



Multiple stressor effects on biological quality elements in the Ebro River: Present diagnosis and predicted responses



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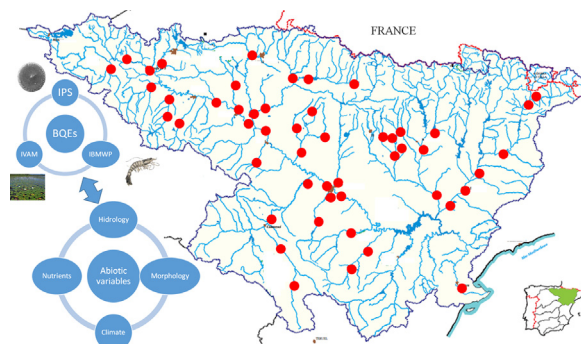
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HIGHLIGHTS

- Biological responses to multiple stressors were modeled in the Ebro River catchment.
- Stressors were modeled for different future socioeconomic and climatic scenarios.
- Increased agriculture, urbanization and nutrients were linked to poor ecological status.
- The scenarios predicted a future deterioration in the ecological status of water bodies in the catchment.

GRAPHICAL ABSTRACT



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ABSTRACT

Multiple abiotic stressors affect the ecological status of water bodies. The status of waterbodies in the Ebro catchment (NE Spain) is evaluated using the biological quality elements (BQEs) of diatoms, invertebrates and macrophytes. The multi-stressor influence on the three BQEs was evaluated using the monitoring dataset available from the catchment water authority. Nutrient concentrations, especially total phosphorus (TP), affected most of the analyzed BQEs, while changes in mean discharge, water temperature, or river morphology did not show significant influences. Linear statistical models were used to evaluate the change of water bodies' ecological status under different combinations of future socioeconomic and climate scenarios. Changes in land use, rainfall, water temperature, mean discharge, TP and nitrate concentrations were modeled according to the future scenarios. These revealed an evolution of the abiotic stressors that could lead to a general decrease in the ecosystem quality of water bodies within the Ebro catchment. This deterioration was especially evidenced on the diatoms

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and invertebrate biological indices, mainly because of the foreseen increase in TP concentrations. Water bodies located in the headwaters were seen as the most sensitive to future changes.

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1. Introduction

Global change affects ecosystems at planetary scale (Briner et al., 2013), and may be particularly important in some world regions. The Mediterranean basin is one of the regions where climate and land use alterations, together with intense economic and social changes, will produce large effects on the ecological quality of water bodies (Cooper et al., 2013; Skoulikidis et al., 2017). Climate patterns and future projections in the Mediterranean region indicate that both mean temperature and monthly distribution of precipitation will change, leading to more severe extreme events consisting on either high rainfall or droughts (Ceballos-Barbancho et al., 2008; González-Hidalgo et al., 2010; López-Moreno et al., 2010). These climate changes, together with snowpack reduction, land use change and effects of dam regulation, have already caused a general decrease in river discharges and a displacement in seasonality (López-Moreno et al., 2011). In particular, the abandonment of agricultural lands is associated with the increase of forested area, with the subsequent increase in evapotranspiration and decrease in runoff (Gallart et al., 2011; Buendia et al., 2016). Riverine water regimes may be further altered because of irrigation schemes that may revert the seasonal hydrological patterns (Piqué et al., 2016). Changes in discharge patterns may be associated with changes in concentrations of nutrients and contaminants (Han et al., 2009; Jeppesen et al., 2011), particularly in urban areas acting as point pollution sources (Brown et al., 2005). Altogether, present and foreseen changes may lead to a decrease in water discharge, alteration of hydrological patterns and progressive deterioration of water quality (Lehner et al., 2006; Meybeck, 2004). These changes are reflected in the structure of biological communities inhabiting the river system (Sabater et al., 2016; Tonkin et al., 2017; Cooper et al., 2013).

The Water Framework Directive (WFD; EC, 2000) establishes that the “good” ecological status of natural water bodies has to be based on the chemical, hydromorphological and biological features, compared with reference conditions (Feio et al., 2014). The biological variables, or Biological Quality Elements (BQEs), use the composition and abundance of taxa to establish class boundaries in the different water bodies. For the diatoms, the IPS index (Indice de Polluosensibilité Spécifique, Cemagref, 1982) characterizes the status of a water body based on diatom community characteristics (Almeida et al., 2014) and the sensitivity value and abundance of the present taxa. The IBMWP¹ is a biotic index which indicates the sensitivity of the aquatic invertebrate community to organic pollution (Alba Tercedor et al., 2002). Finally, the IVAM index is a quality indicator based on macrophytes composition and cover (Moreno et al., 2006).

The objective of this study is to define the impact of different stressors associated with global change on the biological communities in the Ebro catchment (NE Spain). The biological status was described using the three BQEs (diatoms, invertebrates and macrophytes) mentioned above. Stressors are defined anthropogenic disturbances (either abiotic or biotic) which cause potential injurious changes to organisms and communities (Segner et al., 2014; Crain et al., 2008), even with the potential to drive evolutionary processes over geological times when severe conditions persist (Parsons, 2005). The BQEs response was analyzed following a conceptual model that relates the ecological status of water bodies with the most relevant stressors occurring in the River Ebro (i.e. hydromorphological alterations, discharge reduction, loss of riparian cover, nutrient enrichment). Finally, the obtained models were used to define potential changes in the ecological status of the river under future scenarios of land use and climate change.

2. Study area

The Ebro catchment is located in northeast Spain and covers an area of 85,550 km². The Ebro is the longest Mediterranean river in the Iberian Peninsula (total length of 928 km), and it flows from the Cantabrian Range down into the Mediterranean Sea (Fig. 1). The catchment is mainly delimited by the Pyrenees in the north and the Iberian Range in the south. The Ebro shows a high inter-annual and intra-annual water flow variability associated with its intrinsic Mediterranean character. The mean discharge at its mouth (Tortosa) for the period 1912–2012 was 436 m³ s⁻¹, but it ranged from <50 m³ s⁻¹ during the very dry periods to >12,000 m³ s⁻¹ for the highest flood ever recorded (October 1907; Novoa, 1984).

