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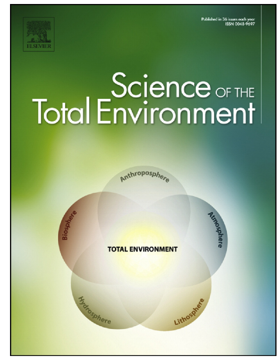

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A diatom-based approach to refine nutrient concentrations compatible with the "good" status of Northern Italy rivers

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Abstract

The identification of ecologically sound thresholds represents an important step toward improving the ecological status of rivers through appropriate measures to contain nutrient loads. The aim of the present study was to estimate phosphorus and nitrogen concentrations compatible with the achievement of the "good" ecological status of rivers from data collected in the Po River District, the largest hydrographic system in Italy. For this purpose, relationships between the diatom index used in Italy for the national assessment of the stream ecological status, the ICMi (Intercalibration Common Metric index), and total phosphorus and nitrate concentrations were analysed using monitoring data collected between 2009 and 2019. The Po River Basin encompasses five distinct river types, from Alpine to Mediterranean to Lowlands, characterized by different anthropogenic pressures and water quality. Through regression analysis between the ICMi and nutrient concentrations, we estimated ranges of the latter values corresponding to a "good" ecological status for each river type. The resulting thresholds are far more stringent than the limits set by the Italian legislation for water quality classification. This is particularly true for total phosphorus, whose threshold value should be roughly halved for all river types. For nitrates, the results are more differentiated according to river type: the estimated thresholds are much more stringent than those currently in use for siliceous Alpine and Mediterranean rivers. Moreover, the availability of such a large database allowed also to assess the influence of one nutrient over the other on the diatom community and to highlight some critical issues in the formulation of ICMi for Mediterranean rivers.

Abbreviations: AIC= Aikake's Information Criterion, BQE= Biological Quality Elements, EC= European Commission, EQR= Ecological Quality Ratio, EU= European Union, H/G= threshold between "high" and "good" status, GAM= Generalized Additive Model, G/M= threshold between "good" and "moderate" status, ICMi= Intercalibration Common Metrix index, IPS= Specific Pollution Sensitivity Index, LIMeco= "Livello di Inquinamento espresso dai Macrodescrittori per lo stato ecologico", i.e., pollution level expressed by macro-descriptors for ecological status, M/P= threshold between "moderate" and "poor" status, $N\text{-}NH_4$= ammonium, $N\text{-}NO_3$= nitrates, P/B= threshold between "poor" and "bad" status, SRP= Soluble Reactive Phosphorus, TI= Trophic diatom index, TN= Total Nitrogen, TP= Total Phosphorus, WFD= Water Framework Directive

Keywords: Water Framework Directive, diatoms, river ecological classification, nutrient thresholds, rivers

1. Introduction

Eutrophication due to phosphorus and nitrogen enrichment associated with an excessive growth of primary producers and changes in species composition is still a major water quality issue across many agricultural or urbanized regions worldwide (U.S. Environmental Protection

Agency, 2016; Kristensen et al., 2018; Le Moal et al., 2019). With respect to this, one of the main steps in the management of surface water eutrophication is the selection of appropriate nutrient criteria. From an ecological point of view, the initial assumption, derived from Vollenweider's classic studies on lakes (1968), is that there is a causal relationship between nutrient loads and aquatic primary producers and that it can be described through linear regression models. In rivers, compared to lakes, the degree of uncertainty is greater because of the multiple pressures acting on the biota and of the high heterogeneity and dynamism that characterize lotic ecosystems. For these reasons, developing pressure-response relationships in rivers is even more challenging, and much less literature is available on river nutrient criteria than for lakes (Poikane et al., 2019).

In the European Union the Water Framework Directive 2000/60 (WFD) provides a common framework for water management and requires achieving the "good" ecological status of all water bodies. However, after 23 years from the adoption of the Directive, the most recent report released by the European Environment Agency (Kristensen et al., 2018) concerning the water assessment, highlighted that 57% of European rivers did not attain the "good" or "high" ecological status. The WFD specifies that to achieve the "good" ecological status, nutrient concentrations should not exceed the levels that allow ecosystems to function and attain the corresponding values of biological quality elements (BQEs – European Commission, 2000, Annex V, Section 1.2). Each EU Member State is responsible for setting nutrient targets for its own river basins. However, the water classification systems based on physico-chemical supporting elements are very variable in Europe along with approaches used to setting nutrient criteria (see for instance González-Paz et al., 2022), resulting in a wide range of concentrations thresholds (Phillips & Pitt, 2016; Poikane et al., 2019), both due to the heterogeneity of river types of individual countries and to the differences in the methodologies adopted. The wide range of nutrient criteria established by the different countries has raised the possibility that some of these may not be fit for purpose, and concerns have been raised about the weak correspondence between nutrients and biological targets (Carvalho et al., 2019). For these reasons, the European Commission has produced guidelines to establish the concentrations of nutrients compatible with the "good" ecological status of rivers (Phillips et al., 2018, recently reviewed by Kelly et al., 2022). These guidelines have already been applied in the Central-Baltic region (Poikane et al., 2021), with the establishment of nutrient threshold values based on the response of macrophytes and diatoms, but there are still no cases of applications in Southern Europe, which has considerable diversification of river types, from the Alpine to the Mediterranean ones. In Northern Italy, in particular, the Po River region shows a wide diversification of river environments, evidenced by the presence of as many as eight river types. This diversification is promoted by an altitudinal development of nearly 5,000 meters, varied orography and climate, and a very extensive range of population density, from a few units of inhabitants per km^2 in some Alpine locations, up to 8000 in large urban centers.

In order to fill this gap, in this study, we analyzed the response of the diatom index, used for ecological water classification, to nutrient concentration using the approach indicated by Phillips et al. (2018) and Kelly et al. (2022), with appropriate modifications where needed. The aim is to establish nutrient boundaries that have a sound ecological basis considering differences in the diatom community composition of river types and can thus help in the attainment of at least "good" ecological status of running waters. Thanks to the application of the WFD, there is now a good availability of chemical and biological data in the Po River region, particularly for benthic diatoms. These organisms are universally recognized as a reliable indicator of the ecological status of rivers and streams due to their wide distribution, presence in all seasons, and key role in the river trophic chain (Bona et al., 2021). Their sensitivity to nutrient concentration, and in particular to phosphorus, is well documented (Shore et al., 2017), as well as the strong relationship between nutrients and phytobenthos metrics (Poikane et al., 2021). Here, we focused on the forms of nitrogen and phosphorus used in Italy for nutrient status classification, namely nitrate (hereafter, $N\text{-}NO_3$) and total phosphorus (hereafter, TP). Obtained results will also be confronted with established numeric nutrient criteria guidelines to evaluate if they are appropriate to meet the “good” ecological status. This research is based on data collected in the Po River region, but the results obtained can also be very significant for many basins of Southern Europe, considering the extension of the study area and the number of hydro-ecoregions and river types included.

2. Material and methods

2.1 Study area

The study was conducted on rivers and streams located in the Po River District (Northern Italy) a territory that comprises the Po River watershed and the watersheds of 8 small fluvial systems (Reno, Fissero Tartaro Canalbianco, Conca Marecchia, Lamone, Fiumi Uniti, Savio, Rubicone and Uso) draining to the North Adriatic Sea. The Po River Basin extends over an area of ~75,000 km^2 and accounts for more than 80% of the investigated area.

The Po River is the longest Italian river (652 km) and flows through Northern Italy, from the Western Alps to the Adriatic Sea. Its basin covers more than 25% of Italy’s surface and includes mountainous areas, foothills, and heavily transformed lowland areas characterized by intensive agriculture (Viaroli et al., 2018), which implies also a high water abstraction for irrigation (Montanari, 2012). The river is fed by a network of more than 140 tributaries with a total length of over 6,700 km. The northern side includes tributaries originating from the Alps and in some cases from deep subalpine lakes, such as the Lake Garda and the Lake Maggiore. The southern slope of the basin includes tributaries of hill or Apennine origin, often affected by hydrologic intermittency for climatic reasons and irrigation withdrawals.

2.2 Database preparation

We used data of diatoms and nutrient concentration collected from rivers in the Po River Basin by the Regional Environmental Protection Agencies between 2009 and 2019 for the evaluation of the ecological status as requested by the WFD. The data were merged into a "biological" (diatom inventories) and an "environmental" database, the latter relating to nitrogen and phosphorus concentrations. The time range corresponds to that of the application of the national standardized diatom index, which in Italy maintains the name of the Intercalibration Common Metric index used in Europe for the intercalibration exercise (ICMi; Mancini & Sollazzo, 2009). The ICM results from the combination of two widely applied diatom indices, the Specific Pollution Sensitivity Index (IPS; Coste,in CEMAGREF, 1982) and the Trophic Index (TI; Rott et al., 1999).

