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1 **Constructed wetlands increase the taxonomic and functional diversity of a degraded floodplain**

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16

17 Running head: Taxonomic and functional diversity in wetlands

18

19 **Abstract**

20 Numerous wetland restoration projects have been implemented in recent years to mitigate the increasing loss of
21 global wetland surface area caused by human activities. Most of these projects have focused on the local
22 recovery of habitats and species diversity, with little evaluation of functional recovery. We aimed at
23 demonstrating that constructing wetlands on a degraded floodplain increases not only the taxonomic, but also the
24 functional diversity of macroinvertebrate assemblages by providing greater water quality to the local fauna. We
25 studied the macroinvertebrate community using taxonomic and functional diversity indices, and the
26 physicochemical characteristics of three wetlands constructed five to 25 years ago, and three relict natural
27 wetlands on the floodplain of a regulated river (Ebro River, NE Spain). Constructed wetlands demonstrated
28 significantly greater taxonomic abundance and richness of macroinvertebrates than natural wetlands. At the
29 functional level, the richness and Shannon diversity of biological traits relating to reproduction, respiration,
30 dispersal and feeding were also greater in constructed wetlands, which is partly explained by low inorganic
31 nitrogen concentration in these habitats. In contrast, a high content of phosphorus and water organic matter led to
32 the lowest values of taxonomic and functional diversity found in natural wetlands. We conclude that it is
33 essential to consider not only taxonomic but also functional aspects at all stages of a restoration project in order
34 to optimize its long-term efficacy to provide and support key species and functions.

35 **Keywords:**

36 Biological traits; created wetlands; macroinvertebrate community; man-made ponds; mixed-effect models;
37 restoration project

38

39 **Introduction**

40 Over 50% of the world's wetland surface has been lost during the last century due to land use changes, flow
41 regulation and wetlands dredging (Mitsch and Gosselink 2007). Faced with this alarming situation, policies and
42 plans for managing the restoration and creation of wetlands have flourished over the last decade (Mitsch and
43 Gosselink 2007). Generally, restoration projects have only considered structural aspects of ecosystems, mainly
44 water quality and species diversity, assuming that the improvement of these aspects is directly linked to the
45 recovery of fundamental ecosystem processes and properties (i.e. ecosystem functions) (Brown and Batzer
46 2001). However, several investigations suggest this assumption may not always hold true (e.g. Grayson et al.
47 1999; Jax 2010; Moreno-Mateos et al. 2012). Analysing and measuring ecosystem functioning should therefore
48 provide an essential view of the overall performance of an ecosystem, and the processes that maintain its
49 structure, which is a powerful and far-reaching tool for the management of wetlands and their ecosystem
50 services (Jax 2010). Consequently, in the last years, a number of indicators have been developed to investigate
51 the functioning of aquatic ecosystems, from hydro-geochemical processes measures (e.g. nutrient cycling,
52 biomass decomposition or hydrologic connectivity) to biological indicators (e.g. primary production, herbivory,
53 predator-prey relationships, biotic resistance to invasive species) (e.g. Balvanera et al. 2005; Cabezas et al.
54 2009c; Español et al. 2013). Yet, ecosystem functioning is rarely addressed either before, during or after the
55 implementation of restoration projects.

56 Macroinvertebrates are considered as excellent quality bioindicators for aquatic ecosystems due to their ubiquity,
57 life-cycles, abundance and diversity of species with varying life history strategies regulated to habitat conditions
58 (Wallace and Webster 1996; Bêche and Statzner 2009; Gallardo et al. 2011); as well as to their contribution to
59 ecosystem functions, including detritus processing, nutrient cycling and food provision to higher trophic levels,
60 among others (Heino 2005). In this sense, macroinvertebrate biological traits such as feeding habits (indicator of
61 resource availability), body size (stability, food web structure), locomotion (capacity to colonize new habitats),
62 reproductive method and life cycle characteristics (resistance and resilience to disturbances) provide direct and
63 indirect information about a given ecosystem structure and function (Gayraud et al. 2003; Bonada et al. 2006;
64 Tachet et al. 2010). The overall diversity of biological traits is directly affected by human-induced disturbances,
65 such as changes in hydrological connectivity (Paillex et al. 2008; Gallardo et al. 2009a, 2009b, 2014), nutrient
66 concentration (Heino 2005, 2008), heavy metal pollution (Dolédéc and Statzner 2008), and changes in land use
67 (Díaz et al. 2008; Vandewalle et al. 2010). Nonetheless, because some traits may be more relevant to indicate

68 restoration success than others (e.g. feeding structure, active/passive locomotion, presence of resistance forms),
69 it is important to investigate the response of individual as well as the whole set of traits to restoration.
70 Macroinvertebrate traits should thus provide a powerful indicator of the ecological state of restored wetlands and
71 their ability to recreate fully functional ecosystems.

72 Remarkably, few studies have applied biological traits to the assessment of the efficacy of restoration projects,
73 mainly focusing on the early years after project implementation (e.g. 0-3 years; Kleef et al. 2006; Ruhí et al.
74 2009; Gallardo et al. 2012a). These studies showed an increase in macroinvertebrate functional diversity during
75 the first few years following wetland restoration due to the arrival of pioneering and opportunistic species with a
76 strong capacity for active dispersal and high reproduction rates (i.e. multivoltine). This observation was
77 attributed to the newly created habitats that provide novel resources and refuge to aquatic communities, thereby
78 reducing species competition (Kleef et al. 2006; Ruhí et al. 2009; Gallardo et al. 2012a). The observed trend in
79 biodiversity is likely to continue in the medium to long term, especially if good standards of water quality and
80 habitat complexity are maintained in the restored wetlands (Reckendorfer et al. 2006). Alternatively, if not
81 submitted to a natural or human-assisted disturbance regime, diversity in constructed wetlands is likely to reach
82 a maximum point after which we would see a significant loss of species and functions due to natural ecological
83 succession (i.e. replacement of opportunistic species by a smaller number of specialist species), habitat
84 homogenization and water eutrophication (Hansson et al. 2005; Kleef et al. 2006; Ruhí et al. 2009, 2012a;
85 Gallardo et al. 2012a). Under this scenario, only species adapted to eutrophic conditions with feeding habits
86 associated with detritus and dead plant remains would survive in the long term, leading to a community
87 assemblage similar to that found in degraded natural wetlands. The balance between these two possible
88 trajectories –and to a great extent the long-term success of restoration projects- depends on a number of factors,
89 including water quality, the availability of habitats and resources, the balance between community succession
90 and renewal processes, and the disturbance regime of restored wetlands. Solid proof that constructing wetlands
91 promotes key ecosystem functions that can be maintained in the long term would certainly provide added value
92 to implementing restoration projects.

93 The present study examines the short and medium-term efficacy of wetland construction projects on a large
94 regulated river floodplain (River Ebro, NE Spain), where natural wetlands are in a degraded state. First, we
95 compared the taxonomic and functional diversity and composition of the macroinvertebrate community in a set
96 of constructed and natural wetlands to investigate if the creation of artificial wetlands provides new and more

97 species and functions to the floodplain. Second, we identified the physicochemical variables responsible for the
98 observed diversity patterns and community composition, so that these strategic variables can be manipulated in
99 future projects to promote greater functionality for restored ecosystems. We particularly hypothesized a higher
100 taxonomic and functional diversity in constructed than in natural wetlands, due to the degradation experienced
101 by natural wetlands and the better water quality provided by constructed wetlands. Consequently, the two types
102 of wetlands should have different community compositions at taxonomic and functional levels, with more
103 pioneering and opportunistic species in constructed wetlands and more eutrophic tolerant species in natural
104 wetlands. We further predicted that the main factors driving the observed changes in diversity patterns and
105 community composition in our study area are those related to eutrophication status. Ultimately, this study aims
106 to emphasize the importance of including functional criteria in ecological restoration projects as a means of
107 advancing towards multi-functional wetlands that maximize the recovery of functions, thereby optimizing the
108 allocation of the limited resources invested in restoration schemes.

109

110 **Material and methods**

111 *Study area*

112 The study area was located in the Middle Ebro River (NE Spain), which has a length of 901 km and a drainage
113 basin of 85,534 km². Historically, extraordinary flood events in the Ebro River have generated a number of
114 natural wetlands in its floodplain, including temporary pools and oxbow wetlands (Ollero 2007). However, since
115 the 1960s, the Ebro River has been extensively affected by an increase in human activity, leading to drastic land
116 use changes (agriculture and urban areas) and the extensive construction of structures to control floods (Cabezas
117 et al. 2008). These pressures have caused the degradation of relict wetlands and the reduction of the river's
118 capacity for creating new natural wetlands on its floodplain (Gallardo et al. 2012a). Consequently, a number of
119 restoration projects have been developed over the last two decades to restore and/or create artificial wetlands
120 along the Ebro River floodplain to mitigate habitat loss and increase local biodiversity.

121 Previous studies have analysed aquatic community changes, sedimentation rates, and aquatic metabolic rates in
122 natural vs. constructed wetlands in the Ebro floodplain. These projects have identified hydrological connectivity,
123 water quality and habitat succession as major drivers of floodplain structure and functionality (e.g. Gallardo et
124 al. 2008, 2012a, 2012b; Cabezas et al. 2008, 2009a, 2009b, 2009c; Español et al. 2013). These studies have also

125 illustrated the benefits of wetland restoration at the local scale. As way of example, only one year after the
126 construction of one of the restored wetlands, Gallardo et al. (2012a) recorded much higher taxonomic and
127 functional diversity values than those of nearby natural wetlands. Studies nevertheless focussed on the early
128 years after restoration, with no further monitoring of the trends observed. This lack of information impairs the
129 capacity of environmental managers to both address the efficacy of past restoration activities and promote the
130 development of future projects.

131 For this study, we selected three riparian areas each comprising one constructed and one natural wetland located
132 no farther apart than 1 km in the floodplain of the Middle Ebro River (NE Spain, 41°39'N, 0°52'W, Fig. 1). In
133 particular, riparian area 1 included wetlands N1 and C1; riparian area 2 included wetlands N2 and C2, and
134 riparian area 3 included wetlands N3 and C3 (Fig. 1). Wetlands C1 and C3 were created through excavation in
135 the surroundings of wetlands N1 and N3, respectively. These wetlands are filled through water seepage from the
136 hillslope aquifer. Riparian vegetation was transplanted on the wetland banks to facilitate shore stabilization and
137 colonization. Wetland C2 consists of an old gravel pit, which was restored through hydrological re-connection
138 and riparian vegetation introduction. These constructed wetlands were created not to be as extant natural
139 wetlands but as a complement, providing new and more diverse habitats to local flora and fauna in the
140 floodplain. The proximity between paired wetlands provided a unique opportunity to investigate the
141 development of constructed and natural reference wetlands that share the same environmental conditions (e.g.
142 hydrological influence, isolation, and wind speed). It should be noted that in this study we use the terms 'natural'
143 as representative of the wetland origin and 'reference' as representative of natural conditions regardless of their
144 environmental quality (i.e. no restoration intervention), as opposed to 'good reference' conditions applied in
145 other studies.

146 The two most representative habitats in each studied wetland were identified and selected as sampling points to
147 cover the wide range of environments available (Table 1), including: (i) areas without vegetation (fine sediment
148 or gravel sediment); and (ii) areas with vegetation (emergent or submerged vegetation).

149

150 *Morphological and Physicochemical characteristics*

151 The average depth (m) of each wetland was measured along transects from shore to centre. Surface area (Ha)
152 was obtained from digitalised aerial photographs. Age (years) was calculated from the date of construction or

153 from first observation according to Cabezas et al. (2008). Triplicate water samples were collected at each
154 sampling point and season directly into 1.5 L PVC bottles previously washed in acid (CLH 0.1 N) at a depth of
155 10 cm, and placed on ice (see total number of samples in Table 1). Total suspended solids (mg/L), total
156 dissolved solids (mg/L) and organic matter (mg/L) content were determined by the gravimetric method, i.e.
157 filtering samples through pre-combusted (450°C, 4 h) Whatman GF/F glass-fibre filters following standard
158 protocols (APHA 1989). Chlorophyll *a* (µg/L) samples were filtered through Whatman GF/F glass-fibre filters,
159 pigments were extracted in 96% ethanol for 24 h, and analysed using the spectrophotometric method (Thermo
160 Helios α; APHA 1989). Filtered water aliquots were stored at -20 °C, and used within one month for the
161 following analyses. Ion chromatography (Metrohm 861 Advanced Compact IC; APHA 1989) was applied to
162 determine dissolved inorganic nitrogen (DIN = NH₄⁺ + NO₂⁻ + NO₃⁻, mgN/L) and sulfate (SO₄²⁻, mg/L)
163 concentration. Soluble reactive phosphorus (SRP, µg/L) was measured by the ascorbic acid method (APHA
164 1989). Total dissolved phosphorus (TDP, µg/L) was also estimated by the ascorbic acid method, but a previous
165 potassium persulfate digestion was performed (90 min, 115 °C) (APHA 1989). Finally, water temperature (°C),
166 pH, conductivity (mS/cm) and dissolved oxygen (mg/L) were recorded *in situ* with portable probes (WTW
167 Multiline P4 and Hach-Lange HQ).

168 Water physicochemical quality of study sites was investigated in two seasons: winter (December 2010) and
169 spring (June 2011), for a total of 72 water samples taken during the study period. These seasonal measures
170 allowed incorporating the potential range of environmental conditions throughout the year, with maximum
171 diversity expected in spring and minimum in winter, as reported in previous studies (e.g. Gallardo et al. 2012a).

172

173 *Taxonomic and functional composition of the macroinvertebrate community*

174 Triplicate macroinvertebrate samples were collected simultaneously to water samples in winter (December
175 2010) and spring (June 2011) at each sampling point using a hand net (frame net 45 x 45 cm, mesh size 500 µm),
176 making a total of 72 samples. The sampling procedure was based on 20 dip-net sweeps in rapid sequence at each
177 sampling point. Samples were preserved *in situ* in 4% formalin. Macroinvertebrate samples were sorted and
178 identified in the laboratory at least to family level, although the majority of samples were identified to genus
179 level (see Appendix 1). It is common practise to use family or even coarser taxonomic resolution for certain
180 groups such as Oligochaeta and Chironomidae that are difficult to identify (e.g. Díaz et al. 2008; Gallardo et al.

181 2009c; Céréghino et al. 2012), although we acknowledge that this level may underestimate species richness in
182 habitats where they dominate.

183 To characterize the functional composition of the macroinvertebrate community, we used 63 categories of 11
184 biological traits defined by Tachet et al. (2010) (see Appendix 2). These biological traits describe different
185 aspects of organism biology, including life cycle characteristics (life cycle duration, potential number of
186 generations per year, aquatic stages), resistance or resilience potential (dispersal, resistance stages, locomotion
187 and substrate relation), general physiological and morphological traits (respiration, body size), and behavioural
188 aspects of reproduction or nutrition (reproduction, food, feeding habits) (Usseglio-Polatera et al. 2000). Tachet's
189 database describes the average affinity (scores 0 – 5) of each genus to each trait category, using a fuzzy coding
190 approach (Chevenet et al. 1994). A score of zero indicates no affinity, while a score of 5 indicates the highest
191 affinity of the taxon to a particular category. Codes for one taxa (*Atyaephira* sp.) not coded in Tachet et al.
192 (2010) were extracted from Gallardo et al. (2014). For taxa identified at higher taxonomic levels than genera,
193 affinity scores were calculated by selecting the most frequent score across all taxa belonging to a particular
194 taxonomic group. This may result in an underestimation of functional diversity of habitats dominated by those
195 families, although according to Dolédec et al. (2000), the overall functional structure of the invertebrate
196 communities is conserved. Because different biological traits confer clear trade-offs (for instance, predators are
197 generally large and univoltine, and small organisms are generally plurivoltine and short-lived), we should expect
198 commonly associated traits to dominate under similar environmental conditions.

199 Finally, taxonomic and functional diversity metrics were computed. At the taxonomic level, we calculated: (i)
200 total abundance of individuals; (ii) total richness of taxa (family level); (iii) Shannon-Wiener diversity index,
201 which incorporates the relative abundance of the different taxa; and (iv) Rao's quadratic diversity index (Botta-
202 Dukát 2005), which takes into account the pairwise dissimilarities among taxa. Likewise, indexes calculated at
203 the functional level included: (i) abundance of individuals for each trait category; (ii) total richness of trait
204 categories (from a total of 63 trait categories); (iii) Shannon-Wiener diversity index; and (iv) Rao's quadratic
205 diversity index. In addition, we calculated the richness, Shannon-Wiener diversity and Rao's quadratic diversity
206 of each of the 11 traits considered in this study (e.g. richness of reproduction modes or feeding habits).

207 Taxonomic and functional metrics were computed using the "vegan" (Oksanen et al. 2008) and "ade4"
208 (Thioulouse et al. 1997) packages of R software, version 2.12.2 (R Development CoreTeam 2007).

209

210 *Statistical analysis*

211 All statistical analyses were based on log-transformed data (with the exception of water pH left untransformed)
212 to normalise distributions and linearize relationships. Still, water physicochemical parameters and diversity
213 metrics showed a non-normal distribution according to a Kolmogorov-Smirnoff test ($P < 0.05$). It is for this
214 reason that the non-parametric Mann-Whitney U test was utilised to identify significant differences between i)
215 pairs of natural and constructed wetlands, and ii) seasons (winter vs. spring). For the same reason, the non-
216 parametric Spearman correlation test was applied to investigate correlations between taxonomic and functional
217 metrics.

218 Linear Mixed Effect models (LME, Laird and Ware 1982) were used to identify the physicochemical variables
219 that control the taxonomic and functional diversity of the study wetlands. This statistical technique was used to
220 avoid the co-dependence effect introduced by repeated measurements over time and riparian area (Demidenko
221 2004). Physicochemical parameters (non-correlated, Spearman rank $\rho < 0.6$) were included as fixed effects in
222 LME models. Sampling season and riparian area were included as random factors. The selection of predictor
223 variables for each model followed a stepwise forward regression selection until all predictors were statistically
224 significant (at $P < 0.05$). The best model was chosen based on the lowest Akaike Information Criteria (AIC) and
225 the highest correlation between predicted and observed values, both of which quantify the goodness of fit of
226 multiple alternative models.