Runoff in the Ebro is regulated by a total of 187 reservoirs, impounding 2/3 of the mean annual runoff. The largest reservoirs are in the lower part of the Ebro and constitute the system Mequinenza-Ribarroja-Flix, with a total storage capacity of around 1.7 km³. Flow regulation has decreased the magnitude of frequent natural floods downstream of the dams (Batalla et al., 2004), and sediment transfer has reduced up to 90% (Vericat and Batalla, 2006; Tena and Batalla, 2013) due to the trapping efficiency of the reservoirs complex. These alterations affect the Ebro delta evolution and have caused ecological consequences to the lowest river segment (Prats et al., 2011; Sabater et al., 2008).

The Ebro basin has a Mediterranean climate with continental characteristics, with semi-arid areas in the center and Atlantic areas in the western part. Average annual precipitation is 622 mm (period 1920–2000), with high monthly and annual variability and highest rainfalls in spring and autumn. The rainfall is irregularly distributed within the catchment, ranging from 900 mm y⁻¹ at the headwaters to 500 mm y⁻¹ in the Mediterranean zone, and with extreme values from 100 mm y⁻¹ to 3000 mm y⁻¹. Average water temperatures range from 13 °C in the headwaters to 17 °C in the lower reach.

A broad spectrum of landscapes can be found within the catchment, including boreal-alpine coniferous forests, mixed deciduous forests, Mediterranean evergreen and mixed forest and shrubs, and semi-arid treeless formations. Historically, the predominant land use was agriculture (vineyards, orchards and corn), but the progressive abandonment of rural life has allowed the recovery of woodland and forest (Gallart et al., 2002). Around half of the population lives in cities mostly located in the central part of the catchment. The Pyrenees and the Iberian plateau (40% of the catchment) have low population densities, with values lower than 5 inhabitants/km². Industrial activities are important around the main cities.

The Ebro River has been deeply altered, particularly in its middle (Ollero, 2007), and lower section (Batalla et al., 2004; Vericat and Batalla, 2006). Riparian vegetation has been largely replaced by agricultural development in the fertile floodplain areas, producing diffuse inputs of nutrients and pollutants (Romani et al., 2010). Mean annual flow records have decreased nearly 40% in the last 50 years, both a result from rainfall decrease, irrigation increase and transformation from agricultural land to forest (Gallart and Llorens, 2004; Buendia et al., 2016). Finally, cities scattered in the basin produce large local inputs of nutrients and pollutants (Sabater et al., 2009). Overall, hydrological alterations and contaminants challenge the ecological status of water bodies in the Ebro and their joint effect could be emphasized according to the loss of dilution capacity (Han et al., 2009), potentially enhanced in future scenarios of climate and land use change.

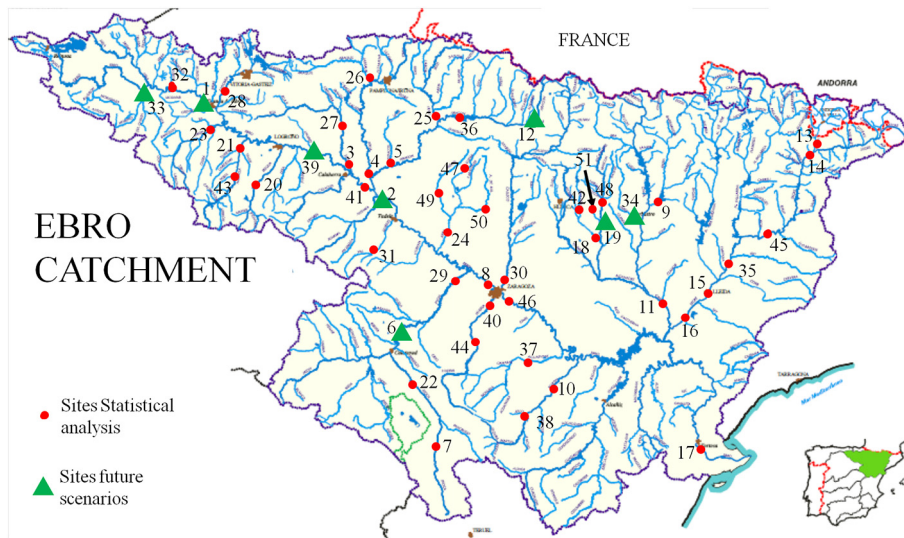


Fig. 1. Area of study. The Ebro basin. Dots represent the sites used for the statistical analysis. A selection of eight sites (green triangles) was used for the assessment of future scenarios. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3. Materials and methods

3.1. Conceptual model

The conceptual model identifies the main drivers, pressures and stressors influencing the ecological status of water bodies in the Ebro (Fig. 2). In this conceptual model, drivers are anthropogenic activities (e.g. agriculture, industry) or climate change phenomena (climate warming, changes in precipitation) that may have an environmental effect; pressures are direct effects of the drivers, and produce environmental modifications (Feld et al., 2016); and stressors are measurable variables resulting from an anthropogenic pressure that adversely affect biological or ecological integrity (Segner et al., 2014). The main drivers, pressures and stressors used in the model were selected based on the existing information (Sabater et al., 2009; Barceló and Petrovic, 2011). Five drivers were considered, i.e. agriculture, climate change, industry, energy and hydropower, and flood protection. Agriculture is a source of diffuse nutrient contamination and modifies the spatial and temporal distribution of river discharge. Climate change alters rainfall patterns and leads to biogeochemical alterations related to the water cycle in the basin. Industrial activities are the origin of point sources of

contaminants and modify the physical and ecological status of the river as a side effect of taking benefit of river waters. Finally, hydroelectric and flood protection infrastructures generate physical and hydrological alterations that affect river discharge and produce hydromorphologic changes.

In our conceptual model, the stressors resulting from these pressures are total phosphorus (TP), nitrate concentration and changes in mean water discharge, water temperature and land use. Water temperature and discharge are considered in the performed statistical analysis presented below. The percentage of agricultural surface and the number of inhabitants were also included as descriptors of land use. Moreover, altitude and hydro-morphological status were considered as a surrogate of natural variability. The hydro-morphological state was measured based on the IHF index (Index of Fluvial Habitat), which includes information concerning sediment characteristics, frequency of riffle areas, shade areas, elements of heterogeneity or aquatic vegetation coverage (Pardo et al., 2002).