The ICM is thus defined as the mean of the Ecological Quality Ratio (EQR) of IPS and TI (Almeida et al., 2014). These metrics, as do most diatom indices, respond strongly to an increase in trophic level related to both wastewater discharges and agricultural land use (Dalu et al., 2017). The limits for the "high" and the "good" quality classes were defined at the end of the intercalibration exercise in the various GIGs. For other quality classes, the limits were calculated following the procedure suggested for other biological elements (Buffagni et al., 2008; Mancini

& Sollazzo, 2009).

All database preparation step, and subsequent statistical analysis were performed in R 4.2.2 (R Core Team, 2022).

For the biological data, the implementation of the database entailed a taxonomic alignment and update based on the most up-to-date sources, which was necessary since the systematic classification of diatoms has been constantly updated over the studied period. All diatom data was then uploaded into OMNIDIA 6.1.5 software (Lecointe et al., 1993) to calculate the Italian normative ICMi.

All the biological monitored stations that did not have a corresponding chemical data station, or vice-versa, were excluded from the database. To associate the water quality with the biological data, we applied the following criterion: the water quality measures carried out within the 3 months preceding or at the latest within 1 month after the biological sampling were taken into consideration (see also below for details). In the event that multiple data were present in this time frame, we considered the mean value. Applying this criterion, the final database consisted of 4086 samples, coming from 1086 sampling stations and including 878 diatom taxa.

To account for intrinsic biogeographic variability, the sampling sites were divided into the following river types provided by the Italian Environment Ministry (D. LGS. 152/2006), namely:

A1 Alpine, calcareous

A2 Alpine, siliceous

C Central (lowland rivers)

M1 Mediterranean, small and very small rivers

M2 Mediterranean, medium and large rivers

M3 Mediterranean, very large rivers

M4 Mediterranean, medium mountain rivers

M5 Mediterranean, temporary rivers

The most represented river type was the Central one, with almost 1700 observations, followed by A2 (largely present in the Alps of the western sector, which is entirely included in the Po Basin) with over 1000 data. The calcareous Alpine type was less represented, as well as, overall, the Mediterranean types (Supplementary Material SM1). To avoid dealing with data belonging to groups that are strongly unbalanced in the number of observations, for subsequent analyses we merged the perennial Mediterranean types in a single set "M1-M4" (identified with "M" hereafter). We left the temporary Mediterranean (M5) as a group apart, due to the strong influence that extreme flow variations can exert on the diatomic community. Each group of sites thus obtained was characterized by having its own ICMi threshold that discriminates between the "good" and "not good" ecological status (in detail: A1= 0.70; A2= 0.64; C= 0.70; M= 0.61; M5= 0.65; European Commission, 2018). River types designated as M share the same threshold.

Most samples were taken in spring, summer and autumn, while winter is less represented, as required by the protocols on the sampling of BQE.

Figure 1 shows the map of the study area with sampling stations classified into river types.

#fig 1 here#

2.2 Regression model between nutrients and diatom ICM index

The first step in investigating the relationship between nutrients and BQEs is the choice of the parameter (nitrogen and/or phosphorus and their chemical forms), the metric (i.e., mean, median, percentile, etc.), and the time interval (annual, seasonal, etc.) to be considered. Across EU Member States there is a wide variety of approaches that have been adopted to define water quality based on nutrient criteria, both in terms of the target nutrient and the metric used (Poikane et al., 2019, 2021). Concerning phosphorus, most EU countries use TP, others soluble reactive phosphorus (hereafter, SRP). Annual averages are used in many cases, but some countries prefer to use the phosphorus corresponding to the growing season. Although it is sometimes necessary to exclude data corresponding to extreme events such as floods, it must be considered that annual averages are calculated on values no more frequent than monthly ones, while most of the nutrient load, especially in basins with high diffuse loads, is released in occasion of episodic impulses difficult to capture in monthly monitoring. Therefore, the choice of nutrient metrics is open to a variety of solutions. In this study, as specified above, we matched the biological data to nutrients concentrations calculated, when possible, as an average of the previous three months, in order to consider the quality of the water over a sufficiently long period of time, but at the same time able to directly affect the biological metric. Regarding the nutrient chemical forms, in this study we opted for those included in the LIMeco index (D.M. 260/2010 – "Livello di Inquinamento espresso dai Macrodescrittori per lo stato ecologico", i.e., pollution level expressed by macro-descriptors for ecological status). In particular, we chose TP and N-NO_3, which are used by the LIMeco index to classify rivers into five ecological levels according to nutrient criteria, from the Level I (highest quality) to Level V (the worst one). According to LIMeco, the thresholds established a priori by the Italian regulations to discriminate between "good" / "moderate" are 0.1 mg/L for TP and 1.2 mg/L for N-NO_3. The concentration thresholds corresponding to Level I were defined on the basis of the concentrations observed in 115 samples taken by IRSA – CNR at 49 reference sites belonging to different river types. In particular, these thresholds correspond to the 75th percentile of N-NO_3 or 90th percentile of TP of the distribution of concentrations of each parameter in the reference sites. The threshold between G/M is derived by doubling the Level I concentrations (D.M. 260/2010).

The choice to use TP and N-NO_3 is not only determined by the fact that they are the chemical forms required by national legislation. There are also scientific reasons and statistical considerations. TP is recognized in the scientific literature on diatoms as the element on which to base the trophic classification of different species (Hofmann, 1994). Moreover, we verified that in our database TP is closely related to SRP values, as indicated by a Pearson correlation of 0.86 (highly positive relationship). TP and SRP variables are thus expected to carry almost the same information. N-NO_3 are used by most European countries (Poikane et al., 2019), followed by total nitrogen (hereafter, TN). In the present study, we assessed the use of N-NO_3 and its relationship with TN. Our analyses confirmed a higher correlation between TN and N-NO_3 (0.85, a high positive correlation) compared to TN and ammonium (hereafter, N-NH_4; 0.62, a moderate correlation). Additionally, the proportion of variance explained by a linear model relating TN to N-NO_3 and N-NH_4 revealed that N-NO_3 has a higher relative importance in explaining total variance in TN (0.72), compared to N-NH_4 (0.25). These analyses were conducted in R using the relaimpo package.

For the chosen nutrients, we then investigated their relationship with the BQE through regression analysis. Regression analysis is the first recommended approach according to the European guidelines, provided that the data span a range of biological quality and BQE has a clear and linear response over the range of interest. Thus, before proceeding with the regression analysis, we explored the water quality and diatom index data. We verified the distribution of nutrients in the different river types, and we compared the distribution of the ICMi data to the G/M boundary established by the regulations for each river type (Mancini & Sollazzo, 2009; and subsequent update European Commission, 2018). We also carried out a data exploratory analysis to verify if the nutrient concentrations cover all the five classes of the ICMi.

Following the guidelines provided by Phillips et al. (2018), to check that the response of ICMi to nutrient was linear, we examined the scatter plot of the data, and we fitted a GAM model (Generalised Additive Model). The nutrient data were log-transformed before the analyses. If the relationship was not linear across the entire range of the data, we fitted a segmented regression to identify where significant changes in the slope of the relationship occur. This allowed us to identify a linear portion of the data, which should at least cover the range of interest in terms of ICMi and nutrient concentrations with respect to the G/M threshold. For the linear portion of the data, we then fitted and compared in terms of Akaike's Information Criterion (AIC) three different regression models relating the ICMi to the nutrients:

RM1: the model that groups all data together, without distinguishing between river types;

RM2: the model that considers all data fitting different straight lines for different river types, with different intercepts, but all characterized by the same slope;

RM3: the model that considers all the data fitting different straight lines for the different river types in terms of both intercept and slope.

In this phase, and for all subsequent regression analyses, to properly manage the pseudo-replicates present in the dataset (due to temporal replicates), a random factor referred to the sampling site was inserted. The general approach continues to be in line with what is proposed by Phillips et al. (2018), but mixed linear models instead of generalized ones were fitted to the data.

