/L	0.69 \pm 0.45	1.22 \pm 0.98	13.95 \pm 3.47*	8.58 \pm 5.58*	3.31 \pm 1.22	3.08 \pm 0.85	1.61 \pm 0.60	1.99 \pm 0.87
Alkalinity (Alk)	meq/L	5.34 \pm 0.35 *	6.02 \pm 1.13 *	6.39 \pm 0.44*	5.32 \pm 0.93*	3.15 \pm 1.01	3.23 \pm 0.32	5.78 \pm 1.01	5.98 \pm 1.31
Phosphate (PO4)	μ g/L	9.28 \pm 8.53	9.70 \pm 9.59	2.68 \pm 1.89*	16.47 \pm 20.48*	5.13 \pm 3.33	3.65 \pm 1.52	182.08 \pm 150.79	160.00 \pm 165.81
Ammonium (NH4)	μ g/L	7.90 \pm 4.21	106.48 \pm 269.98	0.004 \pm 0.014*	10.50 \pm 38.73*	3212.68 \pm 9516.56	176.04 \pm 161.71	199.94 \pm 362.97	239.68 \pm 350.90
Nitrate (NO3)	mg/L	76.88 \pm 15.96 *	47.26 \pm 38.73 *	34.68 \pm 4.85*	10.10 \pm 8.25*	9.23 \pm 8.84	8.71 \pm 3.36	32.25 \pm 12.58	38.66 \pm 16.16
Sulphate (SO4)	mg/L	67.38 \pm 10.79	64.81 \pm 29.66	1410.96 \pm 154.57*	451.18 \pm 421.78*	21.96 \pm 12.33	32.16 \pm 4.22	924.13 \pm 371.81	1009.03 \pm 384.17
Silica oxide (SiO2)	mg/L	13.01 \pm 1.00	11.43 \pm 3.97	18.66 \pm 4.06*	7.75 \pm 4.96*	9.36 \pm 2.92	12.98 \pm 4.33	15.23 \pm 3.16	14.81 \pm 3.50

13

14 Table 3. Taxa and feeding traits that contributed the most to dissimilarity (Av.
 15 Diss/SD>1) between land use types (agricultural use areas vs. forest use areas).
 16 Statistics obtained through a two-way SIMPER analysis with factors date and land use.
 17

	GARONNE		EBRO		BIDASOA		TAJO	
Taxonomic level	(average	dissimilarity	(average	dissimilarity	(average	dissimilarity	(average	dissimilarity
	49.3%)		52.7%)		74.1%)		71.1%)	
	Taxa	Diss/SD	Taxa	Diss/SD	Taxa	Diss/SD	Taxa	Diss/SD
	Copepoda	1.46	Amphipoda	1.39	Oligochaeta	1.67	Ostracoda	1.44
	Amphipoda	1.31	Copepoda	1.22	Amphipoda	1.02		
Functional level	(average	dissimilarity	(average	dissimilarity	(average	dissimilarity	(average	dissimilarity
	38.4%)		37.1%)		41.1%)		53.7%)	
	FFG	Diss/SD	FFG	Diss/SD	FFG	Diss/SD	FFG	Diss/SD
	Deposit feeder	1.60	Deposit feeder	1.44	Deposit feeder	1.38	Deposit feeder	1.54
	Predator	1.52	Shredder	1.43	Shredder	1.24	Scraper	1.09
	Shredder	1.25	Scraper	1.36	Absorber	1.33	Predator	1.04
			Predator	1.34	Filter feeder	1.57		
			Absorber	1.34	Predator	1.47		
				Parasite	1.41			
				Scraper	1.37			

18
 19

20 Table 4. Results from LME models linking physicochemical variables with taxonomic
 21 and functional diversity indices and ecosystem services. All selected explanatory
 22 variables were statistically significant at $P < 0.05$. Total sample size = 160. d^2 =
 23 variance of the random intercept; α = variance of the fixed intercept; ρ = Spearman
 24 correlation coefficient between observed and predicted values, used as a measure of
 25 goodness of fit.

	Explanatory variables	Coefficient	Intercept	Spearman correlation test
<i>Taxonomic diversity indices</i>				
Abundance	% Forest area	0.30	$d^2 = 1.10^2$	$\rho = 0.71$
	NO ₃	0.28	$\alpha = 15.00$	$P < 0.01$
	Temp	- 4.55		
Taxonomic richness	% Forest area	0.09	$d^2 = 0.33^2$	$\rho = 0.57$
	SO ₄	0.06	$\alpha = 3.21$	$P < 0.01$
	Temp	- 0.79		
Taxonomic Shannon diversity index	DOC	0.07	$d^2 = 0.10^2$	$\rho = 0.58$
	% Forest area	0.04	$\alpha = 1.50$	$P < 0.01$
	O ₂	- 0.05		
	Temp	- 0.37		
<i>Functional diversity indices</i>				
Functional richness	% Forest area	0.08	$d^2 = 0.16^2$	$\rho = 0.51$
	PO ₄	- 0.03	$\alpha = 2.99$	$P < 0.01$
	Temp	- 0.54		
Functional Shannon diversity index	DOC	0.07	$d^2 = 0.10^2$	$\rho = 0.61$
	EC	- 0.07	$\alpha = 0.91$	$P < 0.01$
	% Forest area	0.03		
<i>Ecosystem services</i>				
Biogeochemical filtration capacity	% Forest area	0.30	$d^2 = 1.13^2$	$\rho = 0.69$
	NO ₃	0.31	$\alpha = 15.38$	$P < 0.01$
	Temp	- 4.50		
POM breakdown capacity	% Forest area	0.34	$d^2 = 1.11^2$	$\rho = 0.70$
	NO ₃	0.28	$\alpha = 13.86$	$P < 0.01$
	Temp	- 4.11		

26

27

Supplementary Material

[Click here to download Supplementary Material: Appendix_att4.doc](#)