



Estimating river nutrient concentrations consistent with good ecological condition: More stringent nutrient thresholds needed

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ABSTRACT

Nutrient pollution remains one of the leading causes of river degradation, making it important to set thresholds that support good ecological condition, which is the main objective of managing Europe's aquatic environment. A wide range of methods has been used by European member states to set river nutrient thresholds in the past, and these vary greatly among countries, even for similar river types. In some countries, thresholds have been set using expert judgement or the statistical distribution of nutrient concentrations. Application of such thresholds creates problems for planning strategies to achieve good ecological status and for managing transboundary river basins. An alternative approach is to examine the statistical relationship between nutrient concentration and one, or more, biological variables. Such relationships can then be used to inform decisions by water managers. We use such 'ecology-based' approaches (univariate regression and mismatch analyses) to derive nutrient thresholds for several river types in Central Europe. Our analysis focused on soluble reactive phosphorus (SRP) and total nitrogen (TN), two variables which were responsible for significant variation (40–55%) in river benthic floras. In this study, for the first time, river nutrient thresholds are estimated using both macrophytes and phyto-benthos (EQRs) separately and in combination, calculated as the minimum and the average of the EQRs of the two sub-elements. The resulting thresholds supporting good ecological status range from 21 to 42 µg/L SRP and 0.9–3.5 mg/L TN for the low alkalinity lowland river type, and 32–90 µg/L SRP and 1.0–2.5 mg/L TN for the low alkalinity mid-altitude river type. These targets are compared to the values set by member states. We demonstrate that some national nutrient thresholds fall within the range of predicted values if uncertainty is taken into consideration; however, several threshold values considerably exceed this range. Adopting ecology-based nutrient targets should improve sustainable river management where nutrients are the major pressure preventing the achievement of good ecological status.

1. Introduction

Although rivers are critical for providing provisioning (water and food), regulating (water purification, climate resilience) and cultural (recreation and tourism) ecosystem services, they are facing unprecedented threats from urbanization, agriculture and climate change (MEA, 2005; Vörösmarty et al., 2010; Reid et al., 2019) and are heavily

degraded throughout Europe. A recent analysis has shown that less than half (42%) of river stretches in Europe are in high and good ecological status, with the remainder divided between moderate (36%), poor and bad (17%) or unknown (5%) (EEA, 2018). Several pressures affect European rivers, often simultaneously: hydrological and morphological alteration, chemical contamination, microplastics, overexploitation and alien species (Schinegger et al., 2012; Grizzetti et al., 2017; Birk et al.,

Abbreviations: BQE, Biological Quality Element; EQR, Ecological Quality Ratio; SRP, soluble reactive phosphorus; TP, total phosphorus; TN, total nitrogen; WFD, Water Framework Directive.

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Table 1

Summary of datasets used for deriving relationships and nutrient thresholds. SRP = soluble reactive phosphorus; TP = total phosphorus; TN = total nitrogen. The river types are specified in Table 2.

River type code	Number of samples: Macrophytes			Number of samples: Phytobenthos			Range of values and [median]		
	SRP	TP	TN	SRP	TP	TN	SRP ($\mu\text{g/L}$)	TP ($\mu\text{g/L}$)	TN (mg/L)
R-C1	247	369	263	120	79	100	4-421 [28]	10-1400 [140]	0.04-14.6 [2.02]
R-C3	128	366	58	230	67	58	1-370 [40]	4-580 [66]	0.03-4.6 [0.624]
R-C4	432	129	334	436	81	304	1-3440 [66]	3-1460 [160]	0.2-15.5 [1.98]
R-C6	82	82	–	120	82	–	10-1600 [192]	3-1900 [240]	–

2020). The effects are further exacerbated by climate change (Charlton et al., 2018). However, nutrient pollution remains one of the leading causes of river degradation (EEA, 2018; Carvalho et al., 2019). The consequences of nutrient enrichment include increased algal and macrophyte biomass, changed dissolved oxygen and pH regimes, changes in composition and abundance of biological communities and food webs, and altered rates of nutrient and carbon cycling (Dodds and Welch, 2000; Hilton et al., 2006). These effects have major impacts on the delivery of ecosystem services and entail high economic costs (e.g. increases in water treatment costs and degradation of recreational opportunities; McDonald et al., 2016; Grizzetti et al., 2019).

The last decades have seen huge advances in aquatic ecological assessment. The Water Framework Directive (WFD, 2000/60/EC) has been a major driver for this in Europe, requiring assessment of four Biological Quality Elements (BQEs) in rivers (benthic flora, benthic invertebrates, fish fauna and, in large rivers with high retention time, phytoplankton). One hundred and forty river assessment methods have been intercalibrated (i.e. compared and harmonized) and included in the monitoring programs of European countries (Kelly et al., 2009; Poikane et al., 2014, 2020). One half of the methods are based on primary producers and target nutrient enrichment: macrophytes (e.g., Haury et al., 2006), phytobenthos (e.g., Várbbíró et al., 2012), and phytoplankton (e.g., Mischke et al., 2011).

However, although ecological assessment is crucial, it is only a first step toward reaching good status. Once the causes of ecological degradation have been identified, it is then necessary to set management targets for good ecological status and implement a remediation program. Recent reviews of the WFD implementation have identified problems in these later stages (Carvalho et al., 2019).

A potential solution to this difficult problem could involve (i) establishing causal links between stressors and appropriate biological variables. If the stressor of interest is a nutrient, then plant-based BQEs that respond directly to increased nutrients are most relevant to address, rather than higher trophic levels; and, (ii) establishing scientifically sound empirically-based management targets, such as setting nutrient concentrations consistent with good ecological status. Many different approaches have been proposed for setting nutrient targets, ranging from whole-population and reference-population percentiles (Chambers et al., 2008) to linking nutrient targets to predetermined biological outcomes (e.g., phytobenthos biomass; Dodds et al., 1997) and identifying a change point in the nutrient pressure-response models (Smith and Tran, 2010). However, each of these approaches has its own shortfalls, often associated with subjective factors embedded in each of them (Wang et al., 2007; Chambers et al., 2012).

Recently, technical guidance has been developed to enable countries to establish or review thresholds for phosphorus and nitrogen to support good ecological status (Phillips et al., 2018). This guidance describes

statistical methods for determining concentrations appropriate to support ecological status and facilitates the establishment of comparable and consistent boundaries across all European member states. So far, this approach has been applied to lakes (Poikane et al., 2019a), coastal and transitional waters (Salas Herrero et al., 2019) and to synthetic datasets, in order to elucidate the most appropriate approaches under different conditions (Phillips et al., 2019). However, recent analysis revealed that the biggest problems are associated with rivers (Poikane et al., 2019b). A wide range of methods has been used by countries to set river nutrient thresholds, and, consequently, these thresholds are highly variable among countries, even in rivers of a similar type. In some countries, thresholds were set using expert judgement or statistical distributions of nutrient concentrations with no consideration of ecological status. Such nutrient criteria create problems for planning strategies to achieve good ecological status and for managing transboundary river basins.

To date, the problem of setting river nutrient thresholds consistent with good ecological status has received scant attention in the research literature and there has been no investigation of nutrient targets for transnational rivers in Europe. Therefore, the aims of this study are to:

- explore relationships between nutrients and nutrient sensitive-BQEs in rivers of the Central-Baltic ecoregion of Europe;
- derive nutrient thresholds consistent with good ecological status where possible; and,
- compare the thresholds with the nutrient boundaries in use by countries and to discuss the implications of chosen thresholds.

In addition, we provide a comprehensive overview of ecologically-derived nutrient thresholds in rivers that could be useful when setting nutrient targets.

2. Material and methods

2.1. Data

We used data from the Central-Baltic region of Europe, which were collated for the intercalibration of biological assessment methods (Table 1). As required by the WFD, European member states established biological assessment methods for macrophytes and phytobenthos in rivers (Birk et al., 2012). National assessments are expressed as Ecological Quality Ratios (EQR), calculated as a ratio between observed and expected (at reference conditions) metric values, ranging from 1 (near-natural condition) to 0 (the worst possible ecological condition). At first, high-good and good-moderate class boundaries are established by countries following the WFD normative definitions (Annex V, 1.2), afterwards they are intercalibrated and harmonized between countries

Table 2

Description of Central-Baltic ecoregion shared river types (Bennett et al., 2011).

River type	Type code	Catchment area (km^2)	Altitude (m a.s.l.)	Alkalinity (meq/L)	Channel substrate, bank width
Small low alkalinity lowland	R-C1	10–100	<200	<1.0	Sand, 3–8 m
Small low alkalinity midaltitude	R-C3	10–100	200–800	<0.4	Boulders, cobbles and gravel, 2–10 m
Medium-sized mixed alkalinity lowland	R-C4	100–1000	<200	>0.4	Gravel and sand, 8–25 m
Small lowland calcareous	R-C6	10–300	< 200	>2.0	Gravel, 3–10 m

Table 3
Description of member states macrophyte and phytobenthos assessment methods.

Country/ biological quality element	The name of the method	Reference
<i>Macrophytes</i>		
Denmark	Danish Stream Plant Index (DSPI)	Søndergaard et al., 2013
Luxembourg	Biological Macrophyte Index for Rivers (IBMR)	Haury et al., 2006
Netherlands	Revised assessment method for rivers in The Netherlands using macrophytes (includes species composition metrics and growth forms abundance metrics)	Pot and Birk, 2015
Poland	Polish Macrophyte Index for Rivers (MIR)	Szozkiewicz et al., 2006b
UK	Ecological Classification of Rivers using Macrophytes LEAFPACS2 (includes River Macrophyte Nutrient Index, Number of functional groups, Number of macrophyte taxa, and Filamentous algal cover)	Willby et al., 2012
<i>Phytobenthos</i>		
Austria	Multimetric Index (including Trophic index, Saprobic index and Reference index)	Pfister and Pipp, 2010
Luxembourg	Indice de Polluosensibilité Spécifique (IPS)	Coste, in CEMAGREF, 1982
Netherlands	EKR (Ecologische Kwaliteitsratio) based on proportions of positive and negative indicator taxa	Van der Molen, 2004
Poland	Diatom Index for rivers (Avg of Trophic index and Saprobic index)	Picińska-Faltynowicz, 2009; Rott et al., 1997, 1999
UK	Revised Trophic Diatom Index (TDI)	Kelly et al., 2008

in order to ensure that the boundaries are consistent across the EU (Kelly et al., 2009; Birk and Willby, 2010; Poikane et al., 2014). The biological data used in this study were normalised national EQR values (i.e. status class boundaries adjusted to 0.8, 0.6, 0.4 and 0.2; for a detailed description of the normalization procedure, see EC, 2011 (p.78) and Moe et al., 2015). National datasets were grouped into WFD intercalibration common river types, defined by their basin size, altitude, dominant geology, alkalinity and substrate (Table 2).

We examined the nutrients: total phosphorus (TP), soluble reactive phosphorus (SRP) and total nitrogen (TN). Sampling frequencies ranged from single (spot) to annual means based on monthly measurements.

Table 4

Coefficients of determination (r^2) resulting from linear regression analysis between nutrients, Biological Quality Elements, and their combinations. Relationships used for setting of the nutrient thresholds are given in bold. TP = total phosphorus; SRP = soluble reactive phosphorus; TN = total nitrogen; n.s. = relationship non-significant.

