The effect of altered flow regimes on aquatic primary producer communities: Diatoms and macrophytes

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Keywords:	macrophytes, diatoms, periphyton, rivers, hydrological alteration, flow regime, reservoirs, regulated rivers

The effect of altered flow regimes on aquatic primary producer communities: Diatoms and macrophytes

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Abstract

Rivers are intensively managed worldwide through unprecedented flow regime alterations on a global scale. This has led to an increasing interest in the development of quantitative tools to assess the ecological response of organisms to flow alteration. To date, studies reflect a large diversity of responses that make the intensity and prevalence of effects difficult to generalize. The present study analyzed how flow alterations caused by dams affect the structure, composition and traits of diatoms and macrophytes in three Spanish River basins (Cantabric, Ebro and Duero). By using a control-impact design based mainly in hydrological similarity, our results showed consistent patterns of change in diatom and macrophyte communities. Our study showed a shift from resistant traits such as non-colonial diatom forms and crust-forming algae, which are adapted to the extreme events and natural dynamism of unregulated rivers, towards planktonic diatoms, free-floating algae and the mass development of aquatic plants in hydrological altered sites. Both communities shared common thresholds of response to altered hydrological attributes that could be considered in a flow management context. The hydrological indices that impacted diatom and macrophyte communities the most were related with the magnitude and duration of minimum flows. However, our results also show that it is important to consider the inter-relationships between the different hydrological attributes such as the seasonal variability of monthly flows, the magnitude of maximum annual extremes, the timing of flow events, and the frequency and rate of flow changes.

1. Introduction

Rivers are intensively managed in many areas of the world by means of a range of hydraulic infrastructures such as dams, weirs, water intakes, and canals aimed at facilitating water abstraction for a variety of human uses. These water uses are responsible for unprecedented flow regime alterations, occurring at any time of the hydrological cycle

(Peñas & Barquín, 2019; Poff et al., 2010; Sabater et al., 2018). In general, water-stressed ecosystems affected by agriculture or civil uses are characterized by longer low-flow or even dry periods and less frequent and intense peak flows than hydrologically unaltered rivers (Sabater et al., 2018), while energy production generates a higher frequency of peak flows than expected by natural variability (Richter & Thomas, 2007).

Ecological and evolutionary processes in river ecosystems are strongly influenced by many facets of dynamic natural flow regimes (Poff et al., 2010). Indeed, natural flow regimes set a template for contemporary ecological processes, evolutionary adaptations, and native biodiversity maintenance. Under natural conditions, and from an ecological perspective, extreme events such as high flow events or low flow periods exert selective pressures on populations, dictating the relative success of different species. Thus, altered flow regimes are one of the most serious and persistent threats to the ecological sustainability of rivers (Álvarez-Cabria, Barquín & Peñas, 2016; Bunn & Arthington, 2002; Peñas & Barquín, 2019). Flow alteration often involves the simultaneous modification of several components of natural flow regimes. Throughout this manuscript, flow alteration includes changes in flow magnitude, frequency, duration, timing and rate of change (Poff et al., 1997; Richter, Baumgartner, Wigington & Braun, 1997; Peñas & Barquín, 2019; García de Jalón, González & García de Jalón, 2019). Understanding the relationship between ecological responses and specific patterns of flow alteration is needed to help support scientific guidelines for developing flow standards that can be applied to a broad variety of streams and rivers (Poff & Zimmerman, 2010). Reviews of case studies to date (Gillespie et al., 2015; Poff & Zimmerman, 2010; Angus Webb et al., 2013; Sabater et al., 2018) reveal limited quantitative results on ecological responses to flow alteration, only a reduced number focusing on specific groups such as aquatic primary producers.

Primary producers are an essential part of aquatic ecosystems and their role as bioindicators and sensitivity to eutrophication and organic pollution has been extensively studied. In the last decades, there is a growing recognition that flow regime is also a key driver for primary producers in streams and rivers (Franklin, Dunbar & Whitehead, 2008; Bergey et al., 2010; Abati et al., 2016; Abdellah & Saad, 2019). The ecological response of primary producers to flow alteration can be identified in terms of changes in biomass and community structure and composition. For instance, the relative balance between biomass gain and loss processes will be influenced by flow regime (i.e. discharge magnitude, variability, and frequency). In this way, biomass gains occur during periods of higher flow stability, and are further enhanced in altered rivers as a result of the steady flows associated to damming or water abstraction, which promote the accumulation of algae and macrophytes (Biggs, Nikora, & Snelder, 2005; Smolar-Žvanut & Krivograd Klemenčič, 2013; Smolar-Žvanut & Miko, 2014; Riis, Suren, Clausen, & Sand-Jensen, 2008; Ponsatí et al., 2015). Consequently, recent studies suggested that periphyton and its associated metabolism could be used as indicators for monitoring the ecologial effects of increasing minimum flows (Huang et al., 2018). Biomass loss processes, on the other hand, are caused by increased shear stress resulting in breakage and dislodgement of biofilm mats and plants, for example because of high flow events (Franklin, Dunbar, & Whitehead, 2008). However, inconclusive results have also been reported. In the case of benthic algal assemblages, increases as well as decreases in biomass after large floods have been observed (Sabater et al., 2018; Schneider, 2015; Schneider & Petrin, 2017), while macrophyte mass developments occur in some but not other rivers having enhanced winter discharges (Johansen, Brandrud, & Mjelde, 2000). The effect of flow regime on species richness (here identified as local richness or α diversity), community structure and composition is more controversial. Following the Intermediate Disturbance

Hypothesis (IDH), some authors have suggested that a higher species richness would be found at intermediate levels of flow disturbance (Riis et al., 2008). Hence, rivers with altered flow regimes might show communities with lower species richness, as they may enhance or lessen the dynamism of natural rivers. Prolonged periods of reduced flows can reduce the contribution of sediments and produce changes in physicochemical variables favoring high periphyton biomass and species richness (Smolar-Žvanut & Miko, 2014). Tang et al. (2013) also found that low-flow conditions downstream of water abstraction facilities enhanced algal diversity and richness. However, some authors have found that more stable flow regimes were not associated with any effect on taxon richness of periphyton assemblages (Schneider & Petrin, 2017). For macrophytes, increases and decreases in species richness have been reported following river regulation (Abati et al., 2016; Downes, Entwisle, & Reich, 2003). On the other hand, timing of high or low flow during the year has not received much attention, even though the phenology of algae and plants may be strongly affected by these attributes in regulated rivers (Franklin et al., 2008).

Trait-based approaches applied to community ecology have helped to understand the effect of environmental stressors on species assemblies. These approaches avoid the complexity associated with taxonomic and biogeographical differences among sites (Goldenberg Vilar et al., 2014; Hooper et al., 2005; Passy & Blanchet, 2007) and allowing to test, for instance, general relationships of flow velocity with periphyton assemblages (Biggs & Smith, 2002; Biggs, 1990; Biggs, Goring, & Nikora, 1998; Biggs, Smith, & Duncan, 1999; Francoeur & Biggs, 2006; Jowett & Biggs, 1997). However, trait-based approaches applied to the study of ecological effects of hydrological alterations in primary producers is rare (Vieira et al., 2012) and has only recently been considered for diatom communities (Wu et al., 2019).

We believe that general patterns of ecological response of primary producers to flow alteration are difficult to find given two primary reasons. First, the variability of reservoir attributes, which hampers the prediction of their potential hydrological alterations and, second, the magnitude and direction of the alterations, that might depend on the natural characteristics of the impacted river (Abati et al., 2016; Peñas & Barquín, 2019; Poff et al., 2010). Another limitation in the assessment of general patterns of ecological response to flow alteration is that most research to date presents single-case studies, while lacking control or reference sites for natural conditions (Lloyd et al., 2004; Poff & Zimmerman, 2010). Consequently, the ability to predict primary producers' abundance and distribution in relation to hydrological alterations is still relatively limited.

The present study aimed at determining the effect of flow alterations caused by dams on primary producers in rivers, focusing on the response of diatoms and macrophyte communities in a control-impact experimental design. Patterns of ecological response to hydrological alterations can only be found if we consider the attributes driving variability in unaltered flow regimes. In this regard tools and modelling techniques that assure the hydrological and ecological comparability between natural and altered sites, such as hydrological classifications, are needed to assess the degree of hydrological alteration and its effects (Poff et al., 2010). By selecting sites that should show similar hydrological behavior in the absence of perturbations (see: Peñas & Barquin, 2019), we expected to find common responses of primary producers in altered regimes compared to natural flow regime. We hypothesized that changes in the natural flow produced by hydrological regulations (i.e., longer low-flow, less frequent and intense peak flows and seasonal shifts in the magnitude of monthly flows – in our study the independent variables) would favor species (dependent variables) adapted to the new hydrological condition. These changes in community composition would be reflected in the abundance of specific functional groups, favoring loosely attached algal forms and submerged vascular plants. We also expected that the sensitivity of primary producers to flow alteration would allow for the quantification of common thresholds of community changes following shifts in hydrological dynamics in altered sites.

2. Materials & Methods

2.1 Study area

The study was carried out in permanent rivers belonging to three Spanish River basins: the Cantabric, Duero and Ebro basins (Figure 1). The Cantabric basin encompasses several small basins with drainage areas ranging from 30 to 4,907 km^2 covering a total area of 22,000 km^2. Rivers in this area are confined by the Cantabrian Cordillera, a mountain range running parallel to the coast and reaching up to 2,600 m.a.s.l. Rivers are characterized by high slopes and short lengths and lie within the Atlantic climate region (Rivas-Martínez, 2004). The average annual temperature is 14°C and precipitation is abundant throughout the year with a mean of 1,300 mm year^{-1}, presenting maximum rainfalls in December (150 mm month^{-1}) and minimum ones in July (50 mm month^{-1}) (González-Ferreras, Barquín, & Peñas, 2016). However, the amount and distribution of precipitation vary significantly depending on local topography (González-Ferreras, Barquín, & Peñas, 2016). Snow is frequent above 1,000 m.a.s.l. in this area in winter. The Ebro and Duero basins, with catchment areas of 85,530 km^2 and 98,073 km^2, respectively, lie within the Mediterranean region, although both receive temperate and Mediterranean climate influences. The Ebro flows south-eastward from the Cantabrian Mountains into the Mediterranean Sea. The Duero basin is bordered by the Cantabrian cordillera in the north and by the central and Iberian mountain system ranges in the south and northeast, respectively. It flows into the Atlantic sea through Portugal. Annual rainfall is around 600

- 650 mm in these river basins and varies significantly, from 300 mm in the center to 1,800 mm in the highest mountains where snow is also common during winter. The temperature regime also presents oscillations throughout the year with temperatures over 30°C in summer and below 5°C during winter.

2.2 Study design

The analysis of the influence of hydrological alterations on primary producer communities was conducted in 20 permanent river reaches located in northern Spain and following a control-impact experimental design (CI). The first condition for the reaches to be included in the analyses was that hydrological information in both control and altered sites was available, i.e. they had nearby gauge stations. Twelve altered sites (impact sites) were selected attending to the presence of reservoirs upstream. Reservoir purposes varied between reservoirs and were defined as Hydropower (HP), Irrigation (IR), Water Supply (WS), or any combination of them (IR/HP; IR/WS; HP/WS; IR/HP/WS) (Table S1). In this regard, we only considered 1) River reaches affected by a minimum of one and a maximum of three upstream impoundments, and 2) River reaches with a ratio of catchment area at the impoundment - catchment area at the reach above 0.3.