Data on abiotic variables and biotic indicators were obtained from the database available at the web site of the Ebro water authority (Confederación Hidrográfica del Ebro, CHE; www.chebro.es). This dataset includes observations related to physical, chemical and

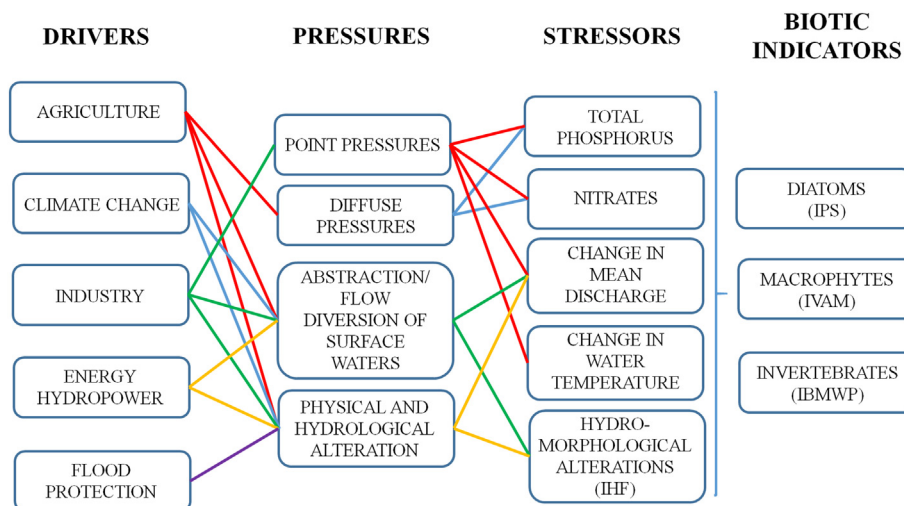


Fig. 2. Conceptual model proposed for the Ebro basin relationship between abiotic stressors and ecological indicators. Different colors are used to improve visualization of the lines linking the elements in the model. (For interpretation of the references to color in this figure legend, the reader is referred to the online version of this chapter.)

ecological parameters in a series of sites within the catchment. The quality of the data was good to fair, depending on the site and the specific variable, and based on the principle of maximal data quality the sites and periods were selected when abiotic stressors and biotic indicators were jointly available (Fig. 1 and Table S1). Each BQE observation in a specific site and given year was paired to the average value of abiotic variables in the 3 previous months (1 to 3 measurements). The BQE data were typically obtained during summer and therefore the values of the abiotic stressors corresponded to mid and late spring conditions. This resulted in three tables (supplementary material, Tables S2–S4), one for each of the three biotic indicators considered and the corresponding values of the abiotic variables (Figure 2).

3.2. Statistical analysis

After data compilation, the response of each BQE was modeled as a function of the abiotic variables. This analysis was carried out using the protocol developed by Feld et al. (2016), which consisted in an initial exploratory analysis to rank all the stressors, environmental descriptors and their interactions in relation with their capacity to predict the focal BQE, followed by the estimation of the predictor's standardized effect sizes (SES) and significance through linear models, where the BQE were the response variables. All the analyses were conducted using the R statistical software (R Development Team, 2017).

Prior to linear model analyses, TP and nitrate concentrations, the number of inhabitants, and mean discharge were log-transformed, and agricultural surface was logit-transformed to reduce skewness, after assessing visually all variable distributions. Then, stressors and environmental descriptors were standardized to a mean equal to zero and standard deviation equal to one and checked for collinearity using Pearson correlation coefficients (r_p). All the stressors were kept as they were not highly correlated ($r_p < 0.65$), except for the IBMWP model where the number of inhabitants was removed to avoid collinearity.

First, a Random Forest analysis was used (*randomForestSRC* R package, Ishwaran et al., 2014) to rank stressors influence and identify potential interactions on IPS, IBMWP and IVAM. The number of trees was set to 3000 and the number of variables used in each split was set to three (i.e., one third of the number of predictors). After Random Forest results and expert knowledge, two candidate interactions were identified: TP \times nitrates, and altitude \times mean discharge. Second, Linear Mixed Models were used (LMM, *lme4* R package, Pinheiro et al., 2017) to create global models to model the response of IPS, IBMWP and IVAM to single and combined stressors. These models included TP, nitrates, agricultural surface, number of inhabitants (excluded in the IBMWP model), IHF, water temperature, mean discharge and altitude as fixed factors. Each model included different pairwise interactions as fixed factors, which were selected based on the Random Forest results. The IPS model included the pairwise interactions agricultural surface \times altitude and number of inhabitants \times altitude, the IVAM model only included the interaction nitrates \times agricultural surface, and the IBMWP model included the interactions nitrate \times agricultural surface and nitrate \times altitude. Site was considered as a random factor to account for repeated measures (Zuur et al., 2007). Single and combined abiotic variables' SES and their significance were then quantified through multi-model averaging (*MuMIn* R package, Bartoń, 2016). This statistical technique ranks all the models generated using all the possible combination of predictors. Then, a set of top models is selected to produce an average model only if the model ranking first is not unambiguously supported (model weight < 0.90). Thus, the models containing all potential combinations of single and combined abiotic variables were ranked using Akaike's Information Criterion (AIC, Akaike, 1973). Those models differing in no more than four AIC units were chosen from the model ranked first (minimum AIC). A natural average method was adopted to conduct the model averaging, which consisted in averaging predictors only over models in which the predictor appears and weighting predictor's SES by the summed weights of these models (Burnham

and Anderson, 2002; p. 152). Candidate models were validated by visually checking their residuals for normality and homoscedasticity (Zuur et al., 2007). For each LMM model, two measures of goodness-of-fit were estimated (Nakagawa and Schielzeth, 2013): marginal goodness-of-fit (r^2_m) indicates the variance explained only by the fixed factors, while conditional goodness-of-fit (r^2_c) shows the variance accounted for by both fixed and random terms. The mean average (based on model weights) of each goodness-of-fit measure for each averaged model is provided. Code and functions run the statistical analysis are available in the supplementary material (Appendix A).

The empirical models obtained for IPS, IBMWP and IVAM were used to analyze future scenarios (Fig. S1, supplementary material). For this purpose it was necessary to evaluate future values of the different variables. Some sites were selected for this analysis aiming to cover different geographical areas (headwaters, lowlands and main Ebro river) and different ecological quality status (good, intermediate, bad). Future scenarios refer to horizon 2050. The definition of future scenarios is detailed in the following section.

