

**BIOMANIPULATION IN RUNNING WATERS:
THE POTENTIAL FOR TOP-DOWN CONTROL BY
CYPRINIFORM FISH IN MITIGATING EUTROPHICATION
EFFECTS IN MEDIUM-SIZED RIVERS**

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1 Preliminary note

Throughout the general part of this dissertation, the first-person singular is used. However, it should be noted that each article of this cumulative dissertation is the work of more than one person. The co-authors of the respective publication are given at the beginning of the specific chapters. In accordance with the applicable doctoral regulations, the author contributions are given in a separate document.

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2 Summary

Cultural eutrophication due to excessive inputs of nutrients seriously threatens aquatic ecosystems worldwide and is one of the major anthropogenic stressors on aquatic biota in European rivers. In streams and shallow rivers, its effects include excessive periphyton growth, which causes biological clogging and thereby oxygen depletion in the hyporheic zone. The result is a serious degradation of habitat quality for benthic invertebrates as well as for the eggs and larvae of gravel-spawning fish. Unlike in standing waters, efficient tools for controlling eutrophication in rivers are lacking. However, top-down control of the food-web by manipulating fish stocks, similar to the biomanipulation successfully applied in lakes, offers a promising approach to mitigating the effects of eutrophication in shallow rivers, especially those in which major reductions in nutrient inputs are not feasible. The overall aim of this thesis was to assess the potential for top-down control by two large cypriniform fish, the common nase (*Chondrostoma nasus*), the only obligate herbivorous fish species in European rivers, and the omnivorous European chub (*Squalius cephalus*), to mitigate the effects of eutrophication in medium-sized rivers. I therefore conducted field experiments on different spatial and temporal scales in the hyporhithral zone of a eutrophic gravel-bed river.

Generally, the results of those experiments revealed the crucial role of fish-mediated top-down effects in river food webs. In a 4-year reach-scale experiment, the key contribution of my thesis, the enhancement of fish densities significantly increased both oxygen availability and water exchange in the upper layer of the hyporheic zone, even though the top-down effects of the fish on periphyton biomass were relatively small. These findings were supported by those of a 4-week mesocosm experiment, which also provided insights into the mechanisms underlying the mitigation of eutrophication effects by nase and chub. The top-down effects of both fish species reduced hyporheic oxygen depletion, suggesting a reduction of biological clogging. The positive effects of herbivorous nase on hyporheic oxygen availability could be attributed to benthic grazing, whereas the reduction of hyporheic oxygen depletion in the presence of omnivorous chub was best explained by the enhanced bioturbation induced by the fish's benthic foraging. Overall, the results of my thesis demonstrate that biomanipulation achieved by enhancing herbivorous and omnivorous fish stocks can mitigate the effects of eutrophication in medium-sized European rivers. The results may be the first step towards the establishment of biomanipulation as a supportive management tool for eutrophication control in running waters and therefore as a strategy to preserve aquatic biodiversity.

3 Zusammenfassung

Eutrophierung infolge übermäßiger Nährstoffeinträge ist eine ernsthafte, weltweite Bedrohung für aquatische Ökosysteme und ist einer der wesentlichen anthropogenen Stressoren auf aquatische Organismen in europäischen Fließgewässern. In Bächen und kleinen bis mittelgroßen Flüssen führt Eutrophierung zu einem übermäßigen Wachstum von Periphyton und dadurch zu einem Verstopfen des hyporheischen Interstitials (biogene Kolmation). Infolgedessen kommt es zu einem Sauerstoffdefizit im Interstitial, wodurch die Habitatqualität für das Makrozoobenthos und für Eier und Larven kieslaichender Fische erheblich beeinträchtigt wird. Anders als in stehenden Gewässern fehlen effiziente Werkzeuge zur Eutrophierungssteuerung in Fließgewässern bisher.

Eine Top-down Steuerung des Nahrungsnetzes durch gezielte Stützung der Fischbestände, vergleichbar mit der erfolgreich in Seen angewendeten Methode der Biomanipulation, ist ein vielversprechender Ansatz zur Reduktion von Eutrophierungseffekten in Fließgewässern – insbesondere in Einzugsgebieten, in denen die Nährstoffeinträge nicht erheblich reduziert werden können. Ziel dieser Arbeit war es, das Potenzial einer Top-down Steuerung zur Reduktion von Eutrophierungseffekten durch zwei großwüchsige karpfenartige Fischarten – die herbivore Nase (*Chondrostoma nasus*) und den omnivoren Döbel (*Squalius cephalus*) – in mittelgroßen Flüssen zu erfassen. Dazu habe ich Freilandexperimente auf unterschiedlich großen räumlichen und zeitlichen Skalen in einem eutrophierten Mittelgebirgsfluss durchgeführt.

