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 phytobenthos (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 27 to 42 µg/L SRP and 1.0–3.5 mg/L TN for the low alkalinity mid-altitude river type, and 32–99 µg/L SRP and 0.9–2.5 mg/L TN for the low alkalinity lowland 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.,...
increases in water treatment costs and degradation of recreational opportunities may have been a major driver for this in Europe, requiring assessment of four central-Baltic ecoregion shared river types (Bennett 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 shortfalls, often associated with subjective factors embedded in each of the 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., phytothems 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 shortcomings, 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 trans-boundary 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 1

| River type | Type code | Catchment area (km²) | 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-C2 | 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 2

| River type | Type code | Catchment area (km²) | 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-C2 | 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 |

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 (Bennett et al., 2011). 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), phytothems (e.g., Várbiro 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., phytothems 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 shortcomings, 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
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 Kelly 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. 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; Szoszkiewicz 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

| 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       | 0.49***    | 0.49***      | 0.52***                                    | 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**                                     |
|            | 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.)                                 |

a p < 0.05; ** p < 0.01
In low alkalinity streams (R-C1 and R-C3), SRP and TN were strongly related to macrophyte and phytobenthos 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. Results**

**3.1. Relationships**

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 phytobenthos 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.

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**Fig. 1.** Relationship between Ecological Quality Ratios (EQR) for macrophytes (upper block) and phytobenthos (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.
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 µg/L SRP (range: 18–58 µg/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 µg/L SRP and 2.21 mg/L TN (Fig. 4), while the high-good class thresholds were 19 µg/L SRP and 0.85 mg/L TN. For the phytobenthos assessment the good-moderate thresholds were at 32 µg/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 µg/L SRP for R-C1 and 70 to 130 µg/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 µg/L SRP for R-C1 and 20–160 µg/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 µg/L SRP or 2 mg/L TN);
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

**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, these two approaches both have 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 setting ecologically-meaningful nutrient thresholds.

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 nutrient concentrations and biota (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 μg/L (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 this approach incorporates information from both organism groups toward the final 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 μg/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 phyto- benthos, 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

| Table 5 |
| Summary of predicted soluble phosphorus (SRP) high-good and good-moderate class boundaries (mg/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 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) |
| --- | --- | --- | --- | --- | --- |
| High-good class boundary |
| 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) |
| Good-moderate class boundary |
| R-C1 | Linear regression | 1.47 (0.59–3.45) | 2.50 (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) |

| 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) |
| --- | --- | --- | --- | --- | --- |
| High-good class boundary |
| 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) |
| Good-moderate class boundary |
| 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) |
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 ($r^2 = 0.40–0.65$, $p < 0.001$, Table 4) while the relationship with TP in these stream types was weak ($r^2 = 0.09–0.20$, $p < 0.001$) 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 ($r^2 = 0.03–0.27$, $p < 0.001$) 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.

![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.](image-url)
### Table 7

Relationships between phylobenthos variables and nutrients. TP = Total phosphorus; SRP = Soluble reactive phosphorus; TN = Total nitrogen; SIN = Soluble inorganic nitrogen; NO$_3$ = Nitrate; TON = Total organic nitrogen; n.s. = not significant.

| Reference | Biological metric vs nutrient metric | Coefficient of determination ($r^2$) |
|-----------|--------------------------------------|-------------------------------------|
| Biagi, 2000 (New Zealand) | Benthic chl-a vs SRP | 0.23/0.29 |
| Chambers et al., 2008 (Ontario and Quebec, Canada) | Benthic chl-a vs TN | 0.46 |
| Chambers et al., 2012 (Ontario and Quebec, Canada) | Benthic chl-a vs SRP | 0.15 |
| Chen et al., 1999 (Ontario and Quebec, Canada) | Benthic chl-a vs SRP | 0.56 |
| Dodds et al., 2002 (Eastern England) | Mean/Max benthic chl-a vs TP | 0.09/0.07 |
| Dodds et al., 2003 (N. America and New Zealand) | Mean/Max benthic chl-a vs TN | 0.35/0.28 |
| Stevenson et al., 2006 (Mid-Atlantic Highlands region, US) | Algal ash-free dry mass vs TP | 0.04 |
| Stevenson et al., 2008 (Mid-Atlantic Highlands region, US) | Algal cover vs SRP | 0.06 |
| Stevenson et al., 2012 (United Kingdom) | Algal cover vs TON | 0.05 |
| Almeida et al., 2014 (Mediterranean region) | ICM (IPS and TI) vs TP | 0.18 |
| Chambers et al., 2012 (Ontario and Quebec, Canada) | Diatom metrics vs SRP | 0.40-0.43 |
| Hlúbiková et al., 2007 (Slovakia) | Diatom indices vs TN | 0.20 |
| Kelly and Whittington, 1995 (UK) | Trophic diatom index (TDR) vs SRP | 0.63 |
| Kelly et al., 2008 (UK) | Trophic diatom index (TDR) vs SRP | 0.35 |
| Lavoie et al., 2008 (Eastern Canada) | Eastern Canadian Diatom Index (IDEC) vs TP | 0.41-0.79 |
| Lavoie et al., 2014 (Eastern Canada) | Diatom similarity metrics vs SRP | 0.04-0.32 |
| Porter et al., 2008 | 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 |
| Rott et al., 2003 (Austria) | Diatom similarity metrics vs SRP | n.s. |
| Smith and Tran, 2010 (New York state, US) | Diatom indices vs TN | 0.02-0.43 |
| Smucker and Vis, 2009 (Ohio, US) | Diatom diversity metrics vs TN | 0.18-0.19 |
| Stevenson et al., 2008 (Mid-Atlantic Highlands region, US) | Diatom indices vs SRP | 0.31-0.55 |
| Vörös et al., 2012 (Hungary) | Diatom indices vs TP | 0.03-0.35 |
| Vilbaste et al., 2007 (Estonia) | Diatom indices vs TP | 0.64 |
| Stevens et al., 2008 (Mid-Atlantic Highlands region, US) | Diatom indices vs NO$_3$ | 0.46-0.55 |
| Zheng et al., 2008 (West Virginia, US) | Diatom indices vs TP | 0.17-0.22 |