Since the AIC value supported the choice of the model that fits straight lines with different intercepts but with the same slope (RM2), for both TP and N-NO_3 (see below, Results section), according to the guidelines we proceeded with a single model for all river types, including river type as a fixed effect and then: (a) regressing ICMi as dependent variable against the nutrient concentration, considered as an explanatory variable; (b) regressing the nutrient concentration against ICMi; (c) performing a Standard Major Axis regression (Legendre, 2013) that assumes uncertainty in both the dependent and the explanatory variables. Among models (a), (b) and (c), we finally chose the most likely one based on a critical analysis of the resulting nutrient thresholds. The goodness-of-fit of the models was assessed by r^2. According to the guidelines (Phillips et al., 2018; Jolicoeur, 1990 in Smith, 2009) a regression model with an r^2 of 0.36 or higher is considered satisfactory. However, there is no clear statistically valid cut-off for what represents a low r^2. For our mixed models, we report both the marginal and the conditional r^2. The marginal r^2 considers the variance of the fixed effects only, while the conditional takes into account both the fixed and random effects variances, i.e., the conditional r2 also includes the proportion of the outcome variation at the sampling site level.

3. Results

3.1 Data Exploratory analysis

Figure 2 shows the distribution of concentrations of TP, N-NO_3, and ICMi, separated by river types.

#figure 2 here #

TP has a high frequency of low values, corresponding to LIMeco Level I, particularly in the Alpine river types (A1 and A2) and in perennial Mediterranean rivers (M). In intermittent rivers (M5), TP mainly ranges between Level I and II. In the Central river type (C) most values fall in Level II, with a significant number of samples in Level V. $N\text{-}NO_3$ concentration has low values (corresponding to LIMeco Level I) in the siliceous Alpine river type (A2) and in M, while concentrations appear shifted toward Level II and III in the calcareous Alpine (A1), C and M5.

For ICMi, the values of type A2 are generally very high, corresponding to the "high" ecological status, in fact the threshold of the latter (0.85) is below the 25th percentile. The medians of samples A1 and M are also above the H/G threshold, which are respectively 0.87 and 0.80. In contrast, a significant number of M5 samples are of "moderate" or lower ecological status, being the G/M equals 0.65. This condition affects most of the C samples, which therefore do not meet the objectives. The G/M boundary for this river type is set at 0.70, which is higher than the median shown in the boxplot. Finally, it is noteworthy that the ICMi of a non-negligible number of samples exceeds the value 1, corresponding to the reference values, reaching in some cases values even higher than 1.5. This is especially the case for river type M.

3.2 Regression analysis between nutrients and ICM index

Before proceeding with the regression analysis, an exploratory analysis about the nutrient data distribution indicated that the available data covered all the five classes of the ICMi and that there was a trend towards a worsening of the index along with an increase in nutrient concentrations (Supplementary Material SM2).

3.2.1 Regression model for total phosphorus

We examined the scatter plot of the data and fitted a GAM model to check the shape of the relationship between TP and ICMi (Figure 3a). After verifying that the relationship was nonlinear across the entire range of data, we fitted a segmented regression to identify where significant changes in the slope of the relationship occurred (Figure 3b).

#figure 3 here #

The blue, green and red lines in Figure 3a indicate the linear relationships resulting from the segmented regression. They clearly identify the different linear portions of the relationship. The two segmentation points identified (0.02 and 0.21) allow enough data to remain above and below the G/M threshold and thus meet the requirements for predicting the corresponding TP value. The data also present nutrient concentration values that meet expectations from the ecological point of view (i.e., in a range of values that are plausible based on current knowledge). The AIC values obtained for the mixed models fitted to this linear portion of the data suggested that the model that considers all data but different intercepts for the river types (RM2; AIC = -3074), should be preferred to the alternative models (AIC = -2772 and -3067 for models RM1 and RM3, respectively).

Finally, critically considering the results obtained through the different regression approaches ((a), (b), (c), see also Supplementary Material SM3), we selected the regression model for TP against ICMi, as shown in Figure 3b.

Table 1 reports the estimated TP concentration thresholds for discriminating between G/M status and the confidence intervals of the threshold for each river type.

#table 1 here #

3.2.2 Regression model for N-NO_3

The same steps seen for TP were followed for N-NO_3. The first step, following the exploratory analysis, is to verify the linearity of the relationship between ICMi and N-NO_3 (Figure 4a).

Again, the identification of the linear region is achieved through the identification of two break points. The segmentation points allow sufficient data to remain above and below the G/M threshold and thus meet the requirements for predicting the corresponding N-NO_3 value. The data also show ecologically plausible N-NO_3 concentration values.

We tested the three models similarly to what we did for the TP. The AIC values were, respectively: -2494; -2703; -2711. The analysis of AICs indicates that for N-NO_3, considering this range of data, it is also possible to continue with the analysis, considering straight lines with the same slope, but different intercepts in the different river types (AIC = -2703). The lowest AIC value would actually be preferable to the model that also includes different slopes (AIC = -2711), but in line with a parsimonious approach to the analysis, even taking into account what was done for phosphorus and the sample size, again it was considered appropriate to proceed with the simpler mixed model, with random factor for the intercept only. The result of the regression analysis for identifying the G/M threshold for N-NO_3 is shown in Figure 4b.

#figure 4 here #

Table 2 reports the estimated thresholds and confidence intervals for each river type.

#table 2 here #

Discussion

With this study, threshold values of TP and N-NO_3 were identified through an ecological criterion, i.e., the response of BQE diatoms to the concentration of these two nutrients, for five different river types. To the best of our knowledge, this result is so far the first one obtained for Southern Europe, encompassing five river types such as Mediterranean and Alpine ones. In the present study, the five river types are characterized by different ecological status reflected in differences in the distribution of ICMi (Figure 3), which highlights several critical values in the Mediterranean intermittent rivers (M5) and in lowland ones (C). In this latter type, the number of samples corresponding to a "not good" status (80%) is much greater than the most recent estimate made for the entire European Union (57%; Kristensen et al., 2018).

Recent studies investigated the range of nutrient targets for “good” ecological status, the approaches used to set these thresholds across EU countries and river types, and assessed the gap between actual nutrient concentrations and these targets. In most cases, these are target values for river broad types extrapolated from limits set by individual Member States that are based on different nutrient fractions and statistical summary metrics (Poikane et al., 2019; Nikolaidis et al., 2022). Nutrient standards using pressure/response relationships, such as those proposed in this study, have so far been estimated for only a few river types, namely small low alkalinity lowland, mid-altitude medium-sized mixed alkalinity, lowland and small lowland calcareous from the Central-Baltic region of Europe (Poikane et al., 2021).

In our study, we followed the approach suggested by the European Commission guidelines, as detailed in Phillips et al. (2018). In particular, we adopted a regression approach that assumes a causal relationship between the nutrients and the ecological status of streams defined by the diatom index (ICMi). The approach led to the identification of the region of data where the relationship among variables can be adequately described by linear regression. In the absence of a clear statistically valid cut-off for r^2 values, the analysis of the outcome of the models reveals that r2 values are higher when we consider not only the fixed part of the model, but also the variation at the random effect level. Our results suggest that a large proportion of the outcome variation reasonably occurs at the site level, and these results confirm the validity of our approach, which slightly modifies the procedure described by Phillips et al. (2018) by introducing a random effect for the sampling site. The mixed approach to modelling was indeed necessary to deal with the dependence among data collected at the same sampling site through time (longitudinal data; Gurka & Edward, 2007, Zuur et al., 2009). Moreover, by including the river types as a fixed effect in the analyses (Harrison et al., 2018), we obtained different estimates of the threshold for each of them. At the same time, we were able to contemporarily exploit all available data. The need to include a random effect in the analyses was also an element that strengthened the decision to adopt regression as an analytical approach. Regression analysis is confirmed as an extremely flexible analytical tool, unique in its ability to correctly model ecological relationships. However, the thresholds derived were also compared with those provided by other types of analysis suggested by Philips et al. 2018 (e.g., categorical analysis, not shown), allowing us to observe a general consistency in the results and reinforcing our conclusions.

Comparison of the resulting ranges with the literature must therefore take the above mentioned methodological differences into account. In Table 3 we report a summary of our results together with those obtained in other European countries for phosphorus. For TP, our boundaries are narrower but generally included in the targets cited by Nikolaidis et al. (2022), although in Mediterranean rivers we obtained slightly less stringent thresholds. The state of the art of European nutrient criteria based on the response of 28 Member States (Poikane et al., 2019) reports a very extended range. Our results are all in all comparable to the European state of the art, with values below 0.05 mg/L, but the boundary for Alpine siliceous type has a much narrower confidence interval. This value corresponds well to the 0.05 mg/L given by Charles et al. (2019) as a threshold between impaired and paired sites based on benthic diatom composition. Moreover, our values range corresponds quite well to that reported in a comprehensive literature analysis performed by Poikane et al. (2021), which cited the range 0.03-0.06 mg/L as the threshold above which algal biomass could reach a nuisance level and diatom community could experience significant shifts in species composition. Poikane et al. (2021) considered SRP instead of TP, finding a G/M boundary for the Low Alkalinity Lowland river type, very similar to our TP range. González-Paz et al. (2022) found two thresholds for SRP, equal to 0.0507 and 0.0264 mg/L, using two different diatom indices and considering 425 sites in Northern Spain.

#Table 3 here#

For N-NO_3, the differences between river types are more pronounced than for TP (Figure 3 and Table 1). Alpine siliceous and Mediterranean perennial types have the most severe values, 0.790 and 0.840 mg/L respectively. The remaining types have a value around 1.3 mg/L. These findings substantially reflect the different content of this nutrient in the two groups discussed above: it is on average much lower in A2 and M (Figure 2). At the European level, although most countries use nitrate as the form of nitrogen for the ecological classification of rivers (Poikane et al., 2019), published literature reports threshold values for TN, with in general marked differences in the thresholds given for different river types. In particular, the lowest thresholds are found for Siliceous Mountain rivers and Lowland rivers, and the highest thresholds are found for Calcareous rivers. Poikane et al. (2019) note that the threshold value given by some countries (5.6 mg/L) for N-NO_3 probably derives from the Drinking Water Directive 80/778/EC so being completely unrelated to ecological status protection criteria.

Our BQE-driven thresholds through regression models were more stringent than those derived from the statistical distribution of nutrient data or expert judgment, in agreement with previous findings (Poikane et al., 2019). In particular, the G/M thresholds of the TP are about half of the value established in Italy for the G/M threshold, which is 0.1 mg/L (Table 1), and even the upper limit of the confidence intervals does not reach that value. Moreover, whilst the same G/M threshold was established for all river types by the Italian regulation, our results clearly indicate that different targets should be identified. N-NO_3 targets (Table 2) do not indicate such a clear difference between the thresholds established with the regression and the threshold established a priori by the Italian regulations, which is 1.2 mg/L. However, also in this case, a differentiation between river types was observed. For some river types (namely, A1, C, and M5), the G/M values are in line with the LIMeco, which is included in the confidence interval obtained with the regression analysis. These are the rivers with the highest average N-NO_3 concentrations, compared to the other two river types. In the latter, on the contrary, the estimated thresholds are significantly lower than the LIMeco value, which is above the confidence interval. It can be hypothesized that interventions aimed at lowering nitrates in river types A2 and M could further increase the number of sites that reach "good" status for BQE diatoms.

The difference between phosphorus and nitrogen targets identified by the normative index LIMeco and those estimated in this work could explain the non-concordance that occurs in many cases between the ecological status defined according to biological indices (ICMi) and the nutrient status of rivers (LIMeco, Bona et al., 2021). Presumably, the higher nutrient thresholds contribute to the failure to achieve the WFD goals, as noted by Poikane et al. (2019). It must also be noted that the stringent limits derive from the model that regresses the nutrient concentration against ICMi, which could overestimate the slope of the regression between the two variables. On the opposite, regressing ICMi against nutrients tends to underestimate it, providing unrealistic results from an ecological perspective (Supplementary Material SM3, Fig. SM3-1 and Fig. SM3-4). However, despite differences in absolute values, the more stringent thresholds are confirmed by an approach based on the geometric mean of the different regressions (see above, models (a), (b) or (c), and Supplementary Material SM3, Figure SM3-1 for TP, and Figure SM3-4 N-NO_3).

In summary, our findings highlight TP concentrations in river type C as the most critical situation, because they are much higher than the estimated G/M boundary. 1090 samples out of 1686 have TP concentrations >0.058 mg/L (estimated G/M threshold). Of these, 807 (about one half) also exceed the upper limit of the confidence interval of 0.098. In these sites, it can be reasonably assumed that a decrease in phosphorus can lead to a significant increase in ecological status.

Finally, it deserves to be noted that Mediterranean rivers show some peculiarities in the relationship between G/M boundaries, effective nutrient concentrations, and ICMi. For M sites, the median observed values of TP and N-NO_3 are well below the thresholds estimated by the model, although the latter is much more stringent than those estimated in the other river types, while at intermittent Mediterranean sites, the G/M threshold is met in a large proportion of samples for both nutrients. Thus, in this respect, no special efforts seem necessary to limit nitrogen and phosphorus inputs in these basins, at least from the point of view of BQE diatoms. It should be noted, however, that the confidence interval for $N\text{-}NO_3$ is much wider than in all other cases, as is the range of variation in the data, despite the smaller number of samples for this river type. Moreover, Mediterranean types deserve further investigation inherent to ICMi: as noted earlier (Falasco et al., 2016; Bona et al., 2021), the reference value for calculating RQEs has not yet been updated, contrary to the other river types. The current reference value (especially for M2, M3 and M5 types) appears to be not very conservative, leading in many cases to an overestimation of ecological status. This is confirmed by the non-negligible number of data well above 1 (which correspond to higher quality than the reference sites), in some cases even 1.5, as shown in Figure 2. The desirable lowering of the reference value will lead to lower ICMi values in the future and the likely lowering of the G/M boundary for the two nutrients.

It is interesting to compare the nutrient thresholds found to be compatible with the 'good' status of diatoms with the literature concerning the ecological significance of phosphorus and nitrogen concentrations. Regarding the classification of diatom species with respect to trophic level, Hofmann (1994) indicates TP ranges corresponding to the different categories, from oligotrophic (TP< 11.8 μg/L) to eutrophic (TP> 46.5 μg/L). Our estimated thresholds lie between the a-meso-eutrophic and eutrophic categories, confirming the need to lower the concentrations established using non-biological criteria. This is also in agreement with Chambers et al. (2012), who reported a TP threshold of about 0.02 to 0.06 mg/ L above which benthic chlorophyll a values increase sharply. For nitrogen, there are no such defined thresholds in the literature. In a multivariate analysis of diatom assemblages coming from five European countries, Fisher et al. (2010) noted that nitrogen concentrations were at least as important as phosphorus concentrations in explaining differences in diatom species composition. Dissolved inorganic forms of nitrogen and phosphorus were more important than total nutrients in determining the differences between samples based on species composition. Moreover, it has been recently observed a strong and negative correlation between nitrate concentrations and the cumulative percentage of diatom taxa classified as threatened at different levels in the diatom Red List, strengthening the idea that among the environmental drivers of diatom distribution, nitrates often stands out as particularly relevant factor (Hofmann et al., 2018; Cantonati et al. 2022).

It should also be highlighted that future studies should take into consideration non- diatoms phytobenthos such as filamentous algae as well (Poikane et al., 2016). Their proliferation is clearly related to the eutrophication process thus being a reliable diagnostic tool of river impairment, especially for lowland types. Unfortunately, these organisms are not currently considered in river phytobenthos assessment systems of most EC countries, including Italy.

In conclusion, this study confirmed the need to provide a sound ecological basis for the target river concentrations of the two main algal nutrients. Although phosphorus is confirmed by our results as the most critical element affecting the assessment of the ecological status, it is of paramount importance to set sustainable management goals that also address nitrogen. In recent decades, measures taken to reduce nutrient loads in the Po catchment have also focused mainly on phosphorus. However, the delay in the concomitant reduction of nitrogen loads has resulted in an extreme imbalance of stoichiometric ratios between nitrogen and phosphorus (Viaroli et al., 2018), which is present also in our dataset. This process requires further studies that relate ecologically-sound thresholds to estimated nutrient loads, taking into account global change scenarios with exacerbating drought and flood events.

