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# Multi-decadal improvements in the ecological quality of European rivers are not consistently reflected in biodiversity metrics

**Supplementary Information 1** – EQR/EQC methods

**Supplementary Table 1:** Methods used to produce the Ecological Quality Ratios and Ecological Quality Classes for each country.

| Country | Method | Method reference |
|---|---|---|
| Austria | Multimetric index using three metrics[a,b] | 1 |
| Belgium | Multimetric Macroinvertebrate Index Flanders[a,b,c] | 2 |
| Bulgaria | Biotic Index[a] | 3 |
| Cyprus | STAR Intercalibration Common Metric Index[a,b,c,d] | 4 |
| Czechia | Multimetric index using river type-specific metrics[a,c] | 5,6 |
| Denmark | Danish Streamfauna Index | 7,8 |
| Estonia | Multimetric index using five metrics[a,b,c,d] | 9 |
| Finland | Finnish Multimetric Index[c] | 7 |
| France | Global Biological Normalized Index[a] | 10 |
| Germany | Multimetric index using river type-specific metrics[c] | 11 |

| | | |
|---|---|---|
| Hungary | Hungarian Multimetric Index[b,c,d] | https://shiny.freshwater-ecology.com/VGT3/Mavige_Modszertani_kezikonyv.pdf; 12 |
| Ireland | Quality Rating System[a,c] | 13 |
| Italy | STAR Intercalibration Common Metric Index[a,b,c,d] | 4 |
| Latvia | Latvian Macroinvertebrate Index[a,c,d] | https://circabc.europa.eu/sd/a/0c2fdd3c-3720-45e4-8684-12dc4fc561c2/LV_river_macroinvertebrates_IC.pdf; 14 |
| Lithuania | Lithuanian River Macroinvertebrate Index[d] | https://e-seimas.lrs.lt/portal/legalAct/lt/TAD/TAIS.296626/asr; 15 |
| Luxembourg | $I_2M_2$[a,b,d] | 10 |
| Netherlands | KRW-maatlatten | 16 |
| Norway | Poorest out of the Average Score Per Taxon index and the River Acidification Macroinvertebrate Index[d] | 7 |
| Portugal | South Portugal macroinvertebrate biotic index[a,c,d] | 17 |
| Spain | Iberian Biological Monitoring Working Party | www.boe.es/buscar/doc.php?id=BOE-A-2015-9806; 18 |
| Sweden | Average Score Per Taxon and the DJ index[d] | 7,19,20 |
| Switzerland | Multimetric index following the German system for a type 3.2 river[c] | 11 |

| | | |
|---|---|---|
| UK | Whalley Hawkes Paisley Trigg (WHPT) Average Score Per Taxon and number of scored taxa[d] | http://wfduk.org/sites/default/files/River%20Invertebrates%20WHPT%20UKTAG%20Method%20Statement%20-%20updated%20May%202021.pdf; 21 |

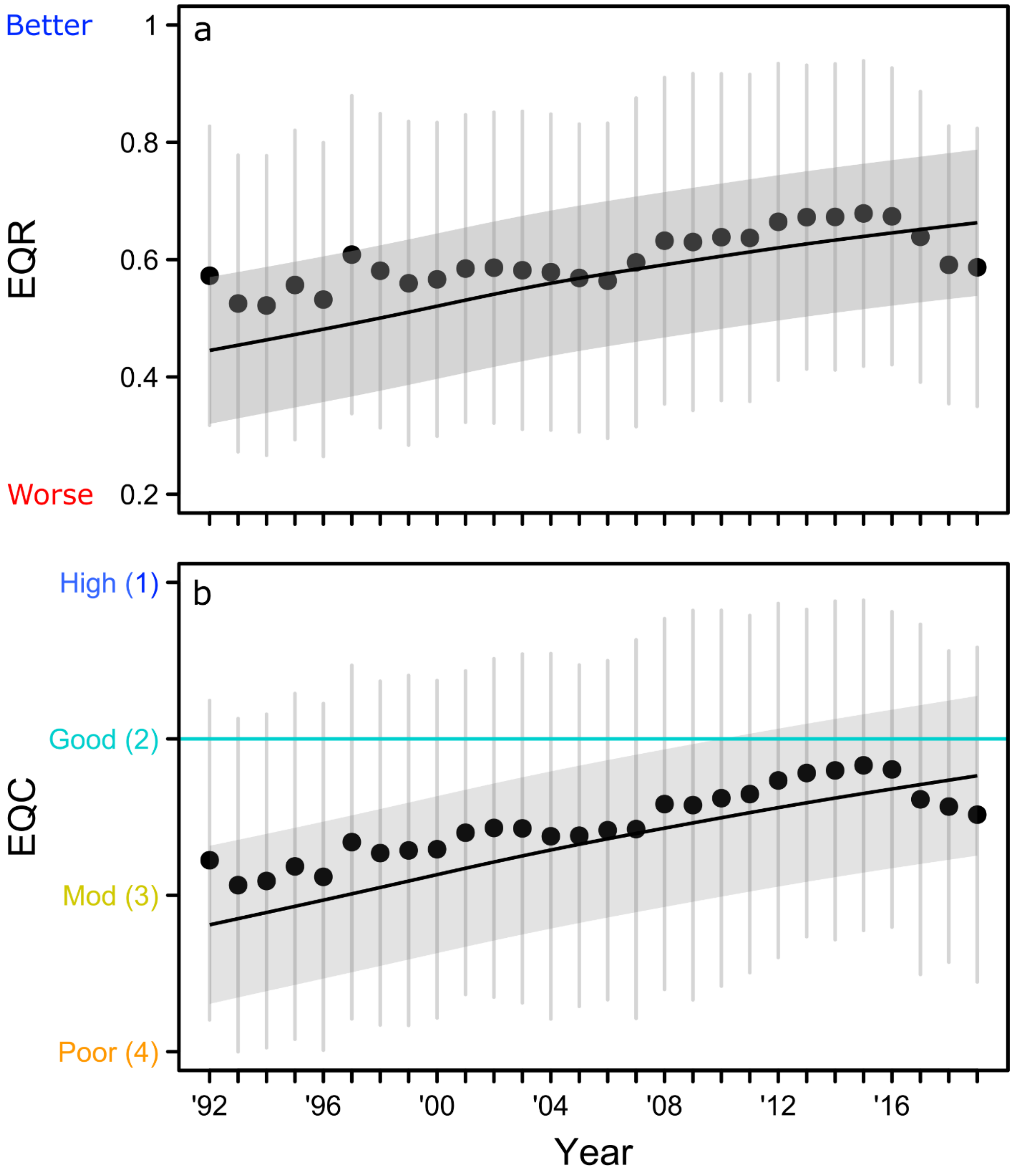
[a] Uses taxon richness
[b] Uses Shannon diversity
[c] Uses EPT richness
[d] Uses an ASPT index

