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Ecological status assessment of clay rivers with naturally enhanced water phosphorus concentrations

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ABSTRACT

The species composition of benthic algae changes as water phosphorus concentrations increase, and these changes can be used for ecological status assessment according to the Water Framework Directive. Natural background phosphorus concentrations in rivers and streams that are unaffected by anthropogenic impacts are usually low. Running waters draining catchments with deposits of marine clay, however, may have enhanced phosphorus concentrations, because the clay is naturally rich in apatite. Almost all clay rich areas have been cultivated for centuries, however, and fertilization has increased the soil phosphorus levels. It has, therefore, been difficult to disentangle natural from anthropogenically enhanced phosphorus in streams draining clay rich areas. We compared water phosphorus concentrations, and the Periphyton Index of Trophic Status PIT, between clay and non-clay, impacted and unimpacted rivers in Norway. We found that water phosphorus concentrations and the PIT index were higher in unimpacted clay rivers than in unimpacted non-clay rivers, indicating that natural phosphorus concentrations in clay rivers are indeed enhanced compared to rivers without deposits of marine clay. In addition, phosphate-P contributed 18–23% to total phosphorus in unimpacted clay rivers, but 33–37% in unimpacted and impacted non-clay rivers and clay rivers affected by agriculture. This indicates that the total phosphorus in unimpacted clay rivers is less bioavailable than in non-clay rivers and in impacted clay rivers. Water total phosphorus concentrations in unimpacted clay rivers significantly increased with catchment clay cover. Based on these findings, we derived new status class boundaries for the PIT index in clay rivers. Clay rivers are suggested to be assessed in only two status classes, i.e., "good or better" or "moderate or worse", respectively. The good/moderate status class boundary for the PIT index was shown to increase with increasing catchment clay cover.

1. Introduction

The species composition and abundance of benthic primary producers in rivers and streams is affected by water nutrient concentrations (e.g., Schneider and Melzer 2003), and phosphorus is often assumed to play an important role. Excess phosphorus, leading to eutrophication, may come from both point (effluent) and diffuse (agricultural) sources (Jarvie et al., 2006). Natural background phosphorus concentrations in rivers and streams vary with river type, but generally are assumed to be low. In Norway, for example, reference values for total phosphorus in running waters (without significant amounts of clay in their catchment) vary between 3 and 11 µg TP/l (Direktoratsgruppen for Vanndirektivet, 2018). Rivers and streams draining areas with deposits of marine clay, however, may have naturally enhanced water phosphorus concentrations (Lyche Solheim et al., 2008). This is important, because the Water Framework Directive (WFD; European Commission, 2000) aims to ensure at least good ecological status for all water bodies in Europe, and enhanced phosphorus concentrations are one of the main reasons for the degradation of water bodies in Europe (Grizzetti et al., 2017). It is, therefore, important to differentiate natural from anthropogenically enhanced phosphorus concentrations.

"Clay rivers", i.e., rivers and streams draining areas with deposits of marine clay, are common in areas where, at the end of the last ice-age, clay from glacial erosion was deposited in the sea, for example along the coasts of Nordic countries and Canada. The clay-rich sediments in the seabed were later exposed during the land uplift that followed the retreat of the huge inland ice cap (Kenney, 1964; Dons, 1977; Kodama, 1979; Ekman, 1996; Jørgensen et al., 2013). In Norway, the elevation of

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this land uplift varies, but the highest so-called 'marine limit', which is the border between areas with and without marine clay deposits, is around 220 m asl. (Dons, 1977). An important characteristic of marine and lacustrine sediment deposits is that they often contain the mineral apatite, formed by diagenesis of phosphate-rich organic debris (e.g., Ptáček, 2016). Analyses of lower soil horizons with little agricultural influence have shown that the marine clay soils in Norway are naturally rich in phosphorus (Øgaard and Krogstad, 2007).

Rivers and streams draining catchments with marine clay deposits should, therefore, have naturally enhanced phosphorus concentrations compared to streams draining areas with poorer soil. Indeed, stream water phosphorus concentrations increased with increasing proportion of clay soils in near-pristine catchments in Norway (Lyche Solheim et al., 2008). Apart from few, near-pristine catchments, however, almost all areas with marine clay have been cultivated for centuries, and fertilization has increased the soil phosphorus levels (e.g., Øgaard, 1995). Hence, most clay soils now contain both natural and anthropogenic phosphorus. In rivers and streams draining areas with deposits of marine clay it has, therefore, been difficult to disentangle natural from anthropogenically enhanced phosphorus.

Phosphorus-rich runoff will cause enhanced total phosphorus concentrations in rivers and streams and is therefore likely to affect benthic primary producers and the ecological status of water bodies, irrespective of whether the phosphorus stems from natural or anthropogenic sources. Following the WFD, the ecological status of a water body is largely defined by its biota. The observed condition of the biota is compared with a reference status, i.e. an "unimpacted" but otherwise similar ecosystem, and the results are categorised into five status classes: high, good, moderate, poor and bad. The good-moderate boundary is the point where "slight" differences in the biota, compared to reference status, turn into "moderate" differences. Since - often costly - measures need to be implemented if good ecological status is not achieved, the boundary between good and moderate status receives much attention in water management. However, since all status class boundaries are defined compared to reference conditions, a correct definition of reference status is of paramount importance for ecological status assessment (Skarbøvik et al., 2020).

Despite the focus of the WFD on biota, physical and chemical parameters, including water nutrient concentrations, are still important. This is because they are so-called supporting elements, based on the link between nutrients and biota. The WFD stipulates that, even if the biota indicate good ecological status, nutrient concentrations must not exceed the levels that ensure the functioning of the ecosystem and the achievement of good ecological status. In other words: the biota must be in good status, and nutrient concentrations must not be too high. Weak linkages between nutrients (pressures) and ecological status (effects on the ecosystem) are therefore a serious concern (Carvalho et al., 2019). For rivers and streams, "macrophytes and phytobenthos" is one of the biological quality elements that needs to be assessed, and Norway uses the "periphyton index of trophic status" (PIT; Schneider and Lindstrøm, 2011) for assessing phytobenthic algae.

Similar to most other indices used for assessment of ecological status according to the WFD, the PIT is not just used for overall classification of a water body, but is also related to a specific stressor. The PIT was developed to assess eutrophication, i.e., the biological response to the overenrichment of a water body with nutrients. The PIT is related to water total phosphorus concentrations, with higher PIT values indicating enhanced phosphorus concentrations (Schneider and Lindstrøm, 2011). Consequently, if rivers and streams draining areas with marine clay do indeed have naturally enhanced total phosphorus (TP) concentrations, and if these concentrations are reflected in higher PIT values, then the reference values for clay rivers need to be higher than for rivers without deposits of marine clay. The dataset from which the PIT was developed, however, included impacted clay rivers, but no clay rivers in reference status (own experience; Eriksen et al., 2015). PIT reference values for all rivers and streams in Norway were, therefore, exclusively based on areas without major deposits of marine clay. This led to concerns that current PIT status class boundaries would be unrealistically low when applied to clay rivers. Defining PIT reference values for clay rivers, however, requires disentangling natural from anthropogenically enhanced phosphorus, which is difficult because almost all areas with deposits of marine clay have been cultivated for centuries.