(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 (Szospkiewicz et al., 2006b; Wiegleb 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 (Kumar et al., 2011), composite pressure gradients (Aguiar et al., 2011; Dodkins et al., 2012) or simple categorical comparisons between impacted and non-impacted sites (Aguiar 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 $r^2 = 0.48-0.56$; 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...
(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; Wilby et al., 2012), and some studies have found stronger relationships with dissolved than with total nutrients (Taylor et al., 2004; Zheng et al., 2008; Fabis 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; Jarvis 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....
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., 2008). Breakpoints in the response of benthic invertebrates metrics; Regression tree analysis and Lowest of 1.05–1.65 mg/L TN.

Smith et al., 2007 (Wadeable rivers of New York State, US) 65 µg/L TP; 0.98 mg/L NO3 – nitrogen; 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

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 invertebrates metrics; Regression tree analysis and Lowest of 1.05–1.65 mg/L TN. |
| Smith et al., 2007 (Wadeable rivers of New York State, US) | 65 µg/L TP; 0.98 mg/L NO3– nitrogen; | 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 invertebrates 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 |

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 phytothens 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 revised. 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 phytothems 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 Várbiro: 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

Aguiar, 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.

Aguiar, F.C., Ferreira, M.T., Albuquerque, A., Rodrigues-González, F., 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., Torres, E., Puccinelli, C., Delmar, F., Dierfing, 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., 2003. Composition and richness of community composition in rivers of different nutrient status. Can. J. Fish. Aquat. Sci. 60 (5), 1190–1191.

Baattrup-Pedersen, A., Wang, J., Brander, K., 2002. Establishing nutrient criteria in streams. Sci. Total Environ. 295 (1–3), 321–332.

Birk, S., Chapman, D., Carvalho, L., Spears, B.M., Andersen, H.E., Argillier, C., Auer, 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., 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., Willby, N., 2010. Toward 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., Smeets, B., Marcus, K., Willby, N., 2010. A method and rationale for deriving nutrient criteria for small rivers and streams in Ohio. Environ. Manag. 45 (4), 842–851.

Birk, S., Chapman, D., Carvalho, L., Spears, B.M., Andersen, H.E., Argillier, C., Auer, 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., Willby, N., 2010. Towards harmonization of ecological quality classification: Establishing common grounds in European macrophyte assessment for rivers. Hydrobiologia 652 (1), 149–163.
Royer, T.V., David, M.B., Gentry, L.E., Mitchell, C.A., Starks, K.M., Heatherly, T., Rott, E., Pipp, E., Pfister, P., 2003. Diatom methods developed for river quality assessment. Water Environ. J. 26 (2), 131–157.

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, BMfL: 1–232.

Rott, E., Hofmann, G., Pall, K., Pfister, P., Pipp, E., 1997. Indikationslisten für Aufwuchsalgen. Teil 1: Saprobielle Indikation. Publ. Wasserwirtschaftskataster, BMfL: 1–232.

Phillips, G., Pitt, J.A., Phillips, G., 2012. The ecological classification of UK rivers using aquatic macrophytes: Implications for nutrient criteria development. J. Am. Water Resour. As. 44 (6), 1521–1536.

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://circap.eucropa.eu/euw/brow se/58a2363a-c5f1-442f-89aa-5ce90a52c7d.

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.

Philips, G., Pitt, J.A., Phillips, G., and their spatial patterns in European running waters. Water Environ. J. 26 (2), 128–151.

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://circap.eucropa.eu/euw/browse/58a2363a-c5f1-442f-89aa-5ce90a52c7d.

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.

Phillips, G., Pitt, J.A., Phillips, G., and their spatial patterns in European running waters. Water Environ. J. 26 (2), 128–151.

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://circap.eucropa.eu/euw/browse/58a2363a-c5f1-442f-89aa-5ce90a52c7d.

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.