Control sites were selected attending to their ecological similarity with impacted sites, and the lack of any perturbation affecting flow dynamics, and used to statistically compare what would have occurred at the impact sites in the absence of the perturbation (Downes et al., 2002). In this study, we mainly focused on hydrological similarity for two reasons: hydrology is a master variable in determining the biotic composition, structure and function of river ecosystems (Poff et al., 1997), and it integrates considerable environmental information, e.g. climate, topography, land cover or geology, in upstream catchments (Peñas et al., 2014). In this context, inductive hydrological classifications group similar rivers attending to flow regime and catchment characteristics that explain the largest part of the hydrological variability. It is important to highlight that the hydrological classification approach is not a fixed and definite product, and the resulting divisions among hydrological classes are not always rigid. Indeed, hydrology's spatial variation must be understood as a continuous gradient of change. We used an inductive hydrological classification that covered the whole Spanish River network and identified the hydrological class of the impacted sites in the absence of perturbations (Peñas & Barquin, 2019). It was developed using 282 flow series recorded at gauges unaffected by impoundments or large upstream abstractions and with under 50% upstream catchment agriculture or forest plantation land cover. In this way, we selected 12 altered (Aguilar, Arbejal, Arlanzón, Carrión, Duero, Ebro, Esla Luna, Nalón, Nansa, Narcea and Porma) and 8 control (Bernesga, Cea, Curueño, Deva, Ega, Omaña, Sella and Tirón) sites, which according to the 20-level hydrological classification developed for Spain, belonged to hydrological classes NC10 and NC13 (NC denotes natural classes; Peñas & Barquín, 2019). However, given their high hydrological similarity (Peñas & Barquín, 2019) classes were treated as one in the statistical analyses.

We assumed that diatom and macrophyte community composition at the control sites would resemble the composition present in the altered sites if the hydrological alterations had never occurred. The similarity of the selected sites in terms of hydrological and catchment characteristics, and the fact that dam operations were the only factor affecting the hydrology of the altered sites, supported this assumption. Altered sites varied in flow alteration along a gradient, allowing for general relationships between flow alteration and ecological response of diatom and macrophyte communities to be inferred. Because other factors such as nutrient concentration and light availability could influence biomass and species composition, we also performed statistical analyses to test for differences in nutrient concentrations and canopy cover between control and altered sites. It was hypothesized that hydrological alterations would be responsible for a shift in diatom and macrophyte community composition and in the relative abundance of functional groups. We expected a stronger ecological response of primary producer communities under increasing levels of hydrological alteration.

2.3 Hydrological data and hydrological indices

The hydrological database used in this study consisted of daily mean flows covering the largest data series from 1959 (although most of them started in 1970) to 2018, available in 20 gauge stations provided by the Ministries of Public Works and Ecological Transition (Spanish Government). The hydrological data of these 20 gauge stations fulfilled the quality criteria established by Peñas et al., (2014). Thus, we eliminated years with: 1) periods of consecutive repeated values; 2) non-natural extreme low flows for short time periods; 3) periods of zero flow values (because all the selected rivers were permanent); 4) non-natural flow magnitude rises and falls; or 5) large differences between two periods, probably due to changes in the flow record method. Years with more than 30 days of missing data were also removed from the analyses. Finally, we discarded those gauges that accounted for less than 20 years. Daily flow series from both natural- and altered-river gauges were normalized by dividing all daily flow values by the mean annual flow. A set of 30 ecologically meaningful hydrological indices (HIs) were calculated for the 20 sites according to Richter et al., (1996). These hydrological indices were divided into six major groups to statistically characterize the annual average and the intra-annual hydrological variation representing the five fundamental attributes of hydrological regimes: magnitude, timing, frequency, duration and rate of change (Poff et al., 2010; Table 1).

2.4 Environmental variables

Environmental and water quality variables, i.e., canopy cover, temperature, pH, conductivity, and water nutrients (NO_2, NO_3, NH_4 and PO_4) were recorded during field surveys in 2017 (Table S2). The percentage of canopy cover of the selected stream reaches was estimated from hemispheric photography. One hemispheric image was collected with a Nikon Coolpix P510 with a fisheye lens in the center of each stream reach (Figure S1). The camera was leveled horizontally, positioned, and oriented towards the magnetic north on a tripod. Canopy cover was determined with the Gap Light Analyzer from the obtained hemispherical images (Estevez et al., 2017; Frazer, Canham, & Lertzman, 1999). Temperature data were obtained from HOBO Pendant® data loggers deployed during 1 week in October 2017. Electric conductivity and pH were measured in the field with a Milwaukee pH55 and EC59 Portable Waterproof pH and EC/TDS Tester. Nutrient variables were determined by continuous flow analysis (FIA and CFA) following standard protocols: UNE-EN ISO 15681-2:2005 for PO_4, UNE-EN ISO 13395:1997 for NO_2 and NO_3, and UNE-EN ISO 11732:2005 for NH_4 at the IHCantabria - Instituto de Hidráulica Ambiental de la Universidad de Cantabria (IH Lab Bio).

2.5 Diatom and macrophyte sampling

The communities of primary producers analyzed in this study belong to the Biological Quality Elements (BQE) required under the European Water Framework Directive for ecological assessments (periphyton and macrophytes). We analyzed diatoms as a proxy for the full periphytic community (Kelly, 2016). Periphyton sampling was conducted in October 2017 by collecting a single composite diatom sample at each site according to UNE-EN 13946: 2014 standard procedure. We selected one longitudinal transect with riffles of about 10 m of length per site and collected 6 cobbles, and sampled an area of approximately 100 cm^2 within the main flow of the river. Zones of slow current (approx. ≤ 20 cm s^{-1}) were avoided as these allow the build-up of loosely attached diatoms, silt, and other debris. Cobbles were collected at a depth of ca. 10 cm to ensure that they were not exposed to air in the previous 4 weeks and that they were placed in the euphotic zone. Areas of heavy shade and those close to the bank were avoided. The upper part of the stone was scrubbed with a dishwasher brush. The dislodged material was decanted into a sample bottle and preserved using formaldehyde. Aliquots of the diatom samples collected (ca. 1-2 mL) were cleaned with 4 – 6 ml of nitric acid (65% v/v) and potassium dichromate ($K_2Cr_2O_7$) at room temperature for 24 – 48 hours. Afterwards, samples were repeatedly centrifuged (1500 rpm) and rinsed with distilled water at least three times to remove oxidation by–products. Permanent slides were prepared from the treated sample, and mounted with a high-resolution diatom resin (Naphrax®, refractive index of 1.74). From each sample, 400 valves were identified at 1000× magnification, except for one site (Arlanzón, 224 valves) due to low diatom concentration in the sample. Taxonomic identification was based on Delgado et al., 2013; Delgado & Pardo, 2015; Krammer & Lange-Bertalot, 1986-1991; Krammer, 1997a; Krammer, 1997b; Krammer & Lange-Bertalot, 2004; Lange-Bertalot, 1993; Lange-Bertalot & Krammer, 1989; Lange-Bertalot, 2001; Levkov, 2009; Novais et al., 2009; Prygiel & Coste, 2000 and Trobajo et al., 2013. Macrophyte surveys were conducted in October 2018 according to UNE-EN 14184:2014 standard procedure for routine water quality monitoring (Meilinger, Schneider & Melzer, 2005). Contiguous sets of survey stretches were sampled by trained surveyors along 80 m river reaches including both swift and slow flowing habitats. The river reaches were waded in zig-zag mode in order to register/record all macrophyte species present along the river channel. Wading was carried out upstream to prevent suspended sediments from interfering with macrophyte observation and identification. During the surveys, all phototrophic organisms included as macrophytes in the European Protocol for sampling macrophytes (all aquatic vascular plants, bryophytes, stoneworts and macro-algal growths) were recorded and the percent cover was estimated in the field. When identification was not possible in the field, samples were taken to the laboratory for confirmation fixed in Kew mixture. One site (Aguilar) could not be sampled in 2018 due to high flow. Macrophyte identifications were based on Bailly, Vergon, & Vadam, 2004; Cirujano, Molina, & Murillo, 2014; Laplace-Treyture et al., 2009; Ordóñez, Carmen, & Collado Prieto, 2003; Talavera & Castroviejo, 1999.

It must be noted that we selected October as the sampling month because it is the unique concurrent period of the year where the altered and controls sites could be compared under similar condition of flow. In this regard, previous hydrological analysis, together with the knowledge of the operational rules of reservoirs, indicated that low flow conditions were present during early October in both type of rivers, as it is the end of the dry period (natural sites) and irrigation period (altered sites).

3. Data analysis

3.1 Hydrologic alterations (HA)

Principal Component Analysis (PCA) was used to summarize the variability in the 30 HIs through the 20 study sites. The broken stick method was used to select the relevant components in the PCA analysis. Statistically significant differences in the HIs between control (8 sites) and altered river gauges (12 sites) were tested by a t-test. In order to assess if differences in diatom and macrophyte community composition between the control and altered sites were due to differences in environmental and water quality variables, we also tested for differences in these parameters between control and altered sites by a t-test after logarithmic transformation to achieve normality when required.

3.2 Primary producer communities

Diatom and macrophyte communities were grouped based on traits. For diatoms, we used ecological guilds, growth forms and cell sizes based on individual cells following Passy, 2007 and Rimet & Bouchez, 2012 (see Table S4). We classified macrophytes into different traits according to organism type: macroalgae, bryophytes or vascular plants, following Fernández-Aláez, Fernández-Aláez, & Bécares, 1999, Rott & Wehr, 2016, and Vieira et al., 2012 (Tables 2 and S4).

For diatom data, percentages of species abundances were calculated, and data were square root transformed prior to the analysis. In order to minimize the noise produced by rare species with very low occurrences, only species whose average cumulative abundance comprised 98% of all observations were kept for the statistical analysis (Potapova & Charles, 2002; Soininen, 2004; Leira & Sabater, 2005; Grenier et al., 2010; Goldenberg et al., 2014). Significant statistical differences between species richness of diatoms and macrophytes and coverage of macrophytes between control and hydrologically altered sites were assessed by t-tests. Non-metric multidimensional scaling (NMDS) was used to summarize the variability of the 20 sites in terms of diatom and macrophyte community structure based on Bray Curtis dissimilarity distance. Statistical differences in community structure between control and altered sites were tested with a permutation analysis of variance (PERMANOVA) based on Bray Curtis (for abundance data) and Jaccard distance (for presence-absence data) similarities. Statistical differences in functional groups between control and altered sites were tested with a t-test. Statistical differences in macrophyte traits between control and altered sites were tested using a Wilcox test, a non-parametric analysis that is better suited for data with non-normal distributions (Quinn & Keough, 2002). The contribution of individual species to overall dissimilarity between control and altered reaches was assessed by similarity percentage analysis (SIMPER) (Oksanen et al., 2013).

We used the Best Subset of Environmental Variables with Maximum (Rank) Correlation with Community Dissimilarities algorithm (BIOENV; Clarke & Ainsworth, 1993; Oksanen et al., 2013) to identify the subset of HIs most influential to diatom and macrophyte community structure. BIOENV determined the correlation between each environmental distance matrix (Euclidean distance) and the community dissimilarity matrix (Bray Curtis distance). The highest correlation identified the combination of HIs that were most strongly related to community composition. In the next step, we performed a Mantel test to assess the level of statistical significance between the BIOENV matrix of selected hydrological indices and diatom and macrophyte community data.

3.3 Effects of HA on primary producers

To further investigate the relationship between hydrological alterations and ecological change, we analyzed the response of diatoms and macrophytes (from the NMDS) to the individual HIs. We selected one HI showing statistical differences between control and altered sites and/or statistically relevant for diatoms and macrophyte communities, from each of the six hydrological attributes analyzed. We also calculated the percent of change in each HI in relation to the control sites using the following formula:

$$AHI_{iy} = \frac{HI_{iy} - \overline{X}\,HI_{icontrols}}{\overline{X}\,HI_{icontrols}} \times 100$$

Where □ $HI_{i\,controls}$ represents the average value of each selected HI in the 8 control sites and HI_{iy} the value of each selected HI in each altered site. In this way, we analyzed the response of diatoms and macrophytes to different aspects of flow alteration, including magnitude, duration, timing, frequency and rate of change. Diatom and macrophyte traits were treated as supplementary variables in the ordination diagrams.