In addition to the problem of the paucity of unimpacted clay rivers that could be used to define reference conditions, an adjustment of PIT boundaries in clay rivers is complicated by the fact that not all of the total phosphorus measured in a water sample is readily bioavailable for primary producers (Krogstad and Løvstad, 1991; Reynolds and Davies, 2001). The PIT was developed using water total phosphorus (TP) concentrations (Schneider and Lindstrøm, 2011), only because sufficient data on other phosphorus fractions were not available. The uncertain and variable bioavailability of TP is likely to have contributed to the observed uncertainty in the TP-PIT relationship, particularly at higher TP concentrations (Schneider and Lindstrøm, 2011). Theoretically, only bioavailable nutrients should affect the biota. Therefore, if – hypothetically - none of the TP in marine clay was bioavailable, then the reference PIT for clay rivers should not be different from non-clay rivers, even if water TP concentrations were enhanced. For this reason, potential differences in bioavailability of TP between clay and non-clay rivers are important.

We therefore asked the following questions:

(i) Are water phosphorus concentrations, and the PIT index, in reference clay rivers different from non-clay reference rivers, and if yes, how much?
(ii) Is there a difference in bioavailability of TP between clay and non-clay, reference and impacted rivers, and if yes, how large is the difference?
(iii) How do catchment clay soils affect water TP concentrations?
(iv) Should PIT status class boundaries in clay rivers be different from non-clay rivers, and if yes, how?

Here we use a Norwegian dataset to answer these questions, but we believe that our approach may serve as an example for all regions with naturally phosphorus-rich clay rivers.

2. Material and methods

2.1. PIT-index

The Periphyton Index of Trophic status PIT (Schneider and Lindstrøm, 2011) is an index reflecting eutrophication developed for use in Norwegian rivers and streams. It is based on indicator values for a total of 153 non-diatom benthic algal taxa and is related to water TP concentrations. PIT boundary values for the assessment of ecological status, according to the WFD, were intercalibrated with other Northern European countries (the so-called "Northern Geographic Intercalibration Group"). For rivers and streams with a calcium concentration above 1 mg/L (concentrations typically observed in clay rivers), the reference PIT is 6.7, the high/good boundary 9.5, the good/moderate boundary 16, the moderate/poor boundary 31, and the poor/bad boundary 46 (Direktoratsgruppen for Vanndirektivet, 2018).

Phytobenthos samples for calculation of the PIT index were generally taken in late summer to early autumn. At each site, samples of non-diatom benthic algae were collected according to European standard procedures (EN 15708:2009) along an approximately 10-m stretch with the aid of a bathyscope. Percent cover of each form of macroscopically visible benthic algae was estimated, and samples were collected and stored separately in vials for species determination. In addition, microscopic algae were collected from ten cobbles/stones with diameters of between approximately 10 and 20 cm, for each site. An area of about 8 × 8 cm from the upper side of each cobble/stone was brushed with a toothbrush to transfer the algae into a beaker containing approximately

1 L of river water, from which a subsample was taken. All samples were preserved with a few drops of formaldehyde. The preserved benthic algae samples were later examined using a microscope (200 to 600 × magnification), all non-diatom algae were identified to species, where possible, and the PIT index was calculated according to Schneider and Lindstrøm (2011).

2.2. Current status assessment of clay rivers in Norway

Clay rivers in Norway are currently defined as having more than 10 mg/L total suspended solids, measured as average monthly or fortnightly water samples taken over one year at regular intervals. (Direktoratsgruppen for Vanndirektivet, 2018). This is the definition we apply in our study. Lyche Solheim et al. (2008) used an additional criterion, i.e., that less than 20% of the total suspended solids were organic (measured as loss of ignition at 550 °C), and recommended that water samples should be taken over a period of three years, due to the large year-to-year variability in the concentration of suspended solids. Eriksen et al. (2015), based on a suggestion by Lyche Solheim et al. (2008), defined clay rivers as rivers where at least one of two criteria was met: (i) average concentration of total suspended solids above 10 mg/L; (ii) percent clay soil coverage in the catchment upstream the sampling site ≥ 20. Although there is an overall positive relationship between these two criteria, there are examples that one but not the other is met (Eriksen et al., 2015), probably due to poor representativeness or low frequency of the water sampling, as well as variations in catchment slope, vegetation cover, and precipitation.

Currently, the PIT status class boundaries in clay rivers in Norway are the same as in other rivers and streams with a calcium concentration above 1 mg/L (Direktoratsgruppen for Vanndirektivet, 2018). There were, however, concerns that these boundaries are unrealistically low when applied to clay rivers. Due to these uncertainties, all status assessment for clay rivers in Norway based on the PIT index is currently reported as "tentative".

With respect to TP, only the boundary between good and moderate status is set for clay rivers. The good/moderate boundary varies with the proportion of marine clay in the upstream river catchment (Direktoratsgruppen for Vanndirektivet, 2018). The higher the proportion of marine clay, the higher the good/moderate boundary for TP. When 20 % of the catchment is covered with marine clay, the good/moderate boundary for TP is 40 μg/L, for 30% clay cover it is 50 μg/L, for 40% clay cover it is 60 μg/L, and for 50% clay cover it is 80 μg/L. For PO_4-P, there is no similar system, but based on empirical data, the good/moderate boundary is set to 10 μg/L (Direktoratsgruppen for Vanndirektivet, 2018).

2.3. Study sites

The data we use here was assembled from different monitoring programmes (Table 1).

Table 1
Datasets used for this study. PIT = periphyton index of trophic status, TP = total phosphorus, SS = total suspended solids.

data set	short name	# sites	available data	data source
A	non-clay reference rivers	74	PIT, TP, PO_4-P in filtered and unfiltered water samples, SS	NIVA's Aquamonitor database
B	clay reference rivers	3	PIT, TP, PO_4-P in filtered and unfiltered water samples, SS	NIVA's Aquamonitor database
C	non-clay impacted rivers	21	TP, PO_4-P in filtered water samples	NIVA's Aquamonitor database
D	clay streams in agricultural catchments	34	TP, PO_4-P in filtered water samples (31 sites), SS	Norway's Vannmiljø database
E	clay streams in forested catchments	15	TP, PO_4-P in filtered water samples (14 sites), SS (14 sites)	NIBIO's JOVA database

(A). Non-clay reference rivers

Within the framework of the project "monitoring of reference rives in Norway", a total of 74 non-clay (i.e., concentration of total suspended solids below 10 mg/L) reference river sites were analysed every second year from May 2017 to December 2021. This means that, for each site, two complete years of data were available. The sites were selected to be virtually without hydromorphological or any other anthropogenic stressors, reflect natural alkalinity gradients that occur in Norway, and were situated across the entire country. However, in some cases "best available" sites had to be chosen. All sampling and analyses were done according to Norwegian standard procedures. Water chemistry samples were taken monthly during each year of analysis and benthic algae samples were taken once in late summer to early autumn of the same year. Total phosphorus (TP) concentrations, as well as PO_4-P concentrations in the filtered (0.45 μm) and unfiltered water samples were analysed according to NS 4724:1984. For data analyses, all results below the detection limit (1 μg/L) were set to 0.5 μg/L. Total suspended solids (SS) were analysed according to NS 4733:1983. Catchment land use and clay cover for each site was derived from http://nevina.nve.no. Results are reported in Moe et al. (2018, 2019), Thrane et al. (2020) and Sandin et al. (2021). All data were extracted from NIVA's Aquamonitor database in January 2022.

