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

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Abstract

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

Keywords:

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

Introduction

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

Macroinvertebrates are considered as excellent quality bioindicators for aquatic ecosystems due to their ubiquity, life-cycles, abundance and diversity of species with varying life history strategies regulated to habitat conditions (Wallace and Webster 1996; Bêche and Statzner 2009; Gallardo et al. 2011); as well as to their contribution to ecosystem functions, including detritus processing, nutrient cycling and food provision to higher trophic levels, among others (Heino 2005). In this sense, macroinvertebrate biological traits such as feeding habits (indicator of resource availability), body size (stability, food web structure), locomotion (capacity to colonize new habitats), reproductive method and life cycle characteristics (resistance and resilience to disturbances) provide direct and indirect information about a given ecosystem structure and function (Gayraud et al. 2003; Bonada et al. 2006; Tachet et al. 2010).The overall diversity of biological traits is directly affected by human-induced disturbances, such as changes in hydrological connectivity (Paillex et al. 2008; Gallardo et al. 2009a, 2009b, 2014), nutrient concentration (Heino 2005, 2008), heavy metal pollution (Dolédec and Statzner 2008), and changes in land use (Díaz et al. 2008; Vandewalle et al. 2010). Nonetheless, because some traits may be more relevant to indicate restoration success than others (e.g. feeding structure, active/passive locomotion, presence of resistance forms), it is important to investigate the response of individual as well as the whole set of traits to restoration. Macroinvertebrate traits should thus provide a powerful indicator of the ecological state of restored wetlands and their ability to recreate fully functional ecosystems.

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

The present study examines the short and medium-term efficacy of wetland construction projects on a large regulated river floodplain (River Ebro, NE Spain), where natural wetlands are in a degraded state. First, we compared the taxonomic and functional diversity and composition of the macroinvertebrate community in a set of constructed and natural wetlands to investigate if the creation of artificial wetlands provides new and more species and functions to the floodplain. Second, we identified the physicochemical variables responsible for the observed diversity patterns and community composition, so that these strategic variables can be manipulated in future projects to promote greater functionality for restored ecosystems. We particularly hypothesized a higher taxonomic and functional diversity in constructed than in natural wetlands, due to the degradation experienced by natural wetlands and the better water quality provided by constructed wetlands. Consequently, the two types of wetlands should have different community compositions at taxonomic and functional levels, with more pioneering and opportunistic species in constructed wetlands and more eutrophic tolerant species in natural wetlands. We further predicted that the main factors driving the observed changes in diversity patterns and community composition in our study area are those related to eutrophication status. Ultimately, this study aims to emphasize the importance of including functional criteria in ecological restoration projects as a means of advancing towards multi-functional wetlands that maximize the recovery of functions, thereby optimizing the allocation of the limited resources invested in restoration schemes.

Material and methods

Study area

The study area was located in the Middle Ebro River (NE Spain), which has a length of 901 km and a drainage basin of 85,534 km^2. Historically, extraordinary flood events in the Ebro River have generated a number of natural wetlands in its floodplain, including temporary pools and oxbow wetlands (Ollero 2007). However, since the 1960s, the Ebro River has been extensively affected by an increase in human activity, leading to drastic land use changes (agriculture and urban areas) and the extensive construction of structures to control floods (Cabezas et al. 2008). These pressures have caused the degradation of relict wetlands and the reduction of the river's capacity for creating new natural wetlands on its floodplain (Gallardo et al. 2012a). Consequently, a number of restoration projects have been developed over the last two decades to restore and/or create artificial wetlands along the Ebro River floodplain to mitigate habitat loss and increase local biodiversity.

Previous studies have analysed aquatic community changes, sedimentation rates, and aquatic metabolic rates in natural *vs.* constructed wetlands in the Ebro floodplain. These projects have identified hydrological connectivity, water quality and habitat succession as major drivers of floodplain structure and functionality (e.g. Gallardo et al. 2008, 2012a, 2012b; Cabezas et al. 2008, 2009a, 2009b, 2009c; Español et al. 2013). These studies have also illustrated the benefits of wetland restoration at the local scale. As way of example, only one year after the construction of one of the restored wetlands, Gallardo et al. (2012a) recorded much higher taxonomic and functional diversity values than those of nearby natural wetlands. Studies nevertheless focussed on the early years after restoration, with no further monitoring of the trends observed. This lack of information impairs the capacity of environmental managers to both address the efficacy of past restoration activities and promote the development of future projects.