4. Definition of future scenarios

4.1. Integrated scenarios

The Integrated scenarios take into account climate, land use and water management changes and are based on various Shared Socio-economic Pathways (SSPs, IPCC, 2017, van Vuuren et al., 2011). Two integrated scenarios were considered to account for the different evolutions of the social and environmental conditions. Each scenario was defined in terms of economic growth, energy consumption, environmental directives, social policies and water management. The two scenarios were denominated as MYOPIC and SUSTAINABLE, and have been developed during the GLOBAQUA project (Navarro-Ortega et al., 2015). The development of these scenarios is explained in detail in the supplementary material (Annex S1).

Land use for the year 2050 was modeled for both scenarios using the iCLUE model, a new version of the CLUE model family (Conversion of Land Use and its Effects) originally developed by Veldkamp and Fresco (1996) and recently reprogrammed by Verweij et al., 2012. The CORINE Land Cover data served as input to the model and was reclassified to thirteen land use classes. The considered categories were: non-irrigated arable land, permanently irrigated land, vineyards, fruit trees and olives, grasslands and pastures, complex cultivation patterns, agriculture with natural vegetation, broad-leaved forest, coniferous and mixed forest, sealed area, transitional vegetation, open spaces with little vegetation and water. The land use maps obtained from this process were used as input for the discharge modeling and the nutrient modeling.

4.2. Climate projections

Climate projections for precipitation and mean air temperature were needed as input for the modeling of nutrients and discharge. These projections were obtained from different Regional Climate Models (RCMs) that dynamically downscale various General Circulation Models (GCMs) at different Representative Concentration Pathways (RCPs 4.5 and 8.5) provided through the EURO-CORDEX initiative (Kotlarski et al., 2014). Due to computational restraints, a subset of GCM-RCM combinations had to be selected for further modeling. A cluster approach was applied (Wilcke and Bärring, 2016), resulting in three main clusters of GCM-RCM combinations. The clustering was based on climate change signals, defined as the difference between the future (2050 horizon, 2036–2065) and reference (1981–2010) period of various variables and several temporal scales (annual and seasonal). The simulations were ranked, based on the proximity to the center of each cluster. In this way, a score sheet was established to identify the highest scoring RCMs. The selection led to three combinations of RCM-GCM that kept most of the spread of the original ensemble over the selected river

basins (Gampe et al., 2016); these RCM-GCM combinations were, therefore, considered for the subsequent impact modeling. The following GCM-RCM combinations have been selected for the impact modeling activities: HadGEM2-ES-RCA4 (hereafter referred to as RCA4), EC-EARTH-RACMO22E (RACMO22E), EC-EARTH-CCLM4-8-17 (CCLM4).

As climate model simulations are prone to biases at the regional scale (Dosio, 2016), a bias adjustment is needed before using the simulated series. A distribution-based scaling approach (Yang et al., 2010) was applied on the selected simulations. The regional reanalysis dataset MESAN (Häggmark et al., 2000) was chosen as reference grid for the adjustment of precipitation and mean air temperature. The resulting bias-adjusted simulations show better agreement with the observations and considerable biases were removed to a large degree.

The resolution of the selected RCMs of 0.11° (~12 km) was not sufficient to cover the processes of hydrological models in heterogeneous terrain. Therefore, the downscaling algorithm SCALMET (Marke, 2008) was applied to further disaggregate the RCM simulations to a 1 km grid. SCALMET uses the lapse-rate approach, which is mass and energy conserving and respects the climatology and main distribution of the original simulations. Topography-dependent patterns obvious in the interpolated observation grids are better represented in the downscaled grids compared to the original bias-adjusted grids. The resulting bias-adjusted and downscaled datasets were used for the modeling of future projections for the variables involved in the conceptual model (Fig. 2): mean discharge, TP and nitrates. Furthermore, projections in air temperature were used as a proxy of changes in water temperature.

4.3. Discharge modeling

The discharge modeling for the application of future scenarios was performed by using the mesoscale Hydrologic Model (mHM), a grid-based distributed hydrological model that simulates canopy interception, snow accumulation and melting, soil moisture dynamics, infiltration, deep percolation, surface runoff, evapotranspiration, storage in the subsurface and groundwater, discharge generation, fast and slow interflow and baseflow (Samaniego et al., 2010; Kumar et al., 2013a). The spatial distribution of the model parameters is obtained from catchment characteristics such as soil types, geological classes and land cover types using a multi-scale parameter regionalization technique (Samaniego et al., 2010). Further details on mHM and the source code can be obtained from www.ufz.de/mhm. The model has been successfully applied in many river basins across the globe (Samaniego et al., 2010; Kumar et al., 2013a, 2013b; Rakovec et al., 2016a, 2016b; Zink et al., 2017; Huang et al., 2017).

The mHM was run at daily time scale with meteorological data from the E-OBS dataset (Haylock et al., 2008) between 1995 and 2014 and calibrated the model against daily discharge at the most downstream site of the eight stations for future scenarios (Fig. 1, Table S1) to obtain the model parameters for present conditions. The model was calibrated with the Dynamically Dimensioned Search (Tolson and Shoemaker, 2007). To capture high, average and low flows, a combination of the Nash-Sutcliffe efficiency (NSE) between observed and modeled discharges and their logarithm was used as objective function. Daily discharge data were obtained from the CHE (www.chebro.es). The model exhibited reasonable skill in capturing the observed dynamics of daily streamflows with NSEs of between 0.5 and 0.66 for the majority of the sub-basins in this study. After calibration of the model parameters, mHM was used to simulate future scenarios of river discharge based on the climate scenarios described in Subsection 4.4.