Acknowledgements

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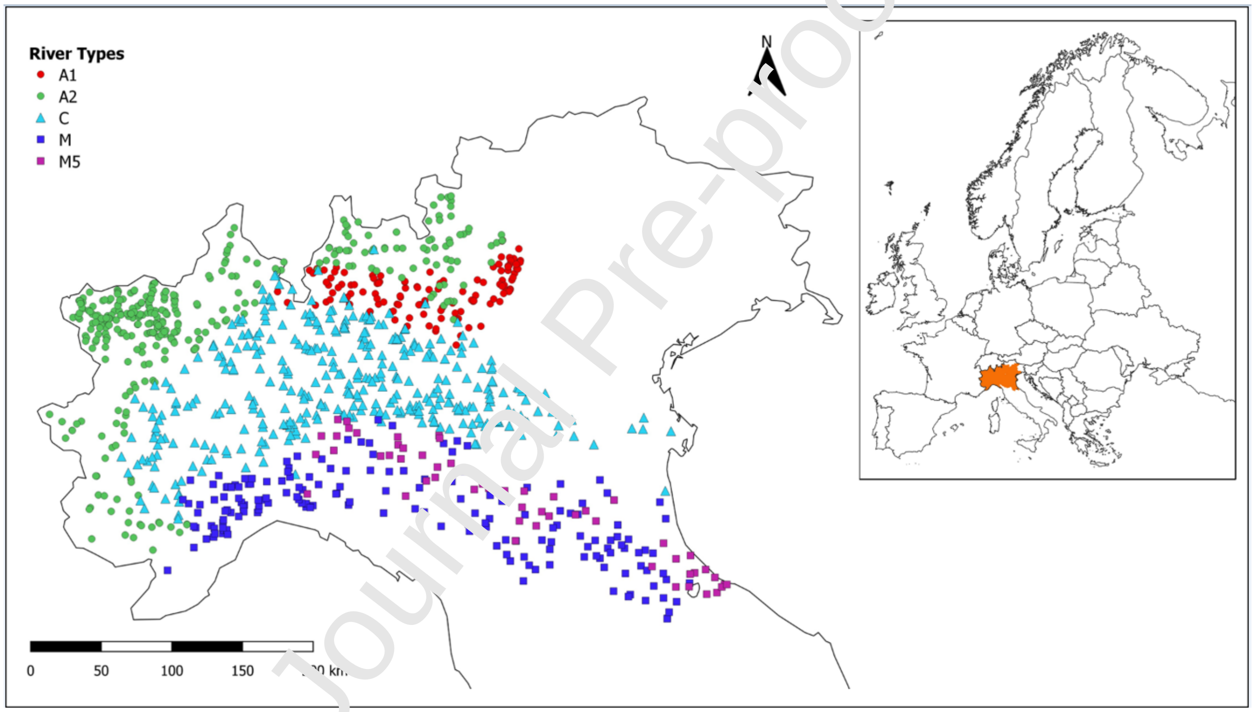


Figure 1. Map of the study area: the sampling sites are divided into river types. The study area is highlighted in orange in the map of Europe.

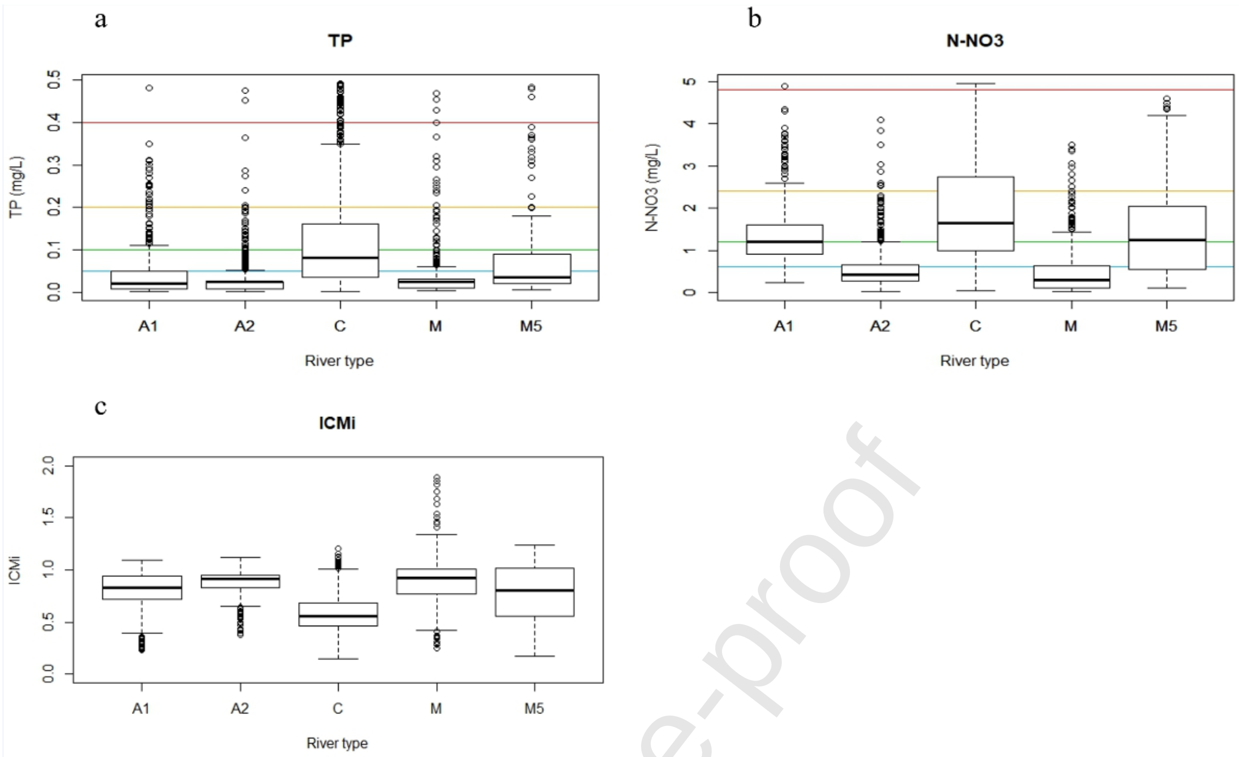


Figure 2. The boxplots illustrate concentrations of (a) TP, (b) N-NO_3, and (c) ICMi values divided into river types. The bottom and top of each box are the 25th and 75th percentiles, the line in the middle is the median, whiskers go from the end of the interquartile range to the furthest observation within 1.5 times the interquartile range. For TP and N-NO_3, blue, green, orange and red lines represent upper LIMeco thresholds for Level 1, Level 2, Level 3 and Level 4 respectively.

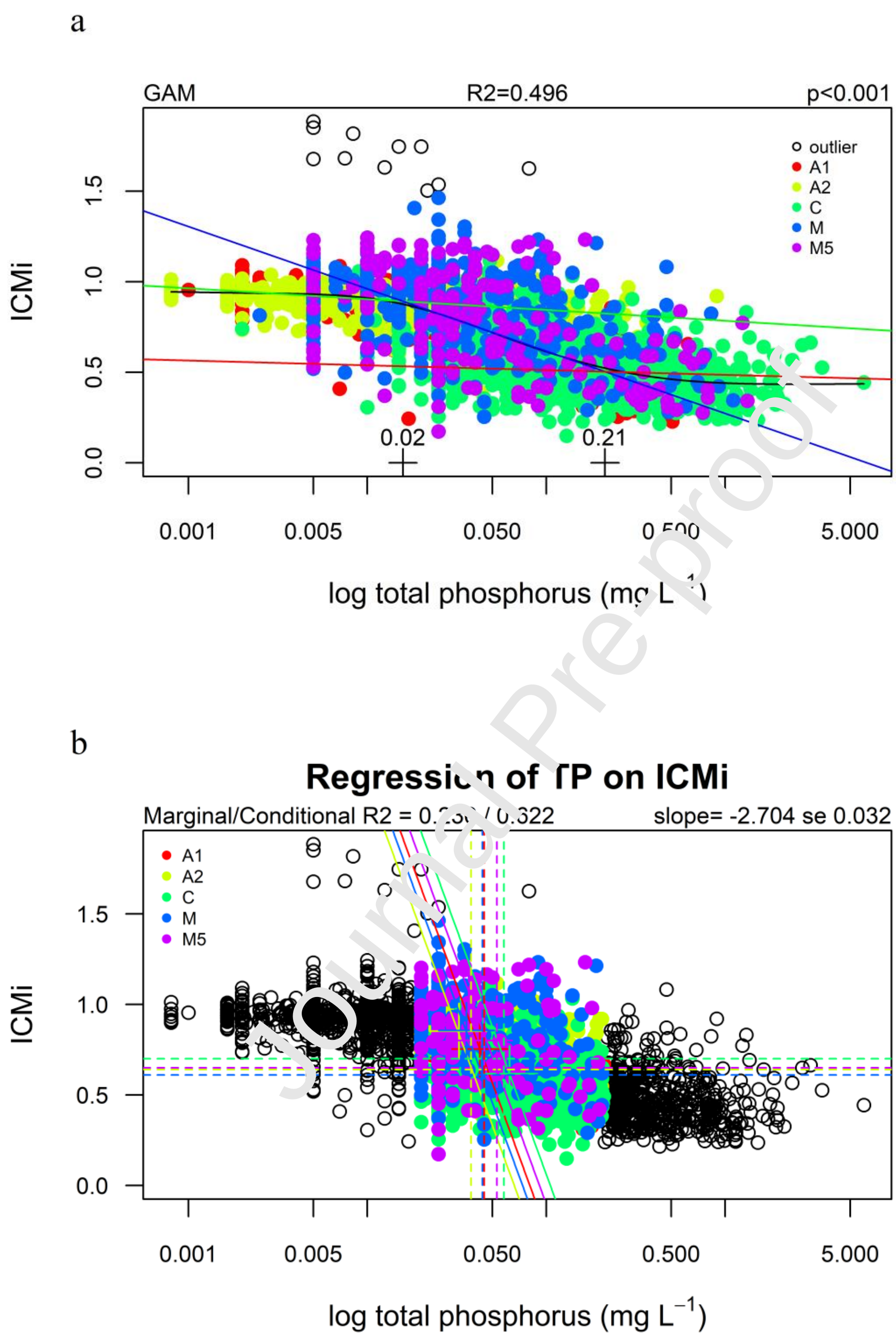
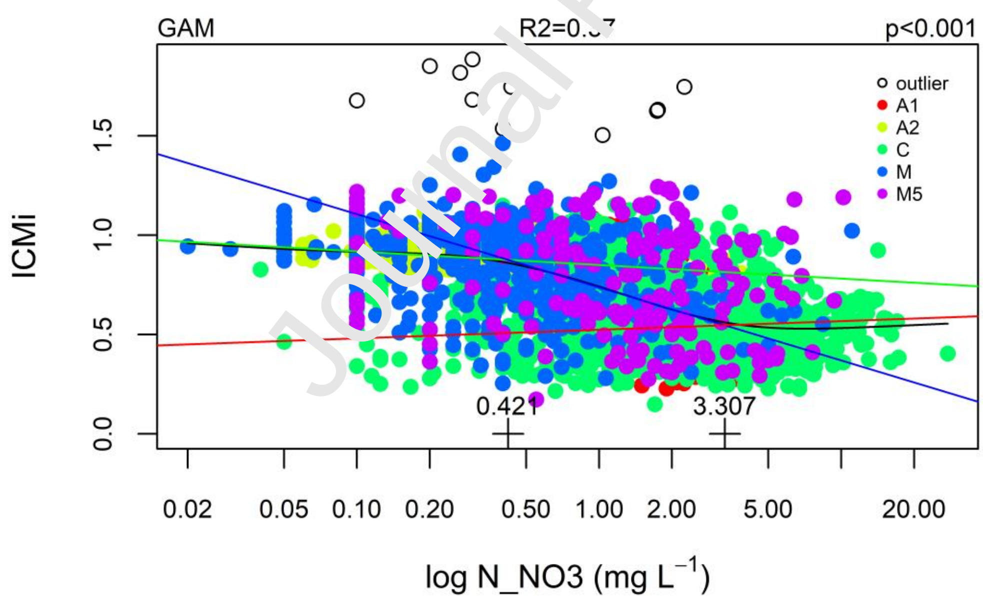