For this study, we selected three riparian areas each comprising one constructed and one natural wetland located no farther apart than 1 km in the floodplain of the Middle Ebro River (NE Spain, 41º39'N, 0º52'W, Fig. 1). In particular, riparian area 1 included wetlands N1 and C1; riparian area 2 included wetlands N2 and C2, and riparian area 3 included wetlands N3 and C3 (Fig. 1). Wetlands C1 and C3 were created through excavation in the surroundings of wetlands N1 and N3, respectively. These wetlands are filled through water seepage from the hillslope aquifer. Riparian vegetation was transplanted on the wetland banks to facilitate shore stabilization and colonization. Wetland C2 consists of an old gravel pit, which was restored through hydrological re-connection and riparian vegetation introduction. These constructed wetlands were created not to be as extant natural wetlands but as a complement, providing new and more diverse habitats to local flora and fauna in the floodplain. The proximity between paired wetlands provided a unique opportunity to investigate the development of constructed and natural reference wetlands that share the same environmental conditions (e.g. hydrological influence, isolation, and wind speed). It should be noted that in this study we use the terms 'natural' as representative of the wetland origin and 'reference' as representative of natural conditions regardless of their environmental quality (i.e. no restoration intervention), as opposed to 'good reference' conditions applied in other studies.

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

Morphological and Physicochemical characteristics

The average depth (m) of each wetland was measured along transects from shore to centre. Surface area (Ha) was obtained from digitalised aerial photographs. Age (years) was calculated from the date of construction or from first observation according to Cabezas et al. (2008).Triplicate water samples were collected at each sampling point and season directly into 1.5 L PVC bottles previously washed in acid (CLH 0.1 N) at a depth of 10 cm, and placed on ice (see total number of samples in Table 1). Total suspended solids (mg/L), total dissolved solids (mg/L) and organic matter (mg/L) content were determined by the gravimetric method, i.e. filtering samples through pre-combusted (450ºC, 4 h) Whatman GF/F glass-fibre filters following standard protocols (APHA 1989). Chlorophyll *a* (µg/L) samples were filtered through Whatman GF/F glass-fibre filters, pigments were extracted in 96% ethanol for 24 h, and analysed using the spectrophotometric method (Thermo Helios α; APHA 1989). Filtered water aliquots were stored at -20 ºC, and used within one month for the following analyses. Ion chromatography (Metrohm 861 Advanced Compact IC; APHA 1989) was applied to determine dissolved inorganic nitrogen (DIN = $NH_4^+ + NO_2^- + NO_3^-$, mgN/L) and sulfate (SO_4^{2-}, mg/L) concentration. Soluble reactive phosphorus (SRP, µg/L) was measured by the ascorbic acid method (APHA 1989). Total dissolved phosphorus (TDP, µg/L) was also estimated by the ascorbic acid method, but a previous potassium persulfate digestion was performed (90 min, 115 °C) (APHA 1989). Finally, water temperature (ºC), pH, conductivity (mS/cm) and dissolved oxygen (mg/L) were recorded *in situ* with portable probes (WTW Multiline P4 and Hach-Lange HQ).

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

Taxonomic and functional composition of the macroinvertebrate community

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

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

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

Finally, taxonomic and functional diversity metrics were computed. At the taxonomic level, we calculated: (i) total abundance of individuals; (ii) total richness of taxa (family level); (iii) Shannon-Wiener diversity index, which incorporates the relative abundance of the different taxa; and (iv) Rao's quadratic diversity index (Botta-Dukát 2005), which takes into account the pairwise dissimilarities among taxa. Likewise, indexes calculated at the functional level included: (i) abundance of individuals for each trait category; (ii) total richness of trait categories (from a total of 63 trait categories); (iii) Shannon-Wiener diversity index; and (iv) Rao's quadratic diversity index. In addition, we calculated the richness, Shannon-Wiener diversity and Rao's quadratic diversity of each of the 11 traits considered in this study (e.g. richness of reproduction modes or feeding habits). Taxonomic and functional metrics were computed using the "vegan" (Oksanen et al. 2008) and "ade4" (Thioulouse et al. 1997) packages of R software, version 2.12.2 (R Development CoreTeam 2007).