4.4. Nutrient modeling

Nutrients (TP and nitrates) in the Ebro basin were estimated as annual loads (t y^{-1}) under the different scenarios in selected reaches within the basin. The pan-European model Green (Grizzetti et al., 2012) was used for this purpose. The basin was divided in 18,568

subcatchments of 4.6 km² size on average. Subbasin reaches were linked in a node-link system that builds the reach network through the basin until its outlet. Nutrient sources comprised fertilization of agricultural land, background deposition, point source loads emitted by urban waste water treatment plants and industries, and emissions from scattered households. The model considers a subbasin retention, which is inversely proportional to annual rainfall in the subbasin and is applied to the diffuse sources in the subbasin, and a reach retention, which is proportional to the reach length and is applied to all load entering the reach. The two retention factors are controlled by two calibration parameters. The dataset used as model input was updated at 2012, and the retention parameters were calibrated against the European Environmental Agency water quality database for the year 2012 at European scale. No local calibration was performed.

The model was run for all the combinations of socioeconomic (MYOPIC and SUSTAINABLE) and climate projections (CCLM4, RACMO22E and RCA4). Baseline conditions were estimated as mean nutrient loads for 1981–2010 using MYOPIC CCLM4 rainfall data, current irrigation amount map, MYOPIC 1995 land use map and 2012 modeled fertilization levels. Fertilization changes were based on expert opinions elicited during workshops for the MYOPIC and SUSTAINABLE scenarios: a decrease of 10% in organic fertilization was applied in any narrative, an increase of 10% of mineral fertilization in future MYOPIC scenarios and a decrease of 15% of mineral fertilization in SUSTAINABLE scenarios. The nutrient loads for future scenarios were assessed as the mean annual load at each reach for the period 2035–2065 (horizon 2050), using rainfall projections and assuming land use and irrigation levels for 2050.

5. Results

Random forest models identified agricultural surface (IVAM and IBMWP), the number of inhabitants (IPS and IVAM) and altitude (IPS and IBMWP) as good predictors of the BQE values. For IPS, the potential

Table 1

Results of the models relating IPS, IVAM and IBMWP to single and combined abiotic variables. Standardized Effect Size (SES) and significance are shown. Significant variables are highlighted in bold. r^2_m : variance explained just by the fixed factors; r^2_c : variance accounted for by both fixed and random terms.

BQE	Abiotic variables	SES	P-value	r^2_m	r^2_c
IPS	Intercept	15.14	0.000	57.0	75.3
	TP	-0.93	0.001		
	Nitrates	0.18	0.538		
	Agriculture	-1.09	0.004		
	Inhabitants	-1.34	0.001		
	IHF	-0.47	0.055		
	Temp	0.18	0.524		
	Qmean	0.33	0.340		
	Altitude	0.33	0.474		
	Agriculture x altitude	-0.44	0.211		
IVAM	Inhabitants x altitude	-0.24	0.437	50.0	57.3
	Intercept	4.50	0.000		
	TP	-0.13	0.265		
	Nitrates	-0.17	0.154		
	Agriculture	-0.46	0.001		
	Inhabitants	-0.52	0.002		
	IHF	0.14	0.168		
	Temp	0.00	0.970		
	Qmean	0.17	0.224		
	Altitude	-0.13	0.407		
IBMWP	Nitrates x agriculture	-0.18	0.151	28.9	50.4
	Intercept	126.66	0.000		
	TP	-9.66	0.038		
	Nitrates	2.82	0.560		
	Agriculture	-0.08	0.991		
	IHF	5.37	0.234		
	Temp	3.00	0.552		
	Qmean	1.65	0.743		
	Altitude	17.67	0.002		
	Nitrates x altitude	5.97	0.226		

interactions identified by random forest were agricultural surface x altitude and number of inhabitants x altitude. For IVAM, nitrates x agricultural surface was identified as potential interaction, while for IBMWP the most important interactions were nitrates x agricultural surface and nitrates x altitude.

Multi-model averaging results highlighted that increasing TP concentration, agricultural surface and number of inhabitants were generally linked to low BQE values (poor ecological status). Specific model results were that increased TP reduced IPS and IBMWP values, while high agricultural surface and number of inhabitants were linked to low IPS and IVAM values (Table 1). Additionally, altitude was positively correlated with IBMWP values. Overall, no interactive effects were found between abiotic variables, but additive effects were dominant for IPS and IVAM. Fixed factors explained 57.0% of

the IPS variance, 50.0% of the IVAM variance and 28.9% of the IBMWP variance. Fig. 3 shows the relationship between the BQEs and the selected abiotic variables to visualize main response patterns of the BQE.

The measured values of IPS, IVAM and IBMWP are displayed in Fig. 4 and plotted against the corresponding values obtained from the models. The points that are modeled less satisfactorily are labeled indicating the site number (Fig. 1, Table S1) and the year (in parentheses). BQEs for sites 14, 23, 29, 34 and 38 were overestimated by several of the models, while in the sites 2, 32 and 51 were underestimated by empirical models. Those overestimated sites are mostly located in the mountain tributaries (14, 38) or in middle height areas (23, 29, 34). The underestimated sites were located at the mainstem of River Ebro (2, 32) in the middle and upper section.