**Supplementary Information 2** – Time series data and sensitivity

**Supplementary Table 2:** Number of sites and the earliest and latest sampling years for each country, the average number of sampling years per site, and average time series length per site.

| Country | Number of sites | Start year | End year | Sampling years | Total length |
|---|---|---|---|---|---|
| Austria | 1 | 2008 | 2019 | 8 | 12 |
| Belgium | 67 | 1992 | 2019 | 9 | 20 |
| Bulgaria | 5 | 2010 | 2019 | 9 | 10 |
| Cyprus | 2 | 2006 | 2019 | 8 | 13 |
| Czechia | 78 | 2000 | 2019 | 9 | 13 |
| Denmark | 248 | 1992 | 2019 | 21 | 23 |
| Estonia* | 10 | 2003 | 2019 | 8 | 10 |
| Finland | 10 | 2000 | 2014 | 15 | 15 |
| France | 265 | 1992 | 2017 | 14 | 22 |
| Germany | 12 | 2000 | 2019 | 10 | 11 |
| Hungary | 84 | 2005 | 2019 | 11 | 12 |
| Ireland | 16 | 2003 | 2019 | 17 | 17 |
| Italy | 5 | 2010 | 2019 | 8 | 8 |
| Latvia | 3 | 1996 | 2015 | 19 | 20 |
| Lithuania | 41 | 2010 | 2019 | 7 | 8 |
| Luxembourg | 20 | 2007 | 2017 | 10 | 11 |
| Netherlands | 46 | 1992 | 2019 | 13 | 20 |
| Norway | 63 | 2003 | 2019 | 11 | 15 |
| Portugal | 2 | 1993 | 2019 | 27 | 27 |
| Spain | 245 | 1992 | 2019 | 17 | 18 |
| Sweden | 91 | 1995 | 2019 | 14 | 14 |
| Switzerland | 1 | 1995 | 2018 | 8 | 24 |
| UK | 37 | 1999 | 2019 | 13 | 13 |

*****Estonia was excluded from our individual GAMMs and RDAs because only two high quality sites were sampled in the earlier years.

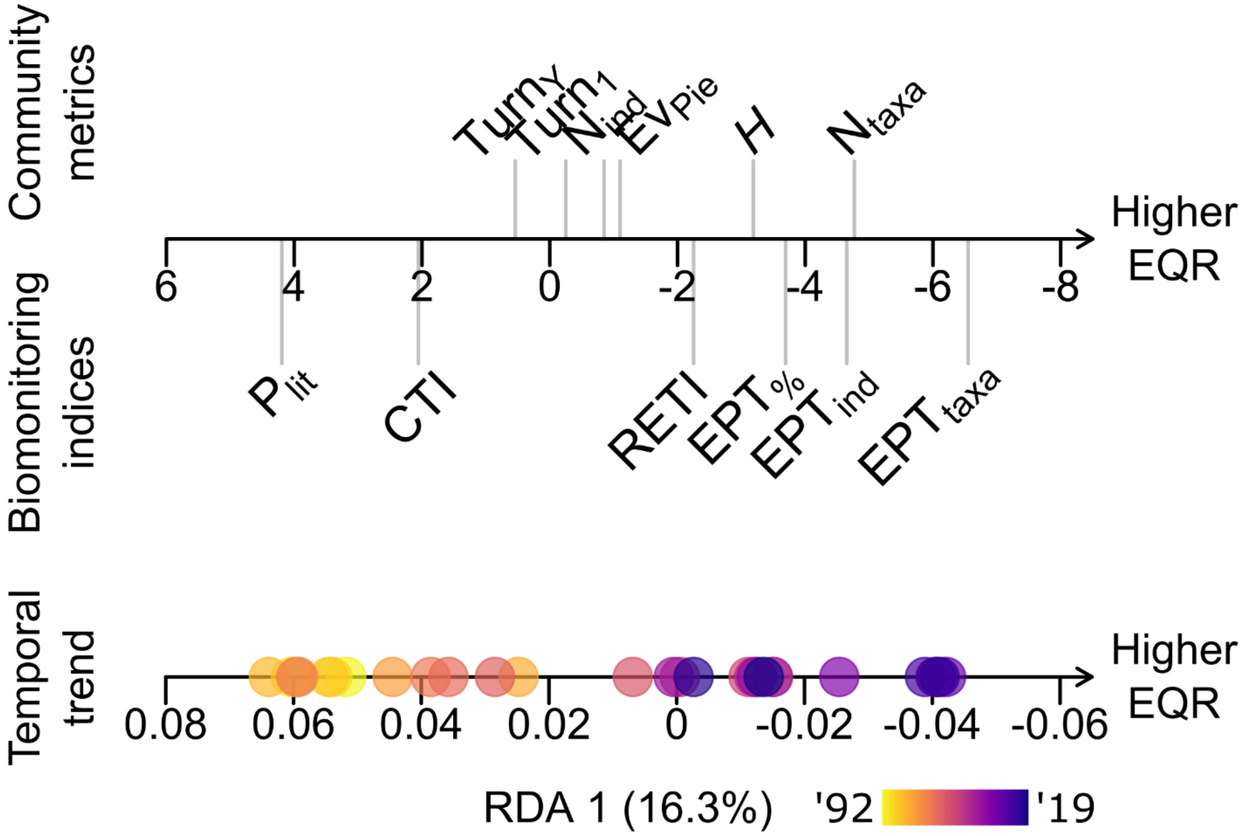


**Supplementary Fig. 1:** Sensitivity of trends in (**a**) Ecological Quality Ratios (EQRs; *n* = 14,361) and (**b**) Ecological Quality Classes (EQCs; 'Mod' = moderate; *n* = 14,361) to the exclusion of countries with datasets shorter than the mean time series length of 18 years (see

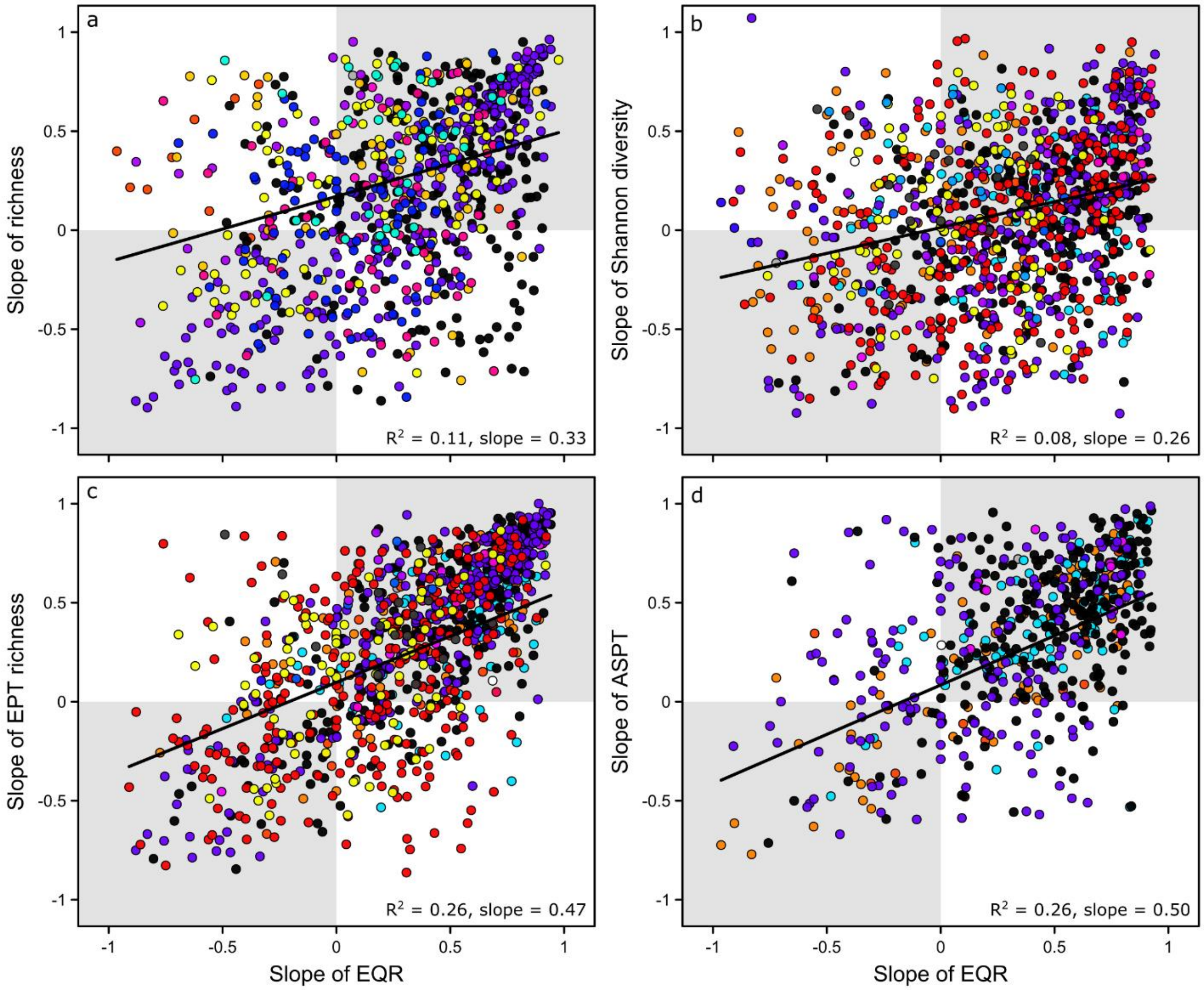
Supplementary Table 2). Black points and vertical grey lines respectively indicate the annual means and standard deviations. Fitted relationships (black lines) and 95% confidence intervals (grey background) were based on the output from generalized additive mixed models. The European Union Water Framework Directive target of a ‘good’ EQC for all waterbodies is indicated by a light blue line in (**b**). Note that the ‘bad’ EQC (class 5) is not plotted.

These results show that the plateau in quality during the 2010s is not driven by adding more countries to the dataset in later years (see differences in time series length in Fig. 4). We also found little influence of individual countries on these trends based on iteratively re-running the analysis with a different country removed in each iteration (i.e., a ‘leave-one-out’ approach).

**Supplementary Information 3** – Circular community metrics and biomonitoring indices



**Supplementary Fig. 2:** Effect of removing sites that calculate Ecological Quality Ratios (‘EQRs’; black arrows) using any of the community metrics or biomonitoring indices involved in our analyses (based on the methods reported in Supplementary Table 1). This analysis includes only sites from Denmark, Estonia, Germany, the Netherlands, and Spain (564 out of 1,365 sites or 41% of the dataset). The results show the same general relationships compared to our analysis of the full dataset (see Fig. 3), specifically that richness is the community metric with the strongest relationship to the EQRs and the EPT indices exhibit the strongest relationships for the biomonitoring indices. We also found the same temporal changes, specifically that the metrics/indices associated with the EQRs tend to show a directional movement from the left to right in the ordination, but there has been little overall change since the early-2010s.



**Supplementary Fig. 3:** Effect of removing sites that calculate Ecological Quality Ratios (‘EQRs’) using (**a**) taxon richness (36% of sites removed), (**b**) Shannon diversity (19% removed), (**c**) the richness of Ephemeroptera, Plecoptera, and Trichoptera (22% removed), and (**d**) the Average Score Per Taxon (ASPT) index (35% removed from a total of 909 sites for which the ASPT index is used). The other community metrics and biomonitoring indices are almost never used to calculate EQRs and so are not plotted because their results would remain the same as in Fig. 6 and Extended Data Fig. 3.

We found that these removals had little overall influence on our results. Specifically, richness still exhibited the most consistent site-level relationship, albeit the relationship was weaker compared to Fig. 6a and fewer sites exhibited matching responses to the EQRS (18% with matching positive or negative slopes that do not overlap 0). The relationship with Shannon diversity was also somewhat weaker compared to Fig. 6b, but we do not present this as a potentially reliable metric in our main text. Similarly, the EPT and ASPT relationships remain generally unchanged (compared to Extended Data Fig. 3), further supporting our conclusion that improving ecological quality is likely caused by improvements in water/habitat conditions.

**Supplementary Information 4** – Country-scale generalized additive mixed models

**Supplementary Table 3:** Coefficients for the effect of year from the generalized additive mixed models of temporal changes in the Ecological Quality Ratio (EQR) for each of 15 countries. Significance ($P < 0.05$) of the smoothed year term in the finalized models was assessed with Wald tests.

| **Country** | ***n*** | **edf** | **F** | ***P*** |
|---|---|---|---|---|
| Belgium | 703 | 1.00 | 32.72 | <0.001** |
| Czechia | 706 | 1.00 | 1.42 | 0.23 |
| Denmark | 5,112 | 1.41 | 65.29 | <0.001** |
| Finland | 150 | 1.00 | 2.57 | 0.11 |
| France | 3,677 | 2.92 | 54.91 | <0.001** |
| Germany | 132 | 1.00 | 2.33 | 0.13 |
| Hungary | 920 | 1.65 | 1.63 | 0.12 |
| Ireland | 269 | 1.00 | 0.002 | 0.97 |
| Lithuania | 293 | 1.00 | 0.005 | 0.94 |
| Luxembourg | 200 | 1.00 | 2.81 | 0.095* |
| Netherlands | 632 | 1.40 | 0.35 | 0.76 |
| Norway | 685 | 2.08 | 6.80 | <0.001** |
| Spain | 4,118 | 1.00 | 92.85 | <0.001** |
| Sweden | 1,284 | 1.00 | 0.47 | 0.49 |
| UK | 467 | 1.00 | 2.01 | 0.16 |