River type	Nutrient	Macrophytes	Phytobenthos	Macrophytes and phytobenthos combined (avg)	Macrophytes and phytobenthos combined (min)
R-C1	TP	0.00 (n.s.)	0.20**	0.03 (n.s.)	0.04 (n.s.)
	SRP	0.41**	0.42**	0.49**	0.48**
	TN	0.40**	0.49**	0.55**	0.65**
R-C3	TP	0.09**	0.14**	0.15**	0.11**
	SRP	0.40**	0.43**	0.48**	0.54**
	TN	0.49**	0.53**	0.46**	0.48**
R-C4	SRP	0.18**	0.06**	0.22**	0.27**
	TP	0.00	0.01 (n.s.)	0.00	0.01 (n.s.)
	TN	0.09**	0.03*	0.08**	0.08**
R-C6	TP	0.08**	0.10**	0.18**	0.12**
	SRP	0.02 (n.s.)	0.00	0.11**	0.04 (n.s.)

* $p < 0.05$; ** $p < 0.01$

Austria provided 90th percentile values; these were halved before being included in the analysis.

A variety of approaches have been adopted for macrophyte assessment (Table 3), all of them including an index of the sensitivity of macrophytes to nutrient enrichment, i.e. the average score of indicative species weighted by their abundance and, in some cases, also the taxon's ecological tolerance (Haury et al., 2006; Szozkiewicz et al., 2006a; Willby et al., 2012). In addition to sensitivity measures, there are several country-specific metrics. The Dutch method includes a metric for the abundance of growth forms (Pot and Birk, 2015), while the UK method includes richness of macrophyte growth forms, macrophyte taxa richness and cover of green filamentous algae (Willby et al., 2012). All indices are directed at eutrophication and significant relationships with nutrients (Birk and Willby, 2010; Birk et al., 2012). Most countries use a measure of phytobenthos (Table 3), with diatom indices based on a weighted-average approach and optimized against gradients of nutrients (Rott et al., 1997, 1999; Kelly et al., 2008) or general degradation (Coste, in CEMAGREF, 1982). An exception is the Dutch index EKR, which is based on proportions of positive and negative diatom indicator taxa (Van der Molen, 2004).

2.2 Methods for estimating nutrient threshold supporting good ecological status

Technical guidance has been developed that describes statistical methods for determining appropriate concentrations to support good ecological status along with a toolkit (Phillips et al., 2018), which provides the statistical models in the form of R scripts (R Core Team, 2019). We selected two approaches described in this guidance, following the experiences gained by testing different approaches with synthetic datasets (Phillips et al., 2019):

- 1) Univariate linear regression between EQR and nutrient concentrations, with nutrient threshold values predicted by the regression models. Type II regression (Ranged Major Axis regression; RMA) was used, which assumes equal uncertainty in measurement of both EQR and nutrients. Segmented regression methods were used to test for linearity within the data set.
- 2) The 'minimization-of-mismatch' approach determines the nutrient concentration that gives the lowest mismatch between classification based on biology and on nutrient concentration. The boundaries predicted by our analysis were compared with the boundaries set by countries (for overview and details see Poikane et al., 2019a).

In addition to estimating nutrient thresholds for macrophytes and phytobenthos separately, the average and minimum of the EQRs of the

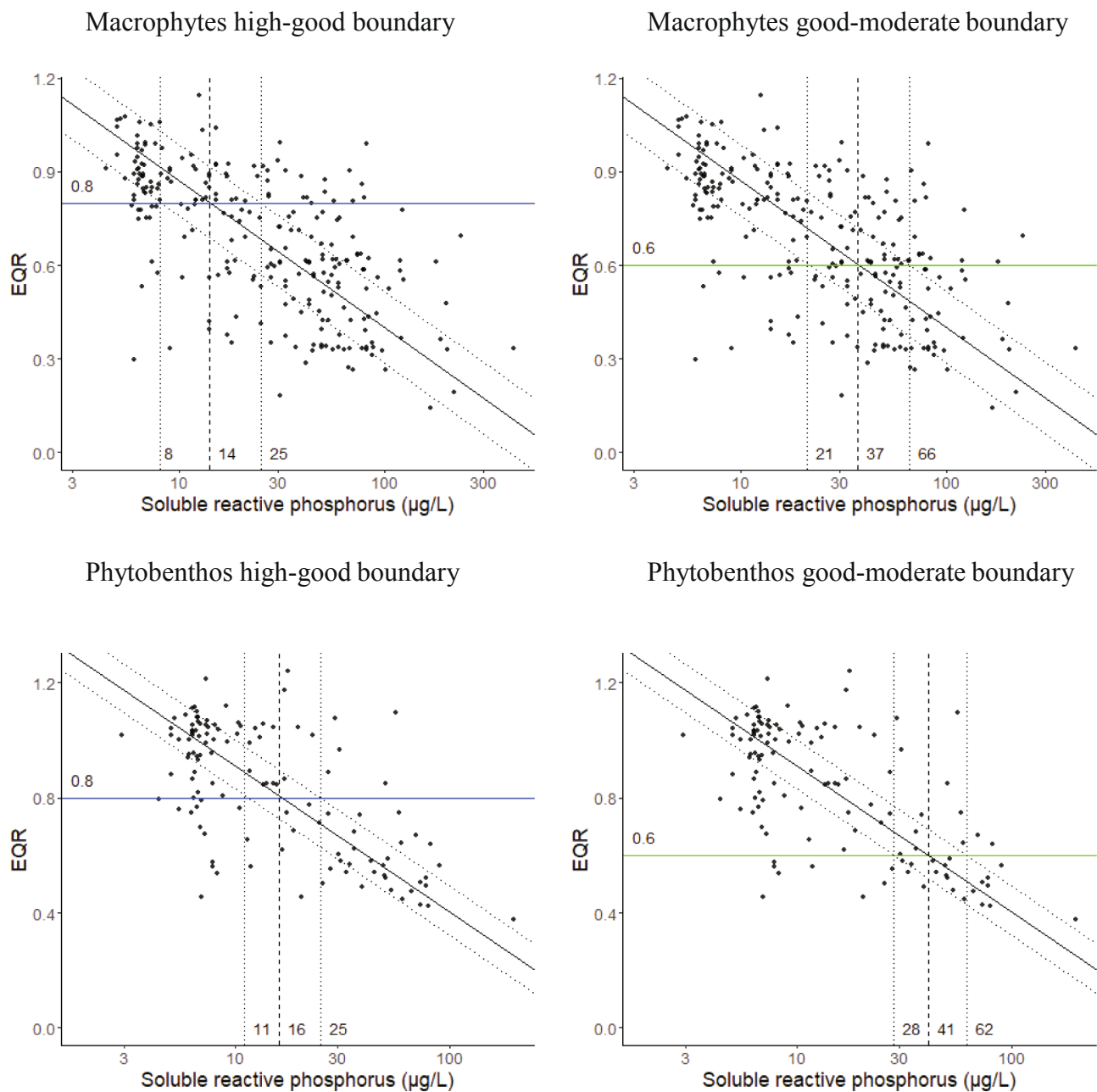


Fig. 1. Relationship between Ecological Quality Ratios (EQR) for macrophytes (upper block) and phytoplankton (lower block) with soluble reactive phosphorus for low alkalinity lowland rivers (Type R-C1) showing high-good boundary (left) and good-moderate boundary (right) values. Solid line shows type II RMA regression, dotted lines show area containing 50% of the data.

two organism groups were used.

3. Results

3.1. Relationships

In low alkalinity streams (R-C1 and R-C3), SRP and TN were strongly related to macrophyte and phytoplankton assessments (both singly and in combination). The relationship with TP was weaker or non-significant, as was the relationship to nutrients in higher alkalinity types. We therefore did not pursue the threshold analysis for this nutrient parameter and these types (Table 4).

3.2. Univariate regression models

For low alkalinity lowland rivers (R-C1), the relationship between SRP and Macrophyte EQR predicted a concentration for the good-moderate boundary of 37 µg/L, with 50% of the data having values between 21 and 66 µg/L (Fig. 1). Results for the high-good boundary were 14 (range 8–25) µg/L SRP. The univariate relationships between SRP and phytoplankton predicted a good-moderate threshold of 41 µg/L SRP (range: 28–62 µg/L) and high-good threshold of 16 µg/L SRP (range: 11–25 µg/L) (Fig. 1). The results for the R-C3 type is included in the [Supplementary material](#).

The relationships between TN and macrophytes predicted a good-moderate threshold of 1.47 mg/L with a range of 0.59–3.45 mg/L and high-good threshold of 0.39 (range: 0.16–0.91) mg/L TN.

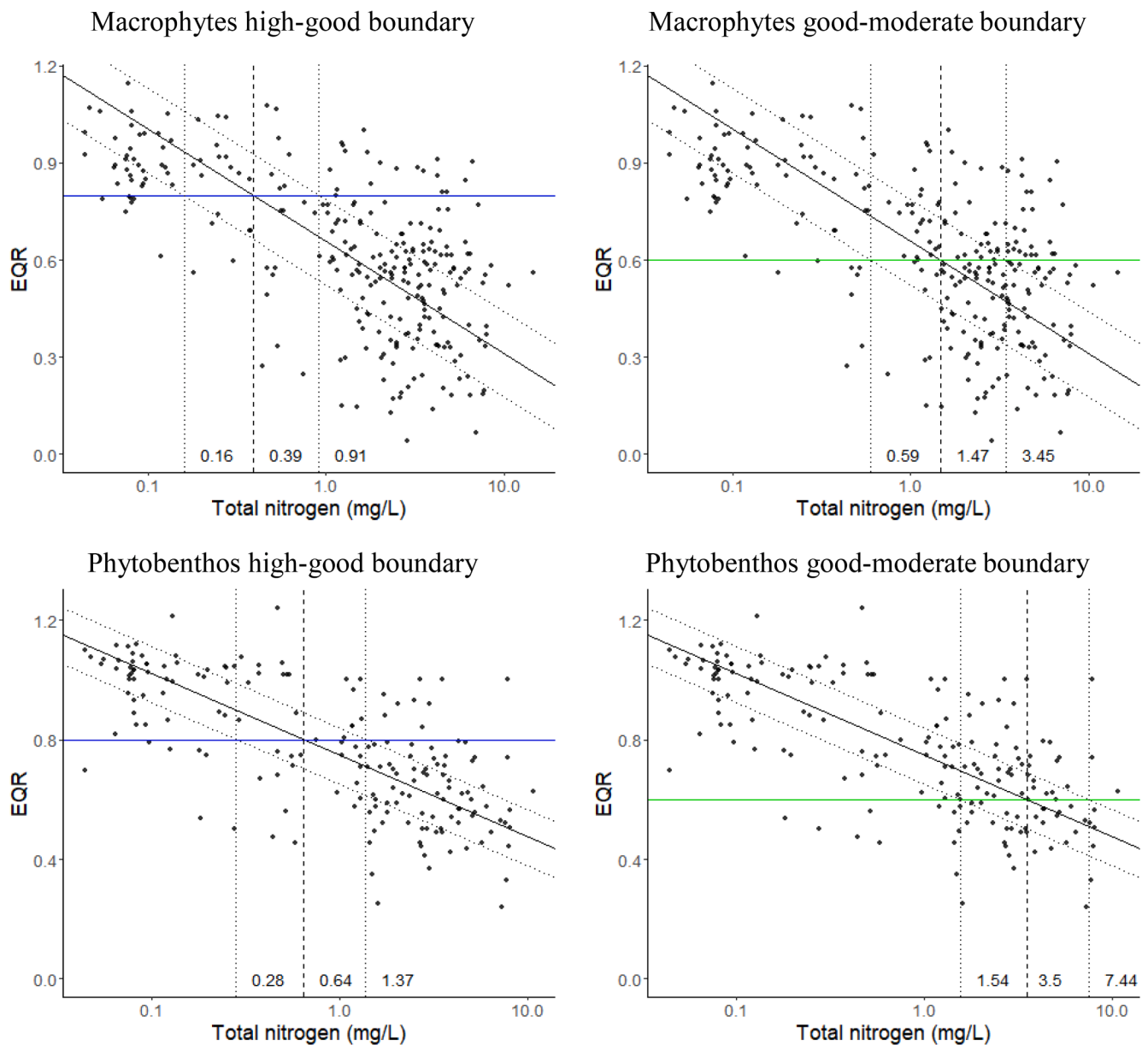


Fig. 2. Relationship between Ecological Quality Ratios (EQR) for macrophytes (upper block) and phytobenthos (lower block) with total nitrogen for low alkalinity lowland rivers (Type R-C1) showing high-good boundary (left) and good-moderate boundary (right) values. Solid line shows type II RMA regression, dotted lines show area containing 50% of the data.