227 Multivariate analyses were performed to evaluate the individual response of each taxon and each of the 11
228 biological traits to physicochemical parameters, thereby avoiding problems related to trade-offs between
229 biological traits. In particular, a Correspondence Analysis (CA) was conducted using macroinvertebrate
230 abundance data to compare the taxonomic composition of natural and constructed wetlands. Likewise, to assess
231 the functional composition of natural and constructed wetlands, we used a Fuzzy Correspondence Analysis
232 (FCA, Chevenet et al. 1994), which links the macroinvertebrate abundance data matrix with the biological traits
233 matrix. We additionally tested correlations between sample scores of the first and second CA and FCA axes and
234 environmental parameters (physicochemical and morphological features: depth, age and area) using non-
235 parametric Spearman correlation tests.

236 Non-parametric analyses of variance (Mann-Whitney U test) were performed using SPSS version 18.0 (©SPSS,
237 Inc., Chicago). LME models were computed using the “nlme” package (Lindstrom and Bates 1990). Correlation

238 (Spearman test) and multivariate analyses (CA and FCA) were performed using the “ade4” package (Thioulouse
239 et al. 1997), all of them in R version 2.12.2 (R Development Core Team 2007).

240

241 **Results**

242 *Morphological and Physicochemical characteristics of natural and constructed wetlands*

243 Natural wetlands were older (50 - 65 years) and had a larger surface area (10 - 70 ha) than constructed wetlands
244 (5 - 25 years, 0.4 - 0.9 ha, respectively), whereas both types of wetlands had similar depths (Table 1). Unlike
245 constructed wetlands, natural wetlands lacked habitats with gravel sediment or submerged vegetation.

246 Concentration values of total suspended solids, chlorophyll *a*, organic matter, and phosphorous compounds (SRP
247 and TDP) were more than twofold in natural wetlands than in constructed wetlands (Table 2). Physicochemical
248 parameters also showed significant variation between seasons (Table 2). For instance, temperature ($Z = -5.14$, P
249 < 0.01 , $N = 72$) and conductivity ($Z = -2.86$, $P = 0.04$, $N = 72$) were significantly higher in spring than in winter;
250 whereas total nitrogen ($Z = -3.39$, $P < 0.01$, $N = 72$) and dissolved oxygen ($Z = -4.07$, $P < 0.01$, $N = 72$) were
251 greater in winter.

252

253 *Taxonomic and functional composition of natural and constructed wetlands*

254 Functional diversity indices showed a significant positive relationship with taxonomic diversity (Spearman test,
255 $\rho > 0.6$; see Appendix 3), and both showed significantly higher values in spring than winter (Table 3).

256 Non-parametric analysis of variance showed significant differences between natural and constructed wetlands
257 for taxonomic and functional metrics. More specifically, the abundance ($Z = -2.37$, $P = 0.02$, $N = 72$) and
258 taxonomic richness ($Z = -3.66$, $P < 0.01$, $N = 72$) were significantly higher in constructed wetlands (Fig. 2).

259 The most abundant family in both types of wetlands was Chironomidae, which accounted for over 50% of
260 individuals. This was followed by Oligochaeta (ca. 20%) and Corixidae (ca. 20%) in natural wetlands, whereas a
261 wider range of macroinvertebrates (e.g. Corixidae, Caenidae, Atyidae, Oligochaeta) co-dominated constructed
262 wetlands (Fig. 3).

263 At the functional level, the Shannon-Wiener diversity of all biological traits together was significantly higher in
264 constructed than in natural wetlands ($Z = -2.22$, $P = 0.03$, $N = 72$, Fig. 2). When analyses were made separately
265 by biological trait, significant differences between constructed and natural wetlands emerged. For instance,

266 constructed wetlands showed highest richness and Shannon-Wiener diversity for most biological traits, overall
267 those related to plurivoltinism, reproduction, dispersal, respiration, food sources and feeding habits (Table 4). In
268 contrast, natural wetlands illustrated greater richness of resistance forms and life-cycle duration categories. More
269 specific differences in the taxonomic and trait profiles exhibited by natural and constructed wetlands can be seen
270 in figures 3 and 4.

271

272 *Physicochemical factors controlling macroinvertebrate assemblages in natural and constructed wetlands*

273 According to LME, phosphorus (TDP and/or SRP) and nitrogen (DIN) compounds were the main
274 physicochemical variables controlling the response of the four taxonomic diversity indices investigated (Table
275 4).

276 Functional richness and Shannon-Wiener functional diversity showed a significant negative response to the
277 content of organic matter in water (Table 4). Likewise, Rao's quadratic functional diversity showed a negative
278 outcome with increasing phosphorus and decreasing nitrogen concentration. Regarding taxonomic and
279 functional assemblages, there were significant differences between natural and constructed wetlands along the
280 first axis of the taxonomic CA (Mann-Whitney U test, $Z = -4.45$, $P < 0.01$, $N = 72$) and the trait-based FCA ($Z =$
281 -4.11 , $P < 0.01$, $N = 72$). Constructed wetlands were characterized by taxa of Odonata (e.g. Coenagrionidae and
282 Corduliidae), Pulmonata (e.g. Physidae) and Ephemeroptera (e.g. Baetidae and Caenidae) orders; while natural
283 wetlands were characterised by Oligochaeta (e.g. Tubificidae) and Heteroptera (e.g. Corixidae) (more details on
284 the invertebrate assemblages of natural and constructed wetlands in figures 3 and 5). At the functional level,
285 dominant biological traits in natural wetlands included large organisms with long life-spans, asexual
286 reproduction, and resistance forms (e.g. cocoons), which were mainly deposit-feeders and predators (Fig. 4). In
287 contrast, constructed wetlands featured taxa characterized by small body size, short life-span, active dispersion
288 and active locomotion forms (e.g. swimmers and fliers), which reproduced via free eggs and clutches and fed
289 through shredding or scraping microphytes (Fig. 4). Both taxonomic and functional assemblages seemed to be
290 influenced by the water's physicochemical characteristics, more specifically the concentration of phosphorus
291 (SRP) and nitrogen (DIN), conductivity, sulfate, organic matter content and chlorophyll a (Figs. 5 and 6).

292

293 **Discussion**

294 In this study, we confirmed our initial hypothesis that ecological restoration of a degraded floodplain increases
295 not only the taxonomic, but also the functional diversity of macroinvertebrate communities in the medium to
296 long term. This is, according to our analysis, because constructed wetlands provide good water quality in terms
297 of nutrient (phosphorous and nitrogen) and organic matter concentration, thereby providing greater resource
298 availability to aquatic communities. These results are relevant since they provide new evidence about ecosystem
299 function changes after restoration, an important aspect that remains largely ignored in restoration projects.
300 Indeed, recent studies have suggested that functional recovery is a multifaceted process highly dependent on the
301 environmental context of the system being restored (Grayson et al. 1999; Moreno-Mateos et al. 2012). Our study
302 ultimately highlights the need to evaluate and support with empirical data the ability of restoration plans to
303 recreate fully functional and sustainable wetlands rather than simply assuming this outcome. Such strong
304 evidence would support wetland restoration and guide future management actions.

305

306 *Do functional and taxonomic diversity change after ecological restoration?*

307 Significantly higher taxa and trait richness of macroinvertebrates were observed in constructed wetlands when
308 compared to degraded natural wetlands, in spite of the striking resemblance of their taxonomic and trait profiles.
309 Our study thus confirms previous results by Gallardo et al. (2012a), who reported much greater taxonomic
310 richness and Shannon diversity in the Ebro constructed wetlands when compared to nearby natural wetlands only
311 one year following restoration. Furthermore, authors reported the occurrence in natural wetlands of several novel
312 species of Odonata never recorded before the construction of wetlands in that riparian area, implying that
313 constructed wetlands could act as a source of new and/or lost species towards natural wetlands. In contrast, Ruhí
314 et al. (2012a) suggested that the local taxonomic and functional diversity is not affected by wetland construction.
315 They observed that during the first three years after restoring a number of wetlands, the local loss of pioneer
316 species was not compensated by the arrival of new taxa with less dispersal capacity and/or fewer special
317 requirements, such as those present in the natural wetlands used as a reference. The apparent contradiction
318 between these two investigations could be explained by their choice of reference conditions. In the case of Ruhí
319 et al. (2012a), reference wetlands were in a relatively good conservation state, featuring high hydrological
320 connectivity and water physicochemical quality. In contrast, natural wetlands used in the present study, and also
321 by Gallardo et al. (2012a), experienced intense degradation due to restrained connectivity with the main river

322 channel, eutrophication and excessive accumulation of fine sediments (Cabezas 2008, 2009c; Gallardo et al.
323 2008, 2012b). It is therefore not surprising that the better water quality offered by constructed wetlands resulted
324 in higher diversity in relation to degraded natural wetlands in our models. Yet this observation is not trivial,
325 since it can greatly support the construction of new wetlands in river floodplains affected by river regulation and
326 accumulation of nutrients, widespread problems that affect not only our study area but also the majority of rivers
327 in Europe and North America (Mitsch and Gosselink 2007).

328 In the long term, differences between natural and constructed wetlands are expected to decline naturally as
329 ecological succession proceeds, and constructed wetlands become progressively degraded due to catchment-
330 scale degradation processes such as river regulation, land-use change and diffuse pollution from agricultural
331 areas (Fairchild et al. 2000; Hansson et al. 2005; Ruhí et al. 2012b). This underlies the necessity of long-term
332 monitoring schemes (> 10 years) to follow changes undergone by both constructed and natural wetlands in order
333 to gain better insight into their fundamental causes (Hansson et al. 2005; Ruhí et al. 2012b), and eventually
334 implement maintenance actions. Such understanding is essential to underpin best practice based on robust
335 scientific evidence (Comin et al. 2005; Wortley et al. 2013).