(B). Clay reference rivers

Three clay reference rivers (Vikka (40 km north of Oslo in South-East Norway), Lundåa (40 km south-east of Oslo in South-East Norway), and Leiråa (80 km north-east of Trondheim in Middle-Norway)) were monitored as part of the same program as described in (A), within the same years and using the same methods. For the three clay rivers, however, both the PIT index and water chemical data were available from three years (in contrast to the non-clay reference rivers in (A), for which only two years of data were available). All data were extracted from NIVA's Aquamonitor database in January 2022.

(C). Non-clay impacted rivers

Data on TP and concomitant PO_4-P concentrations in filtered water samples (0.45 μm) from impacted rivers were compiled from the river monitoring programme in Norway. This programme includes 21 sites, some of which have been analysed monthly since 1990. Data on water TP and PO_4-P concentrations were used from all years where they were available, to provide the best possible estimate of average concentrations. The sites are subject to varying degrees and types of anthropogenic stress, including nutrient input from point and non-point sources, and river regulation. None of the sites were defined as clay rivers. The sites were situated in large rivers across Norway and the most recent report is Kaste et al. (2021). TP and PO_4-P concentrations were analysed according to NS 4724:1984. All data were extracted from NIVA's Aquamonitor database in January 2022.

(D). Clay streams in agricultural catchments

Data from 34 clay streams in agricultural catchments were assembled from regional operational monitoring programmes (European Commission, Directorate-General for Environment, 2012), carried out between 2004 and 2016 in three different river basin sub-districts in South-East Norway (Morsa, Leira-Nitelva and Haldenvassdraget). Sampling frequency differed among sites and varied from 4 times per year over a period of 2 years, to fortnightly over a period of 7 years. The sites were subject to a mixture of anthropogenic pressures, including nutrient input from point and non-point sources. Six of the sites in agricultural catchments had average concentrations of total suspended solids below

10 mg/L and were therefore not classified as clay rivers (although there is some marine clay in their catchment). To ensure a sufficient gradient length, these sites were included for analysing the relationships between phosphorus concentrations and concentrations of total suspended solids, and catchment clay cover, respectively. For characterizing the contribution of PO_4-P to TP in clay streams in agricultural catchments, sites with less than 10 mg/L suspended solids were not used, because they do not qualify as clay rivers. Not all water chemical parameters were consistently analysed at all sampling occasions. For calculating average values per site, all available data were used. For calculating the contribution of PO_4-P to TP, only samples where PO_4-P and TP were measured in the same water sample were used. TP and PO_4-P (filtered water sample, 0.45 μm) were analysed according to NS EN ISO 15681-2. Total suspended solids were analysed according to NS 4733.2-1983 and NS-EN-872.2-2005. Catchment clay cover for each site was derived from a digital map system developed by the Norwegian Water and Energy Directorate (NVE; http://nevina.nve.no). All data were extracted from Norway's Vannmiljø database in January 2022.

(E). Clay streams in forested catchments

Since it is difficult to find clay rivers that are not affected by agricultural activities, there are fewer sites in forested catchments than in agricultural catchments in our dataset. Moreover, 10 out of a total of 15 sites were situated in two adjacent catchments, Lund South and North. All 15 sites are located in South-East Norway, and further described in Vandsemb, (2006) and Greipsland et al., (2017). In the two Lund catchments, monitoring was performed in 2013–2015 with the specific intention to analyse natural clay rivers. Water samples were taken 8–15 times at each site, over the course of at least one year. In the other sites, water samples were taken over a period of at least two consecutive years during 1989 to 2015, and a minimum of 5 samples was taken per year. Data were extracted from NIBIO's JOVA database in January 2022.

These 15 sites represent, to our knowledge, the least impacted clay streams that exist in Norway. Five of the sites in forested catchments had average concentrations of total suspended solids below 10 mg/L and therefore are not classified as clay streams. To ensure a sufficient gradient length, these sites were included for analysing the relationships between phosphorus concentrations and concentrations of total suspended solids, and catchment clay cover, respectively. For characterizing the contribution of phosphate to TP in clay streams in forested catchments, sites with less than 10 mg/L suspended solids were not used. Not all water chemical parameters were consistently analysed at all sampling occasions. For calculating average values per site, all available data were used. For calculating the contribution of PO_4-P to TP, only samples where PO_4-P and TP were measured in the same water sample were used. TP and PO_4-P (filtered water sample, 0.45 μm) were analysed according to NS EN ISO 15681-2. Total suspended solids were analysed according to NS 4733.2-1983 and NS-EN-872.2-2005. Catchment clay cover for each site was derived from http://nevina.nve.no.

3. Results

3.1. Differences in phosphorus concentrations, and the PIT index, between clay and non-clay reference rivers

For both clay and non-clay reference rivers, average concentrations of all phosphorus fractions were higher than median values (Fig. 1, Table S1). This indicates that average values were affected by comparatively few events with high concentrations, probably related to periods with enhanced discharge following rain events. In non-clay reference rivers, average PO_4-P in unfiltered water samples was only slightly higher than in filtered water samples (1.5 compared to 1.1 μg/L, respectively; Fig. 1, Table S1). In contrast, PO_4-P in unfiltered water samples was about four times higher than in filtered water samples of clay reference rivers (44.3 compared to 10.5 μg/L, respectively). This indicates that a substantial amount of PO_4-P was adsorbed to suspended

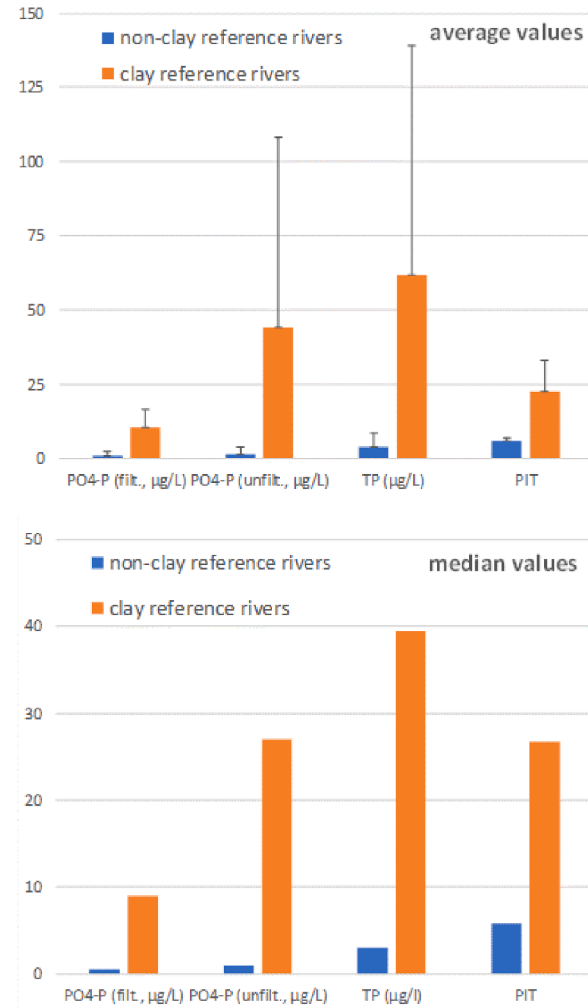

Fig. 1. Average (+ standard deviation) and median PO_4-P concentrations in filtered and unfiltered water samples, TP concentrations, and PIT index in 3 clay and 74 non-clay reference rivers in Norway (datasets A and B, Table 1). Phosphorus concentrations were measured monthly over 2 years at each site (3 years in the clay rivers), while the PIT index was determined twice at each site (three times in the clay rivers). Note different scale for average and median values. PO4-P and TP are given in μg/L, while the PIT index is given as dimensionless number.

solids in clay reference rivers (the average concentration of total suspended solids in non-clay reference rivers was 1.2 mg/L, compared to 50 mg/L in clay reference rivers; data not shown).

Overall, PO_4-P concentrations in both filtered and unfiltered water at the 74 non-clay reference rivers were below 1.5 μg/L, and often at or below the detection limit (which was at 1 μg/L; Table S1, Fig. 1). Total phosphorus concentrations in the non-clay reference rivers were on average 4 μg/L. Maximum concentrations were markedly higher (Table S1), likely related to periods of enhanced discharge after rain events. Overall, phosphorus concentrations in non-clay reference rivers were low. This meets expectations for rivers which were virtually free from anthropogenic impacts. The average PIT index in non-clay reference rivers was 6, i.e., close to the reference value, thus meeting expectations.

Total phosphorus and PO_4-P concentrations in the three clay reference rivers were clearly higher than in non-clay reference rivers (Table S1, Fig. 1). This was true for both average and median values. The average PO_4-P concentration in the filtered water sample was 10.5 μg/L, i.e., 9 μg/L higher than in non-clay reference rivers. The average PO_4-P concentration in the unfiltered water sample was 44 μg/L (43 μg/L higher than in non-clay reference rivers), and the average total phosphorus concentration was 62 μg/L, i.e., 58 μg/L higher than in non-clay reference rivers (Table S1). The average PIT index was 22.7, indicating moderate ecological status. The results therefore indicate that there is a substantial difference in phosphorus concentrations and the PIT index between clay and non-clay reference rivers.

However, catchment land cover also differed between clay and non-clay reference rivers (Table 2). Catchment land cover in non-clay reference rivers was dominated by barren landscape and forest, with only minor contributions from agriculture and urban environments. The catchment of the three reference rivers, in contrast, was dominated by forest, and to a substantial part by agriculture (Table 2). One site, Vikka is situated nearby Oslo airport Gardermoen. Groundwater underneath the runway slowly feeds into Vikka, explaining the high percentage of "urban" environments in its catchment. Groundwater flow is slow, however, such that chemicals used for, for example, de-icing of aircrafts, are expected to be degraded long before reaching the stream (pers. comm. NIVA). Among the three clay sites, Lundåa was the site with least agriculture and urban environments in its catchment.

Vikka, Lundåa and Leiråa were among the least impacted clay rivers which scientists and Norwegian water management authorities were able to find in Norway. They therefore represent "best available" sites. Nevertheless, the higher cover of agricultural (and urban) land use in the catchment of the three clay reference rivers compared to non-clay reference rivers indicates that the enhanced phosphorus concentrations and PIT index observed in those sites (Fig. 1, Table S1) may not exclusively be due to natural reasons, but likely to a combination of natural and anthropogenic sources.

3.2. Contribution of PO_4-P to total phosphorus

Total phosphorus and PO_4-P measured in filtered water samples were the only P-fractions consistently available from clay and non-clay, reference and impacted rivers, and were thus used for comparison. In non-clay rivers, on average, 37% of TP consisted of PO_4-P, both in reference and impacted rivers (Fig. 2). In clay streams in agricultural catchments, the average contribution of PO_4-P to TP was 33%. In contrast, the percentage of PO_4-P was lower in clay reference rivers and in clay streams situated in forested catchments (23% in the three clay reference rivers, and 18% in the clay streams in forested catchments, respectively).

Standard deviations, however, were high, indicating that the contribution of PO_4-P to TP is variable (Fig. 2). Despite this variability, the results indicate that less TP is in form of PO_4-P in clay reference rivers than in impacted and unimpacted non-clay rivers and in impacted clay rivers (*t*-test, $p < 0.001$ for comparing clay streams in forested catchments and reference clay rivers with non-clay reference rivers, streams in agricultural catchments and impacted rivers). This indicates that TP in unimpacted clay rivers is less bioavailable than TP in other rivers. The relationship between TP and the PIT index was originally developed based on impacted and unimpacted non-clay rivers and on clay rivers affected by agriculture, as at the time data from unimpacted clay rivers was unavailable. Consequently, the PIT-TP relationship was

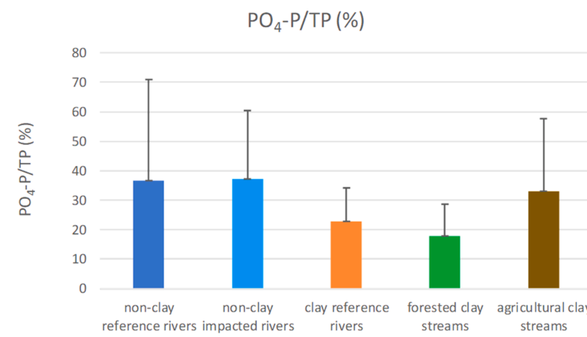

Fig. 2. Contribution of PO_4-P (measured in filtered water samples; + standard deviation) to total phosphorus concentrations; data from 74 non-clay reference rivers (monthly measurements over two years within the period 2017 to 2021), 21 sites in large rivers in Norway (river monitoring programme; monthly measurements over several years from 1990 to 2021), 3 clay reference rivers (monthly measurements over 3 non-consecutive years in the period 2017 to 2021), 28 clay streams in agricultural catchments (> 30 measurements per site in the period 2004−2016), and 9 clay streams in forested catchments (> 20 measurements per site in the period 2007 to 2015). Only data were TP and PO_4-P were measured in the same water sample were used.

built on an average contribution of PO_4-P to TP of 33−37%, while the contribution of PO_4-P to TP in nearly unimpacted clay rivers is, on average, only 18-23%.

3.3. Effect of marine clay on water phosphorus concentrations

As noted above, there are two ways of defining clay rivers, either by average concentrations of suspended solids or by the proportion of marine clay deposits in the catchment (Eriksen et al., 2015). We therefore analysed both these variables.

Water TP concentrations in nearly unimpacted clay streams (clay streams in forested catchments and clay reference rivers) significantly increased with the concentration of suspended solids (Pearson $r = 0.87$, $p < 0.001$), and with catchment clay cover (Pearson $r = 0.93$, $p < 0.001$; Fig. 3). TP concentrations in agricultural catchments also increased with increasing concentration of suspended solids (Pearson $r = 0.69$, $p < 0.001$), and with catchment clay cover (Pearson $r = 0.93$, $p < 0.001$), but were generally higher than in forested catchments with a corresponding concentration of suspended solids, or clay cover, respectively (Fig. 3). This indicates that (i) clay streams in agricultural catchments indeed have higher water TP concentrations than clay streams in nearly unimpacted catchments with a comparable amount of marine clay, and (ii) status class boundaries for TP, and consequently also for the PIT index, should be increased, with increasing amount of marine clay.

Since concentrations of TP significantly increased with both suspended solids (SS) and catchment clay cover, both could theoretically be used to quantify the "clay effect" at a specific site. We suggest, however, using % catchment clay cover, because (i) determination of catchment clay cover was, in this study, straightforward because of the online Norwegian tool Nevina (https://nevina.nve.no/), while determination

Table 2
Catchment land cover for three clay and 74 non-clay reference river sites in Norway. "other" indicates water bodies and glaciers.

	agriculture	urban	forest	wetland	fell (barren landscape)	other
clay rivers						
Vikka	14	45	39	1	0	0
Lundåa	8	0	84	1	0	0
Leiråa	10	0.03	78	10	0	0
average	*11*	*15*	*67*	*4*	*0*	*0*
non-clay rivers ($n = 74$)						
average	*0.30*	*0.01*	*36*	*6*	*49*	*6*
min	*0*	*0*	*0*	*0*	*0*	*0*
max	*2*	*0.2*	*97*	*22*	*96*	*42*

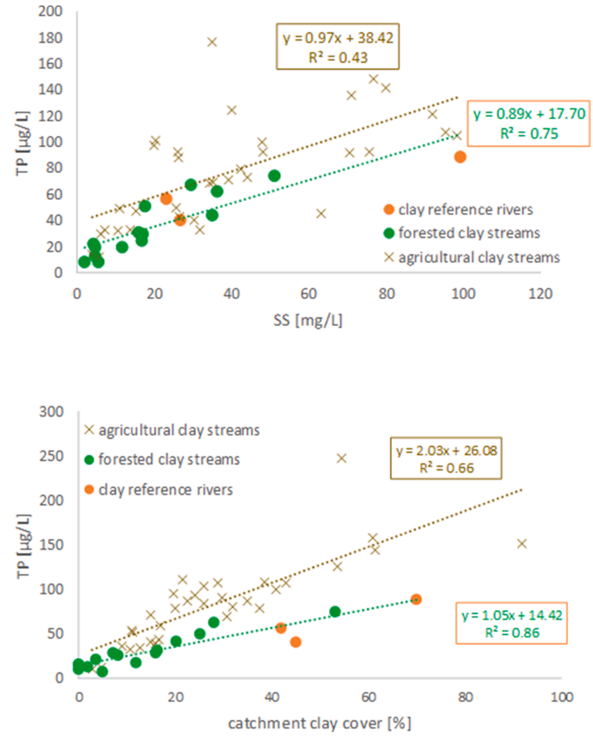

Fig. 3. *Top:* average concentrations of total phosphorus in water samples, plotted against average concentrations of total suspended solids, in clay reference rivers, as well as clay streams in forested and agricultural catchments. Streams with average concentrations of SS below 10 mg/L or less than 20% clay coverage in the catchment do not currently count as clay streams but were included to provide a sufficiently long gradient for suspended solids and catchment clay cover. Data represent average concentrations per site. *Bottom:* same as top, plotted against the proportion of marine clay coverage in the catchment. The regressions for nearly unimpacted clay streams (green lines) were derived from the combined data for forested clay streams and clay reference rivers.

of the average concentration of SS in a stream is costly (at least monthly water samples need to be taken over at least a year), (ii) concentrations of SS are likely to be higher in channelized clay streams compared to reference streams with the same catchment clay cover, due to a lack of natural vegetation along the stream sides (Pilon et al., 2017); if the concentration of SS was used as measure of the "clay effect" at a site, then restoration measures, for example the construction of buffer strips, that reduce both TP and SS would be "penalized" by the need of assessing the restored site against reference values for a lower "clay impact" than the unrestored site (because the concentration of SS at the restored site is likely to be lower than before); this effect would not occur when using catchment clay cover as a measure of the "clay effect" because catchment clay cover remains unchanged by restoration measures such as buffer strips; and (iii) catchment clay cover does not change among years, while the average concentration of suspended solids, depending on weather conditions, may change among sampling years. Hence, using concentrations of suspended solids to quantify the "clay effect" at a site would greatly complicate the interpretation of time-series of ecological status data (because the PIT reference status, which the actual status must be compared with, could change between sampling years if the average concentration of suspended solids changed markedly among years). Indeed, it has been shown that monthly sampling may result in highly uncertain suspended solid concentrations (e.g., Skarbøvik et al., 2012), and more frequent sampling may be needed in order to get reliable average concentrations for suspended solids. For these reasons, we decided to use catchment clay cover for adjusting PIT boundary values.

3.4. Adjusting PIT boundaries

The PIT-index is related to water TP concentrations, and Schneider and Lindstrøm (2011) implicitly assumed that increasing TP concentrations were related to anthropogenic nutrient enrichment. We have shown above, however, that water TP concentrations naturally increase with catchment clay cover in nearly unimpacted catchments (Fig. 3). Consequently, PIT status class boundaries need to be increased by a value that is related to the natural increase in TP.

