

Article

Short-Term Responses of Aquatic and Terrestrial Biodiversity to Riparian Restoration Measures Designed to Control the Invasive *Arundo donax* L.

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Abstract: Invasive species are among the top five causes of biodiversity loss worldwide. Arundo donax has progressively colonized the riparian zones of Mediterranean rivers with detrimental effects on terrestrial and aquatic biodiversity, being catalogued as one of the 100 worst invasive species. In order to control this invasive species and restore native riparian vegetation, different methods have been traditionally used, depending on the environmental, economic and social context. Here, the effect of repeated above-ground removal of A. donax on aquatic and terrestrial communities was assessed by testing two different frequencies of mowing (monthly-intensive and quarterly-extensive), combined with the plantation of native species. Specifically, it was evaluated if riparian vegetation, birds and aquatic macroinvertebrates showed significant responses throughout time and between restoration treatments based on 4-year annual biomonitoring data (2015-2018). Changes in taxonomic diversity and ecological quality indices for the different biological communities were tested using mixed-effect models (LMEs). Similarly, comparisons between restored and reference sites were also performed. LMEs were also applied to assess how riparian variables were related to bird and aquatic macroinvertebrate indices. NMDS and MGLM-Mvabund analyses were performed to detect significant post-treatment differences in taxa composition compared to the initial state and reference sites. During this short-term assessment, increases in riparian and aquatic macroinvertebrate richness and quality indices were found, as well as significant decreases in A. donax height, density and cover, without significant differences between restoration treatments. However, differential effects between extensive (positive-neutral effect) and intensive treatments (neutral-negative effect) were detected for bird richness, density and abundance. After three years of restoration actions, restored sites are still far from reference values in terms of taxa composition, species richness and ecological quality, especially for riparian vegetation and birds. Given the high cost and the great efforts required for restoration, extensive repeated mowing, together with native species plantation, are only recommended on river reaches not fully invaded by A. donax and with a high ecological interest.

Keywords: ecological restoration; biomonitoring; riparian vegetation; macroinvertebrates; birds; biological invasion; alien species; environmental management; Mediterranean rivers; Segura River



1. Introduction

Invasive species are among the most relevant causes of biodiversity loss [1,2]. Multiple and interacting long-standing human pressures in fluvial systems, as channelization, dam construction, riparian deforestation, agricultural and urban development, have favoured the spread of opportunistic and exotic species [3,4]. Such pressures have detrimental effects on native communities, resulting in the impairment of aquatic and riparian habitats worldwide [5–7] due to the alteration of vegetative structure [8], competitive displacement of native riparian vegetation [9], reduction of arthropod and avian diversities and abundances [8,10], among others. Particularly, giant reed (Arundo donax L., Poaceae) has progressively invaded the Mediterranean Basin from its natural distribution (East Europe and Asia) [11]. It has been classified as one of the 100 most dangerous invasive species worldwide due to its high growth and spread rates [12]. In Spain and other Mediterranean countries (e.g., France, Greece, Italy, Malta), the giant reed is widely spread especially in disturbed watercourses where previous riparian fragmentation, mainly associated to agricultural intensification, flow regulation, and fires exist (main drivers of invasion). This riparian fragmentation and deforestation had impoverished native riparian communities, leaving empty niches which have benefited A. donax growth and expansion [13–15]. It was officially described from Spain and south-eastern France three centuries ago by Linneaus [11].

The giant reed is a tall (up to 8 m), erect, robust, fast-growing (5 cm/day under optimum conditions) and perennial hydrophyte [16]. It mainly spreads through vegetative reproduction from thick rhizomes and stem nodes which, carried downstream by high flows and once rooted and established in riparian areas, tends to form large and continuous clonal masses and monospecific stands [16,17]. The stress tolerance of this species has been attributed to its large rhizomes which enable to grow in poor soils and to quickly resprout following disturbances that cause aerial biomass removal [7,18,19]. In disturbed rivers, *A. donax* can outcompete and replace native plant communities causing additional negative effects in riparian habitats by reducing diversity, quality and heterogeneity [16,20] as well as changes in riparian food webs [8,21,22]. In addition, the lack of natural competitors outside its natural distribution range can also contribute to its spread and consolidation [23], which makes extremely difficult to revert this riparian invasion without management and restoration measures.

Nevertheless, the ecological effects of A. donax invasion go beyond riparian vegetation. Riparian zones, as transitional areas between aquatic and terrestrial ecosystems, influence both the structure and functioning of instream and terrestrial associated communities through different processes and functions such as microclimate modification, nutrient and sediment retention, bank stabilization, organic matter supply, ecological corridor, food and habitat provision [24–26]. The spread of A. donax affects these natural processes by altering nutrient cycling, reducing water quality (lower canopy results in less shade to the river, increasing water temperature and decreasing dissolved oxygen) and quantity (higher evapotranspiration rates and less aquifer recharge) [4,9,13,16,27,28], which is especially important in Mediterranean areas in a context of global warming and water scarcity. Furthermore, in comparison with native riparian communities, this invasion has been also associated to fewer opportunities for recreation and navigation (less water discharge and invaded banks), increased flooding risk, riparian fires (high flammability), siltation (greater instream sedimentation which reduces substrate heterogeneity), bank instability and erosion (larger aerial biomass and shallower root system), among others [4,16,29–34]. In addition, A. donax provides low-quality food and habitat for native species since its stems and leaves contain a wide variety of toxic chemicals such as alkaloids, making it unsuitable and unpalatable for vertebrate and invertebrate grazers [35,36]. Finally, native riparian vegetation acts as a buffer for aquatic communities that can modify, incorporate, filter or concentrate a variety of substances, such as nutrients, pesticides or sediments from the surrounding catchment before their incorporation to the aquatic phase, therefore influencing also instream biodiversity patterns. Furthermore, the homogenization of aquatic habitats by A. donax and the low nutritional quality of its leaf litter have negative effects on fishes [29] and aquatic macroinvertebrates [20,37,38].

Riparian galleries constitute key habitats due to their high productivity and heterogeneity, providing important resources as food (e.g., riparian invertebrates, emergent aquatic insects, fruits and seeds), excellent areas for reproduction (e.g., nesting and breeding for aquatic and terrestrial birds and some mammals) and ecological corridors even for strictly terrestrial faunal communities [26]. Among them, birds can be considered relevant bioindicators since they are strongly dependent on habitat structure and riparian condition [39]. In fact, native riparian vegetation constitutes a preferential habitat for many birds during migration and juvenile dispersal [40] and may attract over ten times the number of migratory birds in spring than adjacent upland habitats [41]. Nevertheless, the strong habitat simplification that involves A. donax invasion reduces the number of species that can feed, inhabit and nest on riparian areas [10,20]. A. donax stems are weak and completely vertical, so the lack of a robust horizontal structure impedes most bird nesting. In addition, invertebrates, one of the main food sources for birds, are less diverse and abundant in invaded areas (up to 50% of decline) given the absence of a shrub understory layer [12]. Although the decrease in bird habitat quality following A. donax spread has been well documented [10] and constitutes a matter of concern [22], it has been rarely addressed in the Iberian Peninsula [42,43]. Moreover, to the best of our knowledge the effects of A. donax removal and eradication actions on bird community have not been examined in detail to date.