336

337 *Do functional and taxonomic diversity respond to local habitat conditions?*

338 Our results showed a positive impact of water chemistry (i.e. low content of phosphorus, high nitrogen content,
339 and low content of organic matter in constructed wetlands) over the taxonomic and functional composition of
340 macroinvertebrate assemblages, in line with results observed in other studies (e.g. Heino 2008; Kleinebecker et
341 al. 2010; Gallardo et al. 2008, 2012a).

342 Confirming our second hypothesis, constructed wetlands hosted a greater percentage of pioneer and
343 opportunistic species (i.e. displaying short life-spans, multiple reproductive cycles per year and active dispersal
344 strategies), and greater abundance of taxa with low tolerance to water eutrophication, such as Leptophlebiidae,
345 Cordulliidae, Coenagrionidae and Atyidae (Usseglio-Polatera and Tachet 1994). In contrast, the higher content of
346 phosphorus and organic matter recorded in natural wetlands (i.e. eutrophication), negatively affected diversity
347 indices at both the functional and taxonomic levels. Under severe eutrophication and oxygen reduction
348 conditions, only adapted taxa such as Diptera, Tubificidae and Heteroptera can survive and dominate the
349 invertebrate community of natural wetlands. The presence of adaptive strategies such as the production of
350 cocoons (Verbeck et al. 2008), and predominance of feeding habits based on fine particles and microorganisms

351 (Díaz et al. 2008; Gallardo et al. 2008; Céréghino et al. 2012) further suggest a natural response to
352 eutrophication. These observations contrast with results obtained by Culler et al. (2014), who observed a weak
353 relationship between environmental conditions and the structure and composition of invertebrate communities in
354 recently constructed wetlands. The authors pointed out temporal factors (e.g. seasonal environmental changes)
355 and the wetland's physical structure as the main drivers of the invertebrate community patterns. In our case,
356 taxonomic and functional diversity indexes significantly responded to changes in water chemistry according to
357 regression models considering season and riparian area as random factors. Having said that, we do not disregard
358 the possibility that seasonality and differences in shape and size between our paired study wetlands explain, to
359 some extent, the variability observed in diversity indices.

360 Apart from physicochemical factors, the presence of novel types of habitats (e.g. gravel substrata and submerged
361 vegetation) observed in constructed wetlands could also explain their greater presence of pioneer and
362 opportunistic species. These new and refreshed habitats provide greater substrate heterogeneity (gravels) to
363 attach and hide from predators, as well as provision of new food resources (submerged vegetation), which
364 altogether may favour rapid colonisation (Erman and Erman 1984; Kleef et al. 2006; Gallardo et al. 2012a). This
365 is congruent with the greater presence of scrapers, crawlers and temporarily attached organism observed in
366 constructed wetlands.

367 Other not studied influences, such as hydrological characteristics and habitat heterogeneity, may also have
368 played an important role in shaping the unexplained variance in the taxonomic and functional characteristics of
369 the study wetlands. For instance, several studies have observed a decrease in local biodiversity under conditions
370 of limited hydrological connectivity with the river, due to the restriction of resources and species transfer, and
371 consequent silting up of wetlands with fine sediments and emergent vegetation (Gascón et al. 2005; Jeffries
372 2011; Porst et al. 2012; Reckendorfer et al. 2012; Ruhí et al. 2012a; Gallardo et al. 2014). Likewise, other
373 studies have pointed out habitat heterogeneity, and in particular the diversity of the mineral substrate, as a
374 controlling factor of macroinvertebrate diversity. For example, Paillex et al. (2007) observed an increase of
375 functional and taxonomic diversity indices in areas with greater mineral substrate diversity after hydrological
376 connectivity restoration. All of these additional factors must be considered to establish efficient management
377 tools and improve the success of restoration projects.

378

379 *Concluding recommendations*

380 Results obtained from this study demonstrate the importance of creating and restoring wetlands in degraded
381 floodplains as a means to increase floodplain taxonomic and functional diversity. According to our results, the
382 efficacy of restoration projects can be to some point maximized by controlling water quality, mainly preventing
383 water eutrophication. However, if major large-scale stressors (e.g. climate change, diffuse pollution, land use
384 change) affecting natural wetlands are not tackled; constructed wetlands are likely to progressively degrade and
385 approach a similar ecological state to natural wetlands. Thus maintenance and monitoring plans must be enabled
386 to ensure that increased taxonomic and functional metrics are maintained in the long term.

387 In terms of monitoring biodiversity changes, our results suggest that the abundance, richness, and Shannon-
388 Wiener diversity of taxa and biological traits of macroinvertebrate communities are the most relevant indicators
389 to compare the composition and functionality of natural and constructed wetlands over time. These functional
390 indicators provide complementary information to traditional taxonomic diversity indices, reflecting ecological
391 processes taking place in the ecosystem through organisms in communities and ecosystems (e.g. used resources,
392 food web interactions, resistance ability, dispersal and reproduction).

393 In conclusion, we consider essential that policymakers and stakeholders continue to promote the construction
394 and restoration of wetlands in degraded floodplains. Restoration projects must incorporate both taxonomic and
395 functional aspects from the design to the implementation and monitoring steps to optimize and reinforce their
396 probability of success.

397

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408

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545

546 Table 1. Morphological and habitat characteristics of three natural and three constructed wetlands sampled in the
 547 Ebro floodplain. N = sample size.

548

	Abbr.	Units	Constructed Wetlands			Natural Wetlands		
			C1 (N=12)	C2 (N=12)	C3 (N=12)	N1 (N=12)	N2 (N=12)	N3 (N=12)
Age	Age	(years)	5	25	15	65	50	65
Area	Area	(Ha)	0.53	0.38	0.94	10.33	70.3	35.4
Depth	Dep	(m)	1	1.5	1.7	2.5	0.8	2
Riparian area	Rip		1	2	3	1	2	3
Habitat sampled ⁽¹⁾	Habi		FS, SV	GS, SV	FS, EV	FS, EV	FS, EV	FS, EV

553

554 (1) Habitats sampled were: Fine sediment (FS); Gravel sediment (GS); Emergent vegetation, specifically *Typha*
 555 sp. and *Phragmites* sp. (EV); and Submerged vegetation, specifically *Chara* sp. (SV).

556

557 Table 2. Physicochemical features (mean \pm SD) of three natural and three constructed wetlands located in the
 558 Ebro floodplain. N = sample size. Significant differences between seasons in each type of wetland are indicated
 559 with * ($P < 0.05$, Mann-Whitney U test).

560

561

Physicochemical parameters	Abbr.	Units	Constructed Wetlands		Natural Wetlands	
			Winter (N=18)	Spring (N=18)	Winter (N=18)	Spring (N=18)
Temperature	Temp	(°C)	7.7 \pm 1.8 (*)	22.2 \pm 3.1 (*)	7.5 \pm 1.1 (*)	23.8 \pm 3.3 (*)
pH	pH		7.90 \pm 0.12	8.03 \pm 0.32	8.06 \pm 0.15	7.88 \pm 0.30
Conductivity	Cond	(mS/cm)	4.09 \pm 3.27	4.78 \pm 3.67	2.37 \pm 0.95 (*)	3.02 \pm 1.49 (*)
Dissolved oxygen	DO	(mg/L)	11.91 \pm 0.53 (*)	8.85 \pm 0.96 (*)	11.0 \pm 0.9 (*)	8.9 \pm 1.5 (*)
Total suspended solids	TSS	(mg/L)	9.55 \pm 2.84 (*)	21.42 \pm 10.54 (*)	43.87 \pm 32.28	36.37 \pm 24.50
Total dissolved solids	TDS	(mg/L)	2901 \pm 2362	3477 \pm 2743	1808 \pm 976 (*)	2415 \pm 1263 (*)
Chlorophyll <i>a</i>	Chla	(μ g/L)	1.92 \pm 1.31 (*)	3.91 \pm 1.92 (*)	28.48 \pm 16.90	19.10 \pm 12.33
Organic matter	OM	(mg/L)	2.32 \pm 0.72 (*)	5.98 \pm 3.51 (*)	13.85 \pm 11.35	15.21 \pm 9.97
Dissolved inorganic nitrogen	DIN	(mgN/L)	0.64 \pm 0.22	0.63 \pm 0.80	2.71 \pm 2.27	1.29 \pm 1.87
Sulfate	SO4	(mg/L)	814.5 \pm 604.4	550.7 \pm 272.8	685.2 \pm 601.0 (*)	616.2 \pm 399.2 (*)
Soluble reactive phosphorus	SRP	(μ gP/L)	0.22 \pm 0.13 (*)	1.49 \pm 0.85 (*)	1.90 \pm 0.73	2.58 \pm 1.24
Total dissolved phosphorus	TDP	(μ gP/L)	4.99 \pm 1.84 (*)	7.63 \pm 3.59 (*)	33.87 \pm 40.10	15.39 \pm 8.29

562 Table 3. Diversity (mean \pm SD) of each biological trait in three constructed (CONS) and three natural (NAT)
 563 wetlands. Results from non-parametric analysis of variance (Mann-Whitney U test) between natural and
 564 constructed wetlands are shown in italics (Z ; P). $N = 72$ (36 for natural wetlands and 36 for constructed
 565 wetlands). n.s.= not significant.

