Controls of point and diffuse sources lowered riverine nutrient concentrations asynchronously, thereby warping molar N:P ratios

Katja Westphal1, Andreas Musolff2, Daniel Graeber1 and Dietrich Borchardt1

1 Department Aquatic Ecosystem Analysis, Helmholtz Centre for Environmental Research – UFZ, Brückstr. 3a, 39114, Magdeburg, Germany
2 Department Hydrogeology, Helmholtz Centre for Environmental Research – UFZ, Permoserstr. 15, 04318, Leipzig, Germany
E-mail: katja.westphal@ufz.de

Keywords: nitrogen, phosphorus, stoichiometry, catchment management, long-term monitoring

Abstract

The input of nitrogen (N) and phosphorus (P) into rivers has been reduced in recent decades in many regions of the world to mitigate adverse eutrophication effects. However, legislation focused first on the reduction of nutrient loads from point sources and only later on diffuse sources. These reduction strategies have implications on riverine N:P stoichiometry, which potentially alter patterns of algal nutrient limitation and the functions or community structure of aquatic ecosystems. Here, we use a dataset spanning four decades of water quality for the Ruhr River (Germany) to show that the asynchronous implementation of point and diffuse source mitigation measures combined with time lags of catchment transport processes caused a temporally asynchronous reduction in dissolved inorganic nitrogen and total phosphorus concentrations. This asynchronous reduction increased the molar N:P ratios from around 30 to 100 in the river sections dominated by point sources, reducing the probability of N limitation of algae in favor of P limitation.

The Ruhr River catchment and the environmental policies implemented here illustrate the unintended effects of nutrient control strategies on the ecological stoichiometry at the catchment scale. We urge to assess systematically, whether unintentionally warped macronutrient ratios are observable in other managed river systems and to evaluate their environmental impacts.

1. Introduction

Large-scale mitigation efforts reduced nutrient loads to many rivers in recent decades (Kelly and Wilson 2004, Ibisch et al 2016, Meybeck et al 2018, Westphal et al 2019) to mitigate eutrophication impacts in aquatic ecosystems (Dudgeon et al 2006) and its adverse social and economic consequences (Withers et al 2014). The most critical nutrients of concern are nitrogen (N) and phosphorus (P), which have been excessively released to terrestrial and aquatic ecosystems by intense agriculture and industrial or domestic wastewater inputs (Ibisch et al 2016). The reactive forms of these two nutrients control primary production and thus the eutrophication processes in freshwater and marine ecosystems (Rabalais et al 2009, Dodds and Smith 2016).

At first, targeted nutrient mitigation measures aimed at reducing P inputs from point sources, starting already in the 1970s (Schindler et al 2016). Only later N and P loads from diffuse sources, especially from agriculture, were addressed by policy measures (Ibisch et al 2016). This asynchronous implementation of measures targeting different nutrient sources, but also shifts in their effectiveness altered not only absolute instream N and P concentrations or loads (Paerl 2009) but likely also their stoichiometric proportions.

The stoichiometric N:P ratio is of great importance for nutrient limitation of algal growth (Guildford and Hecky 2000, Keck and Lepori 2012) and potentially impacts the function and community structure of aquatic ecosystems (Peñuelas et al 2013, Meunier et al 2017). The first and most well-known N:P ratio is the Redfield ratio of 16N:1P, which describes the surprisingly consistent ratio of nutrients in marine phytoplankton (Redfield 1960). Various other critical N:P ratios have been proposed in...
recent years to better predict nutrient limitations (Bergström 2010, Keck and Lepori 2012). Knowing the limiting nutrient of in-stream algal growth is crucial to implement effective measures to mitigate adverse environmental impacts.

Alterations in aquatic N:P ratios by human activities were shown at different spatial scales (Penuelas et al 2020) and for long-term time series (Justić et al 1995, Radach and Patisch 2007, Grizzetti et al 2012, Minaudo et al 2015, Burson et al 2016, Oelsner and Stets 2019, Penuelas et al 2020). However, altered N:P ratios of river ecosystems are rarely studied and even less in a Driver-Pressure-State-Impact perspective, although it constitutes a crucial step towards understanding the effects of policy measures on biologically relevant N:P shifts in river ecosystems. We, therefore, analyzed the entire cause-effect-response-chain in a long-term nested record from a central European river catchment (Ruhr River, Germany). The proxies comprise quantitative N and P inputs triggered by sets of policy measures including source control, end-of-pipe treatment and shifting land-use intensities, resulting riverine N and P concentrations, and N:P ratios.

The data record is based on weekly samples from 1989 to 2013 provided by the local river basin management organization and covers a rural-urban impact gradient from upstream to downstream reaches situated at low-mountain to lowland elevations. The time series represents a period of intense, long-term efforts to manage N and P loads and allowed to test the following hypothesis:

Asynchronous mitigation measures targeting nutrients from point and diffuse sources led to sequential reduction of P and N loadings and instream concentrations in the Ruhr River. At the same time, N:P ratios exhibit asynchronous temporal changes of biological relevance.

To test this hypothesis, we focused on three objectives: (1) We evaluated the effects of N and P mitigation measures over time and along the river gradient by using annual average concentrations and concentration-discharge relationships. (2) We determined the temporal and spatial interplay of asynchronous source reduction on N and P concentration (3) We evaluated whether temporal patterns or spatial magnitude of reductions in N and P concentrations have the potential to affect eutrophication via nutrient limitations in relation to molar N:P thresholds as proxies.