Ordinations were performed in CANOCO 5 (ter Braak & Šmilauer, 2012), while all other statistical analyses were conducted using R (R Development Core Team, 2014) with the Vegan package (Oksanen et al., 2013).

4. Results

4.1 Hydrological alterations

Most HIs showed statistically significant differences between control and altered sites. Strong alterations of mean monthly flows were found with the exception of May and October mean flows (M5 and M10). The mean flow of winter months was significantly lower and the mean flow of summer months was significantly higher in altered sites compared to control sites (Figure 2). The magnitude of minimum annual flow (LF indices) was significantly larger in hydrologically altered sites, while the magnitude of maximum flow (HF indices) showed the opposite. Regarding the timing of extreme flow events, we found that the Julian minimum flow (Jmin) was significantly earlier within the year in hydrologically altered sites. The number and duration of high and low flow pulses (nPHigh, meanDPhigh, nPLow and meanDPLow) as well as the rate of flow changes (meanPos and meanNeg) were not statistically different between control and altered sites (Figure 2 and Table S5).

The PCA based on the HIs discriminated between control and altered sites and three principal components were retained based on the broken stick rule. As expected, several HIs exhibited a high degree of inter-correlation (e.g. M1-M12 and HF indices), contributing strongly to the first axis of the PCA (51% of explained variation) (Figure 3). The first axis was negatively correlated to the mean flow of the winter months, HF indices, Jmin and Jmax, and positively with the mean flow of the summer months, as well as with the HIs related to the duration of high pulses (Figure 3A). Ebro, Porma, Aguilar and Duero were the sites with the strongest deviation of this axis in the mean monthly flows, the duration of pulses and the timing of extreme flow events in relation to control sites. By contrast, sites affected by hydroelectric dams (Narcea, Nalón and Nansa, and especially the last two) had the minor deviation in the HIs in relation to control sites. The second axis (12.5 % of explained variation) was mainly related to M10, frequency and duration of low flow pulses (npLow and meanDPLow) and rate of flow changes (meanPos and meanNeg) (Figure 3A). The third axis (12% of explained variation; Figure 3B) was strongly related to the magnitude of minimum annual flows (LF indices). Arlanzón and Aguilar showed the strongest deviation from controls as regards the third axis.

The results of the t-test showed that there were no statistical differences in the environmental variables measured between control and altered sites with the exception of canopy cover, that was higher in control sites (mean= 53.92 ±18.2) than in altered ones (mean= 28.94 ±21.8), (t=2.78, *P*=0.013; Table S6, Table S2). To further investigate these differences, we performed an extra PERMANOVA test (using Bray Curtis similarity). We compared diatom and macrophyte composition between two groups of 10 sites: high (> 40%) and low (< 40%) canopy cover. The sites belonging to the high canopy cover group were all control sites except Sella and three of the altered sites (Arbejal, Arlanzón and Carrión). All the other sites were included in the low canopy cover group. For both communities, the PERMANOVA tests were not significant (F=0.93, *P*=0.48; F=1.31, *P*=0.20 for diatoms and macrophytes, respectively).

4.2 Primary producer communities

A total of 115 diatom species were recorded in the 20 diatom samples. Of all species, 68 had an average cumulative abundance comprising 98% of all observations and were kept for the statistical analysis. No statistical differences were found in the number of diatom taxa between control and altered sites (t = -0.88; df=13.5 P = n.s.).

As for macrophytes, we collected 63 taxa (7 cyanobacteria, 17 algae, 20 bryophytes and 19 vascular plants). Some species of vascular plants were merged to genus level for the statistical analyses. The number of macrophyte taxa was statistically higher in hydrologically altered sites (t = -3.35; df=16.61 $P < 0.01$), mean numbers reaching 8.37 (± 2.13) and 12 (± 2.57) in control and altered sites, respectively.

Average macrophyte cover was statistically lower in control than altered sites (t = -2.19; df=14.4 $P = 0.04$). Mean macrophyte cover in control sites was 30.35% (±14.49), while in altered sites it was 56.12% (±35.02). In general, we found higher macrophyte coverage in hydrologically altered sites, with the exception of Arlanzón and Carrión, two altered sites that presented a relatively lower coverage than the rest of the hydrologically altered sites (16.6% and 14.5%, respectively).

Diatom and macrophyte communities represented in the NMDS indirectly showed the main hydrological alteration gradient (NMDS axis 1; Figure 4A and Figure 4B). The differences in community similarity between control and altered sites were statistically significant (PERMANOVA test based on Bray Curtis distance, Table 3; and PERMANOVA test based on Jaccard distance, Table S3). The species that contributed the most to overall dissimilarity in diatom and macrophyte communities are shown in Tables 4 and 5, respectively. For diatoms, the species that contributed the most to overall dissimilarity were the ones showing changes in their relative abundances while present in both control and altered sites. For example, *Achnanthidium minutissimum* (higher abundance in altered sites) and *Achnanthidium pyrenaicum* (higher abundance in control sites). Among species present only in altered sites it is interesting to highlight planktonic taxa such as *Aulacoseira granulata*, *Cyclotella meneghiniana*, *Discostella pseudostelligera*, *Tabellaria flocculosa* or *Cyclotella atomus*. As regards macrophytes, the species that contributed more to overall dissimilarity presented changes in coverage (macroalgal taxa such *Cladophora sp.* or *Lemanea sp.* with higher abundances at control sites), though there were also species that were only present in either control or altered sites. For example, some macroalgal taxa such as *Audouinella sp.*, or *Oedogonium* sp., or some hydrophytes, were only present in altered sites, while other species (e.g. the macroalgal taxa *Heribaudiella sp.*) were only present in control ones.

The differences in community structure between control and altered sites were also highlighted by statistical differences in functional group abundance. Higher abundances of loosely attached functional groups were found in altered sites within the planktonic guild (e.g. *Cyclotella meneghiniana, Aulacoseira granulata*) (t= -2.27, df= 11.87, *P*= 0.042), colonial growth forms (e.g. species such as *Encyonema, Rhoicosphenia abbreviata, Diatoma vulgaris*) (t= -2.52, df= 14, *P*= 0.025) and large cell sizes (e.g. *Cocconeis placentula var. lineata*, *Cocconeis euglypta*) (t= -3.45, df= 16.04, *P*= 0.003). Within the macrophyte communities, floating filamentous algae (e.g. *Oedogonium sp*. or *Spirogyra sp.*) (w= 16, *P*= 0.014), hydrophytes with floating and submerged leaves (e.g. *Ranunculus sp*. or *Myriophyllum sp.*) (w= 17.5, *P*= 0.03), and algal tufts (*Audouinella sp.*) (w=16; *P*= 0.008) were more abundant in altered sites. In control sites, non-colonial and smaller diatom forms (*Achnanthidium pyrenaicum*, *A. atomoides*, *and Amphora pediculus*) and firmly attached macrophyte species such as crust-forming algae (e.g. *Hildebrandia sp., Heribaudiella sp.*) (w= 84.5, *P*< 0.001), were more abundant.

4.3 Effects of HA on primary producers

Both diatom and macrophyte communities were sensitive to changes in magnitude and duration of minimum annual flows (90LF). Diatoms were also clearly affected by the Julian day of minimum flow (Jmin), the number of low pulses (nPLow) and mean October flow (M10; Table 3). Correlations between diatom and macrophyte community structure and HIs were significant, as shown by the Mantel test (Table 3).

Changes in diatom and macrophyte communities increased with the degree of alteration in the magnitude and duration of minimum annual flows (90LF), their ecological response being already noticeable at levels around 50% of change in this HI (90LF; Figure 5 and Figure 6). Regarding the magnitude of monthly flows, changes in diatom and macrophyte communities were already apparent with a decrease of 25% in winter-spring flows, or an increase of 300% in summer-autumn flows. The maximum annual high extremes (1HF) and the timing of extreme low flow events (JMin) were lower in the altered sites, and changes in diatom and macrophyte communities were noticeable when Jmin exceeded 50% and 14% of change, respectively (Figure 5 and Figure 6). The frequency of pulses (nPLow and nPHigh) and the rate of flow changes (meanPos and meanNeg) were not statistically different between control and altered sites (Figure 2) and we have not found a clear relationship between the degree of hydrological alteration in these HIs and an ecological response of diatoms or macrophytes.

5. Discussion

Our study supports our expectation that hydrological alterations produce a significant shift within diatom and macrophyte communities by selecting species that are adapted to altered hydrological regimes. Consistent ecological changes in stream primary producers were observed, diatom and macrophyte communities sharing common thresholds of ecological response to hydrological alterations across study sites.

5.1 Effect of HA on diatom and macrophyte communities

Although a wide range of studies have assessed the response of periphyton to natural changes in hydraulic conditions such as water velocity and discharge (Biggs et al., 1999; Francoeur & Biggs, 2006; Horner, Welch, Seeley, & Jacoby, 1990), less research has documented the response of periphyton community composition to flow alteration in terms of changes in ecological meaningful hydrological attributes (e.g. flow magnitude, frequency, duration, timing and rate-of-flow change; Bergey et al., 2010; Smolar-Žvanut & Krivograd Klemenčič, 2013; Tang et al., 2013; Huang et al., 2018; Wu et al., 2019). Particularly, only a few studies have analyzed diatom assemblages in relation to the effect of altered flow regimes in rivers (Growns, 1999; Growns & Growns, 2001; Krajenbrink et al., 2019; Wu et al., 2009, 2010). In our study, we found evidences that diatom communities were sensitive to the main indicators of hydrological alteration: an increase in the magnitude and duration of minimum flows (LF indices) and the earlier timing of the minimum flow (Jmin). Jmin is directly linked to seasonal shifts in the magnitude of monthly flows, typically occurring at hydrologically altered sites. Longer periods of low flow enhanced hydrological stability, driving changes in the relative abundance of diatom species responsible for the observed variations in community structure. Specifically, a shift towards planktonic forms as well as larger cell sizes was recorded. The planktonic guild is sensitive to physical disturbance and hence, reaches highest abundances in conditions of minimum flow disturbance. Examples of species with affinity towards hydrologically altered sites in our study were *Aulacoseira granulata*, species of the genus *Cyclotella,* or the high profile species *Diatoma vulgaris*. These results agree with other studies suggesting that diatom assemblages downstream of reservoirs differ from assemblages inhabiting upstream sites, where the occurrence of these species downstream of reservoirs and lake outflows was also reported (Growns, 1999; Growns & Growns, 2001). The higher proportion of larger cell sizes in regulated sites could be due to several reasons. Cell sizes are related to diatom life forms. For example, larger cell sizes are usually planktonic forms, or species attached to the substrate by stalks, or rosette-forming diatoms. These life forms create tall structures that overtop basal-layer pioneer taxa (Morin et al., 2008; Rimet & Bouchez, 2012). In addition, large diatom species have longer cell cycles than smaller ones (Morin et al., 2008). These types of life forms of ¨tall stature¨ as well as longer cell cycles are favored under longer periods of low flow and higher stability, conditions that often occur in regulated rivers. On the other hand, natural disturbance tolerant traits (Wu et al., 2019) as non-colonial forms and motile growth forms were more abundant in control sites. However, an increase in species of the motile guild in sites downstream of reservoirs has been reported in England (Kajenbrink et al., 2019). These taxa are mostly of small sizes and characteristic of fast flowing rivers (Rimet & Bouchez, 2012). Examples of such species in our study were *Achnanthidium pyrenaicum, Amphora pediculus* or *Nitzschia fonticola*. It is worth mentioning that, while low profile species were highly abundant in both control and altered sites, *A. pyrenaicum* was replaced by the pioneer and generalist *Achnanthidium minutissimum* (Watchorn et al, 2008) in the hydrologically altered sites. Our analysis also revealed that diatom communities responded to two other hydrological variables: the mean flow of October (M10) and the number of low pulses (nPLow). Results did not show significant differences in these two HIs between control and altered sites. However, we observed higher values in sites situated along Atlantic rivers compared with those on the Mediterranean side. The Atlantic region has higher mean annual precipitation than the Mediterranean region, a higher abundance of permanent streams and maximum flows occurring during autumn and winter (Delgado & Pardo, 2015). Some species, such as

Navicula gregaria, *Sellaphora seminulum* or *Karayevia oblongella,* which were also present in our study, have shown preferences for Atlantic climate regions (Delgado & Pardo, 2015).