566

Biological Traits	Richness	Shannon diversity	Rao's diversity
1. Maximal potential size	CONS= 4.89 \pm 0.82 NAT= 4.55 \pm 1.25 <i>(Z= - 1.39; P= n.s.)</i>	CONS= 1.30 \pm 0.18 NAT= 1.19 \pm 0.20 <i>(Z= - 2.28; P= 0.02)</i>	CONS= 3.70 \pm 1.95 NAT= 3.66 \pm 1.95 <i>(Z= - 0.10; P= n.s.)</i>
2. Life cycle duration	CONS= 1.92 \pm 0.28 NAT= 1.89 \pm 0.32 <i>(Z= - 0.39; P= n.s.)</i>	CONS= 0.20 \pm 0.22 NAT= 0.36 \pm 0.23 <i>(Z= - 2.95; P< 0.01)</i>	CONS= 0.75 \pm 0.96 NAT= 1.38 \pm 1.12 <i>(Z= - 2.65; P< 0.01)</i>
3. Potential number of cycles per year	CONS= 2.53 \pm 0.51 NAT= 2.17 \pm 0.38 <i>(Z= - 3.20; P< 0.01)</i>	CONS= 0.62 \pm 0.08 NAT= 0.51 \pm 0.22 <i>(Z= - 2.92; P< 0.01)</i>	CONS= 1.16 \pm 1.07 NAT= 1.04 \pm 1.17 <i>(Z= - 0.72; P= n.s.)</i>
4. Aquatic stages	CONS= 3.83 \pm 0.56 NAT= 3.67 \pm 0.68 <i>(Z= - 1.51; P= n.s.)</i>	CONS= 1.08 \pm 0.18 NAT= 1.12 \pm 0.19 <i>(Z= - 1.23; P= n.s.)</i>	CONS= 4.26 \pm 1.94 NAT= 3.50 \pm 2.02 <i>(Z= - 1.59; P= n.s.)</i>
5. Reproduction	CONS= 5.56 \pm 1.20 NAT= 4.86 \pm 1.05 <i>(Z= - 2.99; P= 0.003)</i>	CONS= 1.32 \pm 0.16 NAT= 1.27 \pm 0.19 <i>(Z= - 1.05; P= n.s.)</i>	CONS= 3.37 \pm 1.57 NAT= 3.04 \pm 1.50 <i>(Z= - 1.40; P= n.s.)</i>
6. Dispersal	CONS= 4.00 \pm 0.00 NAT= 3.81 \pm 0.58 <i>(Z= - 2.04; P= 0.04)</i>	CONS= 1.28 \pm 0.07 NAT= 1.09 \pm 0.27 <i>(Z= - 3.27; P< 0.01)</i>	CONS= 1.90 \pm 0.94 NAT= 2.27 \pm 1.20 <i>(Z= - 1.79; P= n.s.)</i>
7. Resistance forms	CONS= 3.06 \pm 0.53 NAT= 3.36 \pm 0.80 <i>(Z= - 2.21; P= 0.03)</i>	CONS= 0.74 \pm 0.15 NAT= 0.70 \pm 0.18 <i>(Z= - 0.61; P= n.s.)</i>	CONS= 1.08 \pm 0.71 NAT= 0.85 \pm 0.65 <i>(Z= - 1.39; P= n.s.)</i>
8. Respiration	CONS= 3.19 \pm 0.92 NAT= 2.69 \pm 0.95 <i>(Z= - 2.23; P= 0.03)</i>	CONS= 0.77 \pm 0.21 NAT= 0.72 \pm 0.28 <i>(Z= - 1.18; P= n.s.)</i>	CONS= 1.51 \pm 1.07 NAT= 1.51 \pm 1.28 <i>(Z= - 0.28; P= n.s.)</i>
9. Locomotion and substrate relation	CONS= 5.56 \pm 0.69 NAT= 5.25 \pm 1.08 <i>(Z= - 1.04; P= n.s.)</i>	CONS= 1.48 \pm 0.09 NAT= 1.39 \pm 0.24 <i>(Z= - 0.27; P= n.s.)</i>	CONS= 3.65 \pm 2.57 NAT= 4.10 \pm 2.58 <i>(Z= - 1.04; P= n.s.)</i>
10. Food	CONS= 7.00 \pm 1.24 NAT= 6.08 \pm 1.64 <i>(Z= - 2.63; P< 0.01)</i>	CONS= 1.63 \pm 0.14 NAT= 1.52 \pm 0.18 <i>(Z= - 2.85; P< 0.01)</i>	CONS= 6.31 \pm 3.58 NAT= 5.20 \pm 3.25 <i>(Z= - 1.37; P= n.s.)</i>
11. Feeding habits	CONS= 6.78 \pm 0.87 NAT= 6.44 \pm 1.78 <i>(Z= - 0.60; P= n.s.)</i>	CONS= 1.71 \pm 0.10 NAT= 1.58 \pm 0.23 <i>(Z= - 2.63; P< 0.01)</i>	CONS= 4.10 \pm 2.14 NAT= 3.60 \pm 1.83 <i>(Z= - 0.87; P= n.s.)</i>

567

568 Table 4. Results from Linear Mixed Effects Models linking physicochemical variables with a number of
 569 taxonomic and functional diversity indices. All selected explanatory variables were statistically significant at $P <$
 570 0.01. d^2 = variance of the random intercept. α = variance of the fixed intercept. ρ = Spearman correlation
 571 coefficient between observed and predicted values of the selected model used as a measure of goodness of fit.

572

DIVERSITY INDICES	Explanatory variables	Slope of explanatory variables	Intercept	Spearman correlation test
Taxonomic				
Abundance total	TDP	-18.87	$d^2 = 401.15^2$	$\rho = 0.84$
	Cond	280.43	$\alpha = -45.34$	$P < 0.01$
Richness total	SRP	-1.48	$d^2 = 2.52^2$	$\rho = 0.46$
	DIN	0.89	$\alpha = 5.38$	$P < 0.01$
	Cond	0.48		
Shannon diversity	SRP	-0.21	$d^2 = 0.25^2$	$\rho = 0.62$
	DIN	0.11	$\alpha = 1.03$	$P < 0.01$
Rao's diversity	SRP	-0.37	$d^2 = 0.57^2$	$\rho = 0.53$
	DIN	0.24	$\alpha = 1.88$	$P < 0.01$
Functional				
Richness total	OM	-0.50	$d^2 = 3.76^2$	$\rho = 0.19$
			$\alpha = 51.19$	$P < 0.01$
Shannon diversity	OM	-0.005	$d^2 = 0.02^2$	$\rho = 0.50$
			$\alpha = 3.52$	$P < 0.01$
Rao's diversity	SRP	-6.34	$d^2 = 8.14^2$	$\rho = 0.68$
	DIN	3.29	$\alpha = 36.46$	$P < 0.01$