Relationships for TN and phytobenthos produced a good-moderate threshold of 3.5 mg/L (range: 1.54–7.44 mg/L) (Fig. 2). The high-good criteria were 0.64 mg/L TN (range: 0.28–1.37) mg/L.

The combined macrophyte/phytobenthos models (calculated as the minimum of the EQRs of the two organism groups) gave the most stringent predictions: good-moderate criteria of 32 $\mu\text{g/L}$ SRP (range: 18–58 $\mu\text{g/L}$) and 1.63 mg/L TN (range: 0.71–4.19 mg/L) (Fig. 3).

The results for both river types and all BQEs are summarised in Table 5 and 6.

3.3. Minimise the mismatch between classifications based on biology and nutrient concentration

For the R-C1 type using macrophytes, the values for good-moderate thresholds were 42 $\mu\text{g/L}$ SRP and 2.21 mg/L TN (Fig. 4), while the high-good class thresholds were 19 $\mu\text{g/L}$ SRP and 0.85 mg/L TN. For the phytobenthos assessment the good-moderate thresholds were at 32 $\mu\text{g/L}$

SRP and 2.86 mg/L TN (Tables 5 and 6).

3.4. Comparison with nutrient boundaries in use by countries

For SRP, countries have set good-moderate class boundaries ranging from 90 to 100 $\mu\text{g/L}$ SRP for R-C1 and 70 to 130 $\mu\text{g/L}$ SRP for R-C3, expressed as annual mean or vegetation season mean (Phillips and Pitt, 2016). Thresholds expressed as annual 90th percentile equal to 160 $\mu\text{g/L}$ SRP for R-C1 and 20–160 $\mu\text{g/L}$ SRP for R-C3.

For TN, countries have set good-moderate boundaries ranging from 2.3 to 10.0 mg/L for R-C1, similar values (i.e. 2.0–10.0 mg/L) for the R-C3 type, expressed as annual mean or vegetation season mean (Poikane et al., 2019b; Phillips and Pitt, 2016).

A comparison with our results (Table 5 and 6) shows the following:

- Several of the reported boundary values are within the range of the predicted values (e.g., 70 $\mu\text{g/L}$ SRP or 2 mg/L TN);

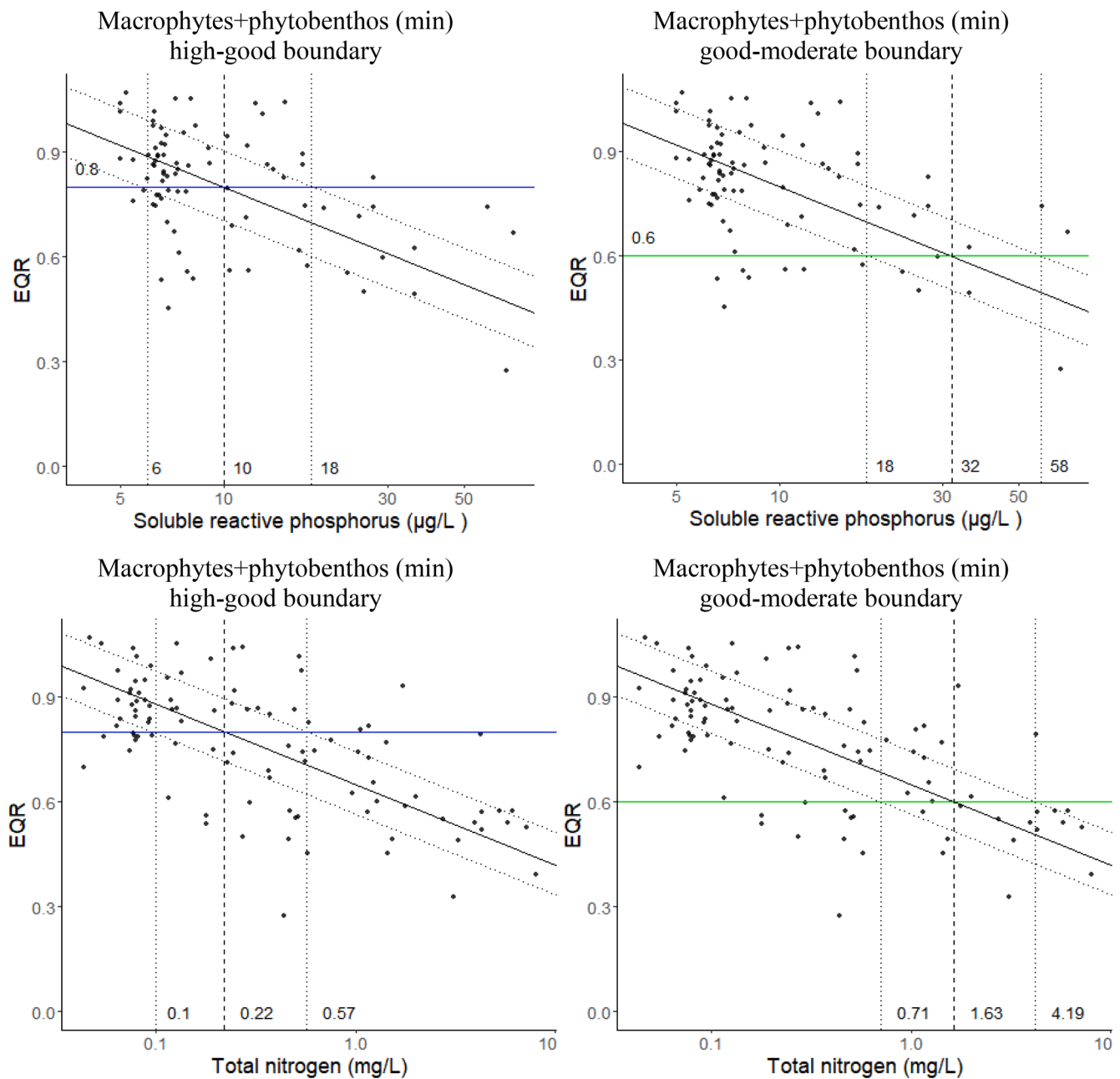


Fig. 3. Relationship between combined macrophyte and phytobenthos Ecological Quality Ratios (EQR) (minimum) with soluble reactive phosphorus (upper block) and total nitrogen (lower block) for low alkalinity lowland rivers (Type R-C1) showing high-good boundary (left) and good-moderate boundary (right) values. Solid line shows type II RMA regression, dotted lines show area containing 50% of the data.

- However, some of these boundary values exceed this range significantly (e.g., 130 µg/L SRP or 10 mg/L TN);
- There is a particularly strong difference for the R-C1 type and SRP values where all values set by countries (90–100 µg/L SRP) are considerably higher than SRP values supporting good ecological status established by our analysis (21–42 µg/L SRP).

4. Discussion

4.1. Setting nutrient thresholds in rivers

Nutrient enrichment may adversely affect both structure and function of stream ecosystems. Therefore, river ecologists and managers need to identify nutrient thresholds that will ensure risks of ecological impairment are low. In this study, we used relationships between

nutrient-sensitive organism groups (macrophytes and phytobenthos) and nutrient concentrations to identify nutrient thresholds consistent with good ecological status. The most robust relationships were found for SRP and TN in low alkalinity rivers explaining 40–55% of variance. The resulting thresholds range from 21 to 42 µg/L SRP and from 0.9 to 3.5 mg/L TN for the low alkalinity lowland river type, and from 32 to 90 µg/L SRP and from 1.0 to 2.5 mg/L TN for the low alkalinity mid-altitude river type.

There are many ways to estimate nutrient threshold levels in rivers. The most common methods include (i) the percentiles approach, e.g. using the 25th percentile of the whole-population (which assumes that the best available values are appropriate) or 75th percentile of reference sites (Stevenson et al., 2008); (ii) approaches based on ecological effects of nutrients to the river ecosystem (Smucker et al., 2013). The percentile approach has been criticized for disregarding ecological response and

Table 5

Summary of predicted soluble phosphorus (SRP) high-good and good-moderate class boundaries ($\mu\text{g/L}$) for low alkalinity lowland rivers (R-C1) and low alkalinity upland rivers (R-C3). The table includes the values predicted by the linear regression model (in brackets: range defined as 25th and 75th percentiles of the residuals of the linear model). For the mismatch analyses the nutrient concentration is shown that yielded the lowest mismatch between classifications based on biology and nutrient concentration (in brackets: the 25th and 75th percentiles of cross-over values calculated from the bootstrap ($n = 500$) replicates).

River type	Method used	Macrophytes	Phytobenthos	Macrophytes and phytobenthos (average)	Macrophytes and phytobenthos (minimum)
<i>High-good class boundary</i>					
R-C1	Linear regression	14 (8–25)	16 (11–25)	12 (7–21)	10 (6–18)
	Mismatch	19 (17–22)	13 (11–15)	11 (10–13)	9 (8–9)
R-C3	Linear regression	12 (5–29)	21 (11–41)	17 (8–29)	8 (4–16)
	Mismatch	14 (12–18)	20 (16–23)	18 (15–25)	9 (7–11)
<i>Good-moderate class boundary</i>					
R-C1	Linear regression	37 (21–66)	41 (28–62)	35 (20–45)	32 (18–58)
	Mismatch	42 (37–49)	32 (24–42)	26 (22–30)	21 (17–28)
R-C3	Linear regression	90 (40–207)	65 (34–123)	78 (37–135)	40 (19–78)
	Mismatch	70 (50–95)	70 (59–87)	81 (62–98)	32 (28–38)

Table 6

Summary of predicted total nitrogen (TN) high-good and good-moderate class boundaries (mg/L) for low alkalinity lowland rivers (R-C1) and low alkalinity upland rivers (R-C3). See Table 5 for further explanation.

River type	Method used	Macrophytes	Phytobenthos	Macrophytes and phytobenthos (average)	Macrophytes and phytobenthos (minimum)
<i>High-good boundary</i>					
R-C1	Linear regression	0.39 (0.16–0.91)	0.64 (0.28–1.37)	0.36 (0.18–0.68)	0.22 (0.10–0.57)
	Mismatch	0.85 (0.61–1.05)	0.71 (0.62–0.83)	0.72 (0.60–0.91)	0.26 (0.20–0.31)
R-C3	Linear regression	0.50 (0.24–1.05)	0.84 (0.43–1.73)	0.70 (0.4–1.7)	0.38 (0.22–0.85)
	Mismatch	0.51 (0.40–0.66)	0.94 (0.78–1.11)	0.60 (0.50–0.80)	0.40 (0.34–0.47)
<i>Good-moderate class boundary</i>					
R-C1	Linear regression	1.47 (0.59–3.45)	3.50 (1.54–7.44)	2.00 (1.00–3.30)	1.63 (0.71–4.19)
	Mismatch	2.21 (1.80–2.40)	2.86 (2.30–3.12)	1.76 (1.500–2.00)	0.90 (0.60–1.10)
R-C3	Linear regression	2.44 (1.18–5.10)	2.48 (1.27–5.12)	2.00 (1.00–4.40)	1.79 (1.02–4.04)
	Mismatch	1.80 (1.30–2.30)	1.72 (1.44–1.89)	1.80 (1.40–2.30)	1.04 (0.96–1.12)

providing over- or underprotective criteria which might mislead river managers (Chambers et al., 2012; Smucker et al., 2013). In contrast, criteria based on ecological effects provide information on nitrogen and phosphorus concentrations associated with low ecological impairment (Hausmann et al., 2016; Charles et al., 2019) and serve as benchmarks for assessing and managing water resources.

The ecology-based approaches fall in two categories: (i) linking nutrient concentrations to a predefined ecological condition (typically, a certain level of the benthic algal biomass or composition-based metric, Chambers et al., 2012; Dodds and Welch, 2000, Charles et al., 2019), and (ii) identifying a change-point in the relationships between nutrients and biological variables (Smith and Tran, 2010; Stevenson et al., 2008; Tibby et al., 2019). However, these two approaches both have drawbacks. Using the first approach, the nutrient threshold will depend on the choice of biological variable and on the definition of acceptable limits. For benthic chlorophyll-a concentrations, for instance, acceptable limits range from 50 to 200 mg/m^2 (Chambers et al., 2012), leading to large differences in corresponding nutrient thresholds (Dodds and Welch, 2000). The change-point approach seems to be more objective; however, the result depends on (i) the analytical approach employed, as the threshold values can differ depending on the choice of statistical method (e.g. non-parametric change-point analysis, regression tree analysis or other; Wang et al., 2007; Smucker et al., 2013) and (ii) biological communities and variables used for analysis (Wang et al., 2007; 2008; Zheng et al., 2008; Chen et al., 2018). In addition, more than one change-point might be identified in some relationships (Stevenson et al., 2008; Smucker et al., 2013), creating a dilemma about the choice of the right threshold. Also, the change-point identified might be located at the very beginning or at the higher end of the nutrient

gradient. For instance, Stevenson et al. (2008) found TP thresholds between 10 and 12 $\mu\text{g/L}$ TP; however, these levels coincide with reference conditions, so protection of all streams with this threshold was deemed impractical.

We followed the former approach in this study, primarily because the WFD requires member states to set thresholds in order to support good ecological status. The main advantage of this approach is that ecological status boundaries are set according to WFD definitions and then harmonised amongst EU member states to give a consistent framework for setting ecologically-meaningful nutrient thresholds.

We found that thresholds differed depending upon whether phytobenthos, macrophytes or a combination was used (Table 4). Differences between thresholds obtained using macrophytes and phytobenthos may occur because they have access to different pools of nutrients (water and/or sediment) and also because they respond at different rates to changes in the nutrient concentration. In theory, the organism group most sensitive to phosphorus should be used, but model strength also needs to be taken into account, as well as a recognition of their different contributions to ecosystem processes. Models that use both macrophytes and phytobenthos are stronger than those that use one or the other. Kelly et al. (2020) recommend the use of the average of the two organism groups rather than the minimum, as this approach incorporates information from both organism groups toward the final threshold.

4.2. Relationships between phytobenthos and macrophyte communities and nutrients in rivers

Pressure-response relationships describe the effect of different levels of pressure (nutrients in this case) on components of an ecosystem and

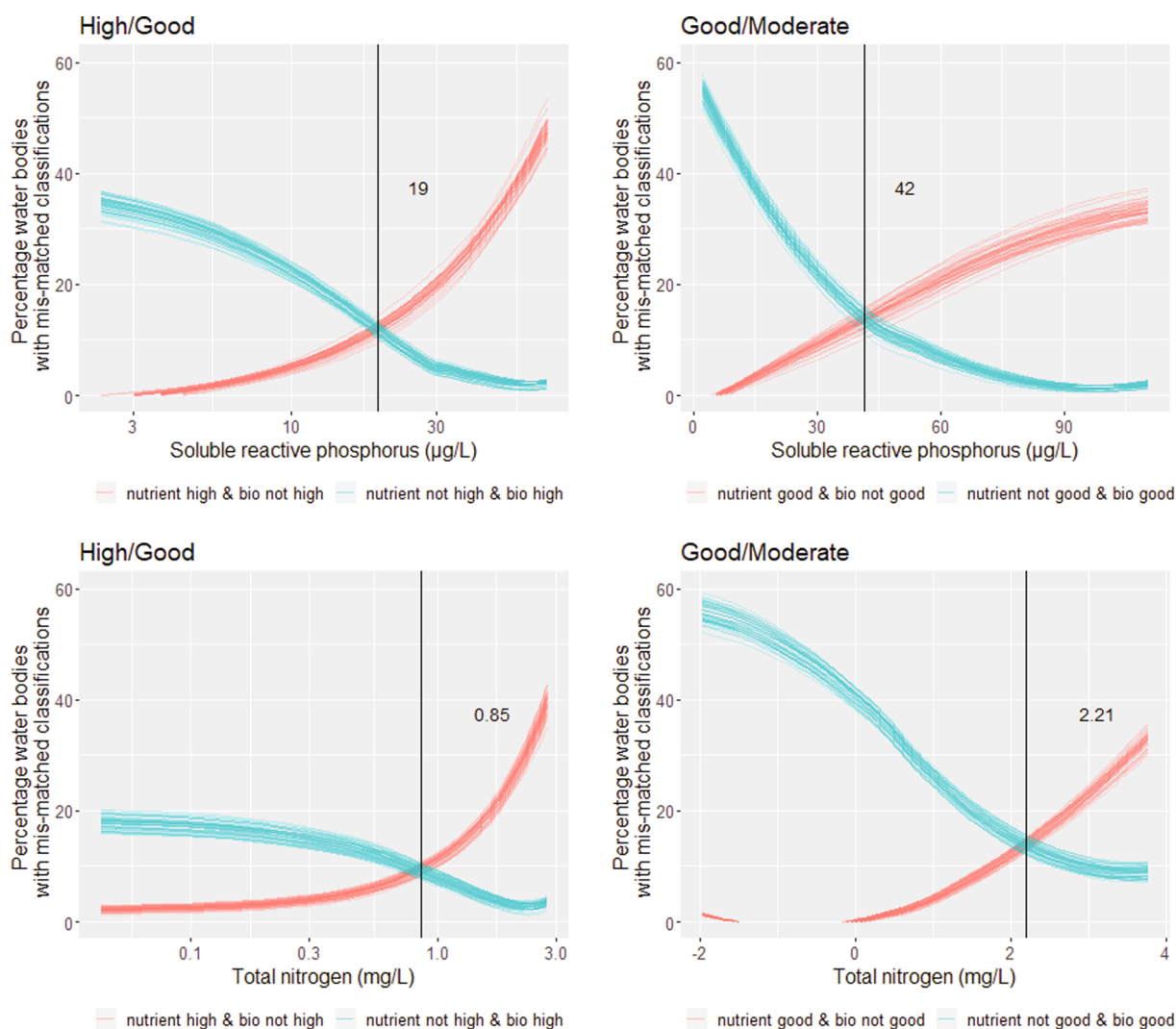


Fig. 4. Percentage of water bodies at low alkalinity lowland rivers (Type R-C1) where either the macrophyte assessments or soluble reactive phosphorus classifications for good ecological status differ in comparison to the level used to set the high-good boundary (left) and good-moderate boundary (right). Vertical lines mark the intersection of curves where mis-match is minimized and equal.

can be used to identify thresholds associated with good ecological condition (Stevenson et al., 2008; Chambers et al., 2012). However, such models tend to have low explanatory power, especially in rivers due to a looser coupling of ecological response to nutrient loading in rivers, than in lakes. The difference between lotic and lentic response is due to a number of factors: physical controls - channel morphology and substrate, flow velocity, flow regime, and light availability (including riparian shading and turbidity) (Dodds et al., 2002; Hilton et al., 2006).

In this study, we found moderately strong relationships between nutrients (SRP, TN) and primary producers (macrophytes and phyto-benthos) in low alkalinity streams R-C1 and R-C3 ($r^2 = 0.40\text{--}0.65$, $p < 0.001$, Table 4) while the relationship with TP in these stream types was weak ($r^2 = 0.09\text{--}0.20$, $p < 0.001$) or non-detectable. In all other river types, characterized by higher alkalinity, relationships between primary producers and all nutrients (SRP, TP and TN) were weak ($r^2 = 0.03\text{--}0.27$, $p < 0.001$) or non-detectable.

Benthic algae play a central role in river eutrophication assessment because of their importance to primary production and strong links to nutrient concentrations (Potapova and Charles, 2007; Smucker et al., 2013; Poikane et al., 2016). Many studies have established relationships between nutrients and phyto-benthos metrics (Table 7). However, studies have found that indices based on species composition, particularly diatom trophic indices, are more strongly linked to nutrients than

biomass or diversity (Porter et al., 2008; Stevenson et al., 2008). The relationship between algal biomass and nutrient concentration is complex, as it is strongly shaped by other factors, such as current velocity, turbidity, riparian shading, grazing and scouring by floods (Biggs, 2000; Dodds et al., 2002; Stevenson et al., 2006, 2008). For these reasons, diatom composition metrics are the most widely-used phyto-benthos variables, also included in our study (Table 3). In general, studies have demonstrated variable results ranging from very weak to strong relationships (Table 7). The range in sensitivity could result from a number of issues: First, diatom composition metrics are calibrated against different gradients depending on the study. For example, some are calibrated to nutrients (e.g. TDI: Kelly et al., 2008), while others are calibrated to general degradation (e.g. IPS, Coste in CEMAGREF, 1982). The latter, whilst often including some correlation with nutrient concentrations, will also reflect other stressors. Both types, in addition, are influenced by natural gradients unrelated to nutrients, particularly current velocity, turbidity (Soininen, 2005) and alkalinity (Kelly et al., 2008; Lavoie et al., 2014). Similarly, metrics such as the TDI are usually calibrated against phosphorus concentration. Stoichiometric calculations generally suggest phosphorus, rather than nitrogen, is more likely to be the limiting nutrient. Therefore, questions need to be asked about the validity of using such metrics for setting nitrogen thresholds. In reality, nitrogen and phosphorus concentrations are often correlated

Table 7

Relationships between phytobenthos variables and nutrients. TP = Total phosphorus; SRP = Soluble reactive phosphorus; TN = Total nitrogen; SIN = Soluble inorganic nitrogen; NO₃ = Nitrate; TON = Total organic nitrogen; n.s. = not significant.