Statistical analysis

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

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

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

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

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

Results

Morphological and Physicochemical characteristics of natural and constructed wetlands

Natural wetlands were older (50 - 65 years) and had a larger surface area (10 - 70 ha) than constructed wetlands (5 - 25 years, 0.4 - 0.9 ha, respectively), whereas both types of wetlands had similar depths (Table 1). Unlike constructed wetlands, natural wetlands lacked habitats with gravel sediment or submerged vegetation. Concentration values of total suspended solids, chlorophyll *a*, organic matter, and phosphorous compounds (SRP and TDP) were more than twofold in natural wetlands than in constructed wetlands (Table 2). Physicochemical parameters also showed significant variation between seasons (Table 2). For instance, temperature ($Z = -5.14$, $P < 0.01$, N = 72) and conductivity ($Z = -2.86$, $P = 0.04$, N= 72) were significantly higher in spring than in winter; whereas total nitrogen ($Z = -3.39$, $P < 0.01$, N = 72) and dissolved oxygen ($Z = -4.07$, $P < 0.01$, N = 72) were greater in winter.

Taxonomic and functional composition of natural and constructed wetlands

Functional diversity indices showed a significant positive relationship with taxonomic diversity (Spearman test, $\rho > 0.6$; see Appendix 3), and both showed significantly higher values in spring than winter (Table 3). Non-parametric analysis of variance showed significant differences between natural and constructed wetlands for taxonomic and functional metrics. More specifically, the abundance ($Z = -2.37$, $P = 0.02$, N = 72) and taxonomic richness ($Z = -3.66$, $P < 0.01$, N = 72) were significantly higher in constructed wetlands (Fig. 2). The most abundant family in both types of wetlands was Chironomidae, which accounted for over 50% of individuals. This was followed by Oligochaeta (ca. 20%) and Corixidae (ca. 20%) in natural wetlands, whereas a wider range of macroinvertebrates (e.g. Corixidae, Caenidae, Atyidae, Oligochaeta) co-dominated constructed wetlands (Fig. 3).

At the functional level, the Shannon-Wiener diversity of all biological traits together was significantly higher in constructed than in natural wetlands ($Z = -2.22$, $P = 0.03$, N = 72, Fig. 2). When analyses were made separately by biological trait, significant differences between constructed and natural wetlands emerged. For instance, constructed wetlands showed highest richness and Shannon-Wiener diversity for most biological traits, overall those related to plurivoltinism, reproduction, dispersal, respiration, food sources and feeding habits (Table 4). In contrast, natural wetlands illustrated greater richness of resistance forms and life-cycle duration categories. More specific differences in the taxonomic and trait profiles exhibited by natural and constructed wetlands can be seen in figures 3 and 4.

Physicochemical factors controlling macroinvertebrate assemblages in natural and constructed wetlands

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

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

Discussion

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

Do functional and taxonomic diversity change after ecological restoration?

Significantly higher taxa and trait richness of macroinvertebrates were observed in constructed wetlands when compared to degraded natural wetlands, in spite of the striking resemblance of their taxonomic and trait profiles. Our study thus confirms previous results by Gallardo et al. (2012a), who reported much greater taxonomic richness and Shannon diversity in the Ebro constructed wetlands when compared to nearby natural wetlands only one year following restoration. Furthermore, authors reported the occurrence in natural wetlands of several novel species of Odonata never recorded before the construction of wetlands in that riparian area, implying that constructed wetlands could act as a source of new and/or lost species towards natural wetlands. In contrast, Ruhí et al. (2012a) suggested that the local taxonomic and functional diversity is not affected by wetland construction. They observed that during the first three years after restoring a number of wetlands, the local loss of pioneer species was not compensated by the arrival of new taxa with less dispersal capacity and/or fewer special requirements, such as those present in the natural wetlands used as a reference. The apparent contradiction between these two investigations could be explained by their choice of reference conditions. In the case of Ruhí et al. (2012a), reference wetlands were in a relatively good conservation state, featuring high hydrological connectivity and water physicochemical quality. In contrast, natural wetlands used in the present study, and also by Gallardo et al. (2012a), experienced intense degradation due to restrained connectivity with the main river channel, eutrophication and excessive accumulation of fine sediments (Cabezas 2008, 2009c; Gallardo et al. 2008, 2012b). It is therefore not surprising that the better water quality offered by constructed wetlands resulted in higher diversity in relation to degraded natural wetlands in our models. Yet this observation is not trivial, since it can greatly support the construction of new wetlands in river floodplains affected by river regulation and accumulation of nutrients, widespread problems that affect not only our study area but also the majority of rivers in Europe and North America (Mitsch and Gosselink 2007).

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

Do functional and taxonomic diversity respond to local habitat conditions?