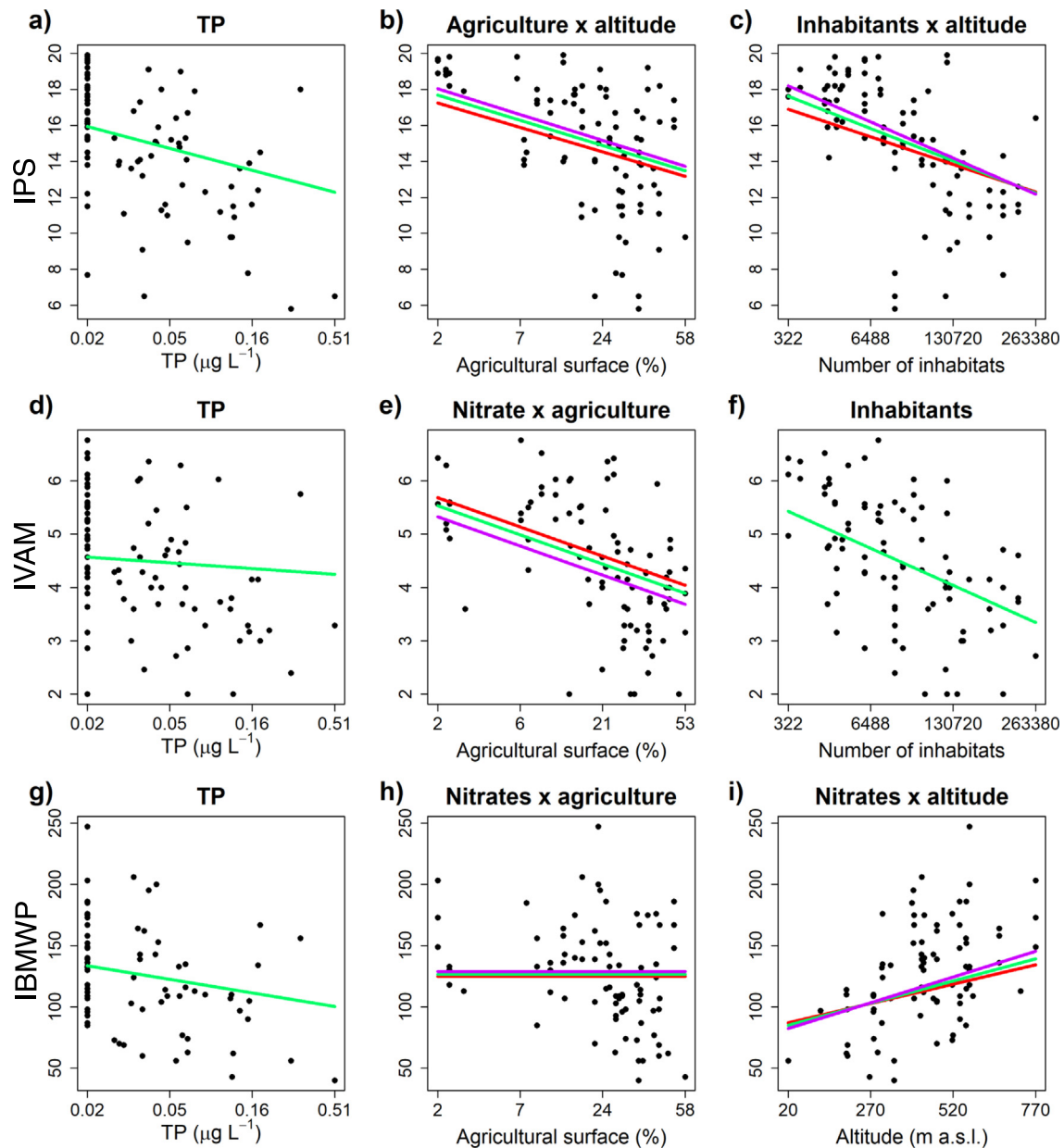


Fig. 3. Response of IPS (a, b, c), IVAM (d, e, f) and IBMWP (g, h, i) to single and combined abiotic variables. Fitted lines are shown for BQE metrics in response to TP (a, e, g), agricultural surface for different altitude levels (b), number of inhabitants for different altitude levels (c), agricultural surface for different levels of nitrates (e, h), number of inhabitants (f) and nitrates for different altitude levels (i). Different colors represent different levels for the variable not shown in the abscise axis (i.e.: b and c: altitude; e, h and i: nitrates): red represents the minimum value within the data set, green represents the mean value within the data set; purple represents the maximum value within the data set. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

5.1. BQEs in future scenarios

The model results for the three climate projections (CCLM4; RACMO22E and RCA4) and the two development scenarios (MYOPIC and SUSTAINABLE) that were tested are shown in Table S5 (supplementary material). RACMO22E climate projection predicts higher discharges and the RCA4 climate projection predicts higher air temperatures. The predicted trends for TP and nitrate concentrations in these scenarios are less consistent. The socioeconomic scenarios predicted slightly higher air temperatures in the MYOPIC than in the SUSTAINABLE, with a large spatial variability in discharge.

When the RCA4 climate projection was aggregated into socioeconomic scenarios, the projected values showed a reduced mean discharge and a higher TP concentration for the MYOPIC and SUSTAINABLE scenarios compared to the baseline (Fig. 5a and b). Nitrates were slightly lower for both future scenarios (Fig. 5c), while water temperature displayed similar values for the scenarios and the baseline (Fig. 5d).

The predicted estimates for the IPS (Fig. 6a), IVAM (Fig. 6b) and IBMWP (Fig. 6c) metrics were of lower values in both the MYOPIC and SUSTAINABLE scenarios relative to the baseline. However, the IVAM predicted values were slightly lower only in the case of the MYOPIC model. In general, the predicted BQE values for the SUSTAINABLE scenario were only slightly higher than those found for the MYOPIC scenario.

6. Discussion

Our analysis indicated that the biological communities in the Ebro basin, represented by diatom, macrophyte and invertebrate biological indices (BQEs), were affected by high nutrient concentrations (mostly phosphorus), agricultural intensification and the increasing number of inhabitants. These pressures showed detrimental additive effects on our biological indices, which suggest unlikely multistressor interactive effects. Our analysis stressed that agriculture and number of inhabitants accounted for higher fraction of the variance than nutrients (higher size effects). This fact may be a consequence of agriculture and population being linked to other impacts (e.g. pesticides and other pollutants, riparian removal, etc.) which could equally affect the BQEs.

Agricultural and urban intensification are key determinants of the ecological status across the Ebro river basin (Sabater et al., 2009) and other geographical areas (Carpenter et al., 1998; Monteagudo et al., 2012; Gutiérrez-Cánovas et al., 2015). Urban settlements occurring along the river network result in considerable phosphorous inputs (Torrecilla et al., 2005), besides other pollutants (e.g. pharmaceuticals, microplastics, Rosal et al., 2010), not considered here but potentially reflected by the number of inhabitants. On the other hand, agricultural intensification is linked to high nitrate (Monteagudo et al., 2012) and pesticide concentrations in running waters (Stehle and Schulz, 2015), especially during summer, when the lower discharge and the most intense irrigation period take place. Agricultural nonpoint sources account for 64% of nitrate loads generated in the central area of the Ebro river basin, while urban and industrial point sources are responsible for 88% of phosphate and 71% of dissolved organic carbon loads (Torrecilla et al., 2005). Rivers exhibiting low flow periods, such as the Ebro or other arid-climate rivers, are particularly sensitive to nutrient and pollutant inputs given their low capacity to dilute chemical stressors and the agricultural intensification in those areas (<http://drylandsystems.cgiar.org/content/worlds-dry-areas>). Particularly, the co-occurrence of higher temperatures, lower flow and higher nutrients can result in primary producer proliferation and eutrophication (Torrecilla et al., 2005).