Given the intensity and variety of the ecological, economic and social impacts linked to the dominance of the giant reed, different methods have been used to control its populations: above-ground (stem cutting) and below-ground (rhizome extraction) mechanical removal, chemical treatments (mainly the controversial glyphosate sprayed or injected [44]), flooding, polyethylene plastic covering and biological control through terrestrial insects [32,45,46]. Most methods are applicable in degraded riparian areas where A. donax dominates completely but not in river reaches where this species coexists with native vegetation. This is especially applicable to protected areas, where less aggressive methods are required to avoid negative collateral effects on native communities and ecological processes. Stem cutting campaigns have been generally performed locally (especially in lower reaches where A. donax forms extensive monospecific stands), at the request of municipalities or as preventive routine management (before autumn rains to avoid hydraulic damages during flash-flood events in Mediterranean rivers), and with scarce coordination or long-term planning, mostly resulting in high costs and poor results [38]. Nevertheless, A. donax clumps are likely to require more than local annual biomass removal, due to the bulk of underground biomass, and the ability of remaining rhizome or stem segments to produce large stands quickly [47]. Thus, river restoration projects should focus on coordinated holistic measures planned at broad scale rather than only local disconnected actions, to develop more effective management strategies [48]. Despite the numerous works addressing how biodiversity responds to different riparian management and restoration strategies [49,50], there is a knowledge gap on the ecological effects of A. donax removal on aquatic and terrestrial associated communities, with the exception of side-effects of chemical treatments as glyphosate [43].

In this context, the LIFE+ RIPISILVANATURA project (see detailed information at www. ripisilvanatura.eu) aims to control invasive alien species by strengthening riparian habitats (specially the habitat 92A0 and 92D0 of European Directive 92/43/CEE) in moderately disturbed reaches of the Segura River (Región de Murcia, SE Spain) where *A. donax* and remnants of native riparian vegetation coexist within or near protected areas. Therefore, this project intends to decrease the density and coverage of *A. donax* while expanding native riparian cover through soft-engineering techniques (repeated above-ground stem removal combined with the plantation of native riparian species) in order to enhance the competition exerted by native riparian species. The rationale behind this restoration strategy is to exhaust *A. donax* rhizome reserves (nutritional storage) by forcing this hydrophyte to constantly replace its stems. Simultaneously, native vegetation gets time to be developed and successfully compete with the giant reed for sunlight and riparian space [32]. Although there are some evidences of the effectiveness of the different control and restoration actions on *A. donax* and other invasive species (e.g., *Saccharum spontaneum*; [51]), very little is known about the performance, success and ecological effects of this particular combination of methods beyond riparian areas [32]. This study consists of a short-term evaluation of the effectiveness of the restoration measures applied to control *A. donax*: repeated mowing with two different frequencies (monthly vs. quarterly) combined with the plantation of native riparian species. It was assessed if riparian vegetation, birds and aquatic macroinvertebrates showed significant responses to these restoration actions and if they reached the ecological values and attributes of non-invaded, well-conserved reference sites. Reductions in *A. donax* cover, height and stem density were predicted. Subsequently, increases in native riparian coverage, diversity and ecological status of riparian and aquatic communities were expected as consequence of control and restoration actions. In the case of birds, it was hypothesized that they could need more time (beyond the project deadline) and a greater development of planted native species to experience significant taxonomic and diversity changes.

2. Materials and Methods

2.1. Study Area

The study was developed in the Segura River basin, a semi-arid Mediterranean catchment located in the South-East of the Iberian Peninsula. In particular, the riparian restorations took place in 52 km along the middle segment of the Segura River including the municipalities of Cieza, Calasparra and Moratalla (Murcia Region, Spain). This area is geologically characterized by the dominance of limestone, sandstone, gypsum and loam substrates and climatically featured by a mean annual precipitation of 300 mm and annual mean temperature of 17 °C. Regarding anthropogenic impacts, this perennial river reach is subjected to intense flow regulation and hydro-morphological alterations [52,53] mainly due to the upstream Cenajo reservoir, the biggest one (437 hm³) in the Segura watershed. The main land use in the area is semi-natural (dominant shrubby landscape) and agriculture (mainly drylands, rice fields, apricot and peach trees), with urban areas being scarce (<2%; Figure 1). Native riparian vegetation in the area was characterized by 92A0 and 92D0 habitats (Habitat Directive 92/43/CEE), showing a mixture of European and Ibero-African flora (Salix spp., Fraxinus angustifolia, Populus spp., Tamarix spp., *Nerium oleander*), which constitutes a distinctive occurrence within the Iberian Peninsula [15,54]. Although historic detailed information about the evolution of invasion in the study area is lacking, native habitats 92A0 and 92D0 have been progressively displaced by A. donax, which already occupies nearly a 40% of the riparian zone within the studied area according to the preliminary evaluation carried out to draft the project (Figure 1).

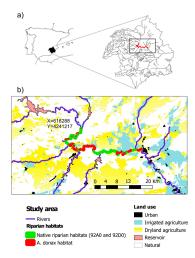


Figure 1. (a) Location of the middle section of the Segura River within the catchment, located in the southeast of the Iberian Peninsula, and (b) distribution of dominant native (green) and exotic (red) riparian species in the study area (showing X and Y coordinates of the upper reach with restoration and control actions within the LIFE+ RIPISILVANATURA project).

In order to prioritize restoration areas and measures with higher expectations of success, the following steps were taken: (i) database and literature searching on native and exotic biodiversity, and ecological quality indices; (ii) field surveys (in the spring of 2015) to complete species inventories, habitat maps and quality assessments; (iii) identification of reference reaches in good or very good riparian and aquatic ecological status; (iv) establishment of scientific and operational criteria for the selection of restoration river reaches: intermediate ecological status, closed to well-conserved natural riparian habitats to enhance connectivity, technically feasible, socially accepted (adjacent landowners and local users) and with potential synergies with other ongoing projects (e.g., LIFE+ RIVERLINK see https://www.chsegura.es/chs/cuenca/segurariverlink/riverlink/ for details); (v) selection of initial method (mechanically or manually) for cutting A. donax depending on the riparian vertical structure as well as native and exotic species abundances; and, (vi) definition of plantation plots based on local environmental features such as ecological status, presence of native vegetation remnants, bank slope, water table, riparian composition and width [55]. The species pool used in restorations (Table 1) mainly arose from the riparian and upland habitats detected in the non-invaded zones of the study area (Mediterranean deciduous broadleaf forests, Habitat Directive 92/43/CEE): 92A0-Salix alba and Populus alba galleries and 92D0 Southern riparian galleries and thickets (Nerio-Tamaricetea and Securinegion *tinctoriae*). Furthermore, seedlings and cuttings for the different species were obtained and produced from native populations to avoid genetic hybridization and increase the probability of survival of the new individuals, given the previous adaptation to local environmental conditions.