Our results demonstrate the sensitivity of diatom communities to the effect of flow alteration even during one single season. It is well known that seasonality influences diatom communities (Soininen & Eloranta, 2004; Goldenberg Vilar et al., 2018; Snell et al., 2019). For example, under spring/summer conditions, water temperature and net radiation, dissolved oxygen and nitrogen availability are key drivers of diatom assemblage composition (Snell et al., 2019). In contrast, winter and autumn diatom communities are associated with other in-stream conditions, primarily turbidity and discharge, precipitation-related climate variations and phosphorus resource availability (Snell et al., 2019). These findings indicate that sampling autumn communities is a good choice to detect in-stream conditions related to hydrological characteristics, while minimizing the effect of secondary variables that have the strongest influence in mid-summer (i.e., temperature, nutrients; Goldenberg Vilar et al., 2018). Krajenbrink et al., (2019) highlighted that the effects of flow regulation on diatom communities were detectable both during spring and autumn, but they found that autumn diatom samples showed a higher sensitivity to the effect of river regulation, as more indicator taxa were found during this season than in spring samples. This is often the case with planktonic taxa, thus, the higher proportion of planktonic taxa in the altered rivers analyzed in our study might be enhanced by the effect of seasonality, and related to the increased production of phytoplankton in the reservoir during summer. Therefore, differences in the relative abundance of species in contrasting seasons (spring or autumn), do not hamper the detection of the effect of river regulation in any of them.

Macrophyte communities also responded strongly to hydrological alterations, showing changes in species richness, coverage and trait dominance, especially as a result of variations in the magnitude and duration of minimum flows (LF indices), as occurred with diatoms. Both species richness and coverage responded positively to the alteration in LF indices, suggesting that longer periods of low flow promoted macrophyte biomass growth and increased richness (Abati et al., 2016; Benítez-Mora & Camargo, 2014; Riis et al., 2008). These results also supported earlier studies, where hydrological alterations were identified as main controllers of vegetation cover (B. J. Biggs, 1996; Riis & Biggs, 2003). The higher macrophyte richness and coverage in altered sites affected community structure in terms of changes in the coverage of species in comparison to the natural communities, but also reflected changes in community composition by the replacement of some species in altered sites (see Table 5). This was the case of some species of hydrophytes such as *Myriophyllum sp*., or *Callitriche sp*. but also that of the red algae *Audouinella sp*. or the filamentous algae *Oedogonium sp.,* only present in altered sites. Macrophyte traits were also different between control and altered sites, with a dominance of floating algal traits, algal tufts and hydrophytes in hydrologically altered sites. Floating algal species such as *Oedogonium sp*. or *Spirogyra sp*. are typical of standing or slow flowing habitats (Abati et al., 2016). Aquatic plants with submerged and floating leaves (*Myriophyllum sp.*, *Ranunculus sp.* or *Potamogeton sp.)* were in general absent in control sites. The presence of these aquatic plants could also be related to the effect of reservoirs acting as a source of propagules for aquatic vegetation (Benítez-Mora & Camargo, 2014). Control sites, were dominated by disturbance resistant traits adapted to the hydrological dynamism of natural flow regimes such as crust-forming green algae (*Gongrosira sp.*), red algae (*Hildebrandia sp.*) or brown algae (*Heribaudiella sp.*), as well as shrub like traits of some macroalgal taxa such *Cladophora spp.* or *Lemanea spp*.

Regarding bryophytes, both coverage and richness were also higher in altered sites and they were dominated by streamers and mat traits, although we did not find significant differences in bryophyte traits between altered and control sites. Streamers are in general abundant in slower currents of streambed in full sunlight while smooth mats are easily found in torrential water impacted zones, in deep shaded microhabitats (Vieira et al., 2012). Examples of these streamers were species of the genus *Fontinalis*, which showed an increase to nearly 100% following river regulation in Norway (Rørslett, Mjelde, & Johansen, 1989). Regarding smooth mats, they were present in control and altered sites. Nonetheless, higher abundances of *Chiloscyphus polyanthos* and *Rhynchostegium riparioides* were found in altered sites while a slightly higher abundance of species of the genus *Brachythecium* was detected in control sites.

It is well known that other variables such as nutrients or temperature changes may also affect diatom and macrophyte community structure and composition (Franklin, Dunbar, & Whitehead, 2008; Krajenbrink et al. 2019;Ponsatí et al., 2015; Rimet, 2012; Schneider & Petrin, 2017). In addition, water chemistry variables may interact with hydrology, scouring of periphyton biomass during floods enhanced under conditions of resource limitation (Biggs, Tuchman & Stevenson, 1999). In our study, some sites showed higher nutrient levels than others, specifically higher orthophosphate and nitrate concentrations in some control sites (Ega and Tirón), or orthophosphate and ammonia in some altered sites (Aguilar and Ebro). However, we did not find statistically significant differences in water quality variables (temperature, pH, conductivity and nutrients) between control and altered sites, which strengthens the idea that hydrological alterations were the main factor triggering the observed shifts in primary producer communities. However, we cannot completely rule out that sites with higher nutrient levels favored the development of certain eutrophic species. On the other hand, canopy cover was significantly lower in altered sites although it also showed a high variability among sites. Canopy cover reduction has been recorded under increasing Gross Primary Production and this could also affect macrophyte composition, enhancing the development of algae or macrophytes with higher light requirements (Burrell et al., 2014), as is the case of filamentous algae of the genus *Cladophora* (Bunn, Davies & Mosisch, 1999). However, we did not find significant differences in diatom or macrophyte community composition between sites with high (> 40%) and low (< 40%) canopy cover. Moreover, we were aware that there might be other factors that could not be controlled and may affect diatom and macrophyte biomass and species composition in this study (e.g. potential grazers).

5.2 Ecological response of stream primary producers to flow alterations. Implications for environmental flow management

Our study revealed that several aspects of flow regimes affected the structure of riverine primary producer communities. We found that not only the magnitude but also the duration of annual minimum flows is an essential attribute of flow regimes that should be considered. A ca. 50% increase in the magnitude of minimum flows of 90 days duration (90LF) triggered community shifts of both diatom and macrophytes selecting for species with specific traits that were outcompeted under highly stable conditions. The relationship between the alteration in the duration of minimum flow and changes in primary producer communities showed a positive relationship, where the highest deviation from baseline conditions (195% of increase in 90LF) paralleled the highest ecological change in diatom and macrophyte communities. We observed that this change of around 50% in 90LF represented an ecological tipping point where both communities experienced shifts in species composition and traits. It has been hypothesized that augmentation of low flows will cause an increase in the abundance of species with preferences for slow-flowing, shallow-water habitats, whereas fluvial specialists or obligate rheophilic species will vary their distribution or decline in richness and abundance (Poff et al, 2010). Proliferation of aquatic macrophytes influences a variety of feedback mechanisms in rivers, including reduced sediment resuspension, reduced phytoplankton biomass via competition for nutrients and sinking (Giblin, 2017), as well as changes in trophic structure (Palmer & Ruhi, 2019). In this study, we found that changes in aquatic macrophytes are also associated with changes in diatom communities. In agreement with our findings, other studies reported that long periods of low flow interspersed with occasional floods contained poorer 'quality' aquatic communities than rivers with low to moderate flow variability (Jowett & Biggs, 2006). This suggests that the interrelation of magnitude and duration of low flows is more important than the magnitude of the low flow per se. As a result, long periods of constant flow, which could result from adherence to a minimum flow, produced the highest impact on primary producer communities and should be avoided. To date, the majority of flow experiments tested the treatment effects of high pulse events and magnitude of minimum flows (Olden et al., 2014) whereas experiments involving the duration of minimum and maximum annual extremes (LF, HF), seasonal variability (M1-M12) and timing of events (Jmin), which are relevant for primary producers, were less common.

As regards the maximum flows, we found that the magnitude of high flow events of short duration (1HF) were reduced in altered sites. Large floods are a major cause of natural disturbance to river ecosystems, dictating the relative success of different species and regulating ecosystem process rates (Poff & Zimmerman, 2010). Diatom and macrophyte communities showed a shift when the magnitude of maximum flow decrease was more than 40%. Similarly, the magnitude of monthly flows and the timing of extreme events also triggered changes in diatom and macrophyte community composition, although this did not occur in all the cases analyzed. For example, the Ebro River showed one of the highest deviations from baseline conditions in terms of magnitude of monthly flows (M3-M9), or the timing of extreme events (Jmin), but the ecological changes observed were not stronger than those found at other altered sites. One possible reason for this lack of relation could be the fact that the main effect of the alteration on the communities analyzed was due to the magnitude and duration of minimum flows, while the Ebro River did not show a strong deviation in these HIs (90LF around 20% of change compared to the controls).

The percent of change in HIs also showed differences among the reservoirs analyzed. For instance, those reservoirs used mainly for hydropower generation (e.g., the Nalón and Nansa rivers), presented only minor alterations to flow regimes, and primary producer communities in these sites showed a high similarity with those of control sites. On the contrary, at the Narcea River, primary producer communities were substantially different to control ones, maybe because of the differing management and operational rules among reservoirs. In this regard, the Narcea river has a large hydropower dam able to capture high flows and store them to generate hydropower when needed. This difference, might have caused the higher number of short low and high pulses in the Narcea station compared to Nalón and Nansa (around 400% increase in number of low pulses and 164% increase in the rate of flow changes) generating a rapidly fluctuating hydrological pattern corresponding to alternating periods of power generation. The rapid fluctuations in water levels associated with hydropower operations can cause considerable ecological changes (Richter & Thomas, 2007) and could be the reason why ecological communities in the Narcea showed low similarities with those of control sites.

6. Conclusions

In our study, diatom and macrophyte communities consistently responded to flow alterations, suggesting that both are sensitive indicators of this type of anthropogenic pressure. We showed a shift from high flow tolerant traits such as non-colonial diatom forms and crust-forming algae, which are adapted to the extreme events and natural dynamism of unregulated rivers, towards planktonic diatoms, free-floating algae and a mass development of aquatic plants in hydrologically altered sites. Both diatom and macrophyte communities shared common thresholds of response to altered hydrological attributes, and these could be considered in a flow management context. Moreover, not only the magnitude of minimum flows proved to be important, but also their duration, enhancing macrophyte biomass and nuisance algal growth. We showed that it is necessary to contemplate the interactions between these attributes (LF indices) and other potentially important attributes for primary producer communities. To test for the effect of these hydrological attributes, the design of manipulative and long-term experiments is encouraged. For example, irrigation dams where LF indices are highest would be benefited by controlled winter releases in terms of mimicking, as far as possible, the magnitude, duration, and seasonality of natural ordinary floods. Hydropower dams, on the other hand, may need a regulation of the frequency and duration of pulses (nPLow and nPhigh) as well as in the rate of flow changes (meanPos and meanNeg) in order to slow down or smooth the rapidly fluctuating daily and sub-daily high and low pulse changes. Long term flow experiments targeting specific hydrological attributes, will lead to effective adaptive management and will undoubtedly advance our mechanistic knowledge regarding the effect of hydrological alterations in ecological communities. Our study contributed to fill this gap by providing insight into the relative sensitivity of ecological endpoints of stream primary producers to flow regulation.