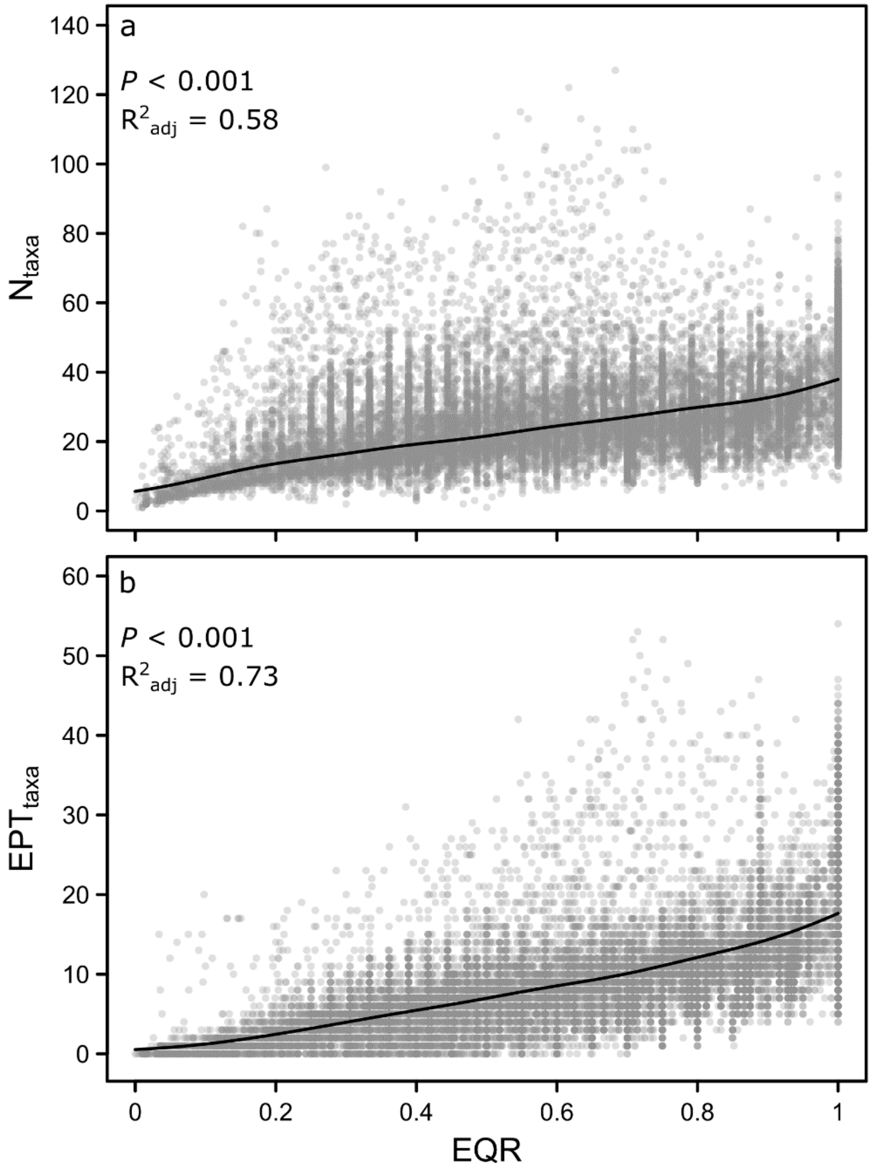
*$P$<0.1 but >0.05 so provides no strong evidence for change but still marginally non-significant
**$P$<0.05 and most are <0.001 so considered fairly strong evidence for change

**Supplementary Table 4:** Coefficients for the effect of year from the generalized additive mixed models of temporal changes in the Ecological Quality Class (EQC) for each of 15 countries. Significance ($P < 0.05$) of the smoothed year term in the finalized models was assessed with Wald tests.

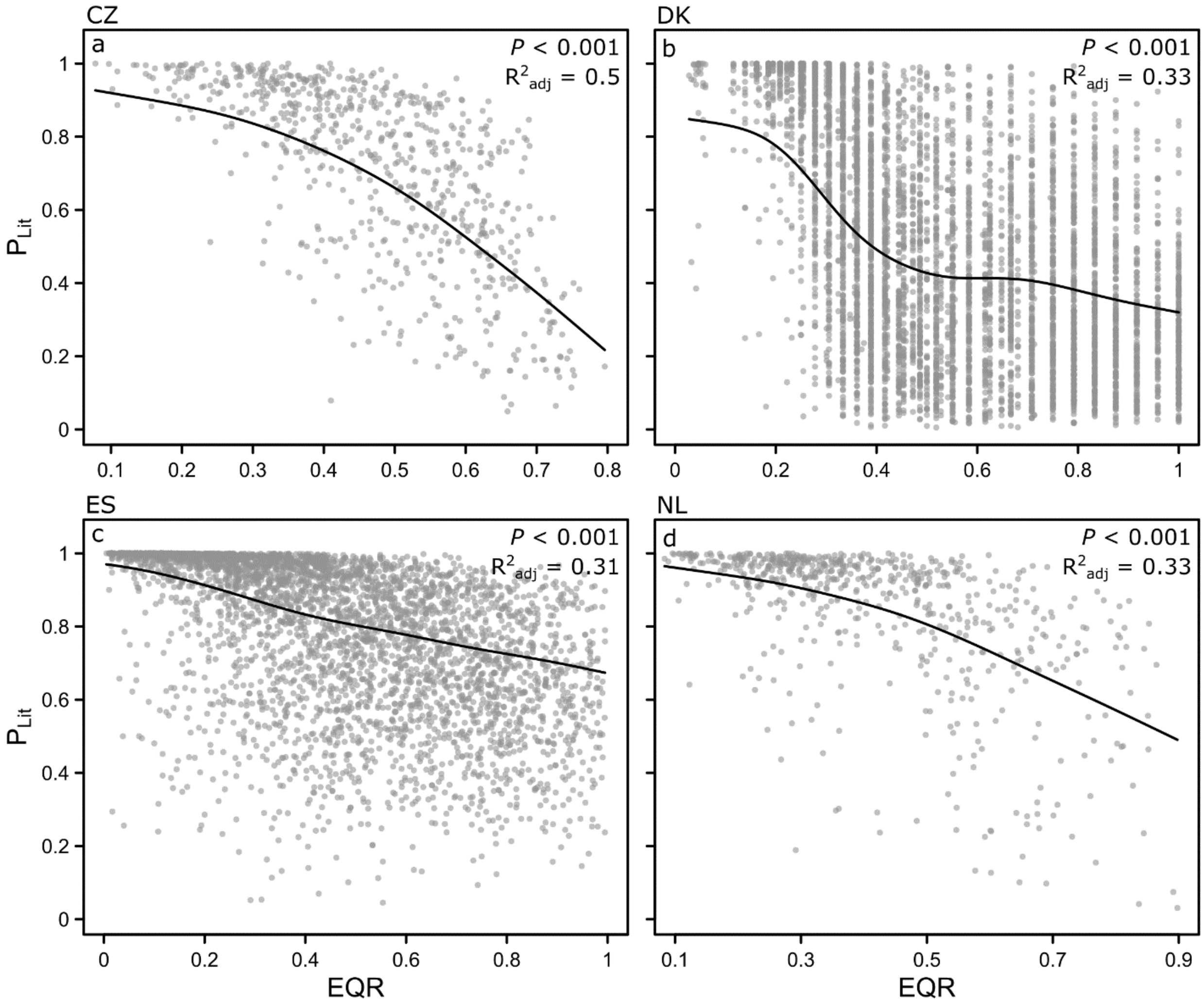
| **Country** | ***n*** | **edf** | **F** | ***P*** |
|---|---|---|---|---|
| Belgium | 703 | 1.00 | 25.76 | <0.001** |
| Czechia | 712 | 2.35 | 1.96 | 0.13 |
| Denmark | 5,112 | 1.00 | 62.91 | <0.001** |
| Finland | 150 | 1.00 | 1.92 | 0.17 |
| France | 3,677 | 2.87 | 56.45 | <0.001** |
| Germany | 162 | 1.00 | 0.016 | 0.90 |
| Hungary | 920 | 1.26 | 3.34 | 0.041** |
| Ireland | 269 | 1.00 | 0.002 | 0.97 |
| Lithuania | 293 | 1.34 | 0.14 | 0.88 |
| Luxembourg | 200 | 1.00 | 1.63 | 0.20 |
| Netherlands | 632 | 1.00 | 0.02 | 0.89 |
| Norway | 685 | 1.00 | 7.42 | <0.001** |
| Spain | 4,118 | 1.00 | 105.58 | <0.001** |
| Sweden | 1,284 | 1.00 | 0.26 | 0.61 |
| UK | 467 | 1.00 | 3.47 | 0.063* |