Trees	Shrubs	Herbs		
Arbutus unedo	Crataegus monogyna	Cladium mariscus		
Celtis australis	Ficus carica	Lonicera biflora		
Fraxinus angustifolia	Genista spartioides	Saccharum ravennae		
Populus alba	Juniperus oxycedrus	Scirpus holoschoenus		
Populus nigra	Nerium oleander	Scirpus maritimus		
Salix alba	Pistacia lentiscus			
Salix fragilis	Rhamnus alaternus			
Tamarix boveana	Rosa canina			
Tamarix canariensis	Salix atrocinerea			
Tamarix gallica	Salix purpurea lambertiana			
Ulmus minor	Salix triandra			
	Sambucus nigra			
	Smilax aspera			

Table 1. Total pool of riparian species used to define each case-specific restoration action.

Finally, this methodological scheme resulted in the selection of 37 riparian patches where soft-engineering restoration actions (removal of above-ground *A. donax* stems) and extensive (quarterly) or intensive (monthly mowing) maintenance have been applied in combination with the case-specific plantation of native riparian vegetation (Figure 2). The first mowing campaigns were done in winter 2015–2016 before the beginning of the growth vegetative season (i.e., spring). After the first mowing, different combinations of native riparian species were planted in late winter (February–March 2016). Subsequent cuts were made with different temporal frequency (monthly vs quarterly) depending on the patch, and including a pause during dormancy period (winter), resulting in a maximum of 8 mowing campaigns until spring 2018. These cuts were done manually (portable electric lawn mower machine) to minimize the ecological disturbance of repeated mowing on autochthonous and planted vegetation but also on aquatic and terrestrial associated communities (cost of control and restoration actions was 430,714 € from a total project budget of 2,454,611 €) Because of the semi-arid climate and high evapotranspiration in the study area, auxiliary irrigation was applied in summer to increase the survival of the saplings of planted native species.



Figure 2. Segura River in Almadenes canyon before the beginning of restoration actions (2015-left side) and after the initial mowing campaigns (2016-right side) performed to remove *A. donax*.

2.3. Biomonitoring and Ecological Indicators

The effectiveness of restoration measures accounting for potential differences between extensive and intensive treatments (frequency of *A. donax* cutting) was assessed through periodical monitoring activities in a BACIPS framework (Before, 2015; After Control-Impact Paired-Sites, 2016–2018). For this purpose, over 35% of the restored river reaches (half of them located in sections with monthly and quarterly mowing, respectively) and 5 reference sites in good or very good ecological state (according to riparian and aquatic habitat quality) distributed along the study area were regularly monitored. One of these reference sites was located upstream to minimize the detrimental effect of the main reservoirs in the basin. Different ecological indicators related to the diversity of riparian (native and exotic plants, birds) and aquatic (macroinvertebrate) groups, as well as ecological quality indices for the different biological communities were considered (Figure S1).

Regarding riparian vegetation, longitudinal transects (1–5 depending on the width of riparian area) were performed in spring, during the growing vegetative season and just before the next mowing campaign in 16 restoration reaches. These transects allowed the estimation of the composition and abundance (semi-quantitative ranging from 1 to 6, corresponding from occasional to dominant, respectively) of riparian species, percentage of native and exotic vegetation cover, and the assessment of riparian quality (Riparian Quality Index-RQI, [56]). In addition, 5 quadrats of 1 m^2 ($1 \times 1 \text{ m}$) were systematically placed along each sampled reach to record the density and height of A. donax (relevant variables influencing competition for space and sunlight, respectively). Riparian bird community was monitored twice per year, in early (15 April-15 May) and late (15 May-15 June) spring, through line transects based on visual and auditory detection [57], which has been recognized as the less biased method to obtain density estimates [58]. This procedure lasted at least 1 h within the first 4 h of sunlight in 14 reaches affected by restoration actions, to obtain annual species richness, density and abundance (Kilometric Abundance Index, [59]). Finally, aquatic macroinvertebrates were annually sampled in late spring (maximum aquatic invertebrate activity) in 15 river reaches with a kick net (500 µm mesh) through a multihabitat standardized protocol where sampling effort was proportional to each habitat occurrence [60]. Kick-samples were pooled into a unique sample per site and preserved in 96% ethanol. In the laboratory, organisms were identified at family level, except for Hemiptera and Coleoptera that were identified at species level. This information was used to calculate the Iberian Biomonitoring Working Party (IBMWP index, [61]) and three richness metrics: total family richness, Coleoptera and Hemiptera species richness as surrogates of the total macroinvertebrate community species richness [62,63]. IBMWP is the official invertebrate biomonitoring index currently used in Spain to assess the ecological status of rivers and assigns to each detected family a score ranging from 1 to 10 according to their known tolerance to pollution. Complementarily, water samples were taken in the same sites to determine pH, water conductivity and temperature (measured in situ), total and volatile suspended solids, and nitrate concentration (photometric method Spectroquant Merck, detection range 0.1–25 mg/L NO₃-N).

2.4. Data Analysis

Changes in riparian vegetation (species richness, quality-RQI, native and exotic cover, averaged A. donax height and stem density per river reach), aquatic invertebrate metrics (IBMWP score, family richness, Coleoptera and Hemiptera species richness) and birds (species richness, density and abundance) among years (2015–2018) and treatments (intensive-monthly vs extensive-quarterly mowing) were tested using linear mixed-effect models (LMEs). LMEs were performed considering "year" and "treatment" as fixed factors and sampling sites as random factors. Goodness of fit was evaluated with Marginal R² associated to fixed effects in LME procedure. Likelihood ratio tests were implemented to compare these models (fixed and random effects) with null ones (only random effects) and detect model significance. If applicable, Tukey-based post-hoc paired comparisons were executed to identify when meaningful responses started. Similarly, LMEs were also performed to compare reference sites with restored ones to check the degree of recovery reached after restoration actions. Complementarily, LMEs were applied to identify the influence of riparian variables on macroinvertebrate and bird indices (considering sampling sites as random factors). In addition, the relationship between water quality (nitrates, conductivity, total and volatile suspended solids) and aquatic macroinvertebrate variables were also studied through LMEs. Homoscedasticity (Levene's test) and normality (Shapiro-Wilk test) of residuals were checked. Logarithmic transformations were applied on response variables if model assumptions were not met to improve linearity and reduce data variability. Non-metric Multidimensional Scaling (NMDS) and multivariate generalized linear models (MGLM-Mvabund) were implemented on abundance (riparian vegetation and birds) or occurrence (aquatic macroinvertebrates) data to detect spatial (treatments) and temporal (years) differences in the taxonomic composition of the different biological communities. The assumption of mean-variance relationships was visually checked for this analysis. All statistical analyses were performed using R statistical software (libraries: "ade4" [64], "car" [65], "lme4" [66], "lmerTest" [67], "multcomp" [68], "mvabund" [69], "MuMIn" [70], "nlme" [71] and "vegan" [72,73]).

3. Results

A total of 134 plant species, 77 aquatic macroinvertebrate families (74 families plus Hydracarina, Ostracoda and Oligochaeta), 24 species of aquatic Coleoptera, 9 species of aquatic Hemiptera and 64 bird species were detected in the study area between 2015–2018 (complete lists available in Table S1). Significant reductions of A. donax height, density and cover, as well as an increase of the riparian quality index (RQI) and species richness were observed, without significant differences between treatments (extensive and intensive maintenance) during the studied period (Table 2, Table S2). No significant differences among years nor treatments were found for native plant cover. Regarding aquatic macroinvertebrates, significant increases in the IBMWP index and richness values (family richness and Hemiptera species richness) were detected after 2017. No significant differences among years or treatments were observed for Coleoptera species richness. In the case of birds, at first glance LMEs did not show significant temporal differences between years for bird density, abundance and species richness (Figure S2). Nevertheless, there was a significant interaction between date and treatment pointing to differential effects between extensive (positive effect) and intensive treatments (neutral-negative effect) on bird community through time (Table 2, Figure 3). All models met normality and homoscedasticity assumptions after the log-transformation of A. donax height and density (diagnostic plots and tests in Figures S3 and S4).