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Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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TABLES

Table 1. Hydrological indices derived from daily flow data at the 20 selected sites. Abbreviations: M=months; HF= High flow; LF= Low flow; J=Julian day; nP= Number of pulses; DP= duration of pulses; Pos= Positive; Neg= Negative.

Flow attribute	**Indices**	**Description**
Magnitude of monthly flow	M1-M12	Mean monthly flow
Magnitude and duration of minimum annual extremes	1LF	Magnitude of minimum annual flow of 1 day duration
	3LF	Magnitude of minimum annual flow of 3 day duration
	7LF	Magnitude of minimum annual flow of 7 day duration
	30LF	Magnitude of minimum annual flow of 30 day duration
	90LF	Magnitude of minimum annual flow of 90 day duration
Magnitude and duration of maximum annual extremes	1HF	Magnitude of maximum annual flow of 1 day duration
	3HF	Magnitude of maximum annual flow of 3 day duration
	7HF	Magnitude of maximum annual flow of 7 day duration
	30HF	Magnitude of maximum annual flow of 30 day duration
	90HF	Magnitude of maximum annual flow of 90 day duration
Timing of extreme flow events	Jmin	Julian day of minimum flow
	Jmax	Julian day of maximum flow
Frequency and duration of pulses	nPHigh	Number of high pulses within a year
	MeanDPHigh	Mean duration of high pulses within a year
	nPLow	Number of low pulses within a year
	MeanDPLow	Mean duration of low pulses within a year

Rate of flow changes	meanPos	Mean of all positive differences between days
	meanNeg	Mean of all negative differences between days

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Table 2. Trait classification used for diatoms and macrophytes with corresponding references

		Trait classification	Reference
Diatoms	Cell sizes	From class 1 to 5	Rimet and Bouchez (2012)
	Ecological guilds	Low profile, high profile, motile and planktonic	
	Growth forms	Non-colonial, filamentous, attached adnate, attached pad, attached stalk, pioneer	
Macrophytes	Algae	Coating, globules, crust, shrub, tuft, floating and cushion	Rott and Wehr (2016)
	Bryophytes	Mats, streamers and fans	Vieira et al. (2012)
	Vascular plants	Helophytes and hydrophytes (including floating and submerged leaves)	Fernández-Aláez et al. (1999)

Table 3. Results from the statistical analysis performed based on diatom and macrophyte community similarity between control and altered sites (PERMANOVA) and statistical tests to unveil the relationship between community structure and HIs (Mantel test and BIOENV). All analysis based on Bray Curtis community similarity.

	Permanova		Mantel test (Pearson)		Model parameters (BIOENV)
	F	*P*	r	*P*	
Diatoms	2.2	0.014	0.50	0.001	90LF, Jmin, nPLow and M10
Macrophytes	1.75	0.046	0.47	0.001	90LF

Table 4. Diatom species with corresponding traits that contribute more to community dissimilarity between control and altered sites based on Bray Curtis dissimilarities. Av. Control and Av. Altered: Average abundances per group in percentages. Contribution: Average contribution to overall dissimilarity in percentage and respective cumulative values (Cumulative).

	Taxa	Guild	Growth form	Size class	Av. Control	Av. Altered	Contribution %	Cumulative %
Affinity towards altered sites	*Achnanthidium minutissimum*	Low	Non-col	1	14.06	30.43	16.57	16.57
	Nitzschia dissipata	Motile	Non-col	4	2.10	3.13	2.86	19.43
	Cocconeis placentula var. lineata	Low	Non-col	5	0.75	2.45	2.10	21.53
	Cocconeis euglypta	Low	Non-col	5	2.25	3.14	2.09	23.62
	Achnanthidium rivulare	Low	Non-col	4	0.00	2.58	2.00	25.63
	Encyonema minutum	High	Colonial	2	1.17	2.29	1.82	27.45
	Rhoicosphenia abbreviata	Low	Colonial	3	0.88	1.91	1.80	29.25
	Navicula tripunctata	Motile	Non-col	4	1.22	2.06	1.77	31.02
	Encyonema silesiacum	High	Colonial	4	1.36	1.88	1.58	32.60
	Diatoma vulgaris	High	Colonial	5	0.33	1.16	0.94	33.53
Affinity towards control sites	*Achnanthidium pyrenaicum*	Low	Non-col	2	34.49	15.90	21.52	55.05
	Amphora pediculus	Low	Non-col	1	9.63	3.36	7.89	62.94
	Gomphonema pumilum	High	Non-col	2	4.68	3.98	5.14	68.08
	Nitzschia fonticola	Motile	Non-col	3	2.42	0.41	4.51	72.59
	Gomphonema minutum	High	Non-col	3	3.19	1.78	2.92	75.52
	Navicula cryptotenelloides	Motile	Non-col	2	2.32	0.89	1.83	77.34
	Achnanthidium atomoides	Low	Non-col	1	1.80	0.72	1.39	78.73
	Navicula wygaschii	Motile	Non-col	3	1.17	0.08	0.92	79.65
	Navicula cryptotenella	Motile	Non-col	3	1.05	0.82	0.91	80.56

Table 5. Macrophyte taxa and traits that contribute more to community dissimilarity between control and altered sites based on Bray Curtis dissimilarities Av. Control and Av. Altered: Average abundances per group in percentages. Contribution: Average contribution to overall dissimilarity in percentage and respective cumulative values (Cumulative).

	Taxa	Organism type	Trait	Av. Control	Av. Impact	Contribution %	Cumulative %
Affinity towards altered sites	*Ranunculus*	Plants	Hydrophytes	0.76	8.95	9.94	9.94
	Diatom mats	Algae	Mats	2.75	5.91	9.87	19.80
	Rhinchostegium riparioides	Bryophytes	Mats	3.89	4.27	6.85	26.66
	Audouinella	Algae	Tuft	0.00	2.83	5.09	31.75
	Oedogonium	Algae	Floating filamentous	0.00	2.83	3.84	35.59
	Dydimosphenia germinata	Algae	Coating	1.25	1.84	3.76	39.35
	Myriophyllum	Plants	Hydrophytes	0.00	4.64	3.26	42.61
	Chiloscyphus polyanthos	Bryophytes	Mats	0.01	1.36	2.51	45.11
	Microspora	Algae	Coating	0.00	1.82	2.39	47.51
	Fontinalis antipyretica	Bryophytes	Streamer	0.64	1.11	2.37	49.88
Affinity towards control sites	*Cladophora*	Algae	Shrub	3.26	2.91	6.19	56.07
	Lemanea	Algae	Shrub	3.25	1.91	5.46	61.53
	Phormidium	Algae	Coating	2.63	2.02	4.82	66.35
	Hildebrandia	Algae	Crust	2.76	0.74	4.29	70.64
	Leptodyctium riparium	Bryophytes	Mats	1.50	0.92	3.21	73.85
	Green algae crust	Algae	Crust	2.04	0.00	2.91	76.76
	Brachythecium	Bryophytes	Mats	1.28	0.55	2.68	79.45
	Heribaudiella	Algae	Crust	1.25	0.00	2.29	81.74

FIGURE CAPTIONS

Figure 1. Geographical context of the study area with sampling locations of control and altered sites within the three basins: Cantabric, Duero and Ebro.

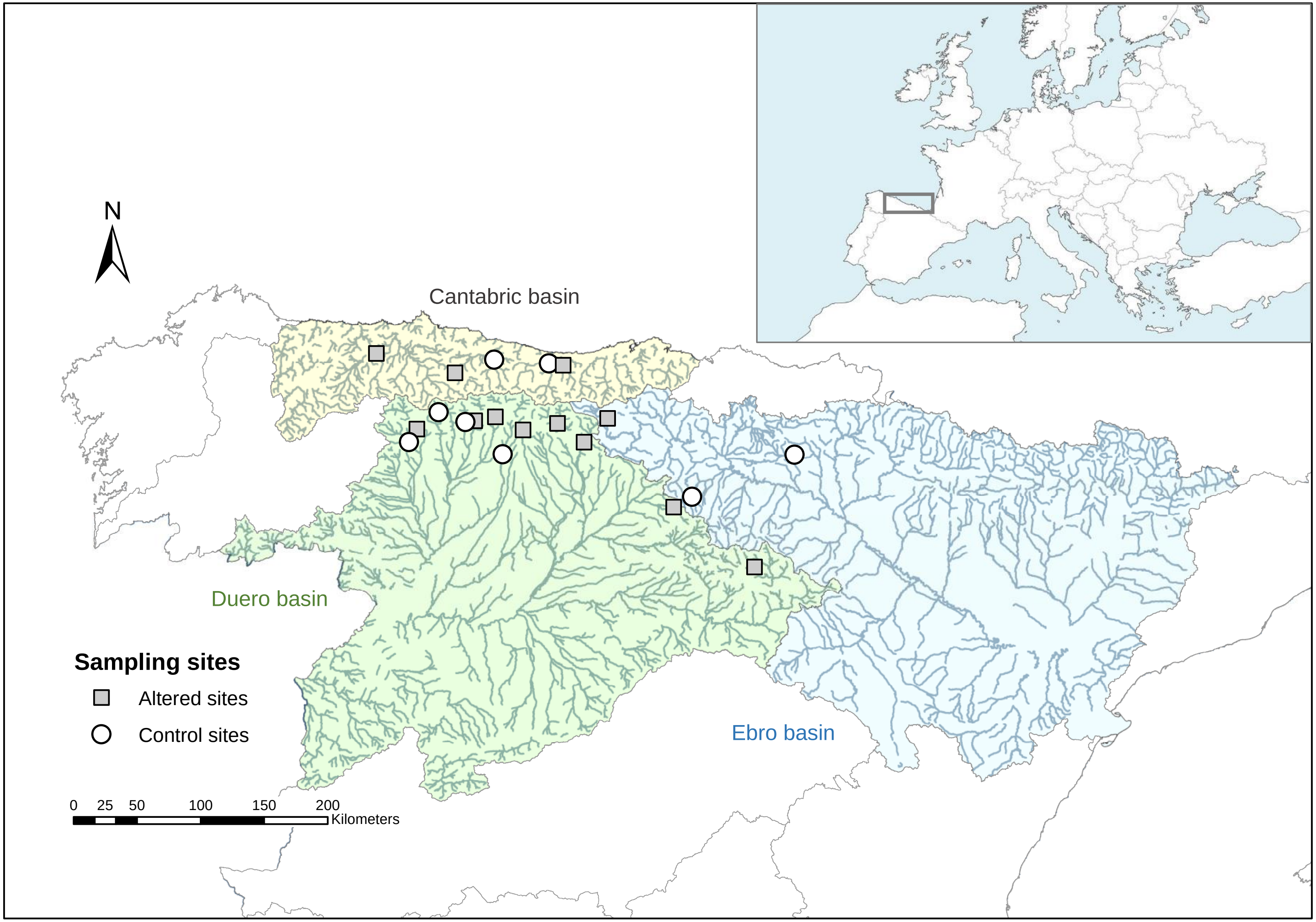
Figure 2. Mean value of hydrological indices (HIs) measured in 8 control (white circles) and 12 altered (grey squares) sites. Codes for hydrological indices are provided in Table 1. Significant statistical differences (t-test $P < 0.05$) of the HIs between natural and altered sites are denoted by asterisks and showed in Table S5. Hydrological indices were segregated according to the hydrological meaningful attribute that they represent: Magnitude of monthly flow; Magnitude and duration of minimum annual extremes; Magnitude and duration of maximum annual extremes; Frequency and duration of pulses; Timing of extreme flow events and Frequency of flow changes.

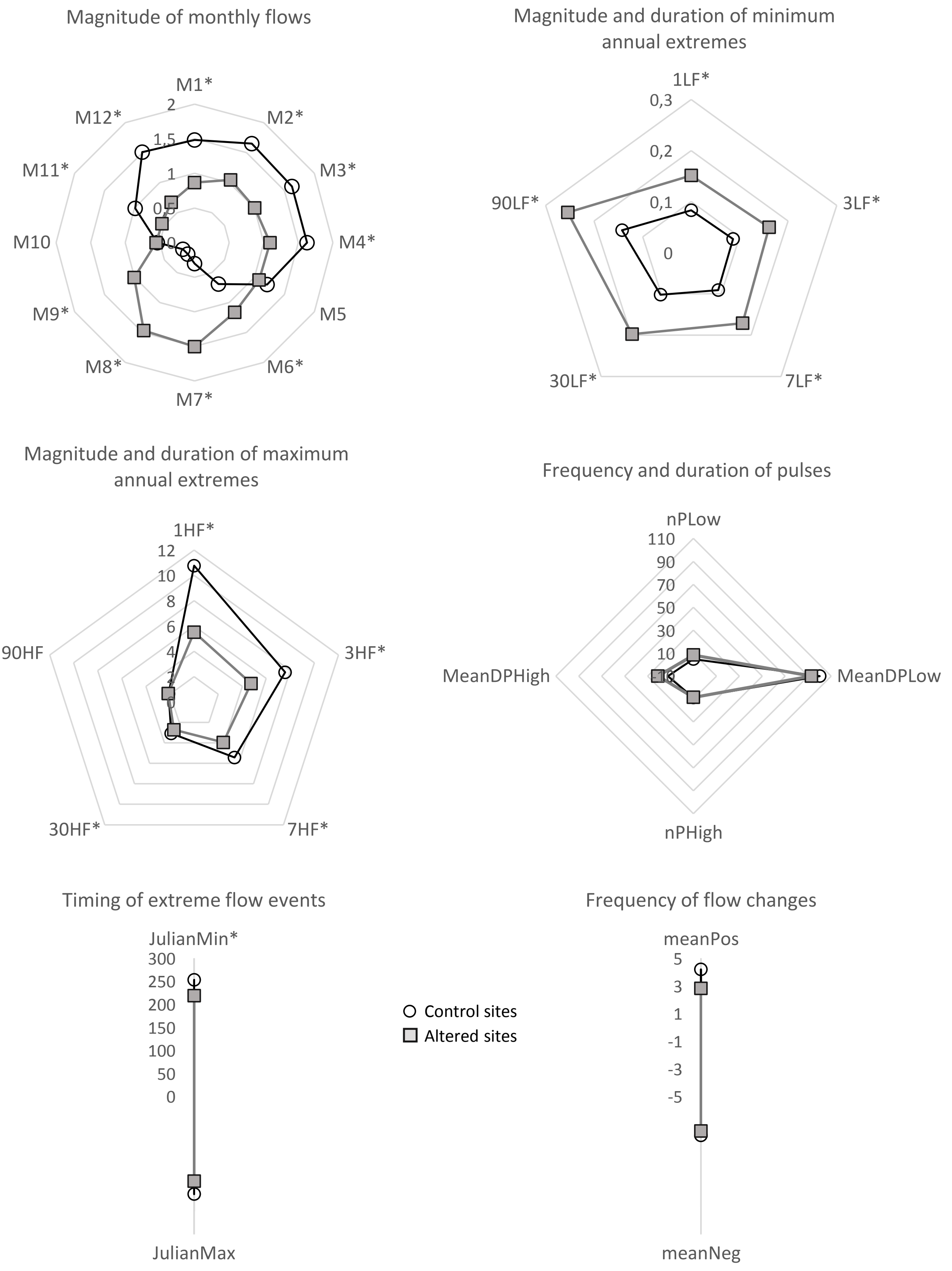
Figure 3. Principal component analysis (PCA) of the 30 HIs used in the study with corresponding percentage of explained variation for axis 1 and 2 (A); and axis 1 and 3 (B).

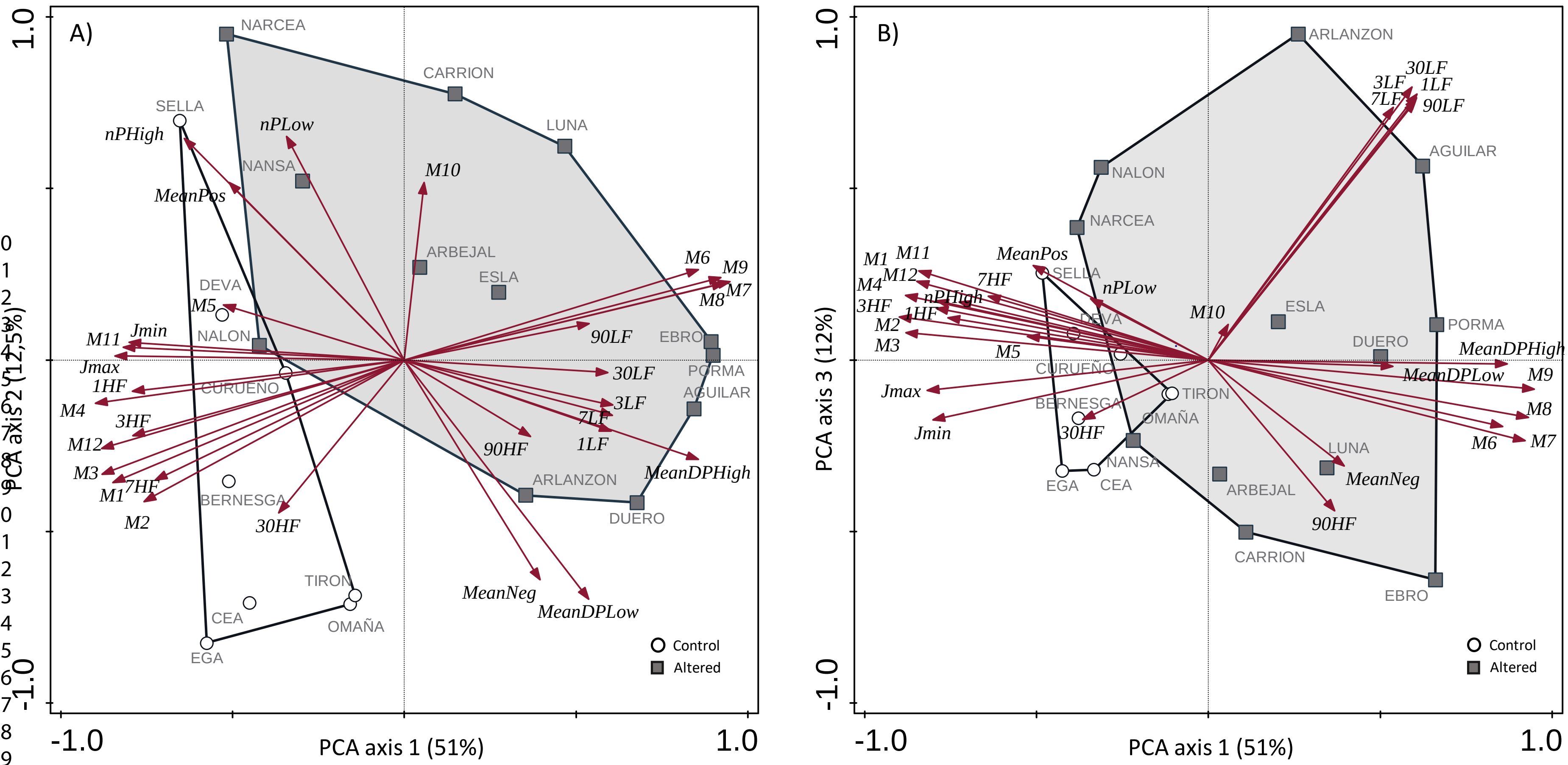
Figure 4. Nonmetric multidimensional scaling (NMDS) based on Bray Curtis dissimilarity distances for diatoms (A) and macrophytes (B). Only significant hydrological indices (selected by BIOENV) and traits that differed between control and altered sites are shown as supplementary variables.

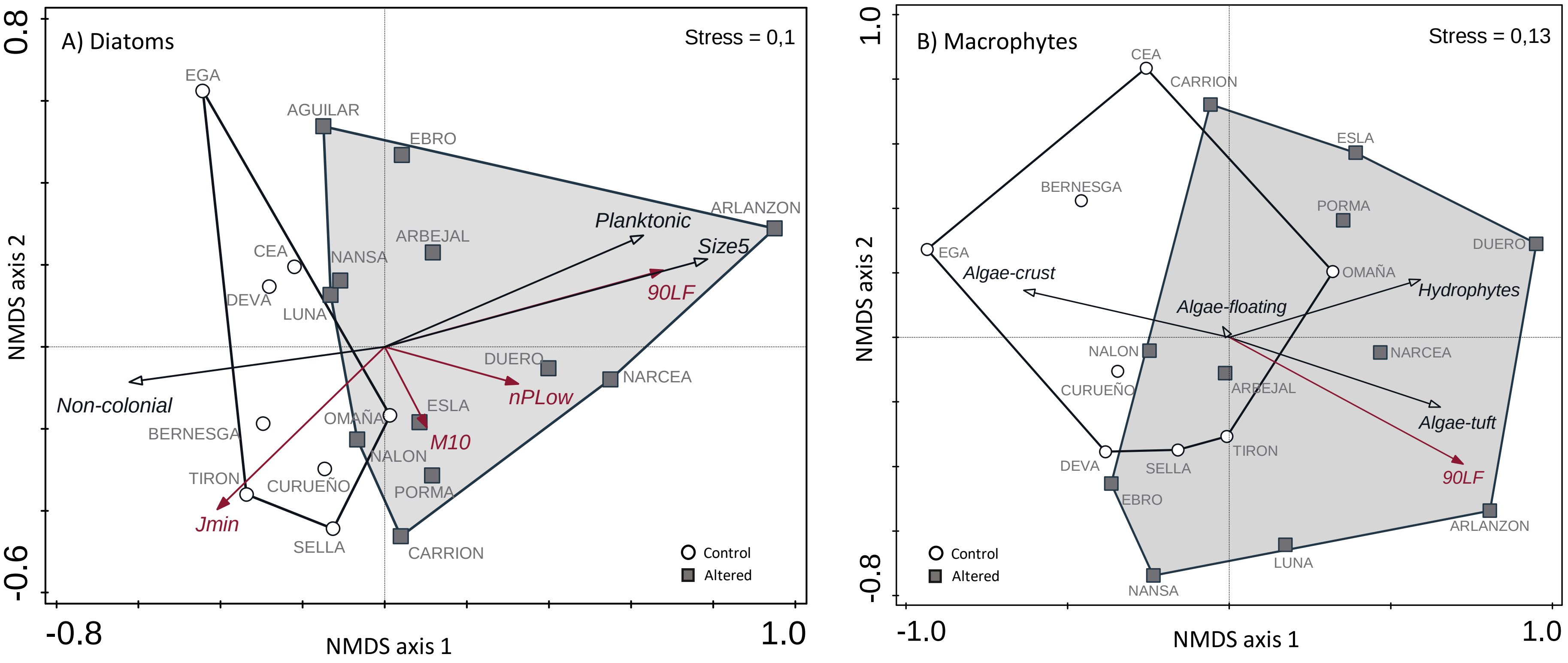
Figure 5. Diatom ecological response with respect to the six major hydrological attributes analyzed. Numbers indicate the percentages of change in the HIs in relation to the controls.