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

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

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

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

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

Concluding recommendations

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

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

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

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

			Constructed Wetlands			**Natural Wetlands**		
	Abbr.	**Units**	**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	35.45
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

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

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

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

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

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

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

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*

Figure 1. Study site location. Riparian areas: Area 1, Area 2, and Area 3. Natural wetlands: N1, N2, and N3. Constructed wetlands: C1, C2, and C3. Black dots correspond to wetlands sampling sites.

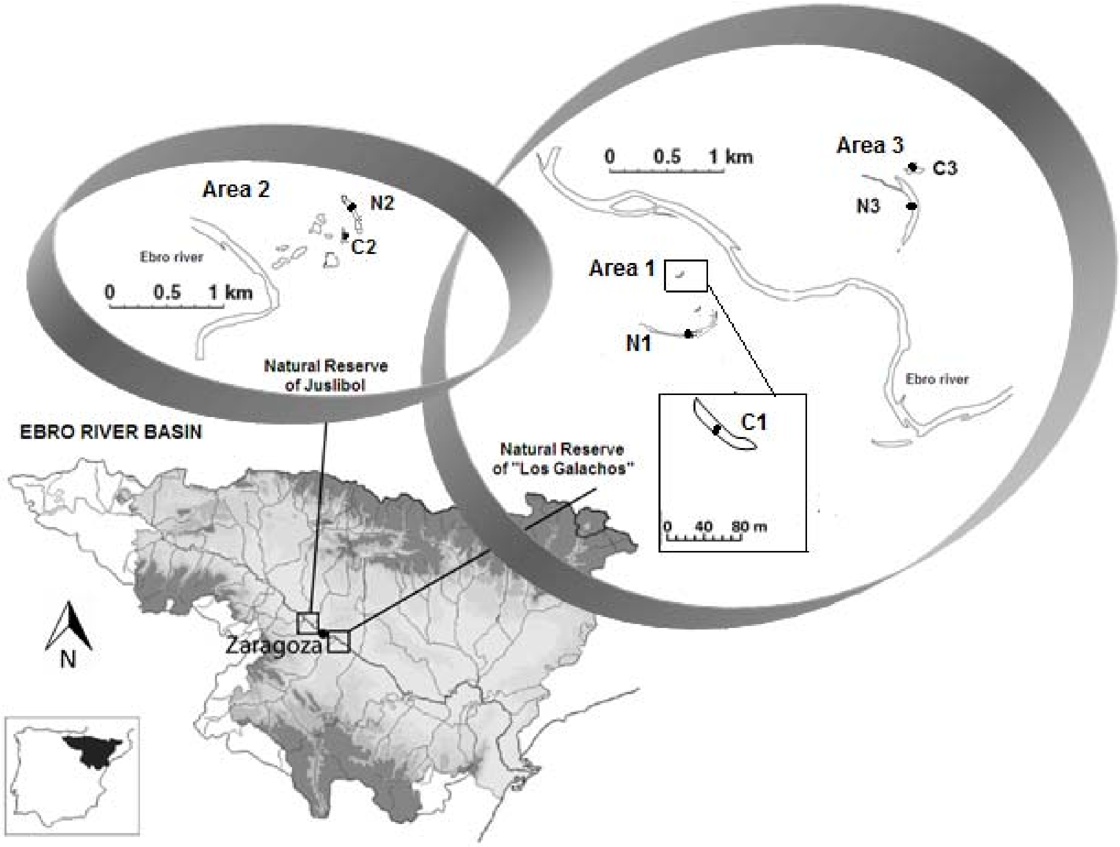


Figure 2. Taxonomic and functional diversity in three constructed and three natural wetlands. Results from non-parametric analysis of variance (Mann-Whitney *U* test) between constructed and natural wetlands are shown in the upper right corner of each graph. Significant differences between constructed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