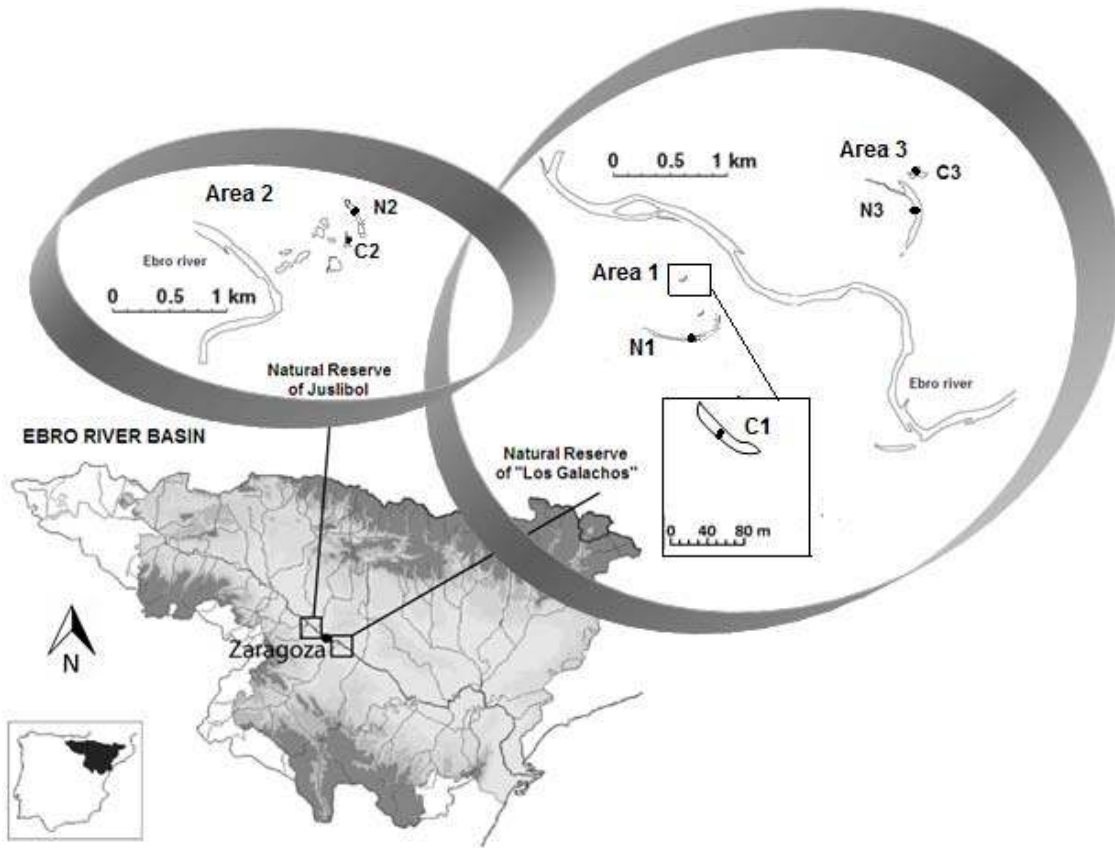
573

574

575 Figure 1. Study site location. Riparian areas: Area 1, Area 2, and Area 3. Natural wetlands: N1, N2, and N3.

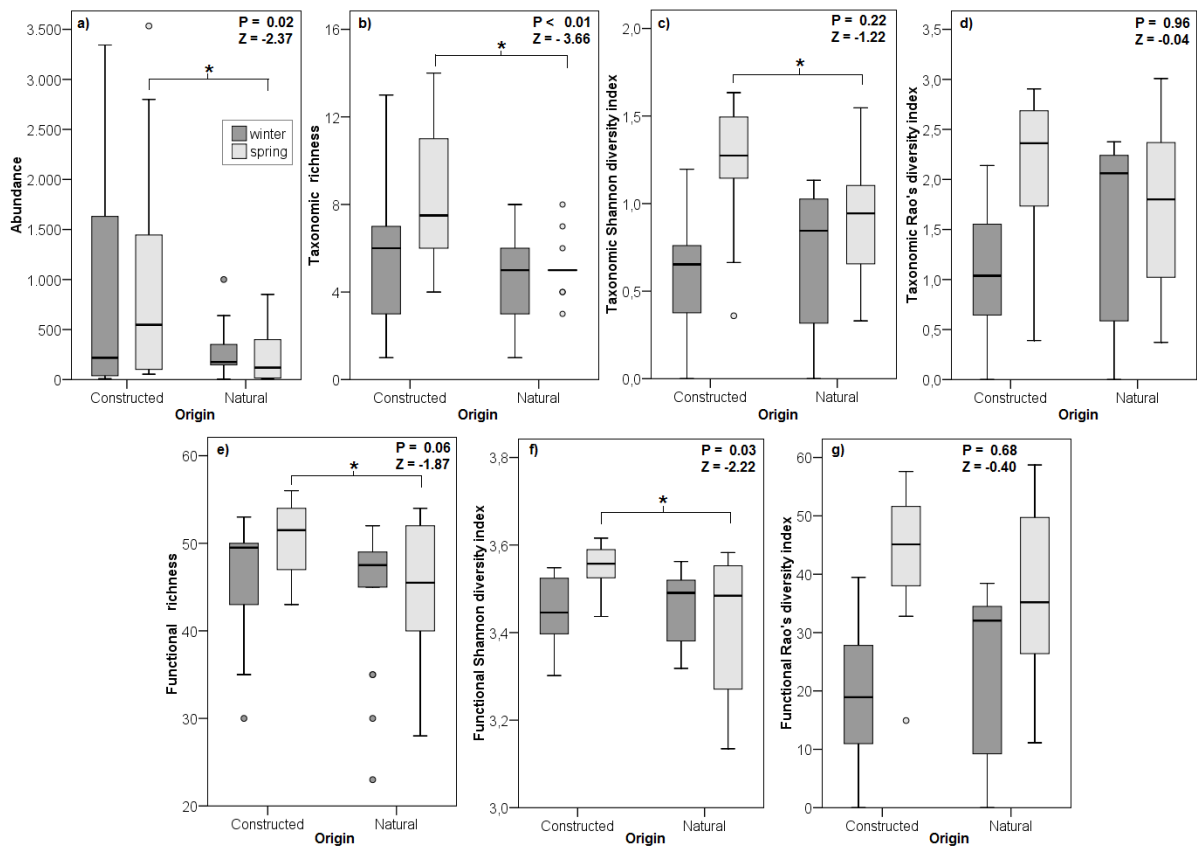
576 Constructed wetlands: C1, C2, and C3. Black dots correspond to wetlands sampling sites.

577



578

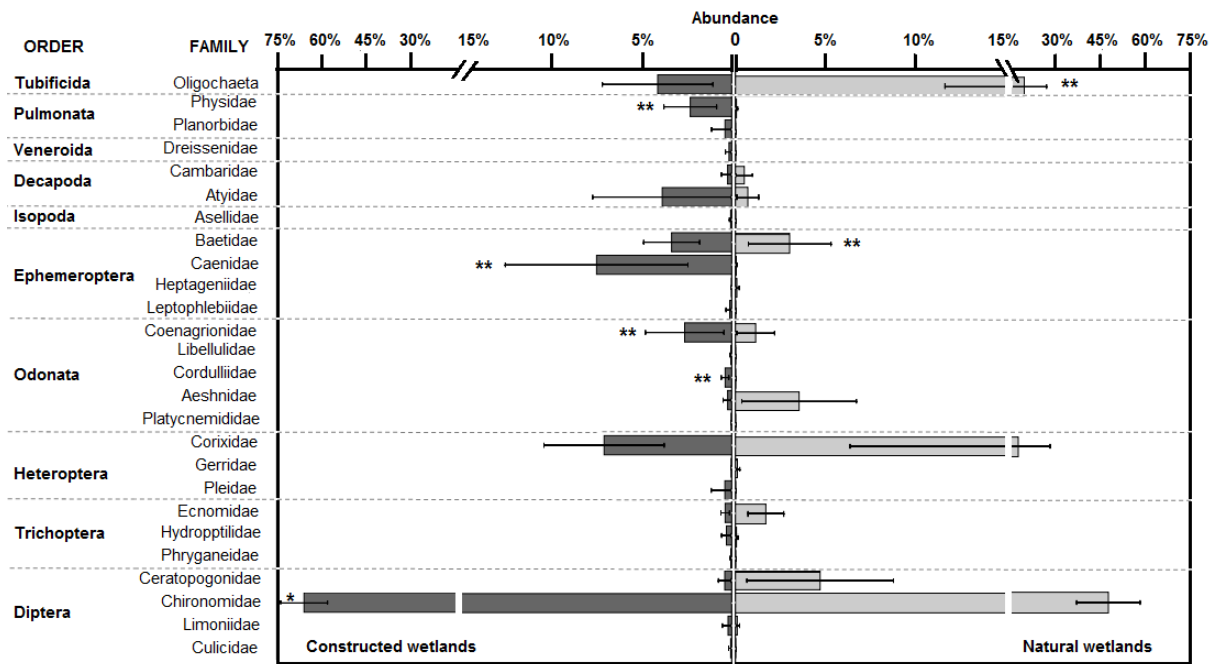
579 Figure 2. Taxonomic and functional diversity in three constructed and three natural wetlands. Results from non-
 580 parametric analysis of variance (Mann-Whitney U test) between constructed and natural wetlands are shown in
 581 the upper right corner of each graph. Significant differences between constructed and natural wetlands in each
 582 season are indicated with * ($P < 0.05$, Mann-Whitney U test). Grey circles show outliers.
 583



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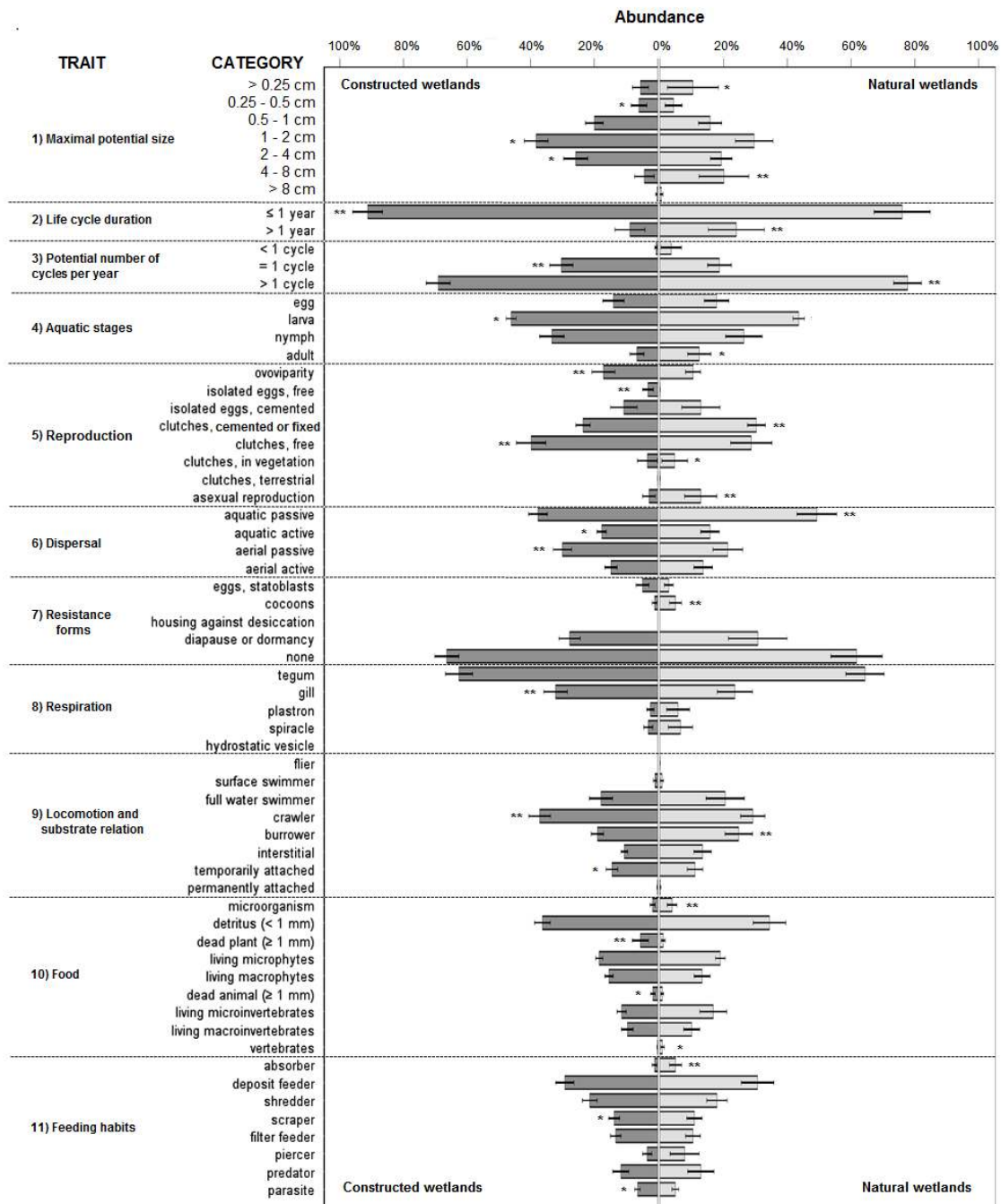
585 Figure 3. Differences in macroinvertebrate assemblages between constructed (left side) and natural wetlands
 586 (right side) of the Ebro floodplain. Bars represent the relative abundance (average percentage \pm SD) of each
 587 family in the macroinvertebrate community. Asterisks on the left side (* $P < 0.05$ or ** $P < 0.01$) indicate
 588 significantly higher absolute abundance (total number of individuals) in constructed than in natural wetlands
 589 (Mann-Whitney U test). Asterisks on the right side indicate significantly higher absolute abundance in natural
 590 than in constructed wetlands (Mann-Whitney U test).