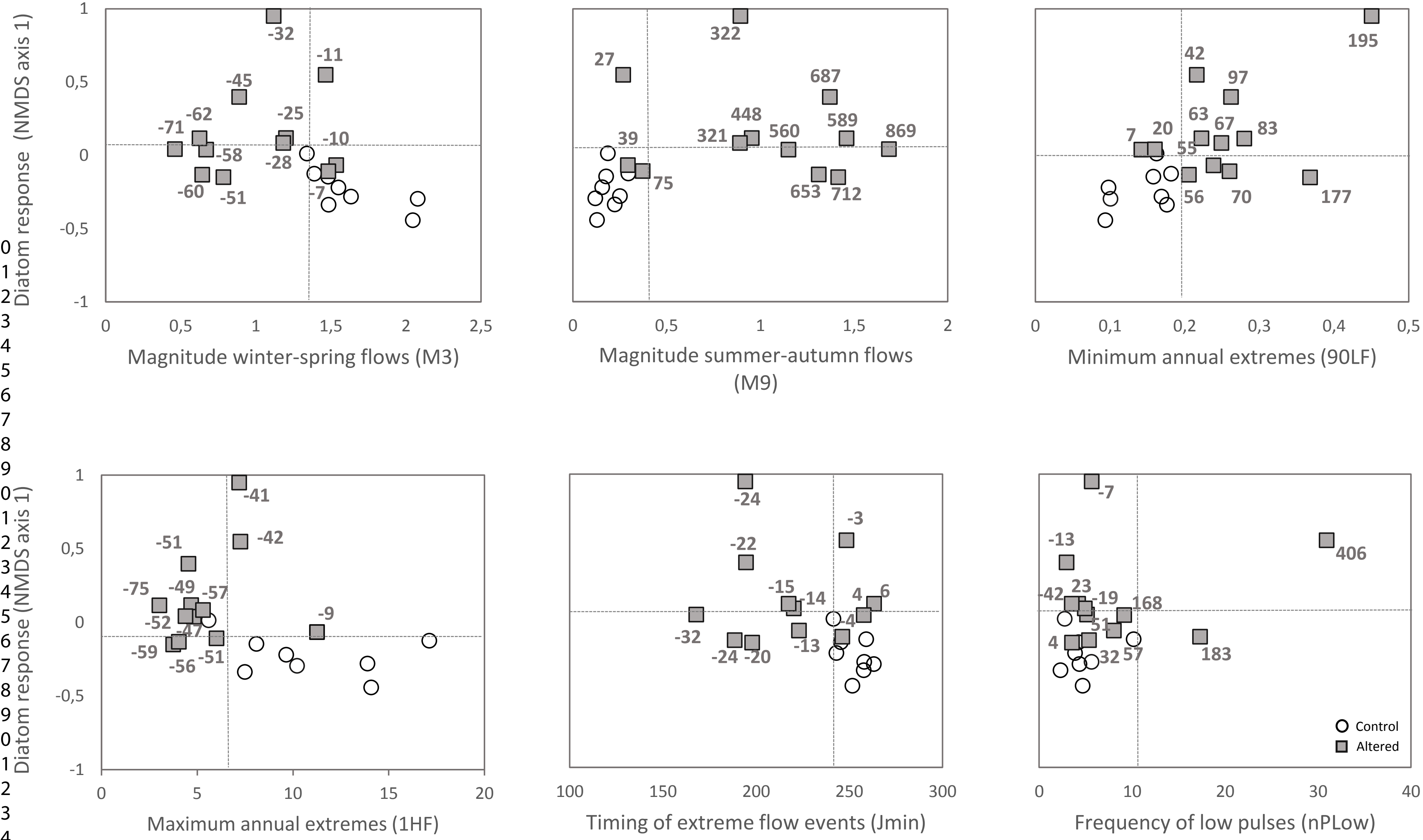
Figure 6. Macrophyte ecological response with respect to the six major hydrological attributes analyzed. Numbers indicate the percentage of change in the HIs in relation to the controls.

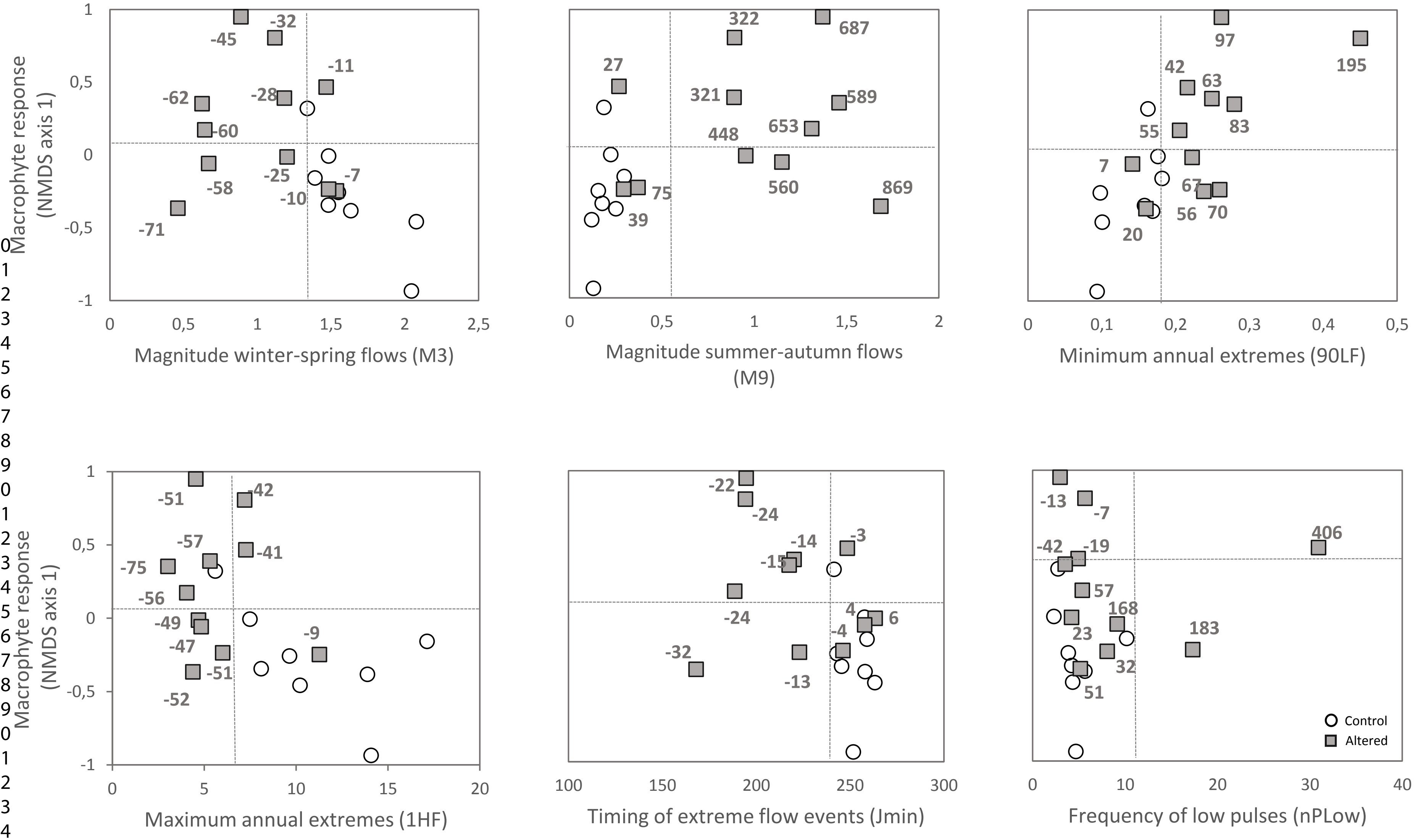

N
Cantabric basin
Duero basin
Ebro basin
Sampling sites
Altered sites
Control sites
0 25 50 100 150 200
Kilometers


Magnitude of monthly flows
M1*
M2*
M3*
M4*
M5
M6*
M7*
M8*
M9*
M10
M11*
M12*
2
1,5
1
0,5
0
Magnitude and duration of minimum annual extremes
1LF*
3LF*
7LF*
30LF*
90LF*
0,3
0,2
0,1
0
Magnitude and duration of maximum annual extremes
1HF*
3HF*
7HF*
30HF*
90HF
12
10
8
6
4
2
0
Frequency and duration of pulses
nPLow
MeanDPLow
nPHigh
MeanDPHigh
110
90
70
50
30
10
-10
Timing of extreme flow events
JulianMin*
JulianMax
300
250
200
150
100
50
0
Control sites
Altered sites
Frequency of flow changes
meanPos
meanNeg
5
3
1
-1
-3
-5


A)
NARCEA
CARRION
LUNA
SELLA
nPHigh
nPLow
NANSA
M10
MeanPos
ARBEJAL
ESLA
M6
M9
M7
M8
DEVA
M5
Jmin
M11
NALON
90LF
EBRO
Jmax
30LF
PORMA
1HF
CURUEÑO
3LF
AGUILAR
M4
3HF
7LF
M12
1LF
90HF
M3
7HF
MeanDPHigh
M1
BERNESGA
ARLANZON
M2
30HF
DUERO
TIRON
MeanNeg
CEA
MeanDPLow
OMAÑA
EGA
Control
Altered
PCA axis 2 (12,5%)
1.0
-1.0
PCA axis 1 (51%)
-1.0
1.0
B)
ARLANZON
30LF
3LF
1LF
7LF
90LF
AGUILAR
NALON
NARCEA
M1
M11
MeanPos
M12
SELLA
7HF
M4
nPLow
nPHigh
3HF
1HF
DEVA
M10
ESLA
M2
PORMA
M3
M5
DUERO
MeanDPHigh
CURUEÑO
MeanDPLow
M9
Jmax
TIRON
BERNESGA
M8
OMAÑA
30HF
Jmin
M6
M7
LUNA
NANSA
EGA
CEA
MeanNeg
ARBEJAL
90HF
CARRION
EBRO
Control
Altered
PCA axis 3 (12%)
1.0
-1.0
PCA axis 1 (51%)
-1.0
1.0


A) Diatoms
Stress = 0,1
NMDS axis 2
0.8
-0.6
NMDS axis 1
-0.8
1.0
EGA
AGUILAR
EBRO
ARLANZON
ARBEJAL
NANSA
CEA
DEVA
LUNA
Planktonic
Size5
90LF
DUERO
NARCEA
Non-colonial
nPLow
ESLA
OMAÑA
M10
BERNESGA
NALON
TIRON
CURUEÑO
PORMA
Jmin
SELLA
CARRION
Control
Altered
B) Macrophytes
Stress = 0,13
NMDS axis 2
1.0
-0.8
NMDS axis 1
-1.0
1.0
CEA
CARRION
ESLA
BERNESGA
PORMA
DUERO
EGA
OMAÑA
Algae-crust
Hydrophytes
Algae-floating
NALON
NARCEA
CURUEÑO
ARBEJAL
Algae-tuft
TIRON
DEVA
SELLA
90LF
EBRO
ARLANZON
LUNA
NANSA
Control
Altered