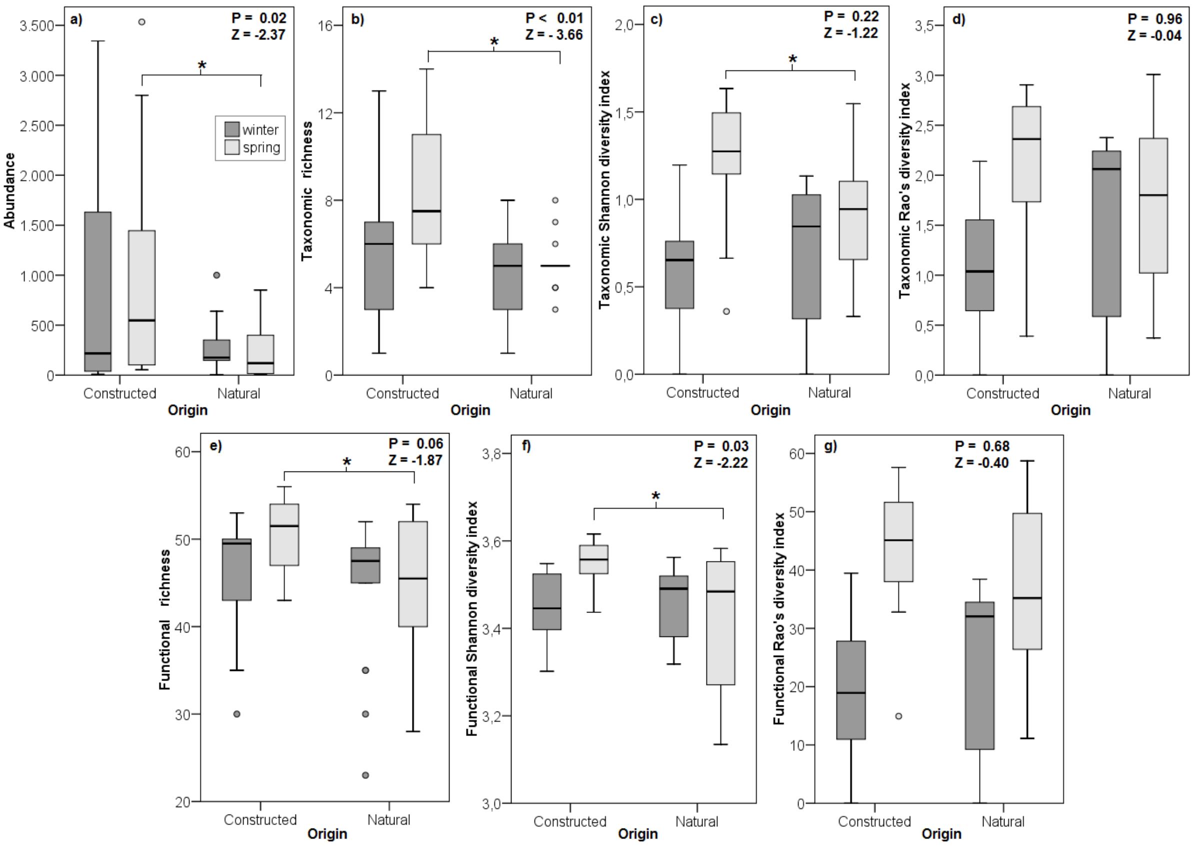


Figure 3. Differences in macroinvertebrate assemblages between constructed (left side) and natural wetlands (right side) of the Ebro floodplain. Bars represent the relative abundance (average percentage ± SD) of each family in the macroinvertebrate community. Asterisks on the left side (* $P < 0.05$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