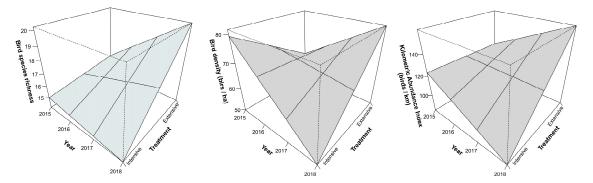


Figure 3. Three-dimensional plots between year, treatment and bird community variables (built using mean values) on significant results for interaction terms tested by linear mixed-effect models (LMEs).

Table 2. Results of linear mixed-effect models (LMEs) on riparian vegetation, aquatic macroinvertebrate and bird community metrics (response variables) in restoration sites. Marginal R^2 (R^2m) and p-values for the whole model (likelihood ratio test) and the different terms (year, treatment and the interaction between them) are shown. Treatments refers to extensive and intensive maintenance. The signs or trends (Tr.) of the relationships are also displayed. Significant results (p < 0.05) have been highlighted in bold. Asterisks depict log-transformed variables.

Response Variable	Model	Model		Year		Treatment		Year: Treatment	
Riparian Vegetation	<i>p</i> -Value	R ² m	Р	Tr.	р	Trend	р	Trend	
Species richness	5.5×10^{-12}	0.33	1.66×10^{-8}	+	0.45	=	0.33	=	
Riparian Quality	0.049	0.08	0.031	+	0.34	=	0.63	=	
Native cover	0.68	-	0.97	=	0.3	=	0.39	=	
A. donax stem density	0.006	0.12	0.017	-	0.11	=	0.12	=	
A. donax height	2.2×10^{-16}	0.73	2×10^{-16}	-	0.9	=	0.07	=	
A. donax cover	0.006	0.08	0.005	-	0.67	=	0.14	=	
Aquat	ic macroinverte	brates							
IBMWP score	0.003	0.26	0.004	+	0.47	=	0.67	=	
Family richness	0.047	0.2	0.013	+	0.8	=	0.94	=	
Coleoptera richness	0.92	-	0.9	=	0.32	=	0.83	=	
Hemiptera richness	4.31×10^{-5}	0.4	9.12×10^{-5}	+	0.65	=	0.05	=	
	Birds								
Species richness	0.048	0.21	0.34	+/=	0.1	=	0.04	Ext (+) ¹ , Int (=)	
Density	0.03	0.11	0.44	+/-	0.58	=	0.02	Ext (+), Int (-)	
Abundance	0.04	0.2	0.49	+/-	0.13	=	0.03	Ext (+), Int (-)	

¹ Ext: Extensive maintenance treatment; ² Int: Intensive maintenance treatment.

According to Tukey post-hoc paired comparisons (Table S3), the riparian metrics (Figure 4) that first responded to restoration actions were riparian richness and *A. donax* height (significant decrease from 2016). Similarly, *A. donax* density started to decrease in 2016 (significant differences between 2015 and 2016, p < 0.05) but this reduction was not consolidated until 2018 (differences 2015–2018, p < 0.05). The riparian quality index (RQI) and *A. donax* cover did not respond until the second (differences 2016–2017, p < 0.05) and third year of restoration actions (differences 2015–2018, p < 0.01), respectively. Similarly, macroinvertebrate-based biomonitoring index (IBMWP), family richness and Hemiptera species richness showed significant responses from 2017 (differences 2016–2017, p < 0.05, p < 0.01 and p < 0.001, respectively) and concordant patterns between 2016–2018 (p < 0.01; Figure 5, Table S4).

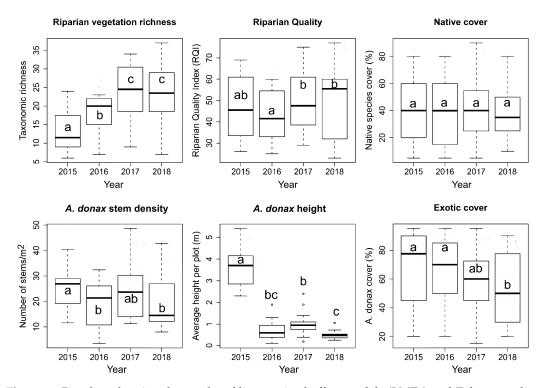


Figure 4. Boxplots showing the results of linear mixed-effect models (LMEs) and Tukey post-hoc paired comparisons relative to the temporal evolution of native and exotic riparian vegetation-related variables in restoration sites. Letters (a, b, c) depict the significant differences found among years (see Table S3). The median is denoted by the bold horizontal line, the box delimits the interquartile range, and the whisker lines extend to the observed maxima and minima, except for the outliers symbolized by points.

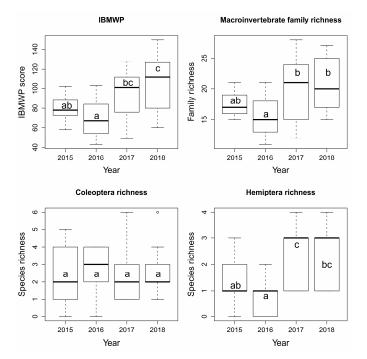


Figure 5. Boxplots showing the results of linear mixed-effect models (LMEs) and Tukey post-hoc paired comparisons relative to the temporal evolution of aquatic macroinvertebrate-related variables in restoration sites. Letters (a, b, c) depict the significant differences found among years (see Table S4). The median is denoted by the bold horizontal line, the box delimits the interquartile range, and the whisker lines extend to the observed maxima and minima, except for the outliers symbolized by points.

Despite the improvement observed in some aquatic and terrestrial community metrics after restoration actions (e.g., Figures 4 and 5), significant differences can be observed between restored and reference sites, especially for riparian vegetation and birds (Figure 6, Table S5). Thus, values obtained along restored reaches in 2018 are still far from those observed for reference ones for riparian richness and quality (Treatment term, p < 0.01), *A. donax* density, native and exotic cover (p < 0.001), IBMWP, bird density (p < 0.05) and abundance (p < 0.01). Comparatively, the richness of riparian species rose in restoration sites but remained stable in reference ones (Year × Treatment term, p < 0.01). Similarly, bird species richness increased in those sites subjected to extensive treatment but decreased in reference ones (Year x Treatment term, p < 0.001). Contrarily, *A. donax* height increased in reference sites but decreased in reference ones, (Year × Treatment term, p < 0.001). All models met normality and homoscedasticity assumptions after the log-transformation of bird density and abundance (diagnostic plots and tests in Figures S5 and S6).

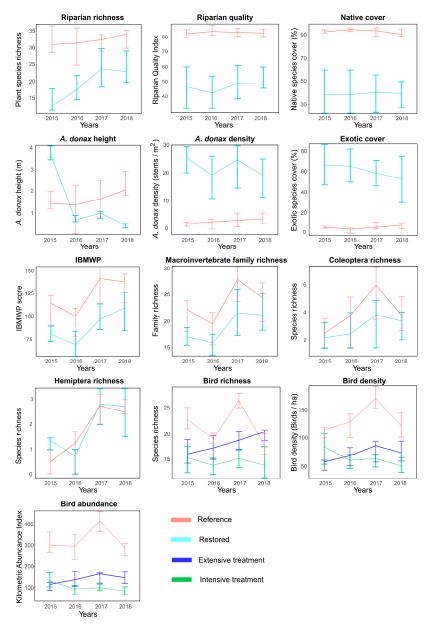


Figure 6. Repeated measures plots comparing the evolution of riparian vegetation, bird and aquatic macroinvertebrate variables between reference and restoration sites. Error bars display the mean, 1st and 3rd quartiles. Note that intensive and extensive treatments are individually displayed only for those bird variables that responded differentially in previous LMEs (i.e., bird variables).

Regarding the relationships between riparian vegetation and faunal communities (explored through LMEs, Figure 7), exotic cover negatively influenced the IBMWP score, ($R^2m = 0.17$, p < 0.05), family richness ($R^2m = 0.11$, p < 0.05) and bird species richness ($R^2m = 0.08$, p < 0.05). Riparian species richness and quality were positively related to Coleoptera (p < 0.05, $R^2m = 0.14$ and $R^2m = 0.19$, respectively) and Hemiptera species richness (p < 0.05, $R^2m = 0.18$ and $R^2m = 0.17$, respectively). In addition, riparian vegetation richness also enhanced bird richness ($R^2m = 0.09$, p < 0.05). *A. donax* stem density was negatively associated with bird species richness ($R^2m = 0.08$, p < 0.05), density ($R^2m = 0.07$, p < 0.05) and abundance ($R^2m = 0.2$, p < 0.001). Finally, no significant relationships were found between water quality (nitrates, conductivity, total and volatile suspended solids) and aquatic macroinvertebrate community variables (p > 0.05).

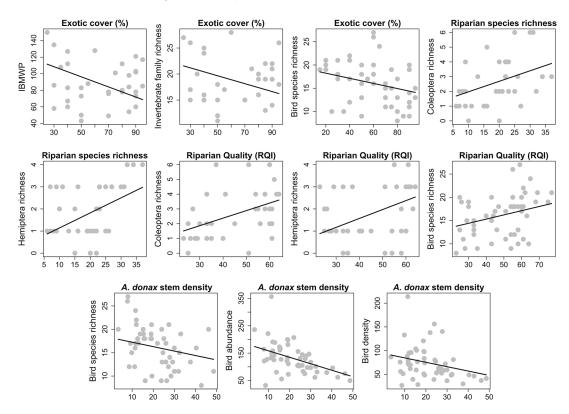


Figure 7. Scatterplot showing significant relationships (p < 0.05) between riparian vegetation-related variables and aquatic macroinvertebrate and bird community indexes according to linear mixed-effect models.

NMDS (Figure S7) and MGLM-Mvabund pointed to temporal (2015–2018) and spatial (reference-restoration sites) differences on taxa composition for riparian vegetation (Table 3), aquatic macroinvertebrates and birds (Table 4). Riparian species assemblages mainly differed between reference and restoration sites: 21 species were more abundant in reference sites (Treatment term p < 0.05). However, 15 of them increased in abundance exclusively in restored sites while *A. donax* followed the inverse pattern (Year × Treatment term, p < 0.05): *Celtis australis, Crataegus monogyna, Daphne gnidium, Fraxinus angustifolia, Genista spartioides, Juniperus oxycedrus, Nerium oleander, Pistacia lentiscus, Populus nigra, Rhamnus alaternus, Rosa canina, Rubia peregrina, Rubus caesius, Sambucus nigra, Salix neotricha, Salix purpurea*. Regarding aquatic macroinvertebrates, significant temporal changes were detected in taxa composition: Aeshnidae, Ancylidae, Corixidae, Elmidae, Gerridae, Melanopsidae, Platycnemididae and Tabanidae expanded while Planorbidae reduced its occurences between 2015 and 2018 both in reference and restoration sites (Year term, p < 0.05). Similarly, the only representant of Cambaridae, the exotic species *Procambarus clarkii* (Girard, 1852) also increased its presence in the study area (Year term, p < 0.05) but was more frequently recorded in restoration than in reference sites (Treatment

term, p < 0.01). Athericidae, Dugesiidae, Leuctridae, Oligoneuriidae and Perlodidae were mostly present in reference sites (Treatment term p < 0.05). Concerning birds, both understory (e.g., *Cetia cetti*, *Luscinia megarhynchos*, *Sylvia atricapilla and Sylvia melanocephala*) and canopy-dwelling species (*Aegithalos caudatus*, *Fringilla coelebs*, *Periparus ater*) were more abundant in reference than restoration sites (Treatment term, p < 0.01). *Jynx torquilla* spread but *Sturnus unicolor* (which was more recurrently detected in restoration sites Treatment term, p < 0.05) declined in the study area throughout the project (Treatment term, p < 0.01). Finally, the abundances of *Columba palumbus*, *Falco tinnunculus* and *Picus viridis* increased in restored but diminished in reference sites (Year x Treatment term, p < 0.05).

Table 3. Evolution of riparian vegetation taxa in reference and restoration sites according to multivariate generalized linear models (MGLM-Mvabund). Temporal (Temp.), spatial and spatio-temporal trends are displayed. Only those species with significant temporal and/or spatial differences are shown. The overall model results for riparian vegetation has been highlighted in bold. ns = non-significant result.