Diatom response (NMDS axis 1)
1
0,5
0
-0,5
-1
-32
-11
-45
-62
-71
-25
-10
-58
-28
-60
-51
-7
0
0,5
1
1,5
2
2,5
Magnitude winter-spring flows (M3)
322
27
687
448
589
560
869
321
39
75
653
712
0
0,5
1
1,5
2
Magnitude summer-autumn flows (M9)
195
42
97
63
67
83
7
20
55
56
70
177
0
0,1
0,2
0,3
0,4
0,5
Minimum annual extremes (90LF)
Diatom response (NMDS axis 1)
1
0,5
0
-0,5
-1
-41
-42
-51
-75
-49
-57
-52
-47
-9
-59
-56
-51
0
5
10
15
20
Maximum annual extremes (1HF)
-24
-3
-22
-15
-14
4
6
-32
-4
-13
-24
-20
100
150
200
250
300
Timing of extreme flow events (Jmin)
-7
-13
406
-42
23
-19
168
51
4
32
57
183
0
10
20
30
40
Frequency of low pulses (nPLow)
○ Control
▨ Altered


Macrophyte response (NMDS axis 1)
1
0,5
0
-0,5
-1
-45
-32
-11
-62
-28
-60
-58
-25
-7
-10
-71
0
0,5
1
1,5
2
2,5
Magnitude winter-spring flows (M3)
322
687
27
321
589
448
653
75
39
560
869
0
0,5
1
1,5
2
Magnitude summer-autumn flows (M9)
97
195
42
63
55
83
7
67
56
70
20
0
0,1
0,2
0,3
0,4
0,5
Minimum annual extremes (90LF)
Macrophyte response (NMDS axis 1)
1
0,5
0
-0,5
-1
-51
-42
-57
-41
-75
-56
-49
-9
-47
-51
-52
0
5
10
15
20
Maximum annual extremes (1HF)
-22
-24
-14
-3
-15
-24
4
6
-4
-32
-13
100
150
200
250
300
Timing of extreme flow events (Jmin)
-13
-7
406
-42
-19
57
168
183
23
32
51
○ Control
▨ Altered
0
10
20
30
40
Frequency of low pulses (nPLow)

Supporting information

Table S1. Control and hydrological altered sites used in the analysis showing the hydrological class (H class), basin, name of Dam, type of dam (IR: Irrigation; HP: Hydropower; WS: Water supply), reservoir drainage area and storage index (storage volume/average annual runoff) of the 20 sites.

Site	H class	Type	Basin	Dam	Reservoir purpose	Area (Km^2)	Storage index
AGUILAR	NC10	Altered	Duero	Aguilar	IR/HP	544	0,82
ARBEJAL	NC10	Altered	Duero	Requejada	IR/HP	220	0,46
ARLANZÓN	NC13	Altered	Duero	Uzquiza/Arlanzón	IR/WS	151	0,63
CARRIÓN	NC10	Altered	Duero	Camporredondo/Compuerto	IR/HP	315	0,72
DUERO	NC10	Altered	Duero	Cuerda del Pozo	IR/HP/WS	544	0,74
EBRO	NC10	Altered	Ebro	Ebro	IR/WS	450	3,71
ESLA	NC13	Altered	Duero	Riaño	IR/HP	611	0,86
LUNA	NC10	Altered	Duero	Barrios de Luna	IR/HP/WS	500	0,84
NALÓN	NC13	Altered	Cantabric	Tanes/Rioseco	HP/WS	328	0,13
NANSA	NC13	Altered	Cantabric	La Cohilla	HP	360	0,06
NARCEA	NC13	Altered	Cantabric	La Barca	HP	1211	0,03
PORMA	NC13	Altered	Duero	Juan Benet -Porma	HP/WS	249	1,64
BERNESGA	NC13	Control	Duero	-	-	-	-
CEA	NC10	Control	Duero	-	-	-	-
CURUEÑO	NC13	Control	Duero	-	-	-	-
DEVA	NC13	Control	Cantabric	-	-	-	-
EGA	NC10	Control	Ebro	-	-	-	-
OMAÑA	NC10	Control	Duero	-	-	-	-
SELLA	NC13	Control	Cantabric	-	-	-	-
TIRÓN	NC10	Control	Ebro	-	-	-	-

Table S2. Environmental and water quality variables measured in the 20 sites in 2017 sampling campaign. T^o: Temperature. NO_2, NH_4 and PO_4 contained several values below detection limit.

Site	**Type**	**Canopy cover**	**Tº**	**pH**	**Conductivity**	**NO_2**	**NO_3**	**NH_4**	**PO_4**
		%	**ºC**		**(µS/m)**	**(µg/L)**	**(µg/L)**	**(µg/L)**	**(µg/L)**
AGUILAR	Altered	3,1	16,0	7,93	502	9,80	268	287	40,5
ARBEJAL	Altered	54,3	16,0	8,18	719	<0,5	142	< 14	< 5
ARLANZÓN	Altered	66,2	13,9	7,55	44,3	<0,5	77,6	< 14	< 5
CARRIÓN	Altered	59,6	10,8	8,22	520	<0,5	297	< 14	< 5
DUERO	Altered	7,5	15,3	7,70	83,6	3,10	63,9	< 14	5,6
EBRO	Altered	13,1	17,9	8,18	543	0,80	68,8	34,1	10,5
ESLA	Altered	9,5	12,1	8,45	113,8	<0,5	225	< 14	< 5
LUNA	Altered	37,2	11,6	8,05	252	<0,5	51,6	< 14	< 5
NALÓN	Altered	20,1	16,8	7,92	210	2,20	278	< 14	< 5
NANSA	Altered	35,6	16,1	7,85	215	<0,5	394	< 14	< 5
NARCEA	Altered	29,1	18,5	8,02	156	<0,5	432	< 14	< 5
PORMA	Altered	12,1	11,8	8,36	139,7	<0,5	363	< 14	< 5
BERNESGA	Control	68,0	12,6	8,34	243	<0,5	38,8	< 14	< 5
CEA	Control	67,0	12,7	8,20	240	<0,5	5	< 14	< 5
CURUEÑO	Control	65,5	12,2	8,43	210	<0,5	82,6	< 14	< 5
DEVA	Control	43,0	16,0	7,96	323	1,30	562	< 14	< 5
EGA	Control	70,8	15,5	8,21	931	3,70	1895	< 14	12,3
OMAÑA	Control	41,1	12,3	7,95	95,6	<0,5	104	< 14	9
SELLA	Control	18,8	16,4	7,97	204	<0,5	363	< 14	< 5
TIRON	Control	57,2	13,3	7,86	726	<0,5	895	< 14	13,9

Table S3. Results from PERMANOVA statistical analysis based on Jaccard distance (presence-absence data) on diatoms and macrophyte communities between control and altered sites.

	Permanova	
	F	P
Diatoms	1.42	0.049
Macrophytes	1.77	0.017

Aguilar
Altered

Arbejal
Altered

Arlanzón
Altered

Carrión
Altered

Duero
Altered

Ebro
Altered

Esla
Altered

Luna
Altered

Nalón
Altered

Nansa
Altered

Narcea
Altered

Porma
Altered

Bernesga
Control

Cea
Control

Curueño
Control

Deva
Control

Ega
Control

Omaña
Control

Sella
Control

Tirón
Control

Figure S1. Hemispheric images taken with fish eye lens for the estimation of canopy cover in the 20 sites.

Table S4. Diatom and macrophyte traits, their categories, description and their expected responses to hydrological disturbances used in this study.

	Traits	Categories	Description and expected responses to hydrological disturbance
Diatoms	Cell size	Size1 (0-100 µm^3)	Smaller cells have higher nutrient uptake rates and growth rates that allow greater resilience to disturbance making them advantage under nutrient-limiting and high disturbance conditions; Larger cells show converse trend (Wu et al., 2019).
		Size2 (100-300 µm^3)	
		Size3 (300-600 µm^3)	
		Size4 (600-1500 µm^3)	
		Size5 (≥ 1500 µm^3)	
	Guilds	High profile	Colonial, filamentous species of tall stature. Adapted to low flow velocities and disturbances.
		Low Profile	Species of short stature attached to the substrate. Adapted to high flow velocities and disturbances.
		Motile	Fast moving species. Adapted to relative high flow velocities and disturbances.
		Planktonic	Solitary or filamentous adapted to lentic environments and resist sedimentation.
	Life forms	Filamentous	Colonial high profile or planktonic taxa that do not resist disturbance.
		Attached mucilage pad	Cells produce mucilage on a pole that stick to substrate or through apical pore fields. Adapted to low -moderate flow velocities and disturbances.
		Attached mucilage stalk	
		Attached adnate	Cells are firmly attached by their valve face or by their girdle view and are more likely to retain under high disturbance condition.
		non-colonial	Single cells, may be adapted to moderate-high flow disturbances.
		Pioneer	Small adnate taxa, most of them belonging to the *Achnanthidium minutissimum* species complex. Resistance to high disturbance.
Macrophytes	Algal life forms	Crust	Flat thallus compacted tiers of cells avoiders of flow-related breaking stress.
		Shrub	Networks extended into flow consisting of single filaments with enhanced anchoring strength with enhance flow resistance.
		Globules	Lobate, mucilagineous globules or slightly calcified adapted to high flow disturbances.
		Tuft	Tuft-like stands (<1 cm) with high extension resistance.
		Coating	Soft gelatinous covers forming a flexible ground layer resistant to low-moderate flow disturbance.
		Floating filamentous	Unbranched filaments of cylindrical cells sometimes attached or free floating. Common in slow-flowing and nutrient-rich conditions.
		Cushion	Loosely attached velvet cushions with low flow resistance.
	Bryophyte life forms	Mats	Branched or unbranched shoots creeping over the substratum and often closely attached to it by rhizoids. Permanently submerged found in torrential water impact zones in deep shaded microhabitats.
		Streamers	Long persisting floating shoots attached in their base to the substrate by rhizoids. Mostly on slower currents of streambed in full sunlight.
		Fans	Shoots with leaves arranged in two lateral ranks and branched in the horizontal plane forming flattened photosynthetic surfaces. Usually in the splashed zone.
	Vascular	Helophytes	Emergent vascular plants. Adapted to low-moderate flow disturbances.

	plants life forms	Hydrophytes	Submerged or floating leaves vascular plants. Adapted to low flow velocities and disturbances.

Table S5. Test for statistical significant differences in the hydrological indices used between control and altered sites (t-test).

Hydrological indices	t	df	*P*
M1	4,05	16,66	<0,001
M2	2,89	15,43	0,011
M3	4,20	17,61	<0,001
M4	3,83	14,86	0,002
M5	1,27	17,97	n.s.
M6	-4,22	17,65	<0,001
M7	-4,90	11,45	<0,001
M8	-5,04	11,17	<0,001
M9	-5,78	11,52	<0,001
M10	-0,24	9,21	n.s.
M11	3,81	17,24	0,001
M12	5,78	12,76	<0,0001
1LF	-2,35	12,78	0,036
3LF	-2,59	12,66	0,023
7LF	-2,84	12,69	0,014
30LF	-3,41	13,19	0,0046
90LF	-4,01	16,31	<0,001
1HF	3,44	10,03	0,006
3HF	3,68	13,36	0,003
7HF	3,77	15,93	0,0017
30HF	2,39	16,31	0,029
90HF	-0,21	17,8	n.s.
Jmin	3,68	13,27	0,0026
Jmax	1,90	13,78	n.s.
nPHigh	0,049	17,97	n.s.
MeanDPHigh	-2,04	13,63	n.s.
nPLow	-1,46	13,78	n.s.
MeanDPLow	0,34	14,32	n.s.
meanPos	0,78	10,78	n.s.
meanNeg	0,27	14,83	n.s.

Table S6. Test for statistical significant differences in the environmental and water quality variables used between control and altered sites (t-test).

Environmental variables	t	df	*P*
Canopy cover	2,78	16,92	0,013
Temperature	-0,88	17,95	n.s
pH	0,76	17,33	n.s
Conductivity*	0,83	16,67	n.s
NO_2	-0,48	17,18	n.s
NO_3*	-0,06	8,58	n.s
NH_4*	-1,35	11	n.s
PO_4*	0,41	16,05	n.s