*$P$<0.1 but >0.05 so provides no strong evidence for change but still marginally non-significant
**$P$<0.05 and most are <0.001 so considered fairly strong evidence for change

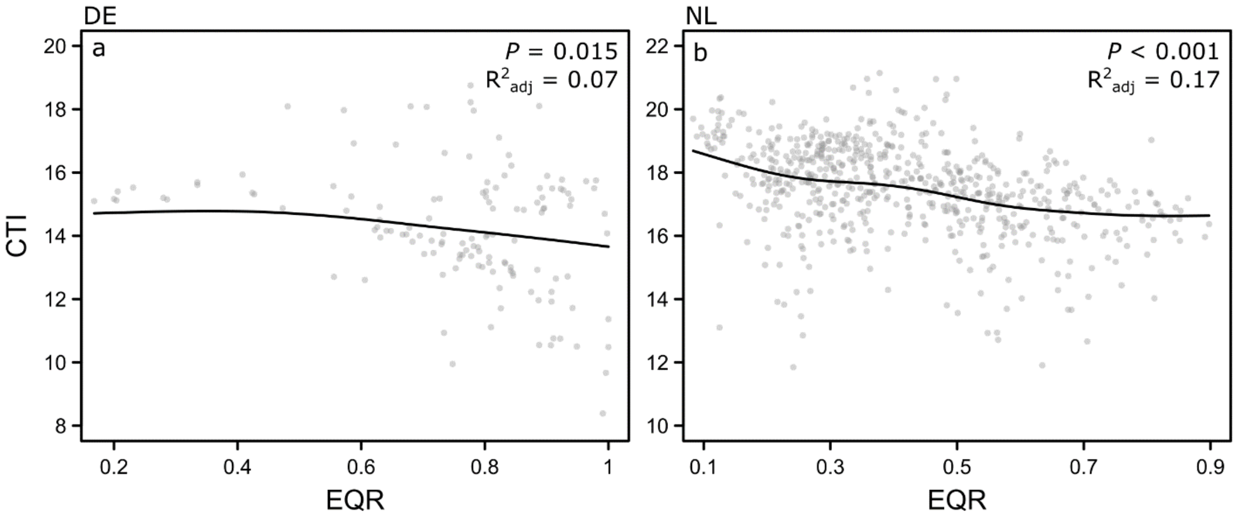
**Supplementary Information 5** – Relationships between ecological quality and individual community metrics or biomonitoring indices.