2. Methods

2.1. Study site
The Ruhr River in western Germany (figure 1) has a length of 219 km and a catchment area of 4478 km². It drains a densely populated area. In 2017, 64 wastewater treatment plants (WWTPs) treated the wastewater of 2.0 million people (Ruhrverband 2019). The middle and lower reaches are hydrologically controlled by five river impoundments in the main channel and by approximately 1200 weirs throughout the entire catchment area (Raschke and Menzel 2005). The catchment exhibits a distinct rural-urban land-use gradient from upstream to downstream (table 1) (Tetzlaff et al 2009, Ruhrverband 2019).

2.2. Data sources
The river basin management organization for the Ruhr River (Ruhrverband) has carried out weekly routine water quality measurements at seven monitoring sites along the main river from upstream to downstream reaches (figure 1). Westphal et al (2019) showed that the monitoring sites of the upper, middle and lower river reaches divide into three distinct water quality types mainly due to increasing proportions of wastewater related to increasing shares of urban areas (table 1), resulting in corresponding effects on water quality along the catchment gradient. Thus, we selected three representative sites for this study: Wildshausen (UP), Westhofen (MID), and Duisburg (DOWN). UP is the most upstream site, whereas DOWN is located just before the confluence with the Rhine River. MID represents the middle course of the Ruhr River, having a medium anthropogenic impact with only a few medium-sized cities upstream. Since 1966, NH₄-N and TP concentrations, as well as discharge, were measured every week. Since 1989, also nitrate (NO₃-N) has been measured at all monitoring sites. Measurements for nitrate (NO₂-N) are only available from 2000 to 2013. We consider them of minor importance due to their low average proportion of 1.6% for NO₃-N relative to the sum of NH₄-N, NO₂-N, and NO₃-N. This proportion was derived based on annual water quality reports published by the Ruhrverband (Ruhrverband 2001, 2014). We, therefore, refer to dissolved inorganic nitrogen (DIN) as the sum of NO₃-N and NH₄-N in the context of this study. TP, NH₄-N, and NO₃-N were determined using the standard protocols DIN EN ISO 17294-2-E29 (2005), DIN EN ISO 10304-1-D20 (2009), and DIN 38406-E 5-1 (1983), respectively.

Table 1. Sub-catchment information.

|            | UP          | MID        | DOWN       |
|------------|-------------|------------|------------|
| Cumulative catchment area [km²] | 752.1       | 2077.2     | 4468.0     |
| Sub-catchment area [km²]      | 752.1       | 1325.1     | 2390.8     |
| Urban area [%]                | 5           | 14         | 20         |
| Agricultural area [%]         | 35          | 34         | 30         |
| Forested area [%]             | 60          | 51         | 49         |
| Population equivalent of WWTPs | 86 450     | 78 250     | 2 935 205  |
Time series data for N-surplus at the federal state level for the entire time series were provided by Häußermann et al. (2019) and Bach et al. (2011). This data set balances N inputs (different fertilizer types, atmospheric deposition, N-binding by legumes, externally produced feed, and co-substrates) and N outputs (plant and animal market products) on an annual basis. Analogous time series data for agricultural P surplus are not available. However, point sources dominate the P transported to the Ruhr River, and agricultural, diffuse P sources are of minor importance (Westphal et al. 2019). We, therefore, reconstructed time series for per-capita TP loads of domestic wastewater (national level) and the share of population equivalents (PE) from different WWTP steps (catchment level) based on archive and literature information. Further references can be found in Westphal et al. (2019).

2.3. Data analysis

In order to address objective (1) we characterized the temporal evolution of annual median concentrations for NO$_3$-N, NH$_4$-N, and TP, and linked them to data on N-surplus, construction and operation of WWTPs and per-capita changes in TP loads. We characterized the long-term evolution of export patterns by determining concentration-discharge relationships using annual slopes b of linear log C-log Q relationships for NO$_3$-N, NH$_4$-N, DIN, and TP, at each monitoring site. These relationships allow to classify into dilution-driven, mobilization-driven or constant export patterns, which link to potential nutrient sources (point and diffuse sources) (Musolff et al. 2015): A slope b of $-1$ indicates that the concentration of the solute varies inversely with discharge, which implies a dilution-controlled behavior in which a constant flux of the solute is diluted by varying discharge (Godsey et al. 2009) as observed for wastewater point sources (Greene et al. 2011). A positive slope is indicative of the mobilization of solutes with higher discharge. However, it can also point to concentration-discharge independence attributed to high reactivity that reduces low flow concentrations more efficiently or a threshold-driven transport of the constituents, mainly related to diffuse sources (Musolff et al. 2015). If solute concentrations do not vary with discharge ($-0.2 < b < 0.2$, Godsey et al. 2009), a chemostatic export pattern prevails that is assumed to originate from diffuse sources (Greene et al. 2011). Thompson et al. (2011) and Musolff et al. (2017) additionally define a chemostatic export pattern by the ratio of the coefficients of variations of concentrations and discharge ($CV_C/CV_Q < 0.5$) since a low slope b does not necessarily imply that concentrations are invariant.

To address objective (2) we visualized the relative importance of both nutrients by plotting the weekly measured TP against DIN and by fitting a segmented regression to the data, which also required a breakpoint analysis, using the ‘segmented’ package version 1.1–0 (Muggeo 2008) of the software R (R Core Team 2018). We further determined the median annual molar N:P ratios over time and for each monitoring site. We based stoichiometric calculations on DIN and
TP because this ratio is the most reliable predictor for nutrient limitation at least in lake and marine systems (Morris and Lewis 1988, Bergström 2010, Kolzau et al. 2014). In the case of N, the largest bioavailable pool is DIN, whereas the best proxy of P bioavailability is TP since this fraction encompasses both externally available dissolved P and internal reserves of particular P derived from luxury consumption (Ptacinik et al. 2010).

Additionally, we conducted trend characterization of the N:P ratios by employing Kendall’s Tau Test based on weekly data and for every monitoring site. Using a Mann-Whitney U-Test, we tested for the equality of molar N:P ratios among monitoring sites, based on weekly available data. Our data met all assumptions of the statistical tests.

For discriminating between N and P limitation, we used a range of N:P thresholds introduced by Keck and Lepori (2012), who found that predictions of N or P limitation were highly uncertain except at extreme N:P molar ratios. N:P ratios of ≤1:1 are associated with a high probability of N limitation of microphytobenthos, whereas N:P ratios >100:1 are associated with a higher probability of P limitation.

We performed all statistical analyses with the software R, version 3.5.2. (R Core Team 2018).

3. Results

3.1. Temporal evolution of nutrient concentrations

Annual median DIN concentrations were highest with 4.51 mg L⁻¹, 5.03 mg L⁻¹, and 5.63 mg L⁻¹ in 1991, 1996, and 1991 at UP, MID, and DOWN, respectively. Concentrations then decreased by 38%, 43%, and 54% (figure S1(a), tables 2 and 3). Note that NO₃-N mainly dominates this pattern due to its significant contribution to DIN (figure S1(a) (available online at stacks.iop.org/ERL/15/104009/mmedia), tables 2 and 3). Agricultural N-surplus rates, likely the primary source for DIN, were highest in 1983 with 146 kg ha⁻¹, and exhibit a clear downward trend since 1987 (figure 2(b)). In total, surplus rates decreased by 26% until 2013 and stabilized since the mid-1990s around values of about 100 kg ha⁻¹ yr⁻¹ (table 2). Annual DIN concentrations and N surplus rates weakly correlated (figure S7).

Concentrations of NH₄-N decreased in the 1970s by 96%, 97%, and 97% for UP, MID, and DOWN, respectively (figure S1(b), table 2). This reduction is in line with the proportion of biological treatment that considerably increased since 1976 in the Ruhr catchment. Since 1996, the proportion of nitrification and denitrification stages in WWTP has also increased sharply (figure S1(d)), efficiently removing NH₄-N from wastewater.

Annual median TP concentrations peaked at all sites in 1976 with maximum annual median concentrations of 0.42 mg L⁻¹, 0.59 mg L⁻¹, and 1.04 mg L⁻¹ at UP, MID, and DOWN (figure 2(c), tables 2 and 3). Since the mid-1990s, annual median TP concentrations have stabilized at <0.1 mg L⁻¹. Per-capita TP loads in domestic wastewater, which are linked to instream TP concentrations, were highest in 1975 with 5 g TP cap⁻¹ d⁻¹ and then decreased by 64% until 2013 (figure 2(d), tables 2 and 3). Since the mid-1990s, per-capita TP loads have stabilized around 1.8 g TP cap⁻¹ d⁻¹. Annual median TP concentrations and annual per-capita loads strongly correlated (figure S8).

Besides the per-capita TP loads, the number of WWTPs in the Ruhr catchment also increased from the mid-1970s to the early 1990s (figure S2). Accordingly, the number of people—expressed as population equivalent (PE)—whose wastewater has been biologically treated increased by a factor of 2.3 since the mid-1970s (figure 2(e)). Since the beginning of the 1990s, also the number of people whose wastewater has been treated with P removing treatment steps such as precipitation or biological P elimination increased 3.4 fold (figure 2(e)).

3.2. Temporal evolution of export patterns

For all three monitoring sites, the C-Q regression slopes for DIN (b_DIN) were mostly in the range of −0.2 and 0.2 (figure 3), pointing to a chemostatic export pattern. However, the Mann-Kendall test revealed a significant positive trend over time for MID and DOWN, but not for UP (table S1), indicating a change of export patterns from chemostatic towards mobilization for the mid and downstream reach. The temporal development for b_DIN is consistent with the development of CV_DIN/CV_Q ratios, remaining below 0.5 (figures S3 and S4). For NO₃-N, the slopes b_NO₃ and CV_NO₃/CV_Q corresponds with DIN (figures S3–S5). Export patterns for NH₄-N were dynamic throughout the time record at all sites with initially dilution-driven (b_NH₄ ≤ −0.2), then chemostatic (−0.2 ≤ b_NH₄ < 0.2) and finally mobilization-driven phases (b_NH₄ ≥ 0.2) (figure S5). Annual CV_NH₄/CV_Q was also variable and increased over time and amounted to ≥0.5 (figures S3 and S4).

For TP, slopes b_TP varied in time at all sites, beginning with a phase of a more dilution-driven export pattern (b ≤ −0.2) and then changing at the beginning of the 1980s towards a chemostatic export pattern with b_TP being within a range of −0.2 and 0.2 (figure 3-TP) and CV_TP/CV_Q ratios below 0.5 (figures S3 and S4).

3.3. Resulting nutrient stoichiometry and limitation

The N:P ratios showed a temporally dynamic behavior at all sites with partly synchronized phases of increasing and decreasing trends in 1994, 2002, and 2009. (figure 4(d), table 4, figure S6). While MID and DOWN sites showed an increase in N:P ratios from an initial low level of N:P ratios between 1988
and 1994, UP started already from a higher level of N:P ratios (table 4). This temporal difference is also reflected by the DIN-TP relationships that revealed a significant break at 0.17 and 0.19 mg TP L\(^{-1}\) for MID and DOWN (figures 4(b), (c), table S2), which divides the DIN-TP relationships into two parts. Both breakpoints correspond to the years 1991/1992 with decreasing TP concentrations but constant DIN concentrations before, followed by a significant decrease in both TP and DIN concentrations (table S3). For UP, the decline of TP concentrations between 1989 and 1992 was not as substantial as for MID and DOWN (table S3). In contrast to MID and DOWN, TP concentrations in UP were already at a lower level before 1992 (figure 4(a)).

In total, annual median N:P ratios have increased from 1989 to 2013 by 19% for UP, 105% for MID, and 25% for DOWN (table 2). Overall mean N:P molar ratios decreased along the river gradient from UP (N:P\(_{UP} = 83 \pm 19\)) to MID (N:P\(_{MID} = 79 \pm 19\)) to DOWN (N:P\(_{DOWN} = 69 \pm 16\)). A Mann-Whitney U-test also revealed that UP and MID have significantly higher N:P ratios than DOWN (table S4).

Considering the thresholds defined by Keck and Lepori (2012), P limitations (N:P ratios ≥ 100) were likely to occur in 33% of the time in UP, 28% of the time in MID, and 21% of the time in DOWN based on weekly data. Especially since the beginning of 2000, N:P ratios have been very close to or above 100, especially for UP and MID (table 4). N limitation with N:P ratios < 1 did not occur.

### 4. Discussion

#### 4.1. Temporal evolution of point- and diffuse-source nutrient contributions

We found significant declines in concentrations of N and P components due to nutrient mitigation measures, mostly related to policies introduced in the 1970s and 1980s in order to control eutrophication.

At all sites, annual slopes b and CV\(_C/CV_Q\) ratios for NO\(_3\)-N and DIN indicated a chemostatic export pattern for most of the time, suggesting that diffuse sources dominated instream NO\(_3\)-N and DIN over the entire time record and hence mitigation measures targeting diffuse sources also caused a decline in NO\(_3\)-N and DIN concentrations. Hence, from 1987 onwards, the reduction of agricultural N-surplus rates led to a reduction of DIN and NO\(_3\)-N concentrations. Interestingly, the decline in N surplus rates started before the adoption of fertilizer limiting regulations such as fertilizer acts (BGBl. I S. 2134 1977, BGBl. IS.1435 1989) or the EU Nitrate Directive (Directive 91/676/EEC 1991) which may also be related to changed land-use practices (Blesh and Drinkwater 2013).

For NH\(_4\)-N, the alterations of C\(_{NH4-Q}\) shapes over time indicate a fundamental shift in sources, as evidenced by the temporal evolution from a...
Figure 2. Upper panels (a) and (c) show the long-term development of annual median DIN and TP concentrations for each monitoring site. The long-term developments of DIN concentrations were related to the N-surplus of North Rhine-Westphalia (b). TP concentrations were related to the potential drivers of annual per-capita TP load in domestic wastewater (d) and the sum of population equivalent (PE) of WWTPs with biological and P elimination treatment steps (e). The solid smooth lines show a non-parametric locally weighted regression (Loess) fitted to the data with 95% confidence interval in lighter colors. Dashed lines mark the turning points of the respective time series.

Figure 3. Long-term development of the annual slope (b) of the linear regression between discharge and concentration in log-log space (b) for DIN and TP for each site. The solid grey, orange, and blue lines show a non-parametric locally weighted regression (Loess) fitted to the data with 95% confidence interval in lighter colors. The two dashed lines mark an area that Godsey et al (2009) defined as chemostatic (independence of concentration from discharge).

more dilution-driven to a more chemostatic export pattern. High slopes and high CV_{NH4}/CV_Q ratios since mid-1990s point to uncoupling effects of concentrations from discharge related to changes in NH4-N sources. This uncoupling is likely due to fast NH4-N turnover in the stream that dominates over mobilization processes (see also Musolff et al 2015).

The reduction of NH4-N concentrations likely links to reduced N loads originating from point sources induced by the increased availability of biological wastewater treatment and the technical upgrading of WWTPs with additional nitrifying and denitrifying treatments steps in the mid-1990s. We attribute these changes to various national and European policies in the 1970s to 1990s (e.g. amend-
Figure 4. (a)–(c): Annual median long-term development of TP versus DIN concentrations at all three monitoring sites. The color gradient from red to blue indicates the course of time. The solid black line is a regression indicating the changing characteristics of the DIN-TP relationships. The grey dots in the background represent the non-aggregated weekly measurements and illustrate the range of concentrations occurring. (d): Long-term development of annual median molar N:P ratios over time from up to downstream. The dashed horizontal lines indicate molar N:P ratios of 1:1 and 100:1. N:P ratios < 1:1 indicate nitrogen limitation. N:P ratios above 100:1 indicate a higher probability for phosphorus limitation (Keck and Lepori 2012). Dashed vertical lines indicated years of a trend change.

Table 4. Site-specific mean N:P ratios, standard deviation (sd), and trend statistics (Kendall’s τ and p-value) for four different periods defined by the years of trend change according to figure 4. Computations are based on weekly data.

|       | 1989–1994 | 1994–2002 | 2002–2009 | 2009–2013 |
|-------|-----------|-----------|-----------|-----------|
| **UP** |           |           |           |           |
| mean  | 87.1      | 78.7      | 104.9     | 106.1     |
| sd    | 61.6      | 32.9      | 76.6      | 54.3      |
| Kendall’s τ | 0.01  | −0.01    | 0.23      | −0.11     |
| p-value | <0.001 | <0.001   | 0.001     | 0.001     |
| **MID** |           |           |           |           |
| mean  | 67.7      | 81.8      | 91.0      | 107.6     |
| sd    | 28.3      | 24.1      | 32.5      | 52.4      |
| Kendall’s τ | 0.48  | −0.115   | 0.264     | −0.162    |
| p-value | <0.001 | <0.001   | <0.001    | 0.001     |
| **DOWN** |          |           |           |           |
| mean  | 75.1      | 81.6      | 78.9      | 78.6      |
| sd    | 60.9      | 38.1      | 31.7      | 34.8      |
| Kendall’s τ | 0.48  | −0.14    | 0.14      | −0.21     |
| p-value | <0.001 | <0.001   | <0.001    | <0.001    |

For TP Westphal et al. (2019) identified decreasing per-capita TP release due to the German Phosphate Limit regulation (BGBl. I S. 664 1980) and technical upgrades of WWTPs due to various administrative regulations in the 1970s as the primary reason for significant concentration reduction. These measures led to a fundamental shift in P sources or transport and retention mechanisms, altering $C_{TP}Q$ shapes.