Generell haben die Ergebnisse dieser Experimente die zentrale Rolle von Top-down Effekten durch Fische in Fließgewässernahrungsnetzen aufgezeigt. In einem vierjährigen großskaligen Experiment, dem zentralen Teil meiner Arbeit, konnte ich zeigen, dass die Stützung der Bestände von Nase und Döbel zu einer deutlichen Verbesserung der Sauerstoffversorgung und des Wasseraustauschs im oberen Bereich des Interstitials geführt hat, und das, obwohl die Top-down Effekte der Fische auf die Periphytonbiomasse vergleichsweise gering ausgeprägt waren. Diese Ergebnisse konnten durch ein vierwöchiges Mesokosmosexperiment gestützt werden, das zugleich wertvolle Hinweise auf die zugrundeliegenden Mechanismen für die Verringerung der Eutrophierungseffekte im Interstitial durch Nase und Döbel geliefert hat. Durch hohe Dichten beider Fischarten wurde das Sauerstoffdefizit im Interstitial verringert, was wahrscheinlich auf eine Reduktion der biogenen Kolmation des Interstitials durch benthisches Grazing der Nasen bzw. durch Bioturbation der Döbel zurückzuführen war. Insgesamt zeigen die Ergebnisse meiner Arbeit, dass eine Biomanipulation durch Stützung der Bestände herbivorer und omnivorer Fische potenziell geeignet ist, um Eutrophierungseffekte in mittelgroßen Flüssen zu reduzieren. Die Ergebnisse könnten somit der erste Schritt sein, um Biomanipulation als unterstützende Maßnahme zur Verringerung von Eutrophierungseffekten in Fließgewässern zu etablieren, und damit zum Erhalt der aquatischen Biodiversität beitragen.

4 General introduction

Aquatic ecosystems have been substantially altered by human populations and their activities, such as agriculture, industrialisation and the urbanisation of catchments (Smith et al., 1999). Cultural eutrophication caused by nutrient inputs from domestic, industrial and agricultural sources (e.g., wastewater discharges, fertiliser application), is one of the major anthropogenic threats to aquatic ecosystems worldwide (Smith, 2003). The excess amounts of nitrogen (N) and phosphorus (P), the key nutrients limiting autotroph growth in aquatic systems (Dodds and Smith, 2016; Hecky and Kilham, 1988), stimulate primary production, leading to blooms of algae and cyanobacteria and thus to a serious degradation of water quality (Smith et al., 1999).

Previous research into freshwater eutrophication largely focussed on lakes and reservoirs (Hilton et al., 2006; Smith et al., 1999). Rivers were long considered to be insensitive to nutrient inputs, whose effects on primary production were thought to be restricted or prevented by factors such as light availability and hydraulic flow (Smith et al., 1999). However, this view was overturned by empirical evidence demonstrating the sensitivity of running waters to anthropogenic nutrient inputs (see Smith et al., 1999 and studies cited therein). In North American streams and rivers, nutrient enrichment of running waters was shown to be a major cause of water quality degradation (Biggs, 2000; Dodds and Welch, 2000). In Europe, an estimated 70% of the land area includes river catchments, which due to nutrient enrichment are at high risk of proliferations of benthic algae and cyanobacteria (periphyton) (McDowell et al., 2020). Indeed, nutrient enrichment has been identified as a major stressor on aquatic biota in European streams and rivers (Dahm et al., 2013; Grizzetti et al., 2017; Hering et al., 2006), with benthic stream assemblages in Europe shown to respond more strongly to elevated nutrient concentrations than pelagic lake assemblages (Johnson et al., 2014).

In nutrient-enriched streams and shallow rivers, intense periphyton growth negatively affects the physical-chemical properties of the surface water by causing large diel fluctuations in oxygen concentrations and in pH (Smith et al., 1999). In addition, eutrophication-driven periphyton blooms adversely affect the ecological functionality of the hyporheic zone, the connecting ecotone between river and groundwater ecosystems (Brunke, 1999; Ibisch et al., 2009). An intact hyporheic zone provides several key ecological functions: it acts as a filter that mediates the exchange of water, nutrients,

organic matter and contaminants; it plays a crucial role in biogeochemical cycling and it buffers against physical and chemical influences (Brunke and Gonser, 1997; Findlay, 1995). Moreover, it serves as an important habitat and refuge for benthic invertebrates and the early developmental stages of gravel-spawning fish (Brunke and Gonser, 1997; Stanford and Ward, 1988; Williams and Hynes, 1974).

The ecological functionality of the hyporheic zone strongly depends on its permeability, with negative effects induced by the clogging of riverbed sediments (Brunke and Gonser, 1997). In general, phases of clogging by sedimentation of fine particles during low-flow conditions alternate with the removal of clogged layers induced by high discharge events (Brunke, 1999). However, clogging of the riverbed sediments is enhanced anthropogenically by fine sediment inputs and/or as a consequence of eutrophication.