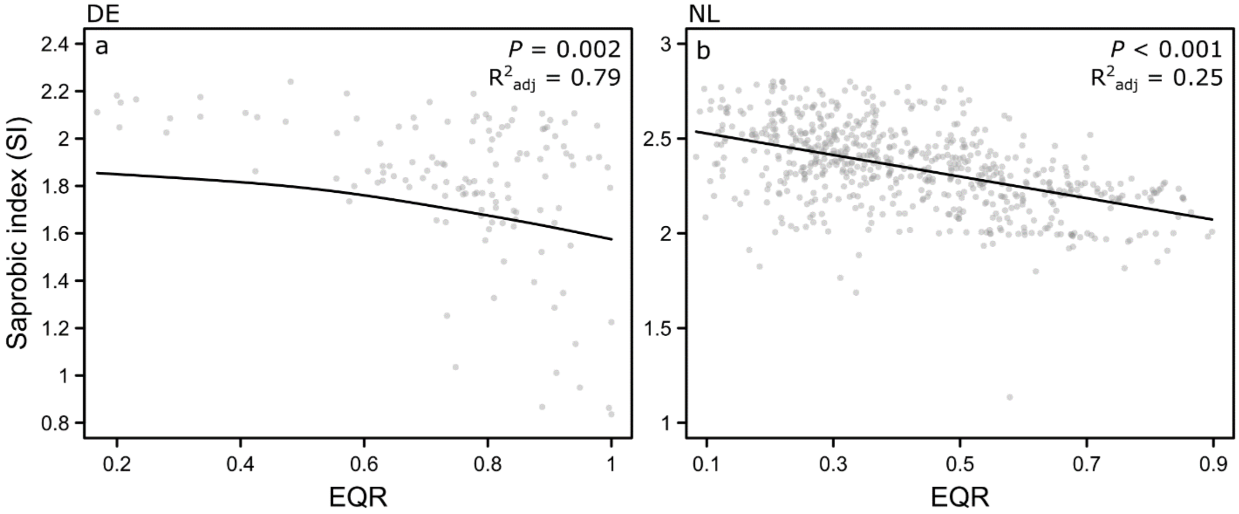
**Supplementary Fig. 4:** Relationships between the Ecological Quality Ratios (EQRs) and (**a**) taxon richness ($N_{taxa}$) and (**b**) the richness of Ephemeroptera, Plecoptera, and Trichoptera taxa ($EPT_{taxa}$) across all countries. Best-fit lines are plotted based on generalized additive mixed models using a Poisson distribution (with a log-link function), a basis dimension of $k = 10$, and thin-plate regression splines. Significance ($P < 0.05$) of the smoothed EQR term in the finalized models was assessed with Wald tests.



**Supplementary Fig. 5:** Relationships between the Ecological Quality Ratios (EQRs) and the proportion of littoral taxa ($P_{lit}$) in (**a**) Czechia (CZ), (**b**) Denmark (DK), (**c**) Spain (ES), and (**d**) the Netherlands (NL). Higher $P_{lit}$ values indicate communities comprised of more littoral taxa. Best-fit lines are plotted based on generalized additive mixed models using a Gaussian (log-link function) or Beta distribution (logit-link function), a basis dimension of $k = 10$, and thin-plate regression splines. Significance ($P < 0.05$) of the smoothed EQR term in the finalized models was assessed with Wald tests.