4.2. Synchronicity and magnitude of changes of riverine N and P concentrations

The riverine response to the implementation of measures for addressing point sources (to control TP and NH$_4$-N) and diffuse sources (to control NO$_3$-N) took place at different times with a time lag of at least 10 years: for point sources mostly in the mid-1970s, and for diffuse sources not before 1987 as the main driver for NO$_3$-N concentration, N-surplus rates, began to decline only then.

In addition to the asynchronous timing of the legislation addressing point and diffuse sources, there is apparent asynchronicity in the response of riverine concentration to mitigation measures. Whereas TP concentrations showed a direct response to reduced per-capita TP loads and enhanced wastewater treatment, DIN concentrations reacted with a time lag to reduced N-surplus rates. Taking the observed maximum annual DIN concentrations, we found a time
lag of 4 years (UP and DOWN) to 9 years (MID). However, the determination of turning points is somewhat imprecise, as no measurements are available before 1989. Riverine concentrations often react with a delayed response to improved N management (Ehrhardt et al. 2018), which is related to various reasons such as long groundwater residence times, that leads to long flush-out times of nitrate from groundwater (Hamilton 2012). Additionally, the long-term accumulation of soil N stocks (Worrall et al. 2015, Dupas et al. 2018) and shifts in precipitation and discharge patterns (Dupas et al. 2016) influence the in-stream-response to improved N management.

Besides asynchronous timing in N and P concentration, there were also differences in the extent and magnitude of N and P reduction. The management of point sources (TP and NH$_4$-N) was more effective than the treatment of diffuse sources (NO$_3$-N). The percentage decrease has been much higher for TP and NH$_4$-N (~86% to 97%) than for NO$_3$-N (~31% to 44%) (table 2). This imbalance in the reduction of N and P was also reported for other riverine systems (Kreiling and Houser 2016, Cozzi et al. 2019, Ibáñez and Penuelas 2019).

4.3. Magnitude and biological relevance of shifts in molar N:P stoichiometry

At the beginning of the time series (figure 4(d), figure S6), we found lower N:P ratios at the MID and DOWN sites (~30 to 40) than for the UP site (~80). The critical difference lies in the level of TP concentrations, which were already at a considerably lower level in UP compared to MID and DOWN, with DIN concentrations being at a similar level in all sites. The rapid decrease in TP concentrations in direct response to the measures described above created a sudden increase of stoichiometric ratios associated with a doubling of molar N:P ratios in MID and DOWN. High TP concentrations before 1989 likely led to extremely low N:P ratios, which probably dropped to a minimum in the mid-1970s when TP concentrations reached its maximum. Therefore, N:P ratios were probably subject to even stronger fluctuations in the course of various eutrophication measures than can be shown here due to the limited length of DIN time series. Similar extremely low N:P ratios between 1960 and 1990 and a sudden shift in the early 1990s were also shown by Wentzky et al. (2018) and Radach and Pätsch (2007). The increase of N:P ratios in their study coincides with the increase of ratios in our study and showed similar trend reversals in mid-1990, early 2000 and again around 2010, suggesting similar drivers across Germany. Here, increasing N:P ratios at the beginning of the time series are likely due to non-simultaneous reductions of N and P loads. These are contrasted by declining N:P ratios at the end of the time series, best explained by now constant TP concentrations but decreasing DIN concentrations. However, shifted discharge patterns may also have played a role for sudden trend reversals, as Green et al. (2007) and Green and Finlay (2010) showed that N:P ratios consistently decline during high flows. At the Ruhr River, extreme rainfall in February caused a significant flood in 2002 across monitoring sites, potentially causing a decline in N:P ratios.

The significant difference in N:P ratios between sites can be best explained by the general lower TP concentrations at UP and MID due to lower urban land use contribution, compared to DOWN with a higher contribution of urbanized areas in the catchment (table 1). Other studies also found such longitudinal urbanization effects due to WTP influence (Choi et al. 2015, Yan et al. 2016, Yun and An 2016) and decreasing N:P ratios along the river gradient were also described for other locations in Europe and Asia (Yin et al. 2004, Dupas et al. 2017).

The shift of nutrient sources in the Ruhr River not only caused substantial changes in stoichiometry but has reduced the probability of N limitation and increased the probability of P limitation over time, in particular from the beginning of the 2000s until 2013 and especially in the up and midstream reaches, where quantitative P limitation occurred in about 30% of the weekly measurements. Besides relative concentrations, absolute concentrations are also relevant as they determine the magnitude of the nutrient effects on biomass (Dodds 2003, Keck and Lepori 2012). Data from more than 200 temperate streams indicated breakpoints at 40 µg TN L$^{-1}$ and 30 µg TP L$^{-1}$, above which chlorophyll concentrations were substantially higher (Dodds et al. 2002). These results are corroborated by Withers and Bowes (2018), which specify values below 0.03 to 0.1 mg SRP L$^{-1}$ for limited algae growth.

In the Ruhr River, N and P concentrations were well above these limits throughout the complete time record despite significant efforts to control eutrophication. Thus, the N:P ratios may have had less influence on the extent of algae growth (see also figure S9), but likely influenced riverine community structure and species diversity (Bowmann et al. 2005, Gafner and Robinson 2007, Li et al. 2010, Penuelas et al. 2020), given the magnitude of the inter-annual fluctuations of N:P ratios in all three sites of the Ruhr River. Phytoplankton diversity may have been depleted, as very few species can compete for the limiting P (Elser et al. 2009). Increased P-limitation of phytoplankton also may have changed the performance of food webs, as P-limited algae are a poor food source for zooplankton due to an unsuitable biochemical composition (Müller-Navarra 1995) or low P content (Elser et al. 2001). Besides structural changes, also functional changes associated with altered N:P ratios were described (Artigas et al. 2008, Schade et al. 2011).