Reference	Biological metric vs nutrient metric	Coefficient of determination (r ²)
<i>Phytobenthos abundance</i>		
Biggs, 2000 (New Zealand)	Mean/max benthic chl-a vs SRP	0.23/0.29
Chambers et al., 2008 (Ontario and Quebec, Canada)	Mean/max benthic chl-a vs SIN	0.12/0.32
	Benthic chl-a TN	0.46
Chambers et al., 2012 (Ontario and Quebec, Canada)	Benthic chl-a TP	n.s.
	Benthic chl-a vs TP	0.15
Chételat et al., 1999 (Ontario and Quebec, Canada)	Benthic chl-a vs TN	0.45
	Benthic chl-a vs TP	0.56
Demars et al., 2012 (Eastern England)	Benthic chl-a vs TN	0.50
	<i>Cladophora</i> biomass vs TP	0.53
Dodds et al., 1997 (N. America and New Zealand)	<i>Melosira</i> biomass vs TP	0.64
	Abundance of filamentous algae vs SRP	0.08
Dodds et al., 2002 (National Stream Water-Quality Monitoring)	Mean/max benthic chl-a vs TP	0.09 / 0.07
	Mean /max benthic chl-a vs SRP	0.05 / n.s.
Smucker et al., 2013 (Connecticut, US)	Mean / max benthic chl-a vs TN	0.35 / 0.28
	Benthic chl-a vs TP	0.03
Stevenson et al., 2006 (North-Central region, US)	Benthic chl-a vs TN	0.06
	Benthic chl-a vs NO ₃	0.08
Stevenson et al., 2008 (Mid-Atlantic Highlands region, US)	Benthic chl-a NO ₂₊₃ (NO _x)	0.14
	Benthic chl-a TP	0.1
Willby et al., 2012 (United Kingdom)	Benthic chl-a vs TP	0.04–0.17
	Benthic chl-a vs TN	0.07–0.19
Stevenson et al., 2008 (Mid-Atlantic Highlands region, US)	<i>Cladophora</i> cover vs TP	0.12–0.45
	<i>Cladophora</i> cover vs TN	0.08–0.30
Willby et al., 2012 (United Kingdom)	<i>Cladophora</i> cover vs TP	0.08–0.30
	Benthic chl-a vs TP	n.s.
Almeida et al., 2014 (Mediterranean region)	Algal ash-free dry mass vs TP	0.04
	Algal cover vs SRP	0.06
Chambers et al., 2012 (Ontario and Quebec, Canada)	Algal cover vs TON	0.05
	Benthic chl-a vs TN	0.07–0.19
Hlúbiková et al., 2007 (Slovakia)	<i>Cladophora</i> cover vs TP	0.12–0.45
	<i>Cladophora</i> cover vs TN	0.08–0.30
Kelly and Whitton, 1995 (UK)	Benthic chl-a vs TP	n.s.
	Algal ash-free dry mass vs TP	0.04
Kelly et al., 2008 (UK)	Algal cover vs SRP	0.06
	Algal cover vs TON	0.05
Lavoie et al., 2008 (Eastern Canada)	Diatom trophic and diversity metrics	
	ICM (IPS and TI) vs TP	0.18
Lavoie et al., 2014 (Eastern Canada)	ICM (IPS and TI) vs SRP	0.37
	ICM (IPS and TI) vs NO ₃	0.06
Porter et al., 2008	Diatom metrics vs TP	0.40–0.43
	Diatom metrics vs TN	n.s.
Porter et al., 2008	Diatom diversity metrics vs TP	n.s.
	Diatom diversity metrics vs TN	0.17
Porter et al., 2008	Diatom indices vs TP	0.27–0.37
	Diatom indices vs TN	0.30–0.34
Porter et al., 2008	Trophic diatom index (TDI) vs SRP	0.63
	Trophic diatom index (TDI) vs TN	0.35
Porter et al., 2008	Trophic diatom index (TDI) vs SRP	0.29
	Trophic diatom index (TDI) vs NO ₃	0.41–0.79
Porter et al., 2008	Eastern Canadian Diatom Index (IDEC) vs TP	0.41–0.79
	IDEC vs TP	0.35
Porter et al., 2008	Diatom indices vs TP	0.04–0.32
	Diatom indices vs SRP	0.03–0.25

Table 7 (continued)

Reference	Biological metric vs nutrient metric	Coefficient of determination (r ²)
Potapova and Charles, 2007	Diatom indices vs TN	0.02–0.28
	High/Low-P indicators vs TP	0.27–0.56
Rott et al., 2003 (Austria)	High/Low-N indicators vs TN	0.16–0.73
	Austrian Trophic Index vs TP	0.72
Smith and Tran, 2010 (New York state, US)	Diatom metrics vs TP	0.03–0.5
	Diatom metrics vs TN	0.02–0.43
Smucker and Vis, 2009 (Ohio, US)	Diatom metrics vs TP	0.01–0.25
	Diatom similarity metrics vs SRP	0.18–0.19
Smucker et al., 2013 (Connecticut, US)	Diatom similarity metrics vs NO ₃	n.s.
	High/Low-P indicators vs SRP	n.s.
Stevenson et al., 2008 (Mid-Atlantic Highlands region, US)	High/Low-P indicators vs NO ₃	0.10
	High/Low-N indicators vs SRP	0.14
Várbró et al., 2012 (Hungary)	High/Low-N indicators vs NO ₃	0.13
	Diatom metrics vs NO ₂₊₃ (NO _x)	0.16–0.42
Vilbaste et al., 2007 (Estonia)	Diatom metrics vs TP	0.31–0.55
	Diatom diversity metrics vs TP	0.10
Zheng et al., 2008 (West Virginia, US)	Diatom metrics vs TP	0.05–0.33
	Diatom indices vs SRP	0.40
Zheng et al., 2008 (West Virginia, US)	Diatom indices vs TN	0.034–0.35
	Diatom indices: IPS vs SRP	0.12–0.18
Zheng et al., 2008 (West Virginia, US)	IBD vs SRP	0.13–0.20
	IPS vs TN	0.18
Zheng et al., 2008 (West Virginia, US)	IBD vs TN	n.s.
	IPS and IBD vs NO ₃	n.s.
Zheng et al., 2008 (West Virginia, US)	IBD vs NO ₃	n.s.
	Diatom indices vs TP	0.17–0.22
Zheng et al., 2008 (West Virginia, US)	Diatom indices vs NO _x	0.46–0.55
	Diatom diversity indices vs TP	0.02
Zheng et al., 2008 (West Virginia, US)	Diatom diversity indices vs NO _x	0.06–0.09

(Kelly et al., 2020) and, whilst this might be helpful for deriving nitrogen concentrations associated with good ecological status, it does not necessarily mean that there is a causal relationship.

Macrophytes respond not only to nutrient concentrations in the water, but also to sediment nutrients, shading, hydrological conditions and substrate (Szoszkiewicz et al., 2006b; Wiegler et al., 2016). In addition, other anthropogenic pressures such as hydrological alteration, herbicide application, and weed-cutting practices may have considerable effect on macrophyte communities (Baatrup-Pedersen et al., 2002, 2003). For these reasons, establishing direct links between macrophyte communities and nutrients has been problematic. Therefore, different approaches have been used, for instance, relationships to land-use metrics (Kuhar et al., 2011), composite pressure gradients (Aguilar et al., 2011; Dodkins et al., 2012) or simple categorical comparisons between impacted and non-impacted sites (Aguilar et al., 2009).

While many studies have tried to establish pressure-response relationships (Table 8), these studies have primarily used macrophyte composition indices that claim to detect nutrient pollution, such as the macrophyte Mean Trophic Rank (Holmes et al., 1999) or IBMR (Haury et al., 2006). Only a few studies attempt to establish relationships with abundance measures (Willby et al., 2012) and these are typically weaker than relationships with composition metrics. The strength of macrophyte-nutrient relationships range from weak or non-significant to moderately strong (up to r² = 0.48–0.56; Willby et al., 2012). In our study, strong relationships were observed (explaining 41 to 49% of the biological variance) whilst in high or mixed alkalinity streams the explained variance was lower (8 to 18%).

The use of dissolved versus total nutrient forms has generated much discussion, with no clear conclusions (Dodds et al., 1997; Dodds and Welch, 2000; Hilton et al., 2006; Wagenhoff et al., 2017). Some studies

Table 8

Relationships between macrophytes variables and nutrients. TP = Total phosphorus; SRP = Soluble reactive phosphorus; TN = Total nitrogen; NO₃ = Nitrate; TON = Total organic nitrogen.

Reference	Biological metric vs nutrient metric	Coefficient of determination (r ²)
<i>Macrophytes trophic indices</i>		
Aguiar et al., 2011 (Portugal, western Iberia)	Mean Trophic Rank (MTR) vs SRP, NO ₃	0.12–0.16
	MTR vs TP, TN	0.09–0.12
	Riparian vegetation index (RVI) vs TP, TN, SRP	0.09–0.12
Demars et al., 2012 (North-east France, Eastern England)	Macrophyte Biological Index for Rivers (IBMR) vs SRP	0.32
	IBMR vs SRP (after removing the effects of pH)	0.08
	IBMR vs NO ₃	0.002
	River macrophyte nutrient index (RMNI) vs SRP and bioavailable P in sediments	n.s.
Demars and Harper, 1998 (England)	Mean Trophic Rank (MTR) vs SRP	0.34
	MTR vs NO ₃	0.18
Fabris et al., 2009 (Germany)	Reference index (RI) vs SRP	0.18
	RI vs TP	0.05
	Trophic index of Macrophytes (TIM) vs SRP	0.41
	TIM vs TP	0.21
Flor-Arnau et al., 2015 (Spain)	Macrophyte Fluvial Index (IMF) vs SRP	0.19
	IMF vs NO ₃	0.20
Suárez et al., 2005 (Spain)	Index of macrophytes (IM) vs SRP	0.16
	IM vs NO ₃	n.s.
Szozskiewicz et al., 2006a (Europe)	IBMR vs TP	0.16–0.49
	IBMR vs SRP	
	MTR vs TP	0.23–0.59
	MTR vs SRP	0.27–0.67
	MTR vs NO ₃	0.06–0.48
Willby et al., 2012 (United Kingdom)	RMNI vs SRP	0.48
	RMNI vs TON	0.58
<i>Macrophyte abundance metrics</i>		
Szozskiewicz et al., 2006a (Europe)	Cover Bryophytes vs TP (mountain streams)	0.37
	Cover Bryophytes vs SRP (mountain streams)	0.38
	Cover Bryophytes vs NO ₃ (South-European sites)	0.35
	Cover Floating free vs SRP (mountain streams)	0.18
Willby et al., 2012 (United Kingdom)	Total cover vs SRP	0.028
	Total cover vs TON	0.040
	Invasive species cover vs SRP	0.012
	Invasive species cover vs TON	0.006

(Dodds et al., 1997; Porter et al., 2008) have found that relationships were generally stronger for total than for dissolved nutrient concentrations. However, several models based on dissolved concentrations of nutrients have been successful (Kelly and Whitton, 1995; Biggs, 2000; Willby et al., 2012), and some studies have found stronger relationships with dissolved than with total nutrients (Taylor et al., 2004; Zheng et al., 2008; Fabris et al., 2009; Almeida et al., 2014). In our study, we found strong relationships with SRP, while the relationships with TP were weaker or non-significant. Two explanations (not mutually exclusive) can be put forward to explain these results: (i) limited P bioavailability (Mainstone and Parr, 2002; Hilton et al., 2006; Charles et al., 2019) and (ii) naive nutrient sampling regimes (Hilton et al., 2006; Jarvie et al., 2006; Lavoie et al., 2008). However, we lack the data to test these hypotheses in the present work.

Similarly, weaker relationships were found in high alkalinity streams. We hypothesize that this was due to over-riding influence of

Table 9

Nutrient thresholds set by different ecology-based methods. BCG = Biological Condition Gradient; TIN = Total Inorganic Nitrogen; NMS = Non-metric Multidimensional Scaling; TITAN = Threshold Indicator Taxa Analysis. See Table 7 for other abbreviations.