Figure 3. Results of GAM model for TP against ICMi**.** (a) Scatterplot of the data with superimposed GAM curve (black line) and identification of the linear portions (coloured lines) of

the relationship between ICMi and log-transformed TP. (b) Selected model to identify the G/M TP threshold. The data used for the linear mixed model (coloured dots) are restricted between 0.02 and 0.21, according to the results of the segmented regression. The continuous lines represent the regression lines; the dashed horizontal lines identify the ICMi boundaries between the G/M status, while the vertical ones identify the corresponding nutrient thresholds for the different river types. Please note that even if the model used to identify the thresholds regresses TP against ICMi, because the purpose of the model is to predict the nutrient concentration that occurs at a given ecological status, the scatterplot has ICMi on the *y*-axis, and the nutrient on the *x*-axis, as this representation reflects the biological relationship between the index and the nutrient, in which the nutrient concentration 'causes' the ecological status.

a



b

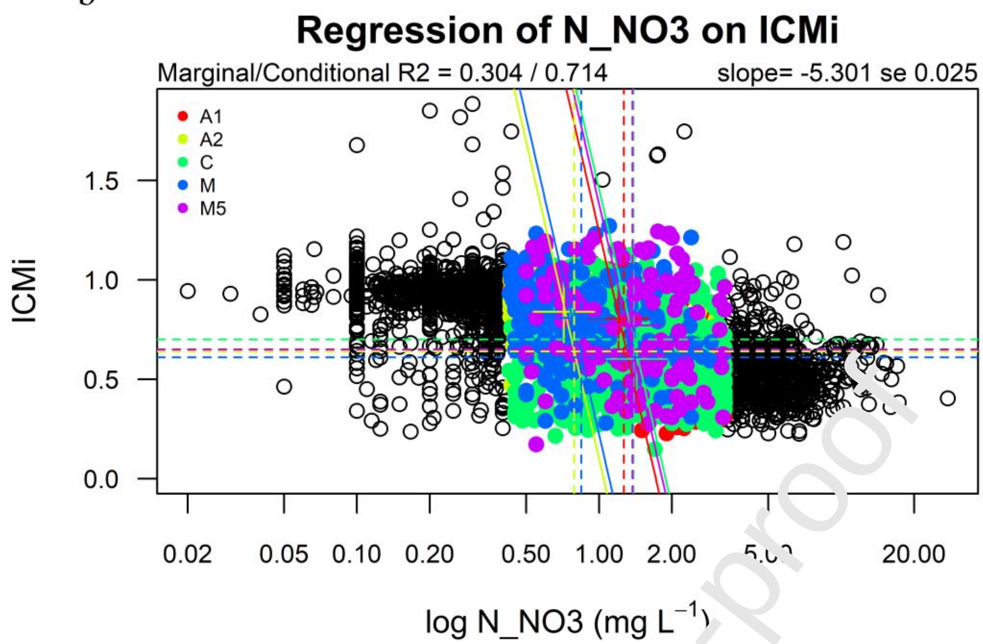


Fig. 4. Results of GAM model for N-NO_3 against ICMi**.** (a) Scatterplot of the data with superimposed GAM curve (black line) and identification of the linear portions (coloured lines) of the relationship between ICMi and log-transformed nitrate; (b) Selected model to identify the G/M N-NO_3 threshold. The data used for the linear mixed model (coloured dots) are restricted between 0.421 and 3.307, according to the results of the segmented regression. The continuous lines represent the regression lines; the dashed horizontal lines identify the ICMi boundaries between the G/M status, while the vertical ones identify the corresponding nutrient thresholds for the different river types. Please note that even if the model used to identify the thresholds regresses N-NO_3 against ICMi, because the purpose of the model is to predict the nutrient concentration that occurs at a given ecological status, the scatterplot has ICMi on the *y*-axis, and the nutrient on the *x*-axis, as this representation reflects the biological relationship between the index and the nutrient, in which the nutrient concentration 'causes' the ecological status.

Table 1 Estimated TP thresholds (and confidence interval) for the G/M boundaries for the different river types. N is the number of records. For comparison, the last row reports the TP threshold adopted in the LIMeco index for the water chemistry classification.

River Type	**N**	**TP Good / Moderate boundary (mg/L)**	**Confidence interval**
A1	218	0.045	0.028-0.065
A2	653	0.038	0.029-0.045
C	1218	0.058	0.033-0.098
M	362	0.044	0.030-0.061
M5	110	0.053	0.031-0.074

TP threshold adopted in the LIMeco index = 0.100 mg/L

Table 2. Estimated $N\text{-}NO_3$ thresholds (and confidence interval) for the G/M boundaries for the different river types. N is the number of records. For comparison, the last row reports the $N\text{-}NO_3$ threshold adopted in the LIMeco index for the water chemistry classification.

River Type	**N**	**$N\text{-}NO_3$ Good/Moderate boundary (mg/L)**	**Confidence interval**
A1	398	1.266	0.947-1.640
A2	566	0.790	0.579-0.938
C	1087	1.385	0.956-2.078
M	237	0.845	0.572-1.146
M5	118	1.374	0.904-2.216

$N\text{-}NO_3$ threshold adopted in the LIMeco index = 1.200 mg/L

Table 3. Comparison among phosphorus concentration thresholds calculated in EC countries.

River Type	**Geographical area**	**Good/Moderate boundary (mg/L)**	**Methodology**	**Reference**
A1	Northern Italy	TP 0.045	Regression with phytobenthos EQR	This study
A2	Northern Italy	TP 0.038	Regression with phytobenthos EQR	This study
C	Northern Italy	TP 0.058	Regression with phytobenthos EQR	This study
M1-M4	Northern Italy	TP 0.044	Regression with phytobenthos EQR	This study
M5	Northern Italy	TP 0.053	Regression with phytobenthos EQR	This study
Range among river types	Europe	TP 0.008-0.660	Various	Poikane et al., 2019
Lowland	Europe	TP 0.040-0.105	Various	Poikane et al., 2019
Mid -altitude	Europe	TP 0.047-0.070	Various	Nikolaidis et al., 2022
Highland	Europe	TP 0.011-0.047	Various	Nikolaidis et al., 2022
Mediterranean	Europe	TP 0.021-0.041	Various	Nikolaidis et a., 2022
Low alkalinity	Europe	SRP 0.021-0.042	Regression with	Poikane et

upland rivers			phytobenthos EQR	al., 2021
Low alkalinity mid altitude	Europe	SRP 0.032-0.090	Regression with phytobenthos EQR	Poikane et al., 2021

Authors contribution

FB: Conceptualization, Funding Acquisition, Methodology, Writing-Original Draft, Writing-review and Editing

EF: Data curation, Visualization, Writing-review and Editing

DN: Funding Acquisition, Writing-review and Editing

MZ: Data curation, Visualization, Writing-review and Editing

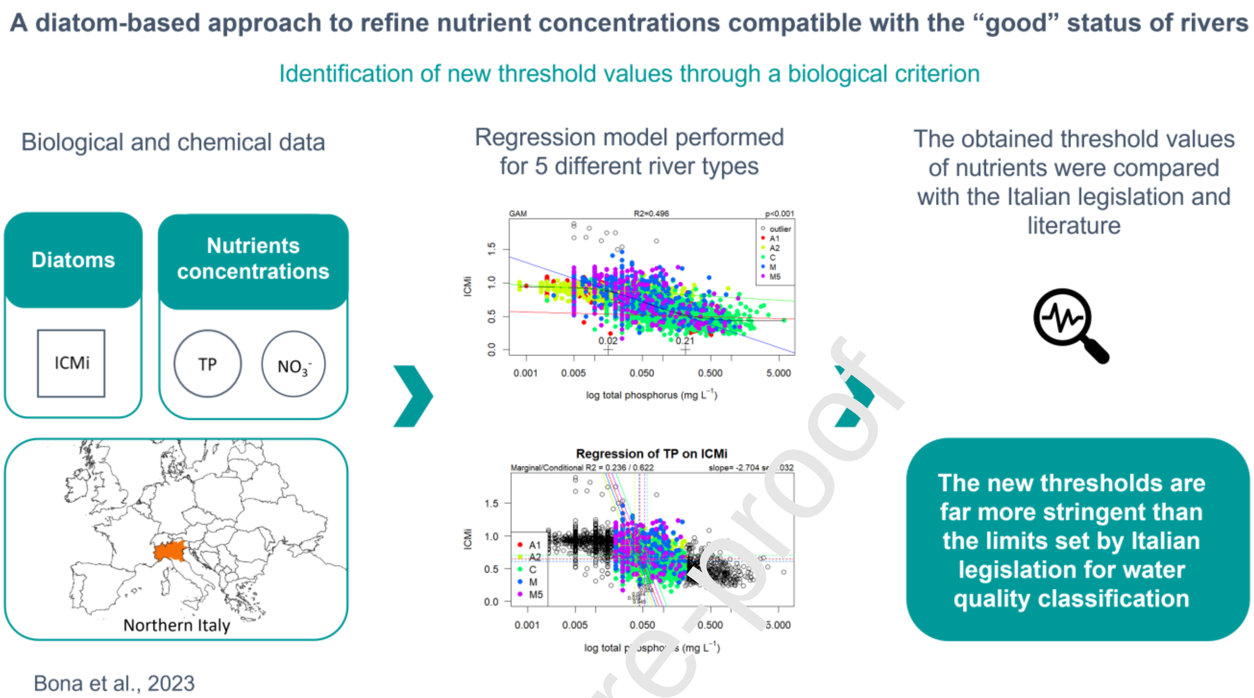
VLM: Formal Analysis, Methodology, Writing-review and Editing

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Graphical abstract



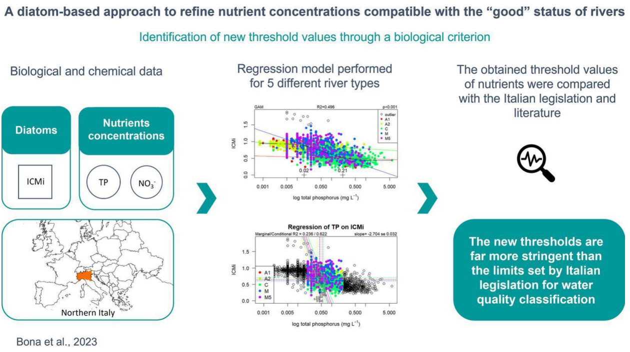
Highlights

Developed regression models to set up ecological-sound nutrient thresholds

Different river types have distinct thresholds estimated with diatom indices

P thresholds should be halved in comparison to current Italian legislation

Nutrient concentrations are critically high in lowland and in intermittent rivers.