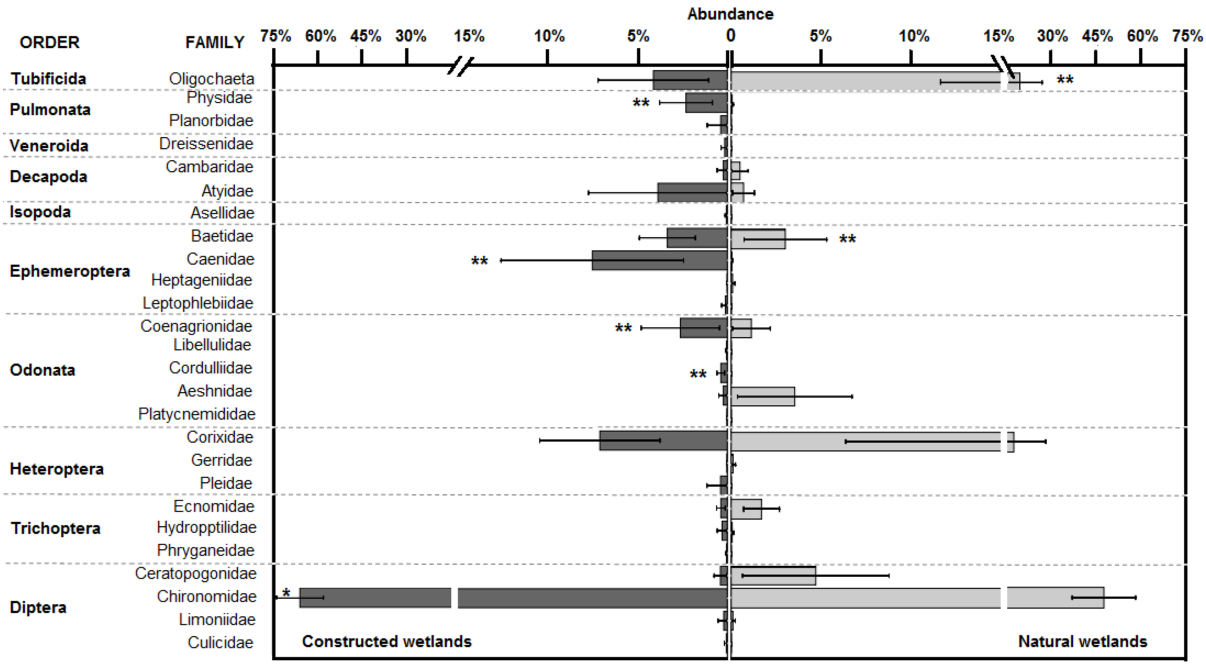


Figure 4. Differences in biological traits abundance between constructed (left side) and natural wetlands (right side) of the Ebro floodplain. Bars represent the relative abundance (average percentage ± SD) of each category for the 11 biological traits evaluated. Asterisks on the left side (* $P < 0.05$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