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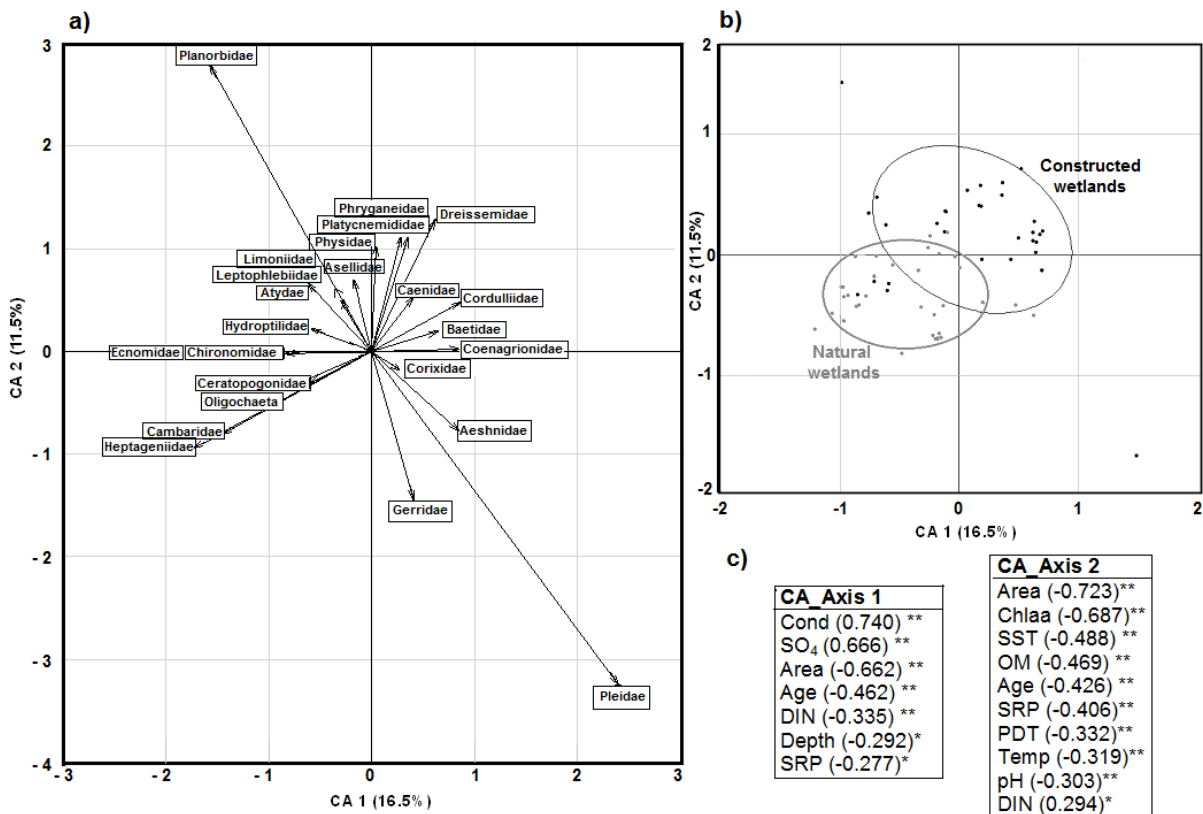
594 Figure 4. Differences in biological traits abundance between constructed (left side) and natural wetlands (right
 595 side) of the Ebro floodplain. Bars represent the relative abundance (average percentage \pm SD) of each category
 596 for the 11 biological traits evaluated. Asterisks on the left side (* $P < 0.05$ or ** $P < 0.01$) indicate significantly
 597 higher absolute abundance (total number of individuals for each trait category) in constructed than in natural
 598 wetlands (Mann-Whitney U test). Asterisks on the right side indicate significantly higher absolute abundance in
 599 natural than in constructed wetlands (Mann-Whitney U test).



600

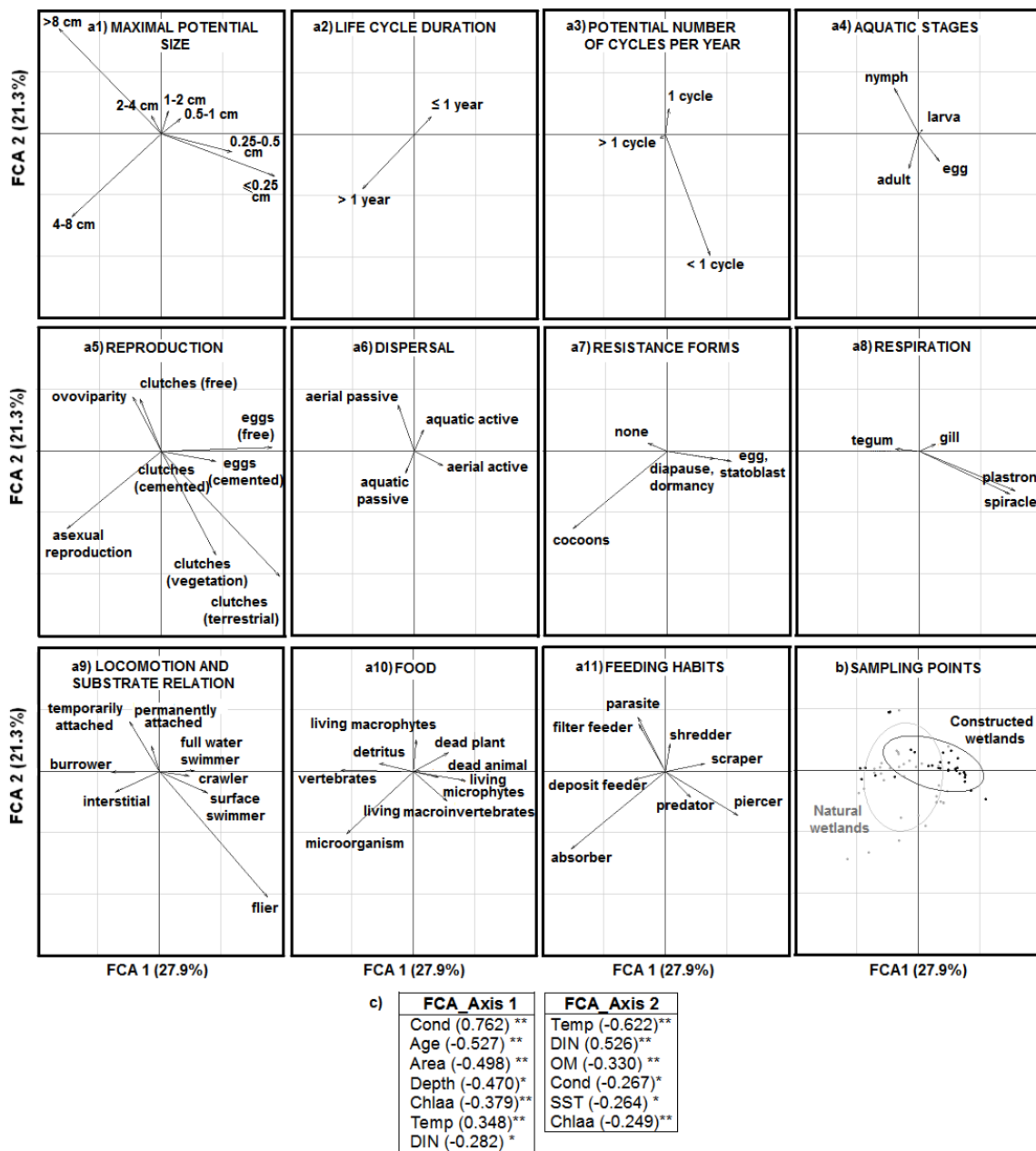
601

602 Figure 5. Results from multivariate analysis performed with data on macroinvertebrate assemblages of three
 603 natural and three constructed wetlands located on the Ebro River floodplain. a) Distribution of macroinvertebrate
 604 families on the first two axes of the taxa-based Correspondence Analysis (CA). b) Plot of sampling points:
 605 constructed wetlands (black dots) and natural wetlands (grey dots). Ellipses encompass 1.5 times variance of
 606 observations in each wetland type. c) Environmental variables (physicochemical parameters and morphological
 607 features: depth, area and age) significantly correlated with the first two axes of the CA (non-parametric
 608 Spearman correlation values ρ are presented in parentheses; * $P < 0.05$; ** $P < 0.01$).