In order to adjust PIT boundaries for clay rivers, we (i) first used the relationship between water TP and catchment clay cover from nearly unimpacted catchments (green line in Fig. 3) to estimate the increase in water TP concentrations due to clay cover. We are aware that these streams are the best-available rather than true reference streams. We are, however, not aware of any completely unimpacted clay stream in a Nordic country for which data on water chemistry and benthic algae are available. (ii) We then added the TP concentration calculated in (i) to the TP concentrations that are related to PIT status class boundaries, separately for the TP corresponding to reference conditions, and to each of the PIT status class boundaries (see Fig. 4). (iii) Lastly, we used the relationship between TP and the PIT (Fig. 4) to determine the median PIT value for the TP concentrations calculated in (ii). Since TP concentrations for the moderate/poor and poor/bad boundaries are outside the range of the published PIT-TP relationship (Fig. 4; Schneider and Lindstrøm, 2011), we only calculated values for reference conditions, high/good and good/moderate boundaries.

When the PIT was developed, however, although clay rivers were included in the dataset these were only from agricultural catchments. No data from nearly unimpacted clay rivers were available in 2011. This means that the PIT-TP relationship was built on an average contribution of PO_4-P to TP of 33−37% (Fig. 2). In contrast, the contribution of PO_4-P to TP in nearly unimpacted clay rivers was lower, on average only 18−23% (Fig. 2). This means, that less of the TP is bioavailable for benthic algae in reference clay rivers, than in the rivers which were used

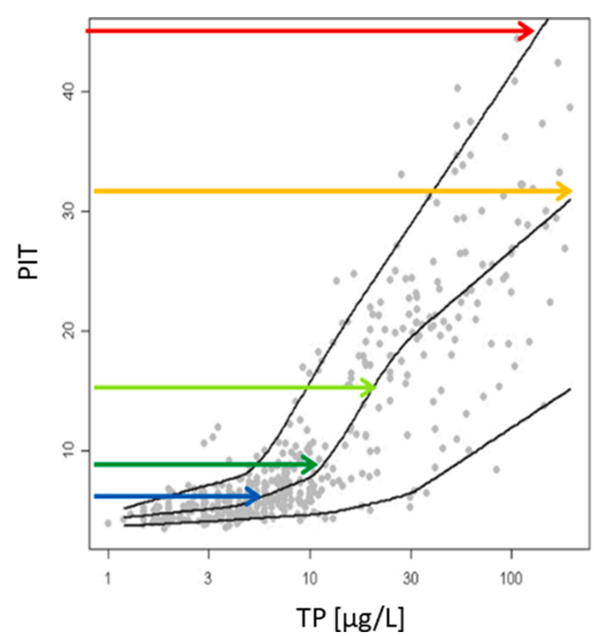

Fig. 4. Relationship between water total phosphorus concentrations and the PIT index; the relationship is based on quantile regression, and the black lines indicate 5th, 50th (median) and 95th percentile. Arrows indicate the PIT reference value for rivers and streams with a Calcium concentration > 1 mg/L (6.7; blue), the high/good boundary (9.5; dark green), good/moderate boundary (16; light green), moderate/poor boundary (31; orange) and poor/bad boundary, respectively (46; red). Figure modified based on Hydrobiologia, Schneider and Lindstrøm, (2011), by permission from Springer Nature.

to set up the PIT-TP relationship (Schneider and Lindstrøm, 2011; Fig. 4). For this reason, only 58% (corresponding to the average between 18/33 and 23/37) of the increase in TP observed with increasing clay cover in nearly unimpacted catchments (Fig. 3), was used for deriving the new PIT boundary values.

For example, the boundary between good and moderate status for non-clay rivers with a Calcium concentration above 1 mg/L is at PIT = 16 (Direktoratsgruppen for Vanndirektivet, 2018). A PIT of 16 corresponds to a TP concentration of 22 μg/L (Fig. 4). If 20% of the catchment is covered with marine clay, then this will lead to an estimated increase in water TP concentrations of 21 μg/L (Fig. 3). Since the TP in reference clay streams is less bioavailable than the TP in the streams used for setting up the PIT-TP relationship, only 58% of these 21 μg/L (=12.2 μg/L) were assumed to contribute to an increasing PIT (see explanation above). A TP concentration of 34.2 μg/L TP (= 22+12.2) corresponds to a PIT of 20 (Fig. 4). The good/moderate boundary for rivers with a catchment clay cover of 20% was therefore set to PIT=20. In this way, we derived PIT reference values, high/good and good/moderate boundaries for catchment clay cover ranging from 10 to 100% (Fig. 5).

The PIT-TP relationship developed by Schneider and Lindstrøm (2011) was based on quantile regression. For this reason, no regression exists for this relationship, and PIT values corresponding to calculated TP concentrations had to be read from Fig. 4. We considered developing a regression from the data presented in Schneider and Lindstrøm (2011) and using it for calculating class boundaries, instead of reading them from a figure. We did, however, decide against it, because Norwegian water management authorities used the original PIT-TP quantile regression for developing a guidance on water TP concentrations that need to be achieved in order to ensure good ecological status in non-clay rivers (Direktoratsgruppen for Vanndirektivet, 2018). Using a different PIT-TP relationship than the one originally published could therefore lead to inconsistencies in status assessment between clay and non-clay rivers.

Since the TP-PIT relationship is logarithmic (Fig. 4), while the increase in TP with catchment clay cover is linear (Fig. 3), reference value, high/good and good/moderate boundaries move closer to each other with increasing catchment clay cover (Fig. 5). This would mean that, in catchments with more than 30% clay cover, the difference between high and moderate ecological status would be less than 3 PIT units. This is considerably less than the observed year-to-year variability in the PIT index in the three reference clay rivers (Fig. 5; note that few indicator taxa were found in Leiråa in 2017 and 2021, such that a valid PIT index could only be calculated for 2019). In order to avoid large year-to-year differences in ecological status for a site, we therefore propose only using the good/moderate boundary for status assessment of clay rivers. This means, that clay rivers are suggested to be assessed in only two status classes, i.e., "good or better" or "moderate or worse", respectively. This approach is in line with the current suggestion for TP concentrations in clay rivers in Norway, which also only sets the boundary between good and moderate status (Direktoratsgruppen for Vanndirektivet, 2018). The good/moderate boundary for the PIT index, as well as expected average TP concentrations at the good/moderate boundary, are given in Table 3.

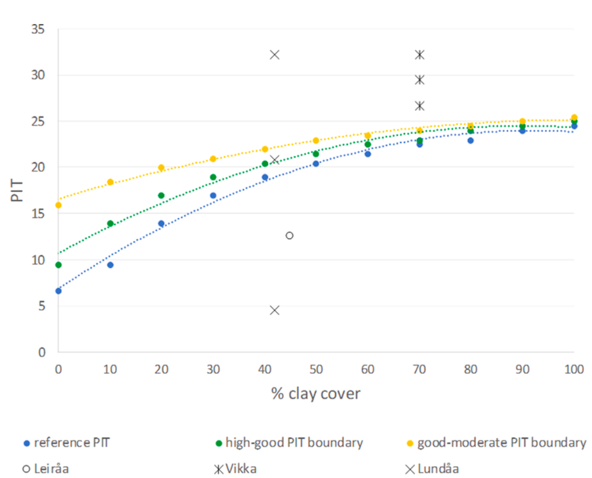

Fig. 5. Reference status, high/good and good/moderate boundaries for the PIT-index in non-clay rivers (0% clay cover), and respective values derived for increasing catchment clay cover, and PIT values in the three clay reference rivers in our dataset. Lines denote polynomial regressions.