Graphics Abstract

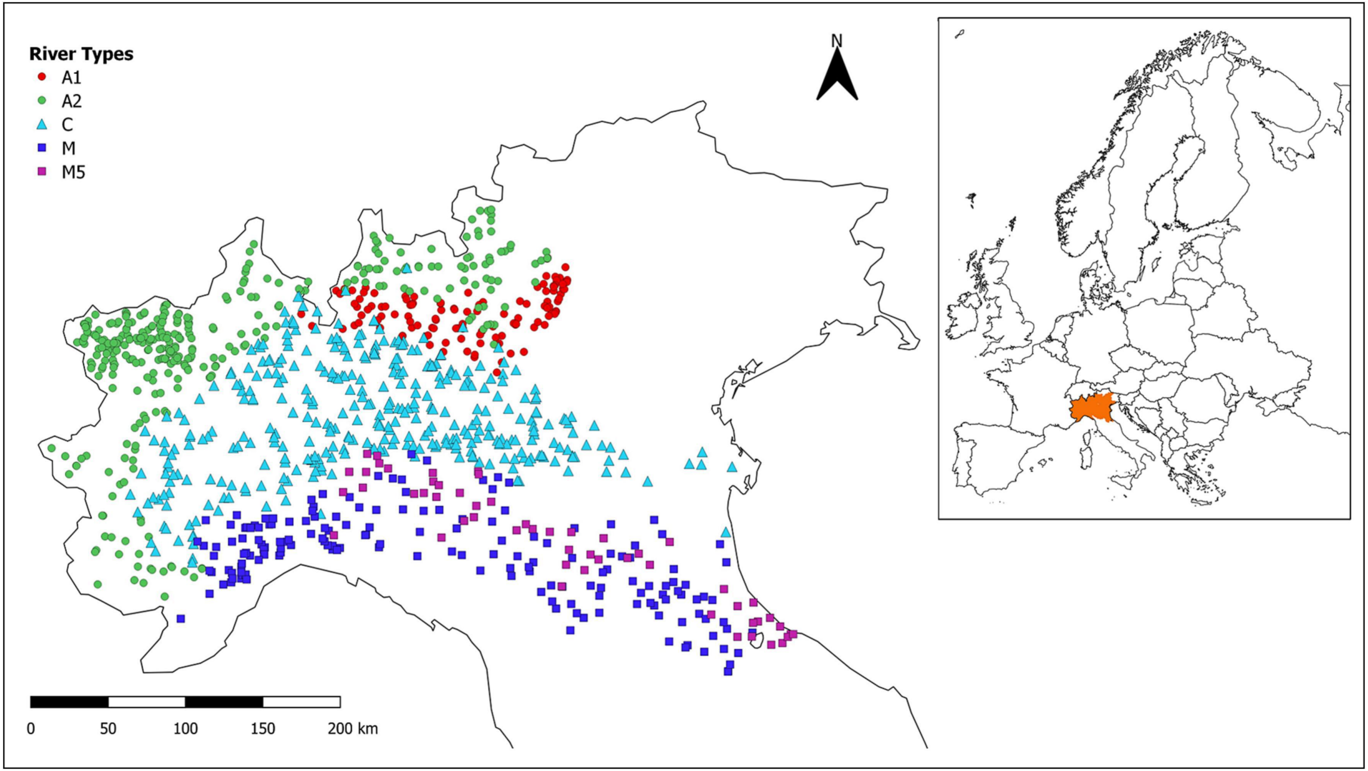


Figure 1

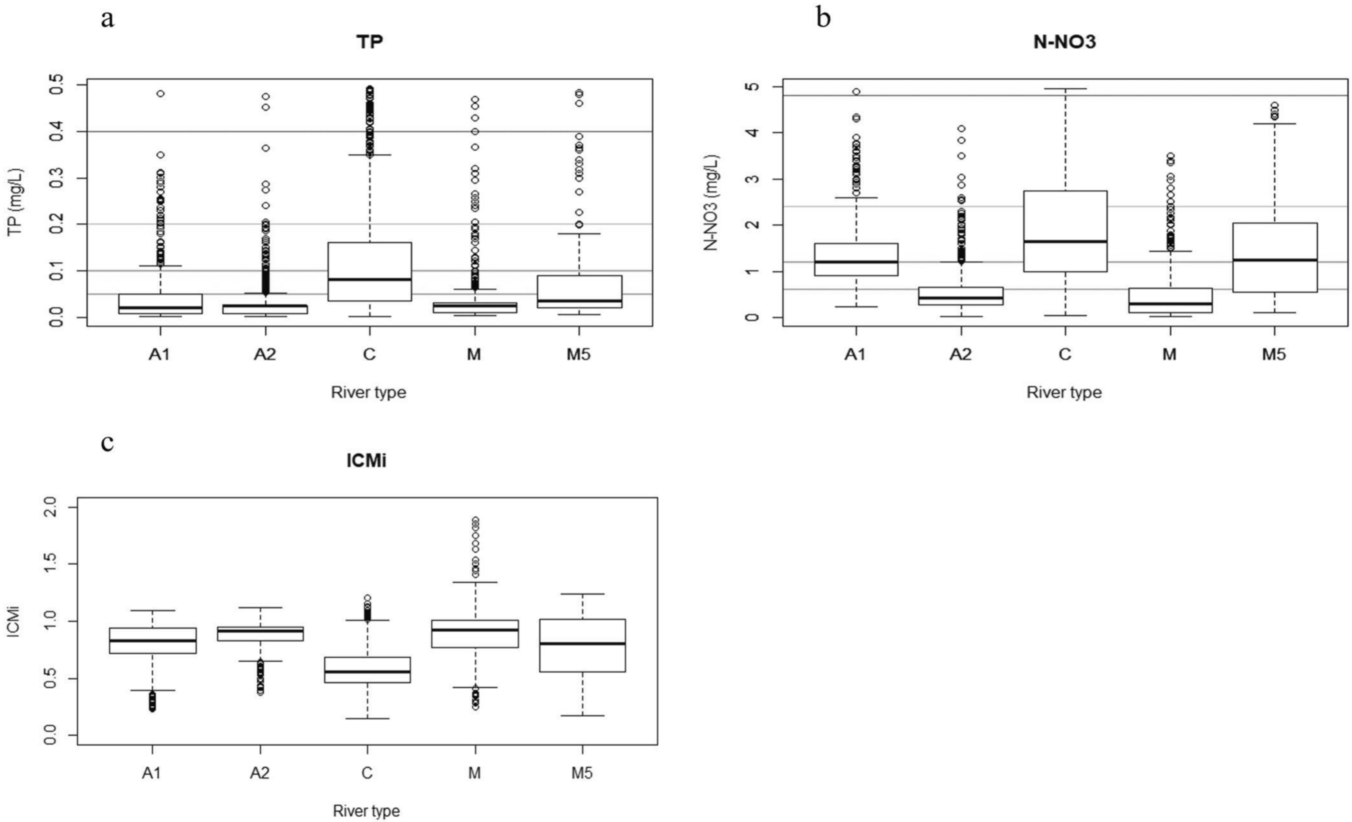
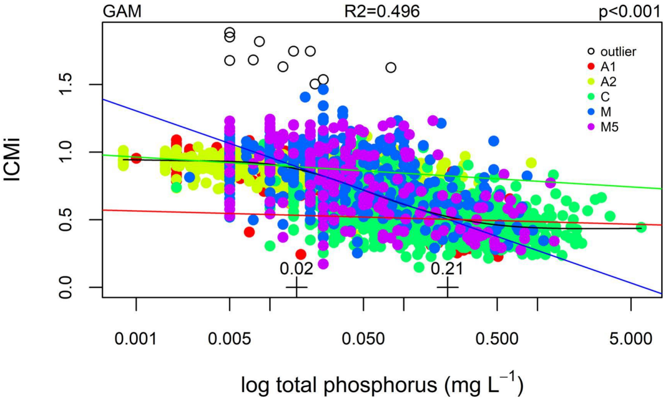

a
TP
TP (mg/L)
0.0
0.1
0.2
0.3
0.4
0.5
A1
A2
C
M
M5
River type
b
N-NO3
N-NO3 (mg/L)
0
1
2
3
4
5
A1
A2
C
M
M5
River type
c
ICMi
ICMi
0.0
0.5
1.0
1.5
2.0
A1
A2
C
M
M5
River type

Figure 2

a


GAM
R2=0.496
p<0.001
outlier
A1
A2
C
M
M5
ICMi
0.0
0.5
1.0
1.5
0.02
0.21
0.001
0.005
0.050
0.500
5.000
log total phosphorus (mg L−1)

b

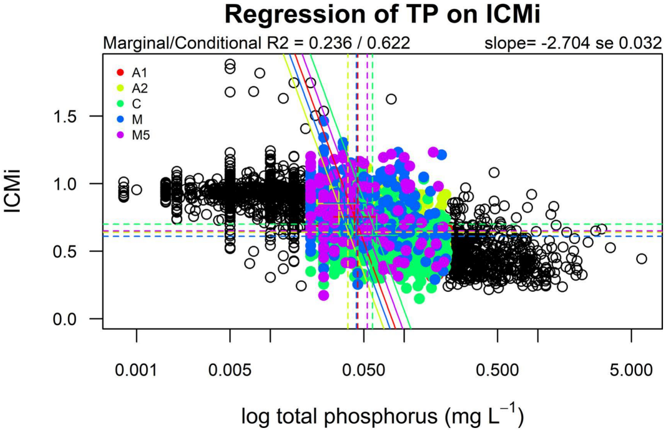

Regression of TP on ICMi
Marginal/Conditional R2 = 0.236 / 0.622
slope= -2.704 se 0.032
A1
A2
C
M
M5
ICMi
0.0
0.5
1.0
1.5
0.001
0.005
0.050
0.500
5.000
log total phosphorus (mg L−1)

Figure 3

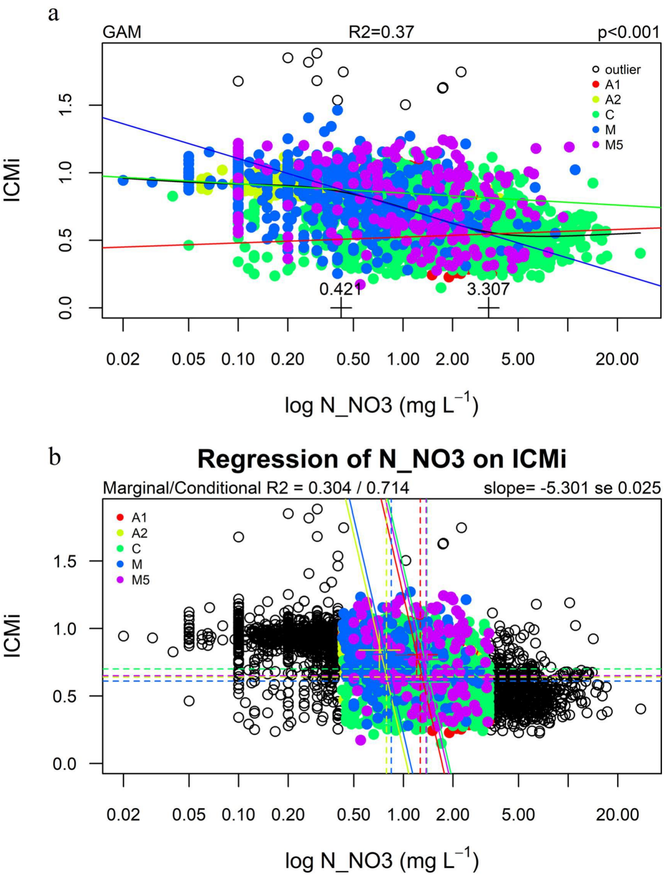

a
GAM
R2=0.37
p<0.001
outlier
A1
A2
C
M
M5
ICMi
0.421
3.307
log N_NO3 (mg L−1)
b
Regression of N_NO3 on ICMi
Marginal/Conditional R2 = 0.304 / 0.714
slope= -5.301 se 0.025
log N_NO3 (mg L−1)

Figure 4