610
611

612 Figure 6. Results from multivariate analysis performed with data on macroinvertebrate assemblages of three
 613 natural and three constructed wetlands located on the Ebro River floodplain. a1 - a11) correlation of 11
 614 biological trait categories with the first two axes of the trait-based Fuzzy Correspondence Analysis (FCA). b)
 615 Sampling points distribution: constructed wetlands (black dots) and natural wetlands (grey dots). Ellipses
 616 encompass 1.5 times variance of observations in each wetland type. c) Environmental variables
 617 (physicochemical parameters and morphological features: depth, area and age) significantly correlated with the
 618 first two axes of the FCA (non-parametric Spearman correlation test, ρ values are presented in parentheses; * $P <$
 619 0.05; ** $P < 0.01$).



620

621 Appendix 1. Macroinvertebrate taxa occurrence (X) in each of three constructed and three natural wetlands

622 studied in the Ebro Floodplain during winter 2010 (W) and spring 2011 (S).

623

Order	Family	Taxa	Constructed wetlands						Natural wetlands						
			C1		C2		C3		N1		N2		N3		
			W	S	W	S	W	S	W	S	W	S	W	S	
Tubificida	Oligochaeta				X	X	X	X	X	X	X	X	X	X	X
Pulmonata	Physidae	<i>Physa</i>			X	X		X			X				
	Planorbidae	<i>Ferrisia</i>													
Veneroida	Dreissenidae	<i>Dreissena</i>			X										
Decapoda	Cambaridae	<i>Procambarus</i>					X				X	X	X	X	
	Atyidae	<i>Atyaephyra</i>			X		X				X				X
Isopoda	Asellidae	<i>Asellus</i>				X									
Ephemeroptera	Baetidae	<i>Baetis</i>	X	X	X	X		X	X	X			X	X	
	Caenidae	<i>Caenis</i>	X	X	X	X		X			X				
	Leptophlebiidae	<i>Thraulodes</i>						X						X	
Odonata	Coenagrionidae	<i>Ischnura</i>	X	X	X	X			X	X				X	
	Libellulidae	<i>Libellula</i>				X									
	Corduliidae	<i>Oxygastra</i>	X	X	X	X									
	Aeshnidae	<i>Boyeria (irene)</i>		X	X	X				X		X			
Heteroptera	Platycnemidae	<i>Platycnemis</i>				X									
	Corixidae	<i>Micronecta</i>	X	X		X		X				X			
	Gerridae	<i>Cymatia</i>								X					X
Trichoptera	Pleidae	<i>Gerris</i>		X								X			
	Pleidae	<i>Plea</i>		X											
	Ecnomidae	<i>Ecnomus</i>			X	X					X		X	X	X
Diptera	Hydroptilidae	<i>Agraylea</i>				X	X						X	X	
	Phryganeidae	<i>Agrypnia</i>			X										
	Ceratopogonidae	<i>Culicoides</i>		X	X	X		X			X	X	X	X	X
	Chironomidae	<i>Tanypodinae</i>				X									
Diptera	Others		X	X	X	X	X	X	X	X	X	X	X	X	X
	Limoniidae	<i>Eriopterini</i>			X			X			X				X
	Culicidae	<i>Culicinae</i>		X											

624

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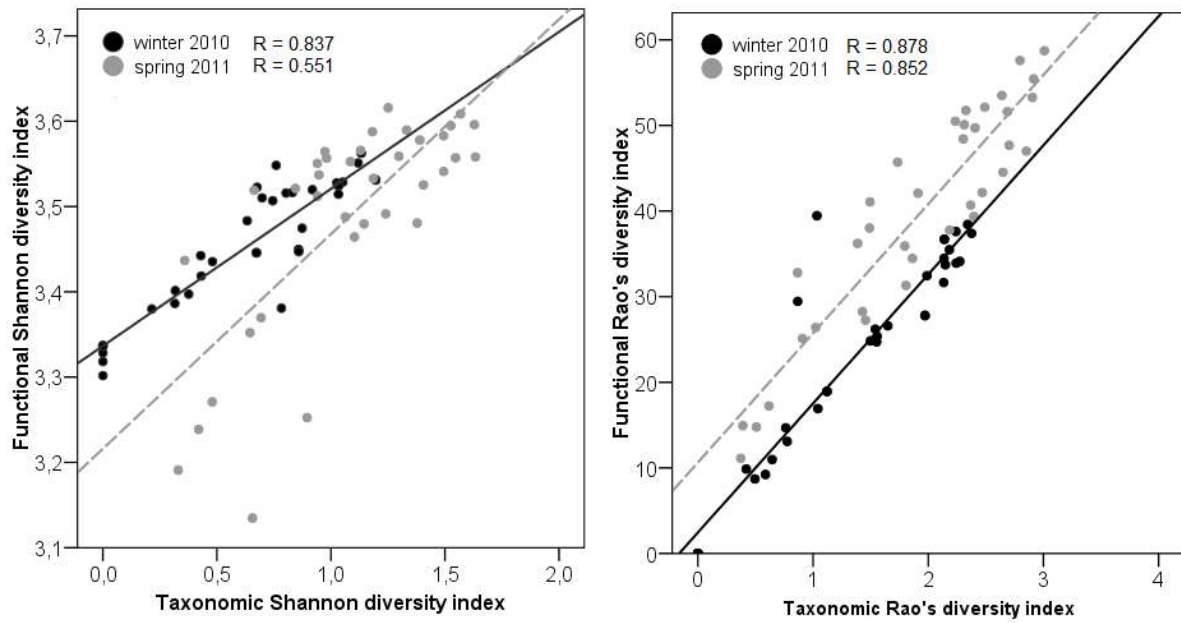
626 Appendix 2. Biological traits and categories for invertebrate taxa considered in the present study. Traits and
 627 categories were those defined by Tachet et al. (2010).

628

Biological Trait	Category
1) Maximal potential size	1.1) > 0.25 cm. 1.2) 0.25-0.5 cm. 1.3) 0.5-1 cm. 1.4) 1-2 cm. 1.5) 2-4 cm. 1.6) 4-8 cm. 1.7) > 8 cm.
2) Life cycle duration	2.1) ≤ 1 year. 2.2) > 1 year.
3) Potential number of cycles per year	3.1) < 1 cycle/year. 3.2) = 1 cycle/year. 3.3) > 1 cycle/year.
4) Aquatic stages	4.1) Egg. 4.2) Larva. 4.3) Nymph. 4.4) Adult.
5) Reproduction	5.1) Ovoviviparity. 5.2) Isolated eggs, free. 5.3) Isolated eggs, cemented. 5.4) Clutches, cemented or fixed. 5.5) Clutches, in vegetation. 5.6) Clutches, terrestrial. 5.7) Asexual reproduction.
6) Dispersal	6.1) Aquatic passive. 6.2) Aquatic active. 6.3) Aerial passive. 6.4) Aerial active.
7) Resistance forms	7.1) Eggs, statoblasts. 7.2) Cocoons. 7.3) Housing/Cells against desiccation. 7.4) Diapause or dormancy. 7.5) None.
8) Respiration	8.1) Tegum. 8.2) Gill. 8.3) Plastron. 8.4) Spiracle (aerial). 8.5) Hydrostatic vesicle.
9) Locomotion and substrate relation	9.1) Flier. 9.2) Surface swimmer. 9.3) Full water swimmer. 9.4) Crawler. 9.5) Burrower (epibenthic). 9.6) Interstitial (endobenthic). 9.7) Temporarily attached. 9.8) Permanently attached.
10) Food	10.1) Microorganism. 10.2) Detritus (< 1mm). 10.3) Dead plant (≥ 1 mm). 10.4) Living microphytes. 10.5) Living macrophytes. 10.6) Dead animal (≥ 1 mm). 10.7) Living microinvertebrates. 10.8) Living macroinvertebrates. 10.9) Vertebrates.
11) Feeding habits	11.1) Scraper. 11.2) Deposit feeder. 11.3) Shredder. 11.4) Scraper. 11.5) Filter feeder. 11.6) Piercer (plants or animals). 11.7) Predator (carver/engulfer/swallower). 11.8) Parasite.

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630

631 Appendix 3. Relationship between functional and taxonomic diversity indices. The black and grey lines
632 represent a linear regression with winter (N = 36), and spring samples (N=32), respectively.



633