Although it would be possible to use regression for calculating the good/moderate boundary for any given catchment clay cover between 1 and 100%, we, for practical reasons, suggest using categories of 10%, as given in Table 3. In order to be conservative, we suggest using the lower category for status assessment of a given site, i.e., sites with a catchment clay cover up to 9% should be treated as non-clay rivers, sites with a catchment clay cover between 10 and 19% should be assessed against the good/moderate boundary for 10%, etc. Because the good/moderate boundary for the PIT index is markedly higher for a catchment clay cover of 10% than 0% (Table 3), our results indicate that rivers and streams should be treated as "clay rivers" from a catchment clay cover above 10%, rather than the current 20% which often corresponds to total suspended solid concentrations above 10 mg/L (Eriksen et al., 2015).

Due to the observed variability in the PIT index of the three clay reference sites (Fig. 5), we suggest that status assessment of clay rivers based on the PIT should be based on average PIT values from three years. The average PIT index for Lundåa was 19.2, and with a catchment clay cover of 42%, this indicates that the site is in "good or better" ecological status. This matches expectations, since Lundåa was indeed the least impacted clay river in our dataset (Table 1). Vikka had an average PIT index of 29.5 and a clay cover of 70%, and therefore is in "moderate or worse" status. In Leiråa, fewer than 2 indicator species were found in two of the three sampling years. For this reason, the site cannot be validly classified yet, and continued attempts to sample benthic algae should be undertaken.

4. Discussion

4.1. Does our approach make sense?

The link between TP concentrations and the PIT good/moderate boundaries for clay catchments proposed here (Table 3), match the earlier suggestions by Lyche Solheim et al. (2008) and Direktoratsgruppen for Vanndirektivet (2018), both of whom suggested TP concentrations at the good/moderate boundary of 40, 50 and 60 μg/L at 20, 30 and 40% catchment clay cover, respectively. Our dataset is not

Table 3
Good/moderate boundary for the PIT index in rivers and streams with a Calcium concentration above 1 mg/L, and TP concentrations expected at the good/moderate boundary (calculated by adding the expected increase in TP from Fig. 3 to the 22 μg/L expected in non-clay rivers at the good/moderate boundary).

catchment clay cover [%]	good-moderate PIT boundary for rivers with a Ca-concentration > 1mg/l	expected TP concentration at the good/moderate boundary [μg/l]
0	16	22
10	18.5	32.5
20	20	43
30	21	53.5
40	22	64
50	23	74.5
60	23.5	85
70	24	95.5
80	24.5	106
90	25	116.5
100	25.5	127

completely independent from these earlier works, because Lyche Solheim et al. (2008) and Direktoratsgruppen for Vanndirektivet (2018) used a subset of the clay streams in forested catchments which we also used. However, our results from the three reference rivers match well with the results from forested catchments, while at the same time substantially extending the gradient length of catchment clay cover (Fig. 3). We therefore feel confident that our results are indeed a good approximation for the near natural increase in average TP concentrations with increasing catchment clay cover.

Our sites in forested catchments and the three clay reference rivers, however, were "best-available" rather than completely unimpacted. The catchments of the three reference sites had between 8 and 14% agricultural land-use (Table 2), and the Lund catchment, where most of the sites in forested catchments lie, is to a small degree used for animal grazing and crop cultivation (pers. observation). The areas in the Lund catchment (dataset D; Table 1), however, were not artificially fertilized with phosphorus during the study period, due to an agreement with the local farmer. While this measure likely did not remove potentially accumulated phosphorus in the soil from earlier years, it nevertheless shows that significant efforts were made to select clay streams that were as little impacted as possible.

According to the Norwegian WFD guidance document, reference values for TP for non-clay rivers are between 3 and 11 μg/L, depending on river type (Direktoratsgruppen for Vanndirektivet, 2018). The reference value for the PIT in non-clay rivers with Calcium concentrations >1mg/L, is 6.7, corresponding to 6 μg/L TP (Fig. 4), while the reference PIT for rivers with Calcium concentrations <1mg/L is 4.9, corresponding to 3 μg/L TP. The average TP concentration measured across all non-clay reference rivers in our dataset was 4 μg/L (Table S1). These numbers were derived independently from each other, but nevertheless are very close to each other. They indicate that the reference TP concentrations in unimpacted non-clay rivers in Norway are indeed between 3 and 11 μg/L.

In contrast, the intercept of the regression line between catchment clay cover and TP for unimpacted clay rivers is 14.4 μg/L TP, and 17.7 μg/L TP for total suspended solids, respectively (green lines in Fig. 3). As this intercept corresponds to zero catchment clay cover (and/or concentrations of suspended solids below the detection limit), you would expect this to match the stated reference values for non-clay Norwegian rivers (3–11 μg/L), but it is in fact between 3 and 15 μg/L higher. We suggest that this difference is due to the - minor but noticeable - agricultural and urban land-use of the catchments in our forested clay streams and clay reference rivers. In other words: the results indicate that our unimpacted rivers were indeed "best available" rather than completely unimpacted, and average TP concentrations were about 3–15 μg/L higher than they would have been if the sites had been completely unimpacted.

For calculating the good/moderate boundary for the PIT in clay catchments, however, we only used the slope of the nearly unimpacted regression, not the intercept. However, it was not only the intercept but also the slope of the regression of TP against catchment clay cover that was higher in agricultural than in nearly unimpacted catchments (the slope is 1 for the green line, compared to a slope of 2 for the brown line in Fig. 3). This matches expectations, because clay soils used for agriculture contain more phosphorus than natural clay soils (Øgaard and Pedersen, 2016). For this reason, and because our unimpacted streams were the best available rather than completely unimpacted, it is reasonable to assume that not only the intercept, but also the slope of the regression for unimpacted clay rivers is higher than it would have been if our sites had been completely unimpacted. We therefore may have overestimated the natural increase in water TP concentrations with catchment clay cover. Since considerable efforts were made to sample the least impacted clay streams in Norway, however, the good/moderate boundary calculated from our data seems to be a goal that is both ambitious and realistic to achieve. Since we suggest using the lower end of the 10% categories as the benchmark against which to compare the observed PIT index (i.e., a site with 48% catchment clay cover should be assessed against the good/moderate boundary given for 40% clay cover; Table 3), the likely overestimation of the natural increase in TP is therefore partially compensated for.