	Yea	ar		Treatment	Year: Treatment		
Таха	<i>p</i> -Value	Temp. Trend	р	Spatial Trend (Greater Value)	p	Spatio-Temporal Trend	
Riparian Vegetation	0.004		0.004		0.001		
Agrostis stolonifera	Ns	=	0.001	Ref	0.006	Ref (+), Rest (=)	
Apium nodiflorum	Ns	=	0.001	Ref	0.012	Ref (–), Rest (=)	
Arundo donax	Ns	=	0.002	Rest	0.002	Ref (=), Rest (-),	
Brachypodium retusum	Ns	=	0.002	Ref	0.006	Ref (+), Rest (=)	
Carex pendula	Ns	=	0.005	Ref	ns	=	
Celtis australis	0.01	+	ns	=	0.042	Ref (=), Rest (+)	
Cistus clusii	Ns	=	0.002	Ref	ns	=	
Clematis vitalba	Ns	=	0.01	Ref	ns	=	
Crataegus monogyna	0.002	+	0.024	Rest	0.002	Ref (=), Rest (+)	
Cyperus fuscus	Ns	=	0.005	Ref	ns	=	
Daphne gnidium	Ns	=	0.007	Ref	0.013	Ref (=), Rest (+)	
Digitalis obscura	Ns	=	0.007	Ref	0.007	Ref (+), Rest (=)	
Dorycnium penthaphyllum	Ns	=	0.001	Ref	0.004	Ref (–), Rest (=)	
Fraxinus angustifolia	0.001	+	ns	=	0.004	Ref (=), Rest (+)	
Genista spartioides	0.007	+	ns	=	0.041	Ref (=), Rest (+)	
Juniperus oxycedrus	Ns	=	0.003	Ref	0.039	Ref (-), Rest (+)	
Mentha suaveolens	Ns	=	0.001	Ref	0.008	Ref (–), Rest (=)	
Nerium oleander	0.001	+	ns	=	0.001	Ref (=), Rest (+)	
Pistacia lentiscus	Ns	=	0.003	Ref	0.035	Ref (=), Rest (+)	
Populus nigra	Ns	=	0.004	Ref	0.009	Ref (=), Rest (+)	
Rhamnus alaternus	Ns	=	0.004	Ref	0.009	Ref (=), Rest (+)	
Rosa canina	0.001	+	ns	=	0.001	Ref (=), Rest (+)	
Rubia peregrina	Ns	=	0.003	Ref	0.005	Ref (=), Rest (+)	
Rubus caesius	Ns	=	0.002	Ref	0.007	Ref (+), Rest (=)	
Sambucus nigra	0.001	+	ns	=	0.001	Ref (=), Rest (+)	
Samolus valerandi	Ns	=	0.001	Ref	ns	=	
Salix neotricha	0.002	+	ns	=	0.001	Ref (=), Rest (+)	
Salix purpurea	0.001	+	ns	=	0.001	Ref (=), Rest (+)	
Smilax aspera	Ns	=	0.001	Ref	0.005	Ref (+), Rest (=)	

Table 4. Evolution of aquatic macroinvertebrate and bird taxa in reference and restoration sites according to multivariate generalized linear models (MGLM-Mvabund). Temporal, spatial and spatio-temporal trends are displayed. Only those species with significant temporal and/or spatial differences are shown. The overall model results for aquatic macroinvertebrates and birds have been highlighted in bold. ns = non-significant result.

	Year			Treatment	Year: Treatment	
Таха	<i>p</i> -Value	Temporal Trend	p	Spatial Trend (Greater Value)	р	Spatio-Tempora Trend
Aquatic Macroinvertebrates	0.002		0.049		0.213	
Aeshnidae	0.04	+	ns	=	ns	=
Ancylidae	0.024	+	ns	=	ns	=
Athericidae	ns	=	0.017	Ref	ns	=
Cambaridae	0.026	+	0.009	Rest	ns	=
Corixidae	0.042	+	ns	=	ns	=
Dugesiidae	ns	=	0.016	Ref	ns	=
Elmidae	0.027	+	ns	=	ns	=
Gerridae	0.012	+	ns	=	ns	=
Leuctridae	ns	=	0.017	Ref	ns	=
Melanopsidae	0.029	+	ns	=	ns	=
Oligoneuriidae	ns	=	0.018	Ref	ns	=
Perlodidae	ns	=	0.017	Ref	ns	=
Planorbidae	0.002	_	ns	=	ns	=
Platycnemididae	0.007	+	ns	=	ns	=
Tabanidae	0.001	+	ns	=	ns	=
Birds	0.012		0.002		0.046	
Aegithalos caudatus	ns	=	0.006	Ref	ns	=
Cetia cetti	ns	=	0.002	Ref	ns	=
Columba palumbus	ns	=	0.011	Ref	0.014	Ref (-), Rest (+
Falco tinnunculus	ns	=	ns	=	0.01	Ref (-), Rest (+
Fringilla coelebs	ns	=	0.004	Ref	ns	=
Jynx torquilla	0.007	+	ns	=	ns	=
Luscinia megarhynchos	ns	=	0.006	Ref	ns	=
Periparus ater	ns	=	0.005	Ref	ns	=
Picus viridis	ns	=	ns	=	0.005	Ref (-), Rest (+
Sturnus unicolor	0.006	_	0.011	Rest	ns	=
Sylvia atricapilla	ns	=	0.003	Ref	ns	=
Sylvia melanocephala	ns	=	0.002	Ref	ns	=

4. Discussion

Repeated mowing in combination with the plantation of native riparian species has partially succeeded in the control of *A. donax* and the recovery of biological communities three years after the start of restoration actions in the middle section of the Segura River. In particular, a significant reduction of *A. donax* height, density and cover, and a parallel increase in riparian quality (RQI) and riparian vegetation richness were detected as consequence of the restoration actions to control *A. donax* and strengthen native plant communities. This improvement of riparian condition was paired with an increase in aquatic macroinvertebrate richness mainly associated to the decrease in *A. donax* cover and the increase in riparian quality and richness. Extensive and intensive treatments based on the differential frequency of mowing exerted similar ecological effects, except for birds which were favoured by the extensive maintenance but not by the intensive one.

The temporal sequence of riparian recovery and associated biological communities seem to follow a reasonable ecological pathway. First, *A. donax* height and density decreased after the first year of implementation of restoration actions as a consequence of repeated mowing. Next, riparian richness started to increase due to the plantation of native riparian species and regeneration of existing plants. This riparian improvement was followed by meaningful changes in aquatic macroinvertebrate richness and IBMWP scores after the second year of restoration actions. Finally, although birds recently started to show increases in density, abundance and richness with extensive treatment, they probably need greater development of native planted species to experience more noticeable changes in species diversity. Despite the initial positive changes observed in riparian vegetation, aquatic macroinvertebrate and bird assemblages after applying *A. donax* control methods and riparian restoration actions, the values reached after three years of restoration actions were still far from reference values, especially those of riparian vegetation and birds. Considering the current modest development of native planted species and the high growth rate and competitive ability of *A. donax*, the prolongation of extensive maintenance could be desirable on those sites on the path of recovery, in order to underpin the positive ecological effects of restoration actions already implemented in the study area. It should be noted that the outcomes obtained after the application of control and restoration actions must be considered as preliminary and taken with caution to avoid over-interpretation. Our results from the Segura River but might not apply to other watercourses in different environmental contexts, so further research beyond Mediterranean catchments would be of high interest to validate our findings over larger geographical extents and increase the knowledge on restoration ecology.

4.1. Riparian Vegetation

Although restored sites did not reach the attributes of reference ones, a general improvement in riparian condition has been observed. Thus, the establishment and consolidation of planted species and other colonizing native species has increased riparian richness in all monitoring sites since the beginning of restoration actions. Thus, riparian plantations have strengthened habitat 92A0 through the increase in richness and abundance of target native riparian species with potential to displace A. donax such as Celtis australis, Crataegus monogyna, Nerium oleander, Fraxinus angustifolia, Populus nigra, Rhamnus alaternus, Rosa canina, Salix neotricha and S. purpurea, among others. This is quite promising since previous studies have demonstrated the effectiveness of willows to successfully compete with A. donax for the space and nutritional resources and, consequently, in depleting its productivity and extension [9]. Although species composition in restored sites turned similar to reference ones, they had reduced values of riparian quality (RQI) and native species cover. Most native saplings were successfully established and exhibited good condition, but their small size and the lack of lateral spread could explain the absence of significant changes in native cover. It seems that woody planted species need more time to develop and outcompete A. donax, occupying progressively the riparian space and intercepting sunlight by closed canopies [45]. Sites where significant declines in A. donax density and coverage have been already observed could be considered preferential to extend restoration actions if necessary.

Non-chemical control treatments are usually based on the removal of the rhizome of *A. donax*. However, the application of this method in sensitive areas is not recommended, due to the strong physical and ecological impact it implies in the initial phases. In this context, although *A. donax* shoots can resprout from rhizomes located at one-meter depth [74,75], repeated mowing can also reduce *A. donax* underground biomass [76]. Given the very high photosynthetic rate of *A. donax*, which enables new stems to become rapidly independent of rhizome reserves [77], coordinated, periodical and scheduled control actions are essential to mitigate the invasion of *A. donax* in Mediterranean rivers. Thus, short time-lags are recommended to exhaust underground nutritional reserves more rapidly [32]. Nevertheless, there were not significant differences between quarterly and monthly mowing on restoration success. Despite the lack of studies assessing the effectiveness of repeated mowing in combination with the plantation of native species, this approach was able to reduce *A. donax* height (-80%), density (-50%) and cover (-35%), which was similar to the results obtained in the evaluation of just repeated mowing [78–80].

The observed ecological trends in response to *A. donax* control and restoration actions should be confirmed based on longer evaluation periods, which would allow extracting more robust conclusions to be considered in further riparian restoration schemes. Final evaluation after the end of the project (2019–2020) will provide additional key data on the survival rates of planted saplings to identify the most successful species outcompeting *A. donax* in habitat 92A0 and 92D0. Although plant species

early established after restoration could be informative on the success of vegetation outcomes [81], long-term (6–10 years) biomonitoring is highly recommended to have a complete view of the processes, effects and durability of the applied measures [81,82].

4.2. Aquatic Macroinvertebrates

The ecological quality (sensu IBMWP), family richness and Hemiptera species richness have experienced meaningful increases after the implementation of restoration actions. Despite flow regulation affect all restored reaches, which could involve reduced IBMWP values [83], most of them reached at least good ecological condition during the last year of the monitoring campaign (2018). The only exceptions were the "Moratalla river mouth" and "La Maestra" reaches, probably due to their proximity to upstream and downstream dams, which cause strong variability in flow intermittence and lentification [84], respectively. Changes in the dominance between native and non-native riparian species can influence the quality, quantity, and timing of allochthonous resources which, in turn, may favour the diversity and structure of invertebrate communities [85,86]. In fact, riparian habitats dominated by exotic species are associated to lower invertebrate density, diversity and evenness than riparian habitats dominated by native vegetation [87]. Namely, A. donax promotes homogeneous and uniform riverbanks and less woody debris, resulting in lower diversity of microhabitats (e.g., tree roots) for aquatic macroinvertebrates. The reduction of A. donax dominance could have boosted the recovery of aquatic macroinvertebrate community. This invasive species reduces insect growth as it constitutes an exceptionally poor resource with an allelopathic potential effect [37]. The higher resource quality of native species debris coupled with a gain of native litter as consequence of restoration actions could have long-term beneficial effects on secondary production of aquatic macroinvertebrates utilizing large-particle organic matter [37]. Particularly, streams in which biotic assemblages are structured by allochthonous organic inputs, shifts from A. donax to native riparian communities could influence higher trophic levels by increasing the relative contribution of shredder macroinvertebrates as a resource for predators [88].

According to our results, the observed temporal trend could be due to the reduction of A. donax cover, the increase of riparian species richness and the improvement on the quality of riparian areas and riverbanks as consequence of restoration actions. Nevertheless, taxa composition and richness followed similar temporal trends in restored and reference sites, suggesting also a general improvement in river condition (9 families expanded but only 1 declined across the study area between 2015 and 2018). Therefore, this general pattern could be related to the good physico-chemical water parameters found along the study area (nitrates < 5 mg/L, water conductivity $< 1000 \mu$ S/cm, total and volatile suspended solids < 5 mg/L; measured at the same time than macroinvertebrates sampling), with the exception of local and punctual disturbances in some sampling sites located near rice fields which affected water quality occasionally. This good physico-chemical state is probably related to the notable reduction of organic pollution in the last decades due to a better management of wastewater and the construction of many water treatment plants along the Segura River basin [89]. However, further conservation and management actions are highly recommended considering that alien invertebrate species as Procambarus clarkii, Corbicula fluminea and Potamopyrgus antipodarum were widely detected during this short-term assessment. In fact, Corbicula fluminea is currently spreading across the Basin [90]. Finally, species of high conservation interest as the endemic mollusk Melanopsis lorcana (which is considered as "vulnerable" in the Spanish red book of invertebrates [91]) has been recurrently recorded during the monitoring period. In fact, its corresponding family (Melanopsidae) increased after restoration measures. Moreover, there were occurrences of species related to well-conserved riparian forests (e.g., Potamophilus acuminatus, Coleoptera) but also other taxa associated to artificial watercourses (e.g., Heliocorisa vermiculata, Hemiptera), which could be indicating that an ecological transition is still underway.

4.3. Birds

Only birds were differentially affected by the frequency of repeated mowing. The extensive treatment was associated with an increase in species richness, density and abundance, whereas the intensive one exerted neutral (species richness) and even negative effects (density and abundance) on bird communities. Furthermore, restored sites under extensive treatment reached near-reference values for bird species richness (but not for abundance and density). Contrarily, the intensive treatment could represent an excessive frequency of mowing (monthly), hindering bird nesting during the critical months of May, June and July, which must be considered in future management and restoration actions. Thus, only extensive treatment (quarterly mowing) should be extended in time to reduce exotic cover without detrimental effects on bird communities. At the moment, 54 bird species have been recorded through transects in the last sampling campaign (2018), and a total of 64 species (Table S1) have been detected in restored reaches during the entire project. This amount is noticeably higher than other monitoring programs in forest habitats in the region (45–56 species; [92]). Bird species richness also fluctuates as a result of seasonal habitat changes and community replacement, particularly due to the seasonal flux of migratory species. During spring and autumn passage, numerous migrant birds concentrate in the Iberian Peninsula along riparian galleries [39,93–95]. Although this is a feature only partially captured by our sampling design, an improvement in the carrying capacity of restored habitats as migration stopovers and corridors is also expectable if treatments are maintained in the mid-term.

Aquatic and riparian bird communities are highly influenced by landscape-scale factors like vertical and horizontal structure of riparian vegetation and adjacent land use [95,96]. *A. donax* invasion is a matter of concern due to the potential negative effects on birds that rely on native riparian vegetation stands for foraging and nesting [27,97]. In particular, giant reed stands in semi-arid Mediterranean areas present a depauperate passerine community in comparison with other similar riparian and reedbed formations, lacking mainly the set of birds that are more selective and adapted to palustrine habitats [98]. This could be due to differences in certain environmental characteristics between native and alien biotopes, as the lower availability of prey (invertebrates) associated with monospecific *A. donax* stands. This probably applies to our riparian habitats, where *Arundo* outcompetes reedbeds of *Phragmites australis* and shrubby formations like willow strips, brambles and different Mediterranean understory and forest communities that provide structural heterogeneity and additional food resources for birds [99].