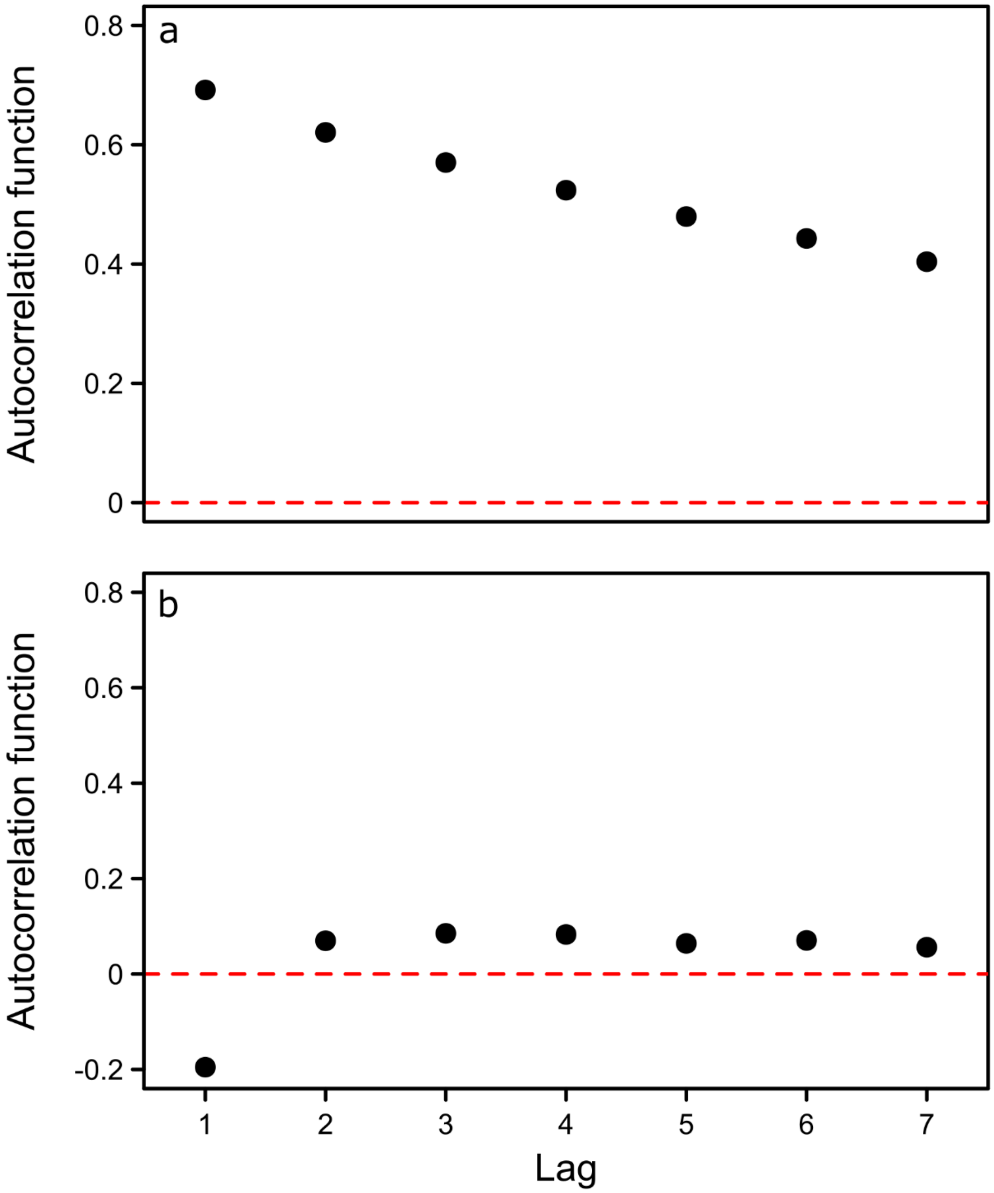


**Supplementary Fig. 6:** Relationship between the Ecological Quality Ratios (EQRs) and the Community Temperature Index (CTI) in (**a**) Germany (DE) and (**b**) the Netherlands (NL). Higher CTI values indicate communities with warmer and wider temperature preferences. Best-fit lines are plotted based on generalized additive mixed models using a Gaussian distribution (log link function), a basis dimension of $k = 10$, and thin-plate regression splines. Significance ($P < 0.05$) of the smoothed EQR term in the finalized models was assessed with Wald tests.

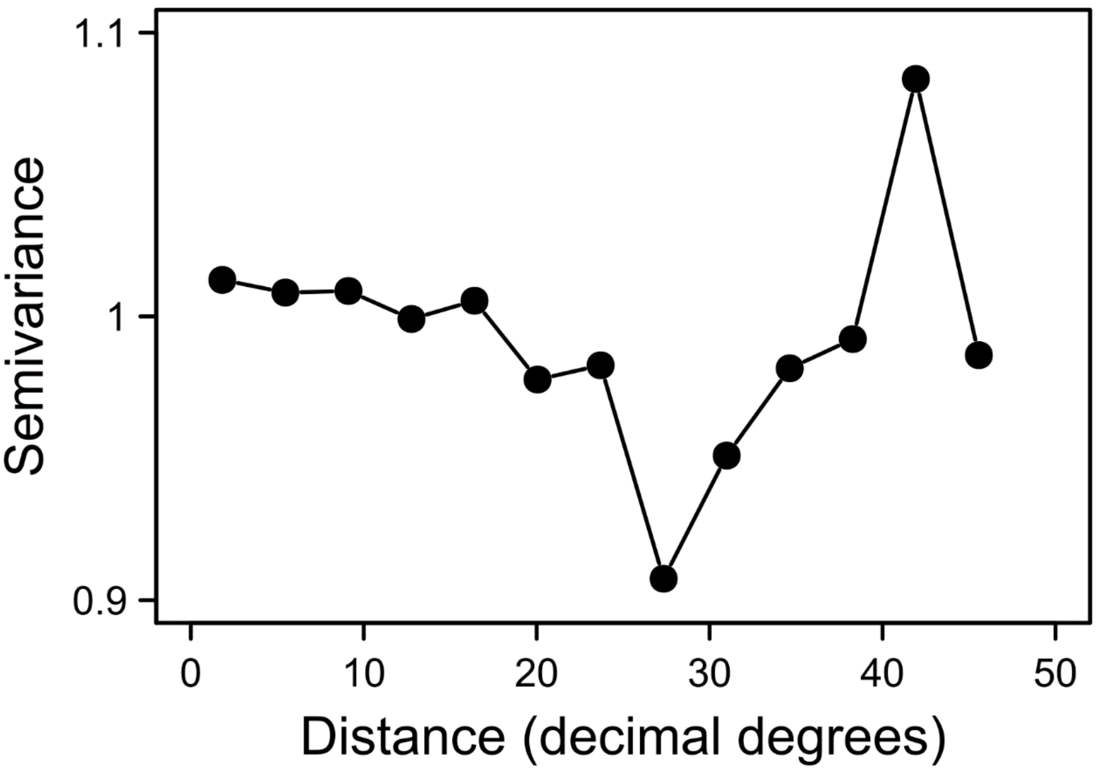


**Supplementary Fig. 7:** Relationships between the Ecological Quality Ratios (EQRs) and the Saprobic Index (SI) in (**a**) Germany (DE) and (**b**) the Netherlands (NL). Higher SI values indicate communities that are more tolerant of organic pollution. Best-fit lines are plotted based on generalized additive mixed models using a Gaussian distribution (log link function), a basis dimension of $k = 10$, and thin-plate regression splines. Significance ($P < 0.05$) of the smoothed EQR term in the finalized models was assessed with Wald tests.

**Supplementary Information 6** – Temporal and spatial autocorrelation



**Supplementary Fig. 8:** Example of temporal autocorrelation in the continent-scale generalized additive mixed model for the Ecological Quality Ratio (EQR; see Fig. 2a). In (**a**), no site-level temporal correlation structure is included in the model so it exhibits relatively high (between 0.4–0.7) autocorrelation across time lags of 1–7 years (all time series have at least seven years of data). In (**b**), adding a first-order autoregressive structure to the model effectively controls for this autocorrelation.



**Supplementary Fig. 9:** Change in the semivariance (i.e., similarity) of the Ecological Quality Ratios among sites at closer versus further geographic distances (in decimal degrees; based on WGS84 latitude and longitude coordinates). The overall change in semivariance with distance is minor (generally between 0.9–1.1) and neither tends to increase or decrease with distance, indicating little spatial autocorrelation.

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