The analysis allowed to capture a wide range of ecological responses to these anthropogenic impacts, considering the spectrum of life histories and stressor sensitivity showed by diatoms, macrophytes and invertebrates (Bonada et al., 2006; Moreno et al., 2006; Sabater et al., 2008). Despite such variability the BQEs based on these organisms responded

roughly similar to the studied stressors. Both diatoms and macrophytes are highly sensitive to nutrient variations because their growth is typically limited by nitrogen and phosphorus availability (Hecky and Kilham, 1988; Elser et al., 2007; Tornés et al., 2007). Thus, their direct

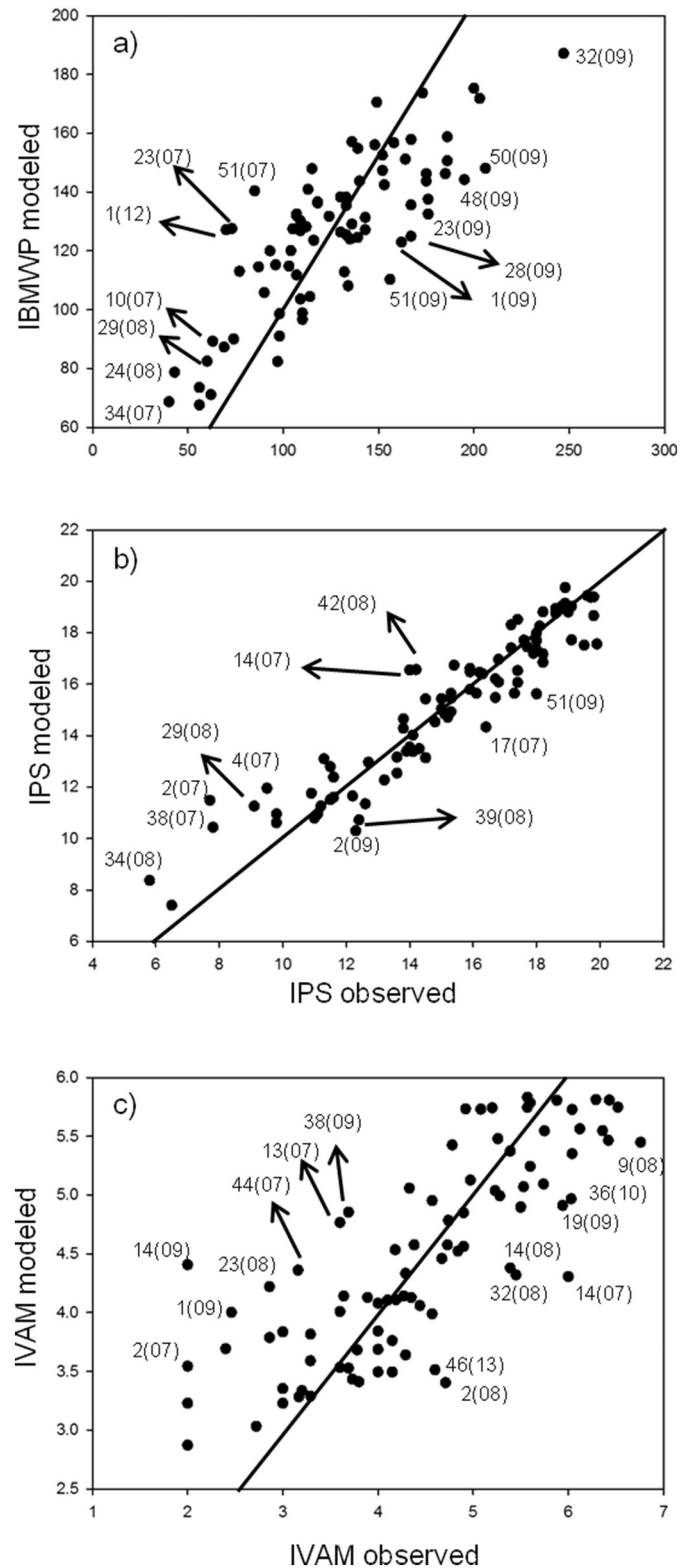


Fig. 4. Comparison between the measured values of the ecological indicators and the values calculated with the models obtained from the statistical analysis: IPS (a), IVAM (b) and IBMWP (c). The labels indicate the site number (Fig. 1) and the year (in parentheses) of those points with the lowest model fit.