Within native plant associations, Mediterranean riparian galleries as habitats 92A0 and 92D0 are key biodiversity hotspots on a regional scale, since they often represent the only well-structured habitat available for bird breeding and foraging within intensively developed landscapes [94]. Moreover, riparian specialist birds share these habitats with forest generalists and ubiquitous species typical of surrounding shrublands and agricultural landscapes [93]. The concept of riparian-obligate and riparian-dependent species [100] is useful since different restoration strategies (local vs landscape-oriented) would deliver improvements in each subset of species [101]. While some riparian-dependent species can be favoured in the initial stages after restoration, recovering the full set of riparian-obligate ones probably needs more time to rebuild the structural complexity they require. Both understory (e.g., Cetia cetti, Luscinia megarhynchos, Sylvia atricapilla, S. melanocephala) and canopy-dwelling species (Aegithalos caudatus, Fringilla coelebs, Periparus ater) were more abundant in reference than restored sites. There was only a negative trend in pioneer rural species inhabiting open habitats (Sturnus unicolor), and an increase of some riparian and facultative birds typical of riparian and upland forests (e.g., Jynx torquilla, Picus viridis). The latter and other species increases in restored but not in reference sites (Columba palumbus, Falco tinnunculus) can be attributed to enhanced foraging opportunities in areas disturbed by control treatments, rather than to a structural improvement of native vegetation as a result of restoration actions. In any case, species with seed dispersal potential were recurrently detected in the study area (e.g., Sylvia atricapilla, Sylvia melanocephala, Turdus viscivorus), which could benefit passive restoration in the long term (as previously demonstrated in burned areas [102]).

Overall, it seems that planted riparian vegetation has not fully developed yet to modify associated bird communities substantially. Nevertheless, mowing campaigns and restoration actions could have enhanced bird diversity through the creation of transient spots of open habitat with animal and plant resources that can be exploited by bird community inhabiting in the remaining tree stratum and adjacent shrubland patches. Tree canopies, which can persist even in river sections partially invaded by A. donax, are the most used habitat by many riparian bird species. Most riparian trees are deciduous, a type of forest limited in the study area to riparian zones due to the climatic restrictions of semi-arid Mediterranean areas. This type of forests hosts particular bird communities [103] that may complement the species typical from conifers and Mediterranean evergreen sclerophyllous forests, enhancing diversity at landscape and regional scale. Moreover, given the greater diversity and abundance of insects in deciduous broadleaf forests [104], these riparian species could result particularly important for birds, especially insectivorous ones. However, despite the importance of these tree canopies, the presence of native understory strata seems also necessary to reach a really diverse community [105]. Accordingly, the plantation of native trees supplemented by shrub and herbs, as done in this project, could promote synergies with existing vegetation and enhance longitudinal, lateral and vertical landscape connectivity with beneficial effects on the riparian bird community in the mid-term.

4.4. Management Implications

Human-driven environmental changes (e.g., land use intensification) disturb native riparian communities adapted to previous local conditions, arising niche opportunities for alien species which can show considerable increases from low densities [106]. The structural and functional changes caused by A. donax in riparian vegetation and associated communities result in detrimental effects on different ecosystem services, such as the provisioning of material and energy, regulation of local climate, extreme events and biogeochemical cycles as well as maintenance of the environment for humans and cultural services [20,29–31]. Given the advanced state of A. donax invasion in the Segura River, the complete removal of this invasive species and successful recovery of native riparian communities seem not feasible without reversing or, at least, mitigating the impacts of invasion drivers. More extensive and ecofriendly agricultural practices and associated environmental management (e.g., creation of buffer areas) could constitute a good base to prevent biological invasions. Intensive agricultural practices usually imply soil over-fertilization and the creation of an abrupt border between riparian forests and crops, which creates an edge effect that benefits generalists, pioneer and invasive species [107]. Burning has been traditionally used by farmers and landowners as a quick control method of A. donax but it has resulted completely ineffective and counter-productive due to the stronger post-fire resprouting exhibited by the giant reed [9]. Flow regime is strongly modified by reservoirs and water abstraction for irrigation in the Segura River basin [52]. This anthropogenic impact leads to reduced flow magnitude, altered frequency and inverted seasonal flow patterns [108], which could have also benefited A. donax expansion [109]. Dam management should implement a more natural high flow timing and frequency to simulate the natural seasonal variation of flow regime, so benefiting ecological integrity and diminishing invasion risks [110,111]. In this context, it should be stressed that the project LIFE + RIPISILVANATURA has attempted, albeit partially, to face invasion drivers through the implementation of complementary actions to restoration measures, such as the removal of unnecessary river embankments to recover lateral connectivity, demarcation of public waters and riparian areas, creation of a land stewardship network, and a public alert system for early detection of fire and invasive species. Social and educational actions were also developed aiming to reach long-lasting successful results: removal of exotic fauna through the involvement of citizens and environmental rangers, environmental voluntary service and awareness campaigns about invasive species (especially students), creation of bird observatories, publication of protocols and handbooks to promote sustainable agricultural practices and optimise riparian management.

The restoration actions performed, based on repeated mowing in combination with native species plantation, are specifically recommended on river reaches not fully invaded by *A. donax* and with

specific ecological interest (e.g., habitats of European interest, protected areas, threatened species, etc.). Nevertheless, this technique is not cost-effective in completely invaded areas. There are promising strategies that could be successfully applied in riparian areas dominated by monospecific stands of *A. donax*, such as plastic layering, a cost-effective, clean and sustainable technique that consists of covering the area recently mowed with an opaque reusable material (preferentially high-density polyethylene) during several months. This technique intercepts sunlight (which helps to exhaust the nutritional reserves of the rhizome) and can increase temperature above 60 °C, which finally produces the massive death of *A. donax* [112]. Regarding technical considerations, an additional mowing effort right before the plantation of native species could have been desirable to weaken *A. donax* to a greater extent and, subsequently, increase the survival rate of native saplings [32]. Finally, the cross-taxon biomonitoring scheme performed here considers the multi-dimensional nature of rivers and expands the assessment to both aquatic and terrestrial communities at river segment scale, which is not common in riparian restoration projects (usually focused on just a specific target community or taxa at meander or local scale [81]).

Supplementary Materials: The following information is available online at http://www.mdpi.com/2073-4441/11/ 12/2551/s1, Table S1: Taxa checklist of riparian vegetation, aquatic macroinvertebrates and birds recorded, Table S2: Table summarizing the mean values and standard deviation of riparian vegetation, birds and aquatic invertebrate indexes through time and between treatments, Tables S3 and S4: Results of Tukey post-hoc paired comparisons for riparian vegetation, bird and aquatic macroinvertebrate-related variables, Table S5: Results of linear mixed-effect models comparing the temporal variation of riparian vegetation, aquatic macroinvertebrate and bird community metrics between reference and restoration sites. Figure S1: Restored river reaches and sampling sites (reference and restoration sites) to monitor the changes of aquatic macroinvertebrates, birds and riparian vegetation. Figure S2: Boxplots showing the temporal variation of bird density, abundance and species richness, Figure S3–S6: Diagnostic plots to check residual's normality and homoscedasticity assumptions for *Arundo donax* density and height, bird density and abundance, including the results of Shapiro-Wilk and Levene's tests, Figure S7. NMDS comparing taxonomic composition before the beginning of restoration actions and the current situation between reference and restoration sites.

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