Reference	Nutrient criteria	Approach to setting criteria
<i>Benthic (or sestonic) algae abundance</i>		
Carleton et al., 2009 (Minnesota, US)	100 µg/L TP; 2.7 mg/L TN	Threshold to prevent nuisance levels of periphyton and cyanobacteria dominance in sestonic algae
Chambers et al., 2008 (Ontario, Canada)	(1) 1.8 mg/L TN; (2) 21 µg/L TP; 0.95 µg/L TN	(1) Benthic chl-a < 100 mg/m ² (2) Sestonic chl-a < 5 mg/L; Predictions from linear regressions
Chambers et al., 2012 (Ontario, Canada)	(1) 1.2 mg/L TN; (2) 14 mg/L TP	(1) Benthic chl-a < 100 mg/m ² ; (2) Sestonic chl-a < 5 mg/L; Predictions from linear regressions
Dodds et al., 1997 (Montana, US)	30 µg/L TP; 0.35 mg/L TN	Thresholds to control nuisance benthic chl-a levels < 100 mg/m ² ; Regression and probabilistic analyses
Dodds and Welch, 2000 (US)	60 µg/L TP; 0.47 mg/L TN	Thresholds to control mean benthic chl-a levels < 100 mg/m ²
Dodds et al., 2002, 2006 (US)	43–62 µg/L TP; 0.54–0.60 mg/L TN	Break-points in nutrient–benthic chl-a regressions (mean and max chl-a)
Miltner, 2010 (Ohio, US)	38 µg/L TP; 0.44 mg/L DIN	Change point in nutrient–benthic chl-a relationship
Royer et al., 2007 (Illinois, US)	70 µg/L TP	Sestonic chl-a < 5 mg/L
Stevenson et al., 2006 (Kentucky, Indiana and Michigan regions, US)	30 µg/L TP; 1.0 mg/L TN	Threshold indicating high probabilities (20–50%) of extensive <i>Cladophora</i> growths (>20% avg. cover)
Wong and Clark, 1976 (Ontario, Canada)	60 µg/L TP	Threshold to limit excessive seasonal growth of <i>Cladophora</i>
<i>Diatom composition</i>		
Chambers et al., 2012 (Ontario, Canada)	22–32 µg/L TP; 0.59 mg/L TN	Thresholds in pressure-response of diatom metrics identified by regression tree analysis
Charles et al., 2019 (New Jersey, US)	50 µg/L TP; 1.0 mg/L TN	Threshold between impaired and unimpaired sites (BCG 4.0) based on diatom taxonomic composition; Visual graph analysis
Hausmann et al., 2016 (Wadeable streams in the US)	50 µg/L TP	Threshold between impaired and unimpaired sites (BCG 3.7) based on diatom taxonomic composition; TITAN
Lavoie et al., 2008 (Quebec, Canada)	20–40 µg/L TP	Diatom index IDEC values show a significant drop
Smith and Tran, 2010 (Large rivers of New York State, US)	(1) 9–20 µg/L TP; 0.41–0.50 mg/L TN (2) 37 µg/L TP; 0.78 mg/L TN	Thresholds shifts in biological community; (1) change points analysis; (2) cluster analysis
Smucker et al., 2013 (Connecticut, US)	(1) 20 µg/L TP; (2) 40 µg/L TP; (3) 65 µg/L TP	(1) Sensitive taxa steeply decline; (2) Diatom community change-points above which most sensitive diatoms lost; TITAN and NMS
Stevenson et al., 2008 (Mid-Atlantic Highlands)	10–20 µg/L TP	Thresholds in Lowess regression and regression tree analysis
Tibby et al., 2019 (South Australia)	30 µg/L TP	Threshold indicating significant decline in sensitive diatom species; TITAN
<i>Benthic invertebrate fauna</i>		

(continued on next page)

Table 9 (continued)

Reference	Nutrient criteria	Approach to setting criteria
Chambers et al., 2012 (Ontario, Canada)	21–63 µg/L TP; 0.59–2.83 mg/L TN	Thresholds in pressure-response of benthic fauna metrics identified by regression tree analysis
Chen et al., 2018 (North-east China)	52–101 µg /L TP; 1.05–1.65 mg/L TN	Breakpoints in the response of benthic invertebrate metrics; Regression tree analysis and Lowess
Smith et al., 2007 (Wadeable rivers of New York State, US)	65 µg/L TP; 0.98 mg/L NO ₃	Threshold between impaired and unimpaired sites; Cluster analysis
Smith and Tran, 2010 (Large rivers of New York State, US)	(1) 11–70 µg/L TP; 0.5–1.2 mg/L TN; (2) 37 µg/L TP; 0.68 mg/L TN	Thresholds shifts in biological community: (1) Change points analysis; (2) Cluster analysis
Wang et al., 2007 (Wisconsin, US)	40–90 µg/L TP; 0.61–1.68 mg/L TN	Threshold at which benthic invertebrate metrics change most dramatically to small changes in nutrient levels; Regression tree analyses
Weigel and Robertson, 2007 (Wisconsin, US)	64–150 µg/L TP; 0.64–1.93 mg/L TN	Breakpoints in nutrient-response relationships; Regression tree analysis
<i>Fish fauna</i>		
Miltner and Rankin, 1998 (Ohio, US)	60 µg/L TP; 0.61 mg/L TN	Threshold beyond which fish community structure is likely to be significantly degraded (based on IBI index)
Wang et al., 2007 (Wadeable streams of Wisconsin, US)	60–90 µg/L TP; 0.54–1.83 mg/L TN	Threshold at which fish metrics change most dramatically to small changes in nutrient levels; Regression tree analyses
Weigel and Robertson, 2007 (Wisconsin, US)	91–139 µg/L TP; 0.64 mg/L TN	Breakpoints in nutrient-response relationships; Regression tree analysis

alkalinity and that inclusion of alkalinity in the model might provide a better solution (Kelly et al., 2008; Demars et al., 2012; Lavoie et al., 2014).

4.3. Nutrient threshold values in rivers

There is a growing body of research efforts worldwide to understand the effects of nutrients on river ecology and to derive ecology-based nutrient thresholds (Table 9). A wide variety of thresholds has been suggested, subject to the biological community and variables used in the analyses, as well as the method applied to set the threshold. The type of river also plays an important role although only a few studies have considered river type when setting thresholds. Despite the differences, some common patterns do emerge: Most studies point to a TP threshold of about 30 to 60 µg/L TP, above which algal biomass may reach nuisance levels and diatom composition changes significantly. There are fewer studies on TN thresholds, and they are more variable, yet most studies converge around a TN threshold of 0.5–2.0 mg/L. For example, Hausmann et al. (2016) defined 52 µg/L TP as a change-point in diatom assemblages, while Tibby et al. (2019) identified thresholds of impairment in Australian watersheds at 30 µg/L TP and Lavoie et al. (2008) at 20–40 µg/L TP in Canadian streams. Similarly, thresholds of 30 µg/L TP and 1.0 mg/L TN were proposed by Stevenson et al. (2006) and 50 µg/L TP and 1.0 mg/L TN by Charles et al. (2019), both based on the response in benthic algal community. Smith and Tran (2010) provide more

conservative guidelines of 37 µg/L TP and 0.7 mg/L TN, based on shifts in biological community structure of benthic macroinvertebrate and diatoms. Similar values (i.e., 30 µg/L TP and 0.35 mg/L TN) are given by Dodds et al. (1997) to control nuisance benthic algal levels, while Chambers et al. (2008, 2012) identify TN thresholds for excessive benthic algal biomass at 1.2 and 1.8 mg/L TN.

These values coincide with the nitrogen planetary boundary values of 1.0–2.5 mg/L N derived to protect aquatic ecosystems from eutrophication or acidification (de Vries et al., 2013). A concentration of 100 µg/L SRP is often quoted as the point above which few changes to the phytobenthos or macrophytes occur (Bowes et al., 2007; Mainstone, 2010) but detailed studies nearly always indicate that significant changes have already occurred before this threshold is reached (Table 9). Our work provided nutrient thresholds in a range of 30–90 µg/L SRP and 1–3.5 mg/L TN, which were broadly comparable with these previous studies.

Comparison with the nutrient limits set by countries sharing these river types shows that several national nutrient thresholds are too high and will not protect sites from becoming ecologically impaired. These thresholds, therefore, need to be revisited. It is not surprising as a recent analysis demonstrated that a large variety of methods has been used for boundary setting, and the boundaries set by expert judgement tend to be higher (=less strict) than those set using contemporary data from the region in question (Poikane et al., 2019b).

5. Conclusions

If water bodies are to achieve good ecological status, establishing scientifically sound nutrient thresholds is of great importance. Examination of pressure-response relationships provide an objective method for establishing nutrient concentration thresholds in support of good ecological status. Our study demonstrates that in some cases it is possible to link nutrient concentrations to assessments of macrophyte and phytobenthos communities for determining nutrient thresholds. However, in other cases the explained variance was low, and further work is needed to consider the effect of other natural and anthropogenic stressors, as well as the nutrient sampling regime.

Finally, comparison of our nutrient thresholds with those used by selected EU member states showed that some states currently use thresholds that are too high. These high thresholds fail to protect rivers from becoming ecologically impaired and require examination and revision.

CRedit authorship contribution statement

Sandra Poikane: Conceptualization, Methodology, Data curation, Writing - original draft, Supervision, Project administration. **Gábor Várbró:** Conceptualization, Methodology, Software, Formal analysis, Investigation, Data curation, Visualization. **Martyn G. Kelly:** Conceptualization, Methodology, Data curation, Writing - original draft. **Sebastian Birk:** Conceptualization, Methodology, Writing - original draft. **Geoff Phillips:** Conceptualization, Methodology, Software, Formal analysis, Investigation, Data curation, Writing - original draft, Visualization.

Declaration of Competing Interest

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.

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Appendix A. Supplementary data

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References

- Aguilar, F.C., Feio, M.J., Ferreira, M.T., 2011. Choosing the best method for stream bioassessment using macrophyte communities: Indices and predictive models. *Ecol. Ind.* 11 (2), 379–388.
- Aguilar, F.C., Ferreira, M.T., Albuquerque, A., Rodríguez-González, P., Segurado, P., 2009. Structural and functional responses of riparian vegetation to human disturbance: Performance and spatial scale-dependence. *Fund. Appl. Limnol.* 175, 249–267.
- Almeida, S.F., Elias, C., Ferreira, J., Tornés, E., Puccinelli, C., Delmas, F., Dörflinger, G., Urbanič, G., Marcheggiani, S., Rosebery, J., Mancini, L., 2014. Water quality assessment of rivers using diatom metrics across Mediterranean Europe: a methods intercalibration exercise. *Sci. Total Environ.* 476, 768–776.
- Baattrup-Pedersen, A., Larsen, S.E., Riis, T., 2002. Long-term effects of stream management on plant communities in two Danish lowland streams. *Hydrobiologia* 481, 33–45.
- Baattrup-Pedersen, A., Larsen, S.E., Riis, T., 2003. Composition and richness of macrophyte communities in small Danish streams - influence of environmental factors and weed cutting. *Hydrobiologia* 495, 171–179.
- Bennett, C., Owen, R., Birk, S., Buffagni, A., Erba, S., Mengin, N., Murray-Bligh, J., Ofenböck, G., Pardo, I., van de Bund, W., Wagner, F., 2011. Bringing European river quality into line: An exercise to intercalibrate macro-invertebrate classification methods. *Hydrobiologia* 667 (1), 31–48.
- Biggs, B.J., 2000. Eutrophication of streams and rivers: Dissolved nutrient-chlorophyll relationships for benthic algae. *J. N. Am. Benthol. Soc.* 19 (1), 17–31.
- Birk, S., Bonne, W., Borja, A., Brucet, S., Courrat, A., Poikane, S., Solimini, A., van de Bund, W., Zampoukas, N., Hering, D., 2012. Three hundred ways to assess Europe's surface waters: An almost complete overview of biological methods to implement the Water Framework Directive. *Ecol. Indic.* 18, 31–41.
- Birk, S., Willby, N., 2010. Towards harmonization of ecological quality classification: establishing common grounds in European macrophyte assessment for rivers. *Hydrobiologia* 652 (1), 149–163.
- Birk, S., Chapman, D., Carvalho, L., Spears, B.M., Andersen, H.E., Argillier, C., Auer, S., Baattrup-Pedersen, A., Banin, L., Beklioglu, M., Bondar-Kunze, E., Borja, A., Branco, P., Bucak, T., Buijse, A.D., Cardoso, A.C., Couture, R., Cremona, F., de Zwart, D., Feld, C., Ferreira, M.T., Feuchtmayr, H., Gessner, M., Gieswein, A., Globovnik, L., Graeber, D., Graf, W., Gutiérrez-Cánovas, C., Hanganu, J., Işkın, U., Järvinen, M., Jeppesen, E., Kotamäki, N., Kuijper, M., Lemm, J.U., Lu, S., Lyche Solheim, A., Mischke, U., Moe, J., Nöges, P., Nöges, T., Ormerod, S., Panagopoulos, Y., Phillips, G., Posthuma, L., Pouso, S., Prudhomme, C., Rankinen, K., Rasmussen, J.J., Richardson, J., Sagouis, A., Santos, J.M., Schäfer, R.B., Schinegger, R., Schmutz, S., Schneider, S.C., Schülting, L., Segurado, P., Stefanidis, K., Sures, B., Thackeray, S., Turunen, J., Uyarra, M.C., Venohr, M., von der Ohe, P., Willby, N., Hering, D., 2020. Impacts of multiple stressors on freshwater biota across spatial scales and ecosystems. *Nat. Ecol. Evol.* <https://doi.org/10.1038/s41559-020-1216-4>.
- Bowes, M.J., Smith, J.T., Hilton, J., Sturt, M.M., Armitage, P.D., 2007. Periphyton biomass response to changing phosphorus concentrations in a nutrient-impacted river: A new methodology for phosphorus target setting. *Can. J. Fish. Aquat. Sci.* 64, 227–238.
- Carleton, J.N., Park, R.A., Clough, J.S., 2009. Ecosystem modeling applied to nutrient criteria development in rivers. *Environ. Manag.* 44 (3), 485–492.
- Carvalho, L., Mackay, E.B., Cardoso, A.C., Baattrup-Pedersen, A., Birk, S., Blackstock, K. L., Borics, G., Borja, A., Feld, C.K., Ferreira, M.T., Globovnik, L., et al., 2019. Protecting and restoring Europe's waters: An analysis of the future development needs of the Water Framework Directive. *Sci. Total Environ.* 658, 1228–1238.
- CEMAGREF, 1982. Etude des méthodes biologiques d'appréciation quantitative de la qualité des eaux. Rapport Q.E. Lyon-A.F. Bassin Rhône-Méditerranée-Corse, 218 pp.
- Chambers, P.A., McGoldrick, D.J., Brua, R.B., Vis, C., Culp, J.M., Benoy, G.A., 2012. Development of environmental thresholds for nitrogen and phosphorus in streams. *J. Environ. Qual.* 41 (1), 7–20.
- Chambers, P.A., Vis, C., Brua, R.B., Guy, M., Culp, J.M., Benoy, G.A., 2008. Eutrophication of agricultural streams: defining nutrient concentrations to protect ecological condition. *Water Sci. Technol.* 58 (11), 2203–2210.
- Charles, D.F., Tuccillo, A.P., Belton, T.J., 2019. Use of diatoms for developing nutrient criteria for rivers and streams: a biological condition gradient approach. *Ecol. Ind.* 96, 258–269.
- Charlton, M.B., Bowes, M.J., Hutchins, M.G., Orr, H.G., Soley, R., Davison, P., 2018. Mapping eutrophication risk from climate change: future phosphorus concentrations in English rivers. *Sci. Total Environ.* 613, 1510–1526.
- Chen, J., Li, F., Wang, Y., Kong, Y., 2018. Estimating the nutrient thresholds of a typical tributary in the Liao River basin. Northeast China. *Sci. Rep.* 8 (1), 1–10.
- Chételat, J., Pick, F.R., Morin, A., Hamilton, P.B., 1999. Periphyton biomass and community composition in rivers of different nutrient status. *Can. J. Fish. Aquat. Sci.* 56 (4), 560–569.
- Demars, B.O., Harper, D.M., 1998. The aquatic macrophytes of an English lowland river system: assessing response to nutrient enrichment. *Hydrobiologia* 384 (1–3), 75–88.
- Demars, B.O., Potts, J.M., Tremolieres, M., Thiebaut, G., Gougein, N., Nordmann, V., 2012. River macrophyte indices: Not the Holy Grail! *Freshwater Biol.* 57 (8), 1745–1759.
- Dodds, W.K., Welch, E.B., 2000. Establishing nutrient criteria in streams. *J. N. Am. Benthol. Soc.* 19 (1), 186–196.
- Dodds, W.K., Smith, V.H., Lohman, K., 2006. Erratum: Nitrogen and phosphorus relationships to benthic algal biomass in temperate streams. *Can. J. Fish. Aquat. Sci.* 63 (5), 1190–1191.
- Dodds, W.K., Smith, V.H., Lohman, K., 2002. Nitrogen and phosphorus relationships to benthic algal biomass in temperate streams. *Can. J. Fish. Aquat. Sci.* 59 (5), 865–874.
- Dodds, W.K., Smith, V.H., Zander, B., 1997. Developing nutrient targets to control benthic chlorophyll levels in streams: A case study of the Clark Fork River. *Water Res.* 31 (7), 1738–1750.
- Dodkins, I., Aguiar, F., Rivaes, R., Albuquerque, A., Rodríguez-González, P., Ferreira, M. T., 2012. Measuring ecological change of aquatic macrophytes in Mediterranean rivers. *Limnologia* 42 (2), 95–107.
- EEA [European Environment Agency]. 2018. European waters. Assessment of status and pressures 2018. EEA report 7/2018. Luxembourg: Publications Office of the European Union.
- Fabris, M., Schneider, S., Melzer, A., 2009. Macrophyte-based bioindication in rivers—A comparative evaluation of the reference index (RI) and the trophic index of macrophytes (TIM). *Limnologia* 39 (1), 40–55.
- Flor-Arnau, N., Real, M., González, G., Sánchez, J.C., Alcaraz, J.L.M., Solà, C., Torras, A. M., 2015. Índice de Macrófitos Fluviales (IMF), una nueva herramienta para evaluar el estado ecológico de los ríos mediterráneos. *Limnologia* 34 (1), 95–114.
- Guzzetti, B., Pistocchi, A., Lique, C., Udiás, A., Bouraoui, F., van de Bund, W., 2017. Human pressures and ecological status of European rivers. *Sci. Rep.* 7 (1), 1–11.
- Guzzetti, B., Lique, C., Pistocchi, A., Vigiak, O., Zulfan, G., Bouraoui, F., De Roo, A., Cardoso, A.C., 2019. Relationship between ecological condition and ecosystem services in European rivers, lakes and coastal waters. *Sci. Total Environ.* 671, 452–465.
- Hauri, J., Peltre, M.C., Trémoilières, M., Barbe, J., Thiébaud, G., Bernez, I., Daniel, H., Chatenet, P., Haan-Archipof, G., Muller, S., Dutartre, A., 2006. A new method to assess water trophy and organic pollution – The Macrophyte Biological Index for Rivers (IBMR): Its application to different types of river and pollution. *Hydrobiologia* 570, 153–158.
- Hausmann, S., Charles, D.F., Gerritsen, J., Belton, T.J., 2016. A diatom-based biological condition gradient (BCG) approach for assessing impairment and developing nutrient criteria for streams. *Sci. Total Environ.* 562, 914–927.
- Hilton, J., O'Hare, M., Bowes, M.J., Jones, J.I., 2006. How green is my river? A new paradigm of eutrophication in rivers. *Sci. Total Environ.* 365 (1–3), 66–83.
- Hlíbková, D., Hindáková, A., Haviar, M., Miettinen, J., 2007. Application of diatom water quality indices in influenced and non-influenced sites of Slovak rivers (Central Europe). *Large Rivers* 443–464.
- Holmes, N.T.H., Newman, J.R., Chadd, S., Rouen, K.J., Saint, L., Dawson F.H., 1999. Mean Trophic Rank: A User's Manual. R&D Technical Report E38. Environment Agency, Bristol.
- Jarvie, H.P., Neal, C., Withers, P.J., 2006. Sewage-effluent phosphorus: A greater risk to river eutrophication than agricultural phosphorus? *Sci. Total Environ.* 360 (1–3), 246–253.
- Kelly, M.G., Whitton, B.A., 1995. The trophic diatom index: A new index for monitoring eutrophication in rivers. *J. Appl. Phycol.* 7 (4), 433–444.
- Kelly, M., Juggins, S., Guthrie, R., Pritchard, S., Jamieson, J., Rippey, B., Hirst, H., Yallop, M., 2008. Assessment of ecological status in UK rivers using diatoms. *Freshwater Biol.* 53 (2), 403–422.
- Kelly, M., Bennett, C., Coste, M., Delgado, C., Delmas, F., Denys, L., Ector, L., Fauville, C., Ferréol, M., Golub, M., Jarlman, A., et al., 2009. A comparison of national approaches to setting ecological status boundaries in phytobenthos assessment for the European Water Framework Directive: results of an intercalibration exercise. *Hydrobiologia* 621 (1), 169–182.
- Kelly, M.G., Phillips, G., Juggins, S., Willby, N.J., 2020. Re-evaluating expectations for river phytobenthos assessment and understanding the relationship with macrophytes. *Ecol. Ind.* 117, 106582.
- Kuhar, U., Germ, M., Gaberšček, A., Urbanič, G., 2011. Development of a River Macrophyte Index (RMI) for assessing river ecological status. *Limnologia* 41 (3), 235–243.
- Lavoie, I., Campeau, S., Zugic-Drakulic, N., Winter, J.G., Fortin, C., 2014. Using diatoms to monitor stream biological integrity in Eastern Canada: an overview of 10 years of index development and ongoing challenges. *Sci. Total Environ.* 475, 187–200.
- Lavoie, I., Campeau, S., Darchambeau, F., Cabana, G., Dillon, P.J., 2008. Are diatoms good integrators of temporal variability in stream water quality? *Freshwater Biol.* 53 (4), 827–841.
- McDonald, R.I., Weber, K.F., Padowski, J., Boucher, T., Shemie, D., 2016. Estimating watershed degradation over the last century and its impact on water-treatment costs for the world's large cities. *Proc. Natl. Acad. Sci.* 113 (32), 9117–9122.
- Mainstone, C.P., 2010. An evidence base for setting nutrient targets to protect river habitat. Natural England Research Report NERRO34. Natural England, Sheffield.
- Mainstone, C.P., Parr, W., 2002. Phosphorus in rivers-ecology and management. *Sci. Total Environ.* 282, 25–47.
- MEA [Millennium Ecosystem Assessment], 2005. Ecosystems and Human Well-being: Synthesis. Island Press, Washington, DC.
- Miltner, R.J., 2010. A method and rationale for deriving nutrient criteria for small rivers and streams in Ohio. *Environ. Manag.* 45 (4), 842–855.

- Miltner, R.J., Rankin, A.E.T., 1998. Primary nutrients and the biotic integrity of rivers and streams. *Freshwater Biol.* 40 (1), 145–158.
- Mischke, U., Venohr, M., Behrendt, H., 2011. Using phytoplankton to assess the trophic status of German rivers. *Int. Rev. Hydrobiol.* 96 (5), 578–598.
- Moe, S.J., Lyche Solheim, A., Soszka, H., Gotub, M., Hutorowicz, A., Kolada, A., Picińska-Fałtynowicz, J., Białokoz, W., 2015. Integrated assessment of ecological status and misclassification of lakes: The role of uncertainty and index combination rules. *Ecol. Ind.* 48, 605–615.
- Pfister, P., Pipp, E., 2010. Leitfaden zur Erhebung der biologischen Qualitätselemente Teil A3—Phytobenthos. Bundesministerium für Land- und Forstwirtschaft, Umwelt und Wasserwirtschaft, Vienna.
- Phillips, G., Kelly, M., Teixeira, H., Salas, F., Free, G., Leujak, W., Solheim, A.L., Várbró, G., Poikane, S., 2018. Best practice for establishing nutrient concentrations to support good ecological status. Technical Report EUR 29329 EN. Publications Office of the European Union, Luxembourg.
- Phillips, G., Pitt, J., 2016. A comparison of European freshwater nutrient boundaries used for the water framework directive: Report to ECOSTAT. Environmental Change Research Centre University College London, UK. <https://circabc.europa.eu/w/browse/58a2363a-c5f1-442f-89aa-5cec96ba52d7>.
- Phillips, G., Teixeira, H., Poikane, S., Salas Herrero, F., Kelly, M.G., 2019. Establishing nutrient thresholds in the face of uncertainty and multiple stressors: A comparison of approaches using simulated datasets. *Sci. Total Environ.* 684, 425–433.
- Picińska-Fałtynowicz, J., 2009. Diatom phytobenthos as a tool for assessing the ecological status of Polish rivers. *Oceanol. Hydrobiol. Stud.* 38, 155–161.
- Poikane, S., Zampoukas, N., Borja, A., Davies, S.P., van de Bund, W., Birk, S., 2014. Intercalibration of aquatic ecological assessment methods in the European Union: Lessons learned and way forward. *Environ. Sci. Pol.* 44, 237–246.
- Poikane, S., Kelly, M., Cantonati, M., 2016. Benthic algal assessment of ecological status in European lakes and rivers: Challenges and opportunities. *Sci. Total Environ.* 568, 603–613.
- Poikane, S., Kelly, M.G., Salas Herrero, F., Pitt, J.A., Jarvie, H.P., Clausen, U., Leujak, W., Lyche Solheim, A., Teixeira, H., Phillips, G., 2019b. Nutrient criteria for surface waters under the European Water Framework Directive: Current state-of-the-art, challenges and future outlook. *Sci. Total Environ.* 695, 133888.
- Poikane, S., Phillips, G., Birk, S., Free, G., Kelly, M.G., Willby, N.J., 2019a. Deriving nutrient criteria to support 'good' ecological status in European lakes: An empirically based approach to linking ecology and management. *Sci. Total Environ.* 650, 2074–2084.
- Poikane, S., Salas Herrero, F., Kelly, M.G., Borja, A., Birk, S., van de Bund, W., 2020. European aquatic ecological assessment methods: A critical review of their sensitivity to key pressures. *Sci. Total Environ.* 740, 140075.
- Porter, S.D., Mueller, D.K., Spahr, N.E., Munn, M.D., Dubrovsky, N.M., 2008. Efficacy of algal metrics for assessing nutrient and organic enrichment in flowing waters. *Freshwater Biol.* 53 (5), 1036–1054.
- Pot, R., Birk, S., 2015. Fitting the revised assessment method for rivers in The Netherlands using macrophytes to the results of the completed Central-Baltic rivers' intercalibration exercise. Oosterheselen, Rijkswaterstaat, Lelystad.
- Potapova, M., Charles, D.F., 2007. Diatom metrics for monitoring eutrophication in rivers of the United States. *Ecol. Ind.* 7 (1), 48–70.
- Reid, A.J., Carlson, A.K., Creed, I.F., Eliason, E.J., Gell, P.A., Johnson, P.T., Kidd, K.A., MacCormack, T.J., Olden, J.D., Ormerod, S.J., Smol, J.P., 2019. Emerging threats and persistent conservation challenges for freshwater biodiversity. *Biol. Rev.* 94 (3), 849–873.
- R Core Team, 2019. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>.
- Rott, E., Hofmann, G., Pall, K., Pfister, P., Pipp, E., 1997. Indikationslisten für Aufwuchsalgen. Teil 1: Saprobielle Indikation. Publ. Wasserwirtschaftskataster, BMFLF: 1–73.
- Rott, E., Van Dam, H., Pfister, P., Pipp, E., Pall, K., Binder, N., Ortler, K., 1999. Indikationslisten für Aufwuchsalgen. Teil 2: Trophieindikation, geochemische Reaktion, toxikologische und taxonomische Anmerkungen. Publ. Wasserwirtschaftskataster, BMFLF: 1–248.
- Rott, E., Pipp, E., Pfister, P., 2003. Diatom methods developed for river quality assessment in Austria and a cross-check against numerical trophic indication methods used in Europe. *Algol. Stud.* 110, 91–115.
- Royer, T.V., David, M.B., Gentry, L.E., Mitchell, C.A., Starks, K.M., Heatherly, T., Whiles, M.R., 2008. Assessment of chlorophyll-a as a criterion for establishing nutrient standards in the streams and rivers of Illinois. *J. Environ. Qual.* 37 (2), 437–447.
- Salas Herrero, F., Teixeira, H., Poikane, S., 2019. A novel approach for deriving nutrient criteria to support good ecological status: Application to coastal and transitional waters and indications for use. *Front. Mar. Sci.* 6, 255.
- Schinegger, R., Trautwein, C., Melcher, A., Schmutz, S., 2012. Multiple human pressures and their spatial patterns in European running waters. *Water Environ. J.* 26 (2), 261–273.
- Smith, A.J., Bode, R.W., Kleppel, G.S., 2007. A nutrient biotic index (NBI) for use with benthic macroinvertebrate communities. *Ecol. Ind.* 7 (2), 371–386.
- Smith, A.J., Tran, C.P., 2010. A weight-of-evidence approach to define nutrient criteria protective of aquatic life in large rivers. *J. N. Am. Benthol. Soc.* 29 (3), 875.
- Smucker, N.J., Becker, M., Detenbeck, N.E., Morrison, A.C., 2013. Using algal metrics and biomass to evaluate multiple ways of defining concentration-based nutrient criteria in streams and their ecological relevance. *Ecol. Indic.* 32, 51–61.
- Smucker, N.J., Vis, M.L., 2009. Use of diatoms to assess agricultural and coal mining impacts on streams and a multi-assembly case study. *J. N. Am. Benthol. Soc.* 28 (3), 659–675.
- Soininen, J., 2005. Assessing the current related heterogeneity and diversity patterns of benthic diatom communities in a turbid and a clear water river. *Aquat. Ecol.* 38 (4), 495–501.
- Stevenson, R.J., Hill, B.H., Herlihy, A.T., Yuan, L.L., Norton, S.B., 2008. Algae-P relationships, thresholds, and frequency distributions guide nutrient criterion development. *J. N. Am. Benthol. Soc.* 27 (3), 783–799.
- Stevenson, R.J., Rier, S.T., Riseng, C.M., Schultz, R.E., Wiley, M.J., 2006. Comparing effects of nutrients on algal biomass in streams in two regions with different disturbance regimes and with applications for developing nutrient criteria. *Hydrobiologia* 561, 149–165.
- Søndergaard, M., Lauridsen, T.L., Kristensen, E.A., Baatrup-Pedersen, A., Wiberg-Larsen, P., Hansen, R.B., Friberg, N., 2013. Biologiske indikatorer i danske søer og vandløb: Vurdering af økologisk kvalitet. Aarhus Universitet, DCE - Nationalt Center for Miljø og Energi.
- Suárez, M., Mellado, A., Sánchez-Montoya, M., Vidal-Abarca, M., 2005. Propuesta de un índice de macrófitos (IM) para evaluar la calidad ecológica de los ríos de la cuenca del Segura. *Limnetica* 24 (3–4), 305–318.
- Szoszkiewicz, K., Zbierska, J., Jusik, S., Zgola T., 2006a. Opracowanie podstaw metodycznych dla monitoringu biologicznego wód w zakresie makrofitów i pilotowe ich zastosowanie dla części wód reprezentujących wybrane kategorie i typy. Etap II, tom II – rzeki. Institute of Environmental Protection, Agricultural Academy, Warsaw.
- Szoszkiewicz, K., Ferreira, T., Korte, T., Baatrup-Pedersen, A., Davy-Bowker, J., O'Hare, M., 2006b. European river plant communities: The importance of organic pollution and the usefulness of existing macrophyte metrics. *Hydrobiologia* 566, 211–234.
- Taylor, S.L., Roberts, S.C., Walsh, C.J., Hatt, B.E., 2004. Catchment urbanization and increased benthic algal biomass in streams: Linking mechanisms to management. *Freshwater Biol.* 49, 835–851.
- Tibby, J., Richards, J., Tyler, J.J., Barr, C., Fluin, J., Goonan, P., 2019. Diatom–water quality thresholds in South Australian streams indicate a need for more stringent water quality guidelines. *Mar. Freshw. Res.* doi:10.1071/MF19065.
- Van der Molen, D.T. (ed.), 2004. Referenties en concept-maatlatten voor rivieren voor de Kaderrichtlijn Water. STOWA-rapport 2004/43. STOWA, Utrecht: 365 pp.
- Várbró, G., Borics, G., Csányi, B., Fehér, G., Grigorszky, I., Kiss, K.T., Tóth, A., Ács, É., 2012. Improvement of the ecological water qualification system of rivers based on the first results of the Hungarian phytobenthos surveillance monitoring. *Hydrobiologia* 695 (1), 125–135.
- Vilbaste, S., Truu, J., Leisk, Ü., Iital, A., 2007. Species composition and diatom indices in relation to environmental parameters in Estonian streams. *Large Rivers* 307–326.
- de Vries, W., Kros, J., Kroeze, C., Seitzinger, S.P., 2013. Assessing planetary and regional nitrogen boundaries related to food security and adverse environmental impacts. *Curr. Opin. Environ. Sustain.* 5 (3–4), 392–402.
- Vörösmarty, C.J., McIntyre, P.B., Gessner, M.O., Dudgeon, D., Prusevich, A., Green, P., Glidden, S., Bunn, S.E., Sullivan, C.A., Liermann, C.R., Davies, P.M., 2010. Global threats to human water security and river biodiversity. *Nature* 467 (7315), 555–561.
- Wagenhoff, A., Liess, A., Pastor, A., Clapcott, J.E., Goodwin, E.O., Young, R.G., 2017. Thresholds in ecosystem structural and functional responses to agricultural stressors can inform limit setting in streams. *Freshwater Sci.* 36 (1), 178–194.
- Wang, L., Robertson, D.M., Garrison, P.J., 2007. Linkages between nutrients and assemblages of macroinvertebrates and fish in wadeable streams: implication to nutrient criteria development. *Environ. Manage.* 39 (2), 194–212.
- Weigel, B.M., Robertson, D.M., 2007. Identifying biotic integrity and water chemistry relations in nonwadeable rivers of Wisconsin: toward the development of nutrient criteria. *Environ. Manage.* 40 (4), 691–708.
- Wiegand, G., Gebler, D., van de Weyer, K., Birk, S., 2016. Comparative test of ecological assessment methods of lowland streams based on long-term monitoring data of macrophytes. *Sci. Total Environ.* 541, 1269–1281.
- Willby, N., Pitt, J.A., Phillips, G., 2012. The ecological classification of UK rivers using aquatic macrophytes. UK Environment Agency Science Reports. Project SC010080/R1. Environmental Agency, Bristol.
- Wong, S.L., Clark, B., 1976. Field determination of the critical nutrient concentrations for Cladophora in streams. *J. Fish. Res. Board Can.* 33, 95–96.
- Zheng, L., Gerritsen, J., Beckman, J., Ludwig, J., Wilkes, S., 2008. Land use, geology, enrichment, and stream biota in the eastern ridge and valley ecoregion: Implications for nutrient criteria development. *J. Am. Water Resour. As.* 44 (6), 1521–1536.