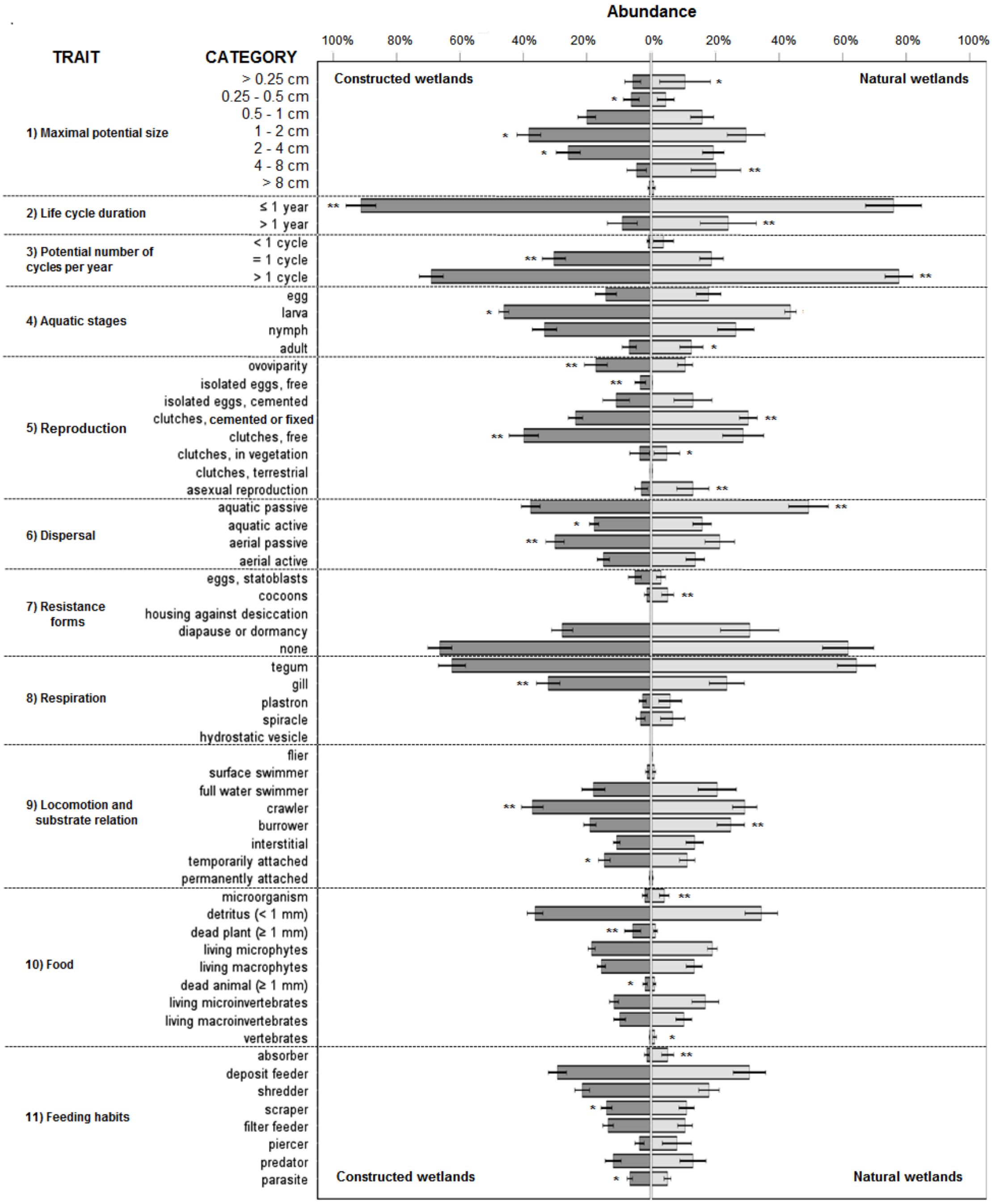


Figure 5. Results from multivariate analysis performed with data on macroinvertebrate assemblages of three natural and three constructed wetlands located on the Ebro River floodplain. a) Distribution of macroinvertebrate families on the first two axes of the taxa-based Correspondence Analysis (CA). b)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

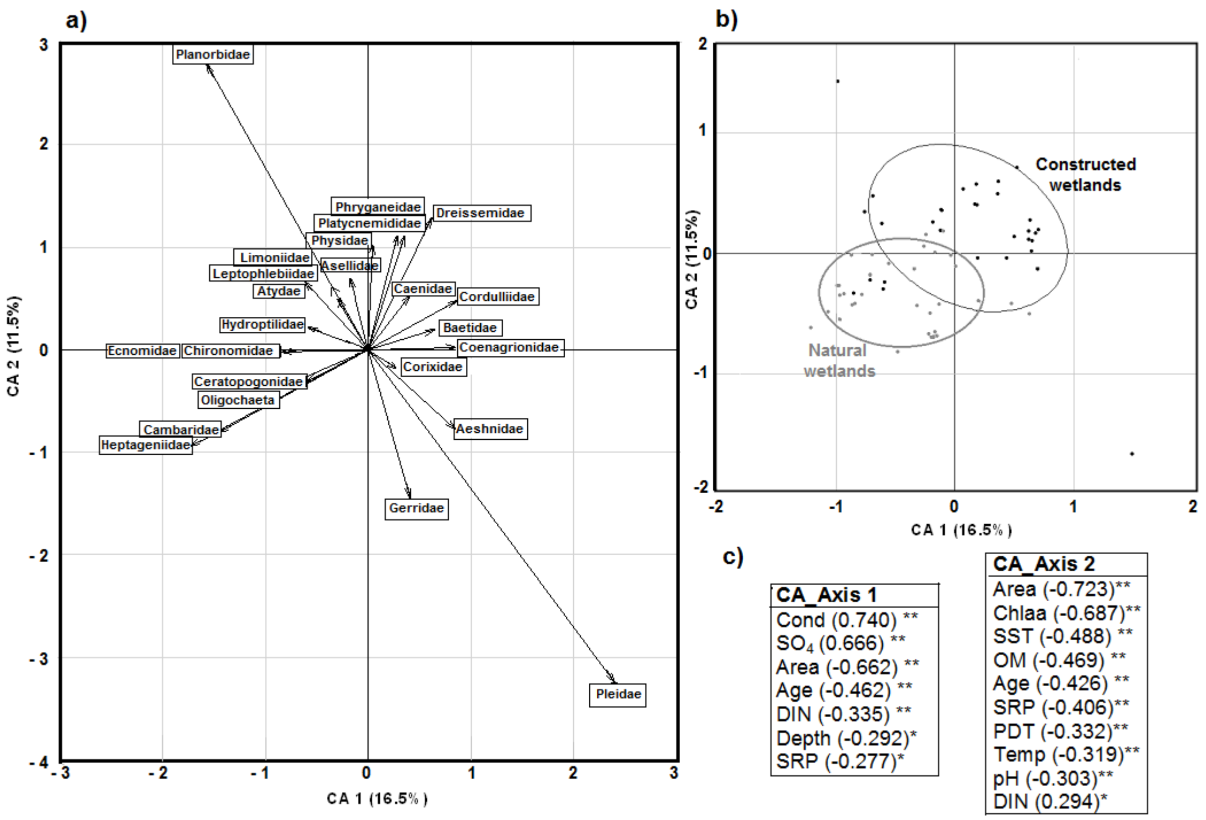
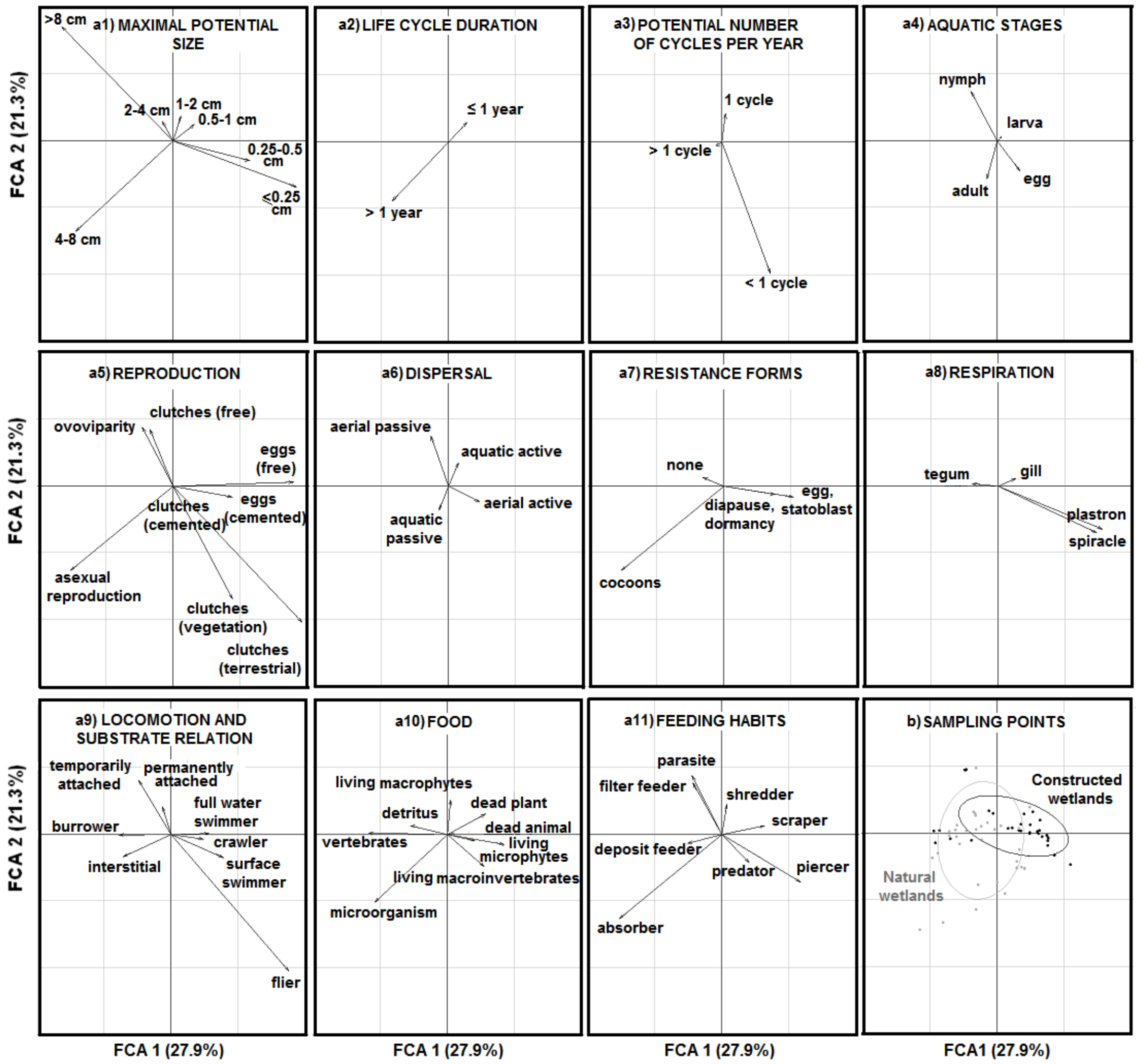


Figure 6. Results from multivariate analysis performed with data on macroinvertebrate assemblages of three natural and three constructed wetlands located on the Ebro River floodplain. a1 - a11) correlation of 11 biological trait categories with the first two axes of the trait-based Fuzzy Correspondence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



c)

FCA_Axis 1
Cond (0.762) **
Age (-0.527) **
Area (-0.498) **
Depth (-0.470)*
Chlaa (-0.379)**
Temp (0.348)**
DIN (-0.282) *

FCA_Axis 2
Temp (-0.622)**
DIN (0.526)**
OM (-0.330) **
Cond (-0.267)*
SST (-0.264) *
Chlaa (-0.249)**

Appendix 1. Macroinvertebrate taxa occurrence (X) in each of three constructed and three natural wetlands studied in the Ebro Floodplain during winter 2010 (W) and spring 2011 (S).

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

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

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) Ovoviparity. 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.

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