However, these structural and functional changes related to altered N:P ratios are complex and not
well understood yet (Yan et al. 2016). We, therefore, urge to systematically assess whether similar asynchronous developments and unequal reductions in N and P, and similar shifts in N:P ratios and a higher probability of P limitation, can be observed in other river systems due to similar changes in environmental policy. It is essential to assess the extent of the environmental impact of such and similar sequences of the described environmental policy decisions in order to determine whether specific stoichiometric-based freshwater management is required, e.g. that synchronize of N and P measures in time and acknowledge the natural constraints of catchments leading to substantial time lags in nutrient transport. Synchronous N and P reduction measures will be of particular benefit for those regions that experience rapid economic growth without adequate environmental protection, leading to significant degradation of water quality in freshwaters, and which will soon address these problems with more stringent environmental legislation.

Our study also highlights the need for efficient controls of diffuse nutrient sources beyond current practices and the avoidance of further N accumulation in anthropogenic landscapes (Pael 2009, Ehrhardt et al. 2018, Ellermann et al. 2018). Our findings are also relevant for the freshwater-to-marine continuum, as upstream nutrient management actions in the form of a P-only reduction strategy leads to an intensification of downstream N-controlled phytoplankton blooms in coastal waters, particularly in those with increased N loadings (Pael 2009).

Acknowledgments

We thank the Ruhrverband for providing the data set and the anonymous reviewers for their helpful comments on improving the manuscript. The authors would also like to thank Olaf Büttner for his support with GIS-based analyses.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

Artigas J, Romani A M and Sabater S 2008 Relating nutrient molar ratios of microbial attached communities to organic matter utilization in a forested stream Fund. Appl. Lim. 173 255–64
BGBl. I S. 2134 1977 Düngemittelgesetz: DüMG
BGBl. I S. 664 1980 Verordnung über Höchstmengen für Phosphate in Wasch- und Reinigungsmitteln: Phosphathöchstmengenverordnung - PhHöchstMengV
BGBl I S. 3017 1976 Wasserhaushaltsgesetz: WHG
Bach M, Godlinski P and Greif J-M 2011 Handbuch Berechnung der Stickstoff-Bilanz für die Landwirtschaft in Deutschland
Jahre 1990 - 2008 (Braunschweig: Julius Kühn-Institut, Federal Research Centre for Cultivated Plants)
Bergström A-K 2010 The use of TN: TP and DIN:TP ratios as indicators for phytoplankton nutrient limitation in oligotrophic lakes affected by N deposition Aquat. Sci. 72 277–81
BGBl. I. S. 1435 1989 Gesetz zur Förderung der bäuerlichen Landwirtschaft. LaFG
Blesh J and Drinkwater L E 2013 The impact of nitrogen source and crop rotation on nitrogen mass balances in the Mississippi River Basin Ecol. Appl. 23 1017–35
Bowmann M F, Chambers P A and Schindler D W 2005 Changes in stoichiometric constraints on epilithon and benthic macroinvertebrates in response to slight nutrient enrichment of mountain rivers Freshwater Biol. 50 1836–52
Burson A, Stomp M, Akil L, Brussaard C P D and Huisman J 2016 Unbalanced reduction of nutrient loads has created an offshore gradient from phosphorus to nitrogen limitation in the North Sea Limnol. Oceanogr. 61 869–88
Choi J-W, Han J-H, Park C-S, Ko D-G, Kang H-I, Kim J Y, Yun Y-J, Kwon -H-H and An K-G 2015 Nutrients and seostic chlorophyll dynamics in Asian lotic ecosystems and ecological stream health in relation to land-use patterns and water chemistry Ecol. Eng. 79 15–31
Cozzi S, Ibáñez C, Lazár L, Rambault P and Giani M 2019 Flow regime and nutrient-loading trends from the largest south European watersheds: implications for the productivity of mediterranean and Black Sea's coastal areas Water 11 1
DIN 38406-E 5-1 1983 German standard methods for the examination of water, waste water and sludge; cations (group E); determination of ammonia-nitrogen (E 5) (DIN)
DIN EN ISO 10304-1-D 20 2009 Water quality—Determination of dissolved anions by liquid chromatography of ions—Part 2: determination of chloride, fluoride, nitrate, nitrite, phosphate and sulfate (ISO 10304-1:2007); German version EN ISO 10304-1:2009 (DIN)
DIN EN ISO 17294-2-E29 2005 Water quality—Application of inductively coupled plasma mass spectrometry (ICP-MS)—Part 2: determination of 62 elements (ISO 17294-2:2000); German version EN ISO 17294-2:2004 (DIN)
Dodds W and Smith V 2016 Nitrogen, phosphorus, and eutrophication in streams IW 6 155–64
Dodds W K 2003 Misuse of inorganic N and soluble reactive P concentrations to indicate nutrient status of surface waters J. N. Am. Benthol. Soc. 22 171–81
Dodds W K, Smith V H and Lohman K 2002 Nitrogen and phosphorus relationships to benthic algal biomass in temperate streams Can. J. Fish. Aquat. Sci. 59 865–74
Dudgeon D et al 2006 Freshwater biodiversity: importance, threats, status, and conservation challenges Biol. Rev. Camb. Philos. Soc. 81 163–82
Dupas R, Jomaa S, Musolff A, Borchardt D and Rode M 2016 Disentangling the influence of hydroclimatic patterns and agricultural management on river nitrate dynamics from sub-hourly to decadal time scales Sci. Total Environ. 571 791–800
Dupas R, Minaudo C, Gruau G, Ruiz L and Gascuel-Odoux C 2018 Multidecadal trajectory of riverine nitrogen and phosphorus dynamics in rural catchments Water Resour. Res. 54 5327–40
Dupas R, Musolff A, Jawitz J W, Rao P S C, Jäger C G, Fleckenstein J H, Rode M and Borchardt D 2017 Carbon and nutrient export regimes from headwater catchments to downstream reaches Biogeoosciences 14 4391–467
Directive 91/676/EEC 1991 Council Directive of 12 December concerning the protection of waters against pollution caused by nitrates from agricultural sources: Nitrates Directive - ND Directive 91/271/EEC 1991 Urban Waste Water Treatment Directive: UWWT
Ehrhardt S, Kumar R, Fleckenstein J H, Attigiger S and Musolff A 2019 Trajectories of nitrate input and output in three nested
catchments along a land use gradient *Hydrol. Earth Syst. Sci.* **23** 3503–24

Ellermann T et al 2018 Nitrogen deposition on Danish nature *Atmosphere* **9** 447

Elser J J, Andersen T, Baron J S, Bergström A-K, Jansson M, Kyle M, Nydick K R, Steger L and Hessen D O 2009 Shifts in lake N:P stoichiometry and nutrient limitation driven by atmospheric nitrogen deposition *Science (New York, N.Y.)* **326** 835–7

Elser J J, Hayakawa K and Urabe J 2001 Nutrient limitation reduces food quality for zooplankton: daphnia response to seston phosphorus enrichment *Ecology* **82** 898–903

Gafner K and Robinson C T 2007 Nutrient enrichment influences the response of stream macroinvertebrates to disturbance *J. N. Am. Benthol. Soc.* **26** 92–102

GMBS S 1989 Rahmen-Abwasser-Verwaltungsvorschrift über Mindestanforderungen an das Einleiten von Abwasser in Gewässer: Rahmen-AbwasserVvU

Godsey S E, Kirchner J W and Clow D W 2009 Concentration-discharge relationships reflect chemostatic characteristics of US catchments *Hydrol. Process.* **23** 1844–64

Green M B and Finlay J C 2010 Patterns of hydrologic control over stream water total nitrogen to total phosphorus ratios *Biogeochemistry* **99** 15–30

Green M B, Nieber J L, Johnson G, Magnen J and Schafer B 2007 Flow path influence on an N:P ratio in two headwater streams: a paired watershed study *J. Geophys. Res.* **112** n/a

Greene S, Taylor D, Mcelarney Y R, Foy R H and Jordan P 2011 An evaluation of catchment-scale phosphorus mitigation using load apportionment modelling *Sci. Total Environ.* **409** 2211–21

Grizetti B, Bouraoui F and Aloe A 2012 Changes of nitrogen and phosphorus loads to European seas *Glob. Change Biol.* **18** 769–82

Guilford S J and Hecky R E 2000 Total nitrogen, total phosphorus, and nutrient limitation in lakes and oceans: is there a common relationship? *Limbol. Oceanogr.* **45** 1213–25

Hamilton S K 2012 Biogeochemical time lags may delay responses of streams to ecological restoration *Freshw. Biol.* **57** 43–57

Haußermann U, Bach M, Klement L and Breuer L 2019 Stickstoff-Flächenbilanzen Für Deutschland Mit Regionalgliederung Bundesländer Und Kreise—Jahre 1995 Bis 2017: Methodik, Ergebnisse Und Minderungsmaßnahmen (Dessau-Roßlau: UBA)

Ibáñez C and Petuelle J 2019 Changing nutrients, changing rivers *Science* **365** 637–8

Ibisch R, Austnes K, Borchart D and Boteler B 2016 European Assessment of Eutrophication Abatement Measures across Land-Based Sources, Inland, Coastal and Marine Waters (Magdeburg, Germany: UFZ)

Justič D, Rabalais N N, Turner R E and Dortch Q 1995 Changes in nutrient structure of river-dominated coastal waters: stoichiometric nutrient balance and its consequences *Estuar. Coast. Shelf Sci.* **40** 339–56

Keck F and Lepori F 2012 Can we predict nutrient limitation in streams and rivers? *Freshw. Biol.* **57** 1410–21

Kelly M G and Wilson S 2004 Effect of phosphorus stripping on water chemistry and diatom ecology in an eastern lowland river *Water Res.* **38** 1559–67

Kolzau S, Wiedner C, Rücker J, Köhler J, Köhler A and Dolfman A M 2014 Seasonal patterns of nitrogen and phosphorus limitation in four German lakes and the predictability of limitation status from ambient nutrient concentrations *PLoS One* **9** e06065

Kreiling R M and Houser J N 2016 Long-term decreases in phosphorus and suspended solids, but not nitrogen, in six upper Mississippi River tributaries, 1991–2014 *Environ. Monit. Assess.* **188** 454

Li Y, Li D, Tang J, Wang Y, Liu Z and He S 2010 Long-term changes in the Changjiang Estuary plankton community related to anthropogenic eutrophication *Aquat. Ecosyst. Health Manage.* **13** 66–72

Meunier C L, Boersma M, El-Sabaawi R, Halvorsen H M, Herstoff E M, van de Waal D B, Vogt R J and Litchman E 2017 From elements to function: toward unifying ecological stoichiometry and trait-based ecology *Front. Environ. Sci.* **5** E2604

Meybeck M, Lestel L, Carrel C, Bouleau G, Garnier J and Mouchel J M 2018 Trajectories of river chemical quality issues over the Longue Durée: the Seine River (1900–2010) *Environ. Sci. Pollut. Res. Int.* **25** 23468–84

Minaudo C, Meybeck M, Moatar F, Gassama N and Curie F 2015 Eutrophication mitigation in rivers: 30 years of trends in spatial and seasonal patterns of biogeochemistry of the Loire River (1980–2012) *Biogeochemistry* **125** 2549–63

Morris D P and Lewis W M 1988 Phytoplankton nutrient limitation in Colorado mountain lakes *Freshw. Biol.* **20** 315–27

Muggeo V 2008 Segmental: an R package to fit regression models with broken-line relationships *R News* **8** 20–5

Müller-Navarra D C 1995 Biochemical versus mineral limitation in Daphnia *Limbol. Oceanogr.* **40** 1209–14

Musolff A, Fleckenstein J H, Rao P S C and Jawitz J W 2017 Emergent archetype patterns of coupled hydrologic and biogeochemical responses in catchments *Geophys. Res. Lett.* **44** 4143–51

Musolf A, Schmidt C, Selle B and Fleckenstein J H 2015 Catchment controls on solute export *Adv. Water Resour.* **86** 135–46

Oehser G P and Stets E G 2019 Recent trends in nutrient and sediment loading to coastal areas of the conterminous U.S.: insights and global context *Sci. Total Environ.* **654** 12250–40

Paerl H W 2009 Controlling Eutrophication along the Freshwater–Marine Continuum: dual Nutrient (N and P) Reductions are Essential *Estuar. Coast.* **32** 593–601

Penuelas J, Janssens I A, Ciais P, Obersteiner M and Sardans J 2020 Anthropogenic global shifts in biospheric N and P concentrations and ratios and their impacts on biodiversity, ecosystem productivity, food security, and human health *Glob. Change Biol.* **26** 1962–85

Petuelle J et al 2013 Human-induced nitrogen-phosphorus imbalances alter natural and managed ecosystems across the globe *Natur Commun.* **4** 2934

Ptacnik R, Andersen T and Tamminen T 2010 Performance of the Redfield ratio and a family of nutrient limitation indicators as thresholds for Phytoplankton N vs. P Limitation *Ecosystems* **13** 1201–14

R Core Team 2018 R: A language and environment for statistical computing *R Foundation for Statistical Computing* (Vienna, R Foundation for Statistical Computing)

Rabalais N N, Turner R E, Diaz R J and Justic D 2009 Global change and eutrophication of coastal waters *ICES J. Mar. Sci.* **66** 1528–37

Radach G and Patsch J 2007 Variability of continental riverine freshwater and nutrient inputs into the North Sea for the years 1877–2000 and its consequences for the assessment of eutrophication *Estuar. Coast.* **30** 66–81

Raschke M and Menzel T 2005 Ergebnisbericht Ruhr: Wasserrahmenrichtlinie in NRW—Bestandsaufnahme (Düsseldorf: MUNLV)

Redfield A C 1960 The biological control of chemical factors in the environment *Sci. Prog.* **11** 150–70

Ruhverband 2001 Ruhrgebietbericht 2000 (Essen: Ruhverband)

Ruhverband 2014 Ruhrgebietbericht 2013 (Essen: Ruhverband)

Ruhverband 2019 Ruhrgebietbericht 2018 (Essen: Ruhverband)

Schade J D, Macneill K, Thomas S A, Camille McNeely F, Welter J R, Hood J, Goodrich M, Power M E and Finlay J C 2011 The stoichiometry of nitrogen and phosphorus spiralling in heterotrophic and autotrophic streams *Freshw. Biol.* **56** 424–36
Schindler D W, Carpenter S R, Chapra S C, Hecky R E and Orihel D M 2016 Reducing phosphorus to Curb Lake Eutrophication is a success Environ. Sci. Technol. 50 8923–9

Tetzlaff B, Vereecken H, Kunkel R and Wendland F 2009 Modelling phosphorus inputs from agricultural sources and urban areas in river basins Environ. Geol. 57 183–93

Thompson S E, Basu N B, Lascurain J, Aubeneau A and Rao P S C 2011 Relative dominance of hydrologic versus biogeochemical factors on solute export across impact gradients Water Resour. Res. 47 W00J03

Wentzyk V C, Tittel J, Jäger C G and Rinke K 2018 Mechanisms preventing a decrease in phytoplankton biomass after phosphorus reductions in a German drinking water reservoir-results from more than 50 years of observation Freshw. Biol. 63 1063–76

Westphal K, Graeber D, Musolff A, Fang Y, Jawitz J W and Borchardt D 2019 Multi-decadal trajectories of phosphorus loading, export, and instream retention along a catchment gradient Sci. Total Environ. 667 769–79

Withers P, Neal C, Jarvie H and Doody D 2014 Agriculture and eutrophication: where do we go from here? Sustainability 6 5853–75

Withers P J A and Bowes M J 2018 Phosphorus the pollutant Phosphorus: Polluter and Resource of the Future—Removal and Recovery from Wastewater ed C Schaum (London: International Water Association) pp 1–33

Worrall F, Howden N J K and Burt T P 2015 Time series analysis of the world’s longest fluvial nitrate record: evidence for changing states of catchment saturation Hydrol. Process. 29 434–44

Yan Z, Han W, Peñuelas J, Sardans J, Elser J J, Du E, Reich P B and Fang J 2016 Phosphorus accumulates faster than nitrogen globally in freshwater ecosystems under anthropogenic impacts Ecol. Lett. 19 1237–46

Yin K, Song X, Sun J and Wu M C S 2004 Potential P limitation leads to excess N in the pearl river estuarine coastal plume Cont. Shelf Res. 24 1895–907

Yun Y-J and An K-G 2016 Roles of N:P ratios on trophic structures and ecological stream health in lotic ecosystems Water 8 22