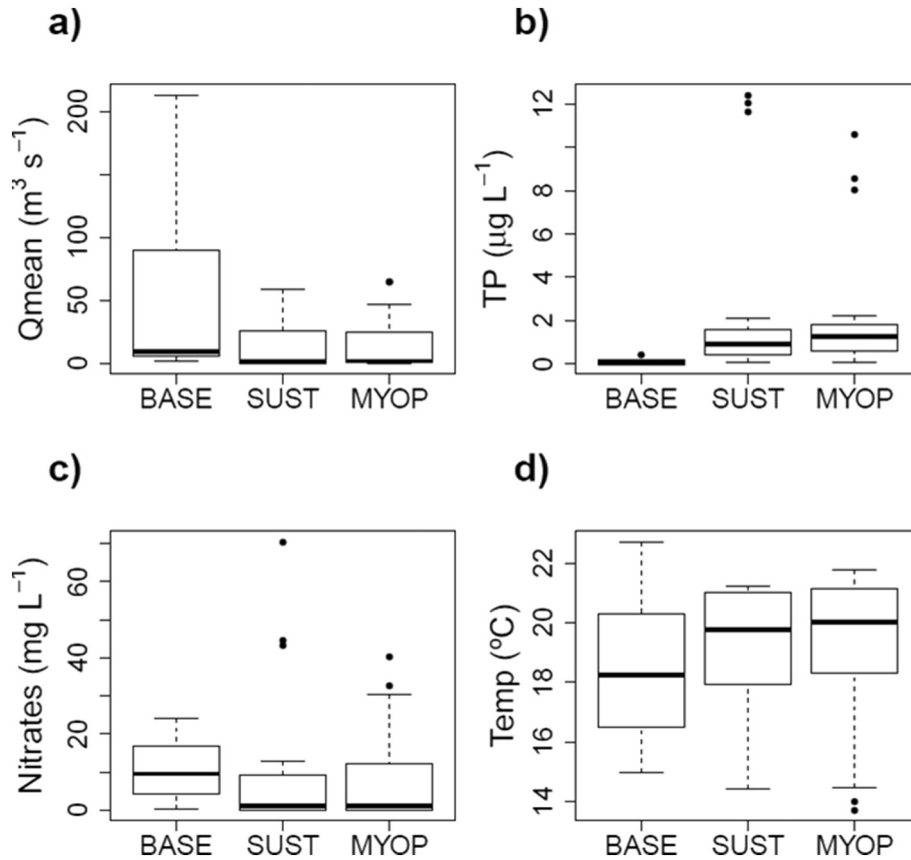


Fig. 5. Predicted values for discharge (a, Qmean), Total Phosphorus (b, TP), Nitrates (c) and air temperature (d). BASE: baseline (current conditions). SUST: sustainable scenario MYOP: myopic scenario. For each of the SUSTAINABLE and MYOPIC scenarios, we included the values predicted for CLM4, RACMO22E and RCA4 climatic models.

relationship with nutrients may explain their strong responses to higher phosphorous, agricultural and urban intensification, and their higher explained variance compared to the invertebrate model. Increased nutrient availability favor fast growing diatoms and macrophytes, where such adapted organisms tend to dominate the community and reduce diversity (Kelly, 2012; Moreno et al., 2006; Licursi et al., 2016). A greater primary producer biomass is generally linked to nutrient enrichment, as observed for the Ebro River, where benthic chlorophyll in the main channel ranged between 7 and 700 mg m⁻² during the low flow period (Sabater et al., 2008). On the other hand, primary producers can control nutrient concentration when light and temperature are sufficiently high and water regulation avoids high discharge peaks (Sabater et al., 2008; Artigas et al., 2012). Thus, when such conditions prevail in the middle and lower Ebro river sections and tributaries (late spring and summer), macrophytes form large masses covering >40% of the bottom and water transparency is high (Ibáñez et al., 2008). Invertebrates were also negatively correlated

to nutrient (P) enrichment, but probably this relationship reflects their response to general impairment of the system (Matthaei et al., 2010). Invertebrates could also be sensitive to changes in basal food or habitat heterogeneity (Mundie and Simpson, 1991; Wang et al., 2007; Astorga et al., 2014); for instance, nutrients can have various indirect effects on invertebrates such as changing palatability of coarse detritus (Graça, 2001) or the composition and biomass of invertebrate trophic groups (Wipfli et al., 1998). Remarkably, the invertebrate index increased with altitude indicating a gradient of natural variation from lowlands to headwaters, as pollution sensitive organisms tend to be dominant in cold, well oxygenated waters at mid-higher altitudes (Clarke et al., 2008; Sánchez-Montoya et al., 2010).

Our predictions using socio-economic and climate scenarios showed a consistent decrease in water discharge and increasing phosphorus concentration, which were linked to a mean decrease in the diatom and invertebrate based BQEs. These changes appeared in the different scenarios tested, which coincided to show a future increase in

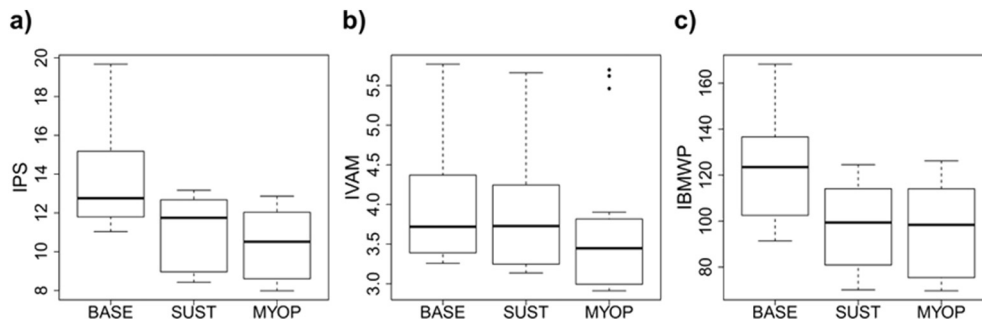


Fig. 6. Predicted values for IPS (a), IVAM (b) and IBMWP (c) at the eight sites considered for the future scenarios. BASE: baseline, current conditions. SUST: sustainable scenario MYOP: myopic scenario. For each of the sustainable and Myopic scenarios, the values predicted for CLM4, RACMO22E and RCA4 climatic models were included.

agricultural and urban pressure together with a decrease in the available water resources. Some of these patterns are already visible in Mediterranean and arid-climate rivers, where river discharge has significantly decreased in parallel to growing human occupation. In the particular case of the Ebro, the separate analysis in many sub-catchments, has shown a rather general pattern of water resources decrease (López-Moreno et al., 2011; Buendía et al., 2016). The decrease in water resources adds to the strong regulation in the basin, which has influenced the basin's hydrology causing a decrease in flood frequency and magnitude (Batalla et al., 2004). The supply-demand (S:D) imbalance is growing with rising agricultural demands and growing human density. The S:D analysis performed by Boithias et al. (2014) showed that water scarcity could be a general problem for the basin, particularly in the lower and agricultural plains, and that this could be aggravated if the extension of irrigated areas would not decrease, which indeed it is not planned (Ebro Basin Management Plan 2015–2021). These predictions of lower water flow and more intense human activity in the basin were correlated to the decrease of diatoms and invertebrate indices, in all future scenarios. The Ebro basin may therefore show poorer ecological status in the future, despite the current implementation of waste water treatment plants across the basin (Torrecilla et al., 2005; Oscoz et al., 2008), and other sanitation efforts. The analysis indicates that the increasingly lower dilution capacity of the river together with the higher arrival of phosphorus inputs will challenge these efforts to maintain or improve the ecological status of the Ebro.

7. Conclusions

Increased total phosphorus, agriculture and urbanization, were linked to poor ecological status of the Ebro River. The river is expected to experience further degradation in the future in response to land-use intensification and climate change. Results suggest that agriculture and urban intensification can be managed independently, but that efforts to achieve a good ecological integrity in the Ebro catchment need to consider all these factors, so effective mitigation and restoration measures can be implemented in a scenario of shrinking water resources.

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