Our approach of inferring the good/moderate boundary for the PIT in clay rivers was based on average concentrations, instead of median values. Median values, however, for different phosphorus fractions were consistently lower than average values (Fig. 1). This likely is explained by short-term periods of higher flow which typically coincide with increased phosphorus concentrations in rivers and streams (Cassidy and Jordan, 2011). Although we generally avoided taking water samples during very high flow conditions, periods with somewhat enhanced flow can, for practical reasons, not be avoided. It is unknown, however, if benthic algae are better related to median rather than to average phosphorus concentrations. Since Norwegian guidance documents with respect to the WFD use average values for water chemical parameters, we decided to follow the same approach. With increasing amount and quality of the data in the future, it will eventually be possible to compare these two approaches or, potentially, use PO_4-P instead of TP values. Likewise, it is known that parameters other than catchment clay cover affect water TP concentrations, for example catchment slope and precipitation pattern (Mutema et al., 2015). Currently, however, not enough data exist to disentangle the effect of these parameters from each other.

4.2. Bioavailability of TP

The adjusted good/moderate boundary for PIT in clay rivers was set taking into account the typical lower bioavailability of TP in unimpacted clay rivers, compared to other river types in Norway. This argument was based on our finding that the average contribution of PO_4-P to TP in nearly unimpacted clay rivers was 18-23%, compared to 33-37% in all other rivers (Fig. 2). Much research has been done in recent decades on the bioavailability of different phosphorus fractions in water and soil (e.g., Sharpley et al. 1991, Bjorkman and Karl, 1994, Ekholm, 1994), and the results show that there is no "one size fits all" answer with respect to which P fraction best characterizes bioavailable P. There is consensus that TP generally overestimates bioavailable phosphorus (Ellison and Brett, 2006), while PO_4-P generally underestimates the amount of phosphorus that is available to algae (Hatch et al., 1999). Bioavailable phosphorus is often approximated by soluble reactive phosphorus (SRP; Hatch et al., 1999; Reynolds and Davies, 2001). Li and Brett (2013) have shown, however, that several organic and inorganic phosphorus fractions classified as non-reactive (i.e., that would not be measured as SRP), have high bioavailability. The same authors also found that apatite had low bioavailability. This contrasts with findings by Krogstad and Løvstad (1991), who showed that a major part of the natural phosphorus in marine clay in Norway, which to a large degree consists of apatite (Krogstad and Øgaard, 2008), is bioavailable for primary producers.

Studies on the bioavailability of various P fractions are generally performed with bioassays, i.e., by incubating algal cultures with a water sample, and determining algal growth after a defined period, often 7-14 days (e.g., Krogstad and Løvstad, 1991; Ellison and Brett, 2006). For this reason, culturing conditions, for example the duration of the experiment, the choice of the algal species, or water pH may affect the results (low pH, which is characteristic for many freshwater ecosystems in Norway, may enhance bioavailability of apatite because of dissolution of calcium phosphate). In addition, bioassays are useful for determining the fraction of P that is available to algae over several days to weeks, such as in the case of lake phytoplankton. In rivers and streams, however, the water flow will transport any phosphorus that is not taken up by benthic primary producers further downstream. For this reason, we argue that for benthic algae in rivers and streams, in contrast to lakes, the bioavailable fraction of water TP consists of the part which is "immediately bioavailable". Phosphorus attached to particles is not immediately available to primary producers, and a variety of physical, chemical and biological processes influence the longer-term bioavailability of this P fraction (Ellison and Brett, 2006). Bjorkman and Karl (1994) found that PO_4-P appeared to be the preferred and, apparently, universal form, for the tested microorganisms and phytoplankton. It is reasonable to assume that this also is true for benthic algae, and that PO_4-P, measured in filtered water samples, therefore is a good approximation for the P that is immediately available to benthic algae at a river or stream site.

Aristi et al. (2017), however, have shown that benthic algal biofilms may trap sediments and recycle some of the trapped phosphorus. This means that some of the phosphorus bound to clay particles could eventually become available to benthic algae in streams. If this is true also in clay streams, we may have underestimated the total amount of phosphorus that is available to benthic algae in clay streams. It is reasonable to assume, however, that the amount of sediment that gets trapped in algal biofilms will vary with stream flow velocity as well as with algal biomass and growth form. No data exist that would enable quantifying how much of the natural phosphorus that is contained in marine clay may be trapped in benthic algal biofilms and become usable for various species of stream benthic algae.

Overall, our results indicate that on average 37% of water TP is in form of PO_4-P and is therefore likely to be immediately bioavailable to benthic primary producers in non-clay streams. This may seem like a high proportion, particularly in unimpacted reference rivers, but is in line with earlier studies that showed that SRP represents between 25 and 75% of TP in soil and water samples in Norway (Krogstad and Løvstad, 1989). In contrast, PO_4-P contributed only 18–23% to TP in unimpacted clay streams, likely because the PO_4-P was bound to clay particles (Edzwald et al., 1976), thereby explaining the high contribution of PO_4-P in unfiltered water samples (Fig. 2). In clay streams in agricultural catchments, however, PO_4-P contributed 33% to TP, probably because clay in agricultural soils contains more water-soluble P than unimpacted deeper clay or clay in forested catchments (Øgaard and Pedersen, 2016).

Bilotta and Brazier (2008) summarized the effects of suspended solids on freshwater organisms and found that suspended solids mostly reduced primary production, biomass and filament length of benthic algae, but could also lead to stimulated growth and filament length. Abrasive damage by suspended solids may be a reason why there generally are fewer benthic algal species in clay rivers than in other river types of comparable size (own experience). This likely contributed to the observed high variability of the PIT index among years (Fig. 5). Within the framework of various monitoring programs, PIT values will in the future be collected from clay streams with different degrees of anthropogenic impact. Such data, together with catchment clay cover and land cover, can eventually be used to validate the proposed PIT good/-moderate boundaries, and analyse the effect of nutrient abatement measures in areas with marine clay on benthic algal communities and the PIT index.

5. Conclusions

Rivers and streams draining areas with deposits of marine clay can have naturally enhanced water phosphorus concentrations. This raised concerns that current PIT status class boundaries would be unrealistically low when applied to clay rivers. We here showed that water P concentrations and the PIT index indeed were higher in unimpacted clay rivers than in non-clay rivers, but also that the TP in unimpacted clay rivers is less bioavailable than in other river types. By comparing impacted with unimpacted clay rivers, we showed that natural water TP concentrations increase with catchment clay cover. We used these results to develop new status class boundaries for the PIT in clay rivers. These status class boundaries were shown to increase with increasing catchment clay cover. Since the boundaries between high, good and moderate ecological status were considerably less than the observed year-to-year variability in the PIT index, we propose only using the good/moderate boundary for status assessment of clay rivers. This avoids unrealistic year-to-year differences in ecological status for a site. Clay rivers are therefore suggested to be assessed in only two status classes, i.e., “good or better” or “moderate or worse”, respectively.

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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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.envadv.2022.100279.

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