Excessive periphyton growth in nutrient-enriched streams and shallow rivers causes two types of biological clogging of the hyporheic zone: external and internal. External clogging by benthic algal mats on the riverbed reduces the water exchange between surface water and the hyporheic zone (Ibisch et al., 2009). Following the detachment of algal mats, internal clogging by infiltrated dead algal cells further reduces streambed permeability (Ibisch and Borchardt, 2002). In addition, internal clogging increases biological oxygen demand in the hyporheic zone, by enhancing the decomposition of decaying algae (Hartwig and Borchardt, 2015). The result of both types of clogging is a depletion of oxygen in the hyporheic zone, which inhibits nitrification and leads to the accumulation of ammonium (Triska et al., 1990). This in turn strongly reduces habitat quality for the eggs and larvae of gravel-spawning fish and for sensitive invertebrates, such as juvenile freshwater mussels (Geist and Auerswald, 2007; Hübner et al., 2009).

Climate change is expected to enhance the severe consequences of eutrophication in running waters because (i) periphyton growth rates increase with increasing water temperature (Kazanjian et al., 2018) and (ii) the low-flow conditions of rivers and streams will become more severe and more persistent, as predicted for most parts of Europe (Arnell, 1999; Feyen and Dankers, 2009). This would favour the development of thick periphyton mats and prolong clogging of the hyporheic zone. Given the severe impacts of eutrophication on the ecological functionality of the hyporheic zone and its expected intensification with ongoing climate change, methods to control eutrophication

in running waters are urgently needed to preserve biodiversity and protect already endangered species.

There are two principal strategies to controlling eutrophication: a reduction of its sources and a mitigation of its effects. The reduction of P inputs is essential for controlling eutrophication given that P is the limiting nutrient in most European rivers (McDowell et al., 2020). In the past four decades, successful reductions of P inputs from point sources (e.g. through enhanced wastewater treatment) and diffuse sources (e.g. through riparian buffer strips) have been achieved; however, in many cases, nutrient management measures have failed to reduce excessive algal growth (Jarvie et al., 2013). The reasons include inadvertent inputs from riparian buffer strips, which although designed to reduce P inputs from non-point sources may gradually become a P source due to their gradual accumulation and subsequent release of “legacy” P derived from past land use activities (Jarvie et al., 2013). Another difficulty is that the reduction of nutrient inputs is unlikely to substantially decrease periphyton biomass accrual unless nutrient concentrations in the river are reduced below algal-growth-limiting threshold concentrations (e.g. the threshold level of total phosphorus in temperate streams and rivers derived from a regression analysis: $43 \mu\text{g L}^{-1}$; Dodds et al., 2006, 2002). Reducing nutrient concentrations to limiting levels is expensive and difficult to achieve, especially in catchment areas that are densely populated and include a high level of agricultural activity (Bowes et al., 2012). In streams, light limitation by the riparian canopy may be an effective tool to control eutrophication during the summer months (Burrell et al., 2014; Ghermandi et al., 2009). However, as the river channel widens downstream and the degree of shading decreases, periphyton growth is not exceedingly light-limited during the vegetation season of medium-sized rivers (Vannote et al. 1980).

Another possibility is to mitigate the effects of eutrophication through top-down control of the food web by manipulating fish stocks. In fact, biomanipulation is a well-established technique that has been used to control eutrophication effects in standing waters (Benndorf, 1990; Hansson et al., 1998), but whether it can also be used in running waters is unknown. The most common biomanipulation method in lakes is the active removal of zooplanktivorous fish or the stocking of piscivorous fish in order to reduce predation pressure on zooplankton communities and thereby enhance their grazing on phytoplankton (Hansson et al., 1998). However, this approach cannot be applied to

running waters, because their food-web structure differs from that of standing waters, including a lack of phytoplankton and zooplankton. In rivers, the retention (travel) time of the water is the key factor determining whether the food web is based on phytoplankton or periphyton as the dominant primary producer (Hilton et al., 2006; O'Hare et al., 2018). Planktonic communities play a role only in the food webs of the slow-flowing lower reaches of large rivers or in impounded rivers with long-retention times (Hilton et al., 2006; Vannote et al., 1980). In fast-flowing streams and rivers, where the retention time is short, benthic periphyton attached to the substrate is the dominant primary producer (Hilton et al., 2006; Vannote et al., 1980). Consequently, the main consumers of autotrophic biomass in fast-flowing streams and rivers are benthic grazers such as herbivorous fish and macroinvertebrates (Hillebrand, 2009; Vannote et al., 1980). An increase in benthic grazing therefore requires either an increase in the stock of herbivorous fish or a reduction of predation pressure on benthic invertebrate grazers. Benthic grazing generally exerts strong top-down effects on periphyton in running waters (Feminella and Hawkins, 1995; Hillebrand, 2009). Strong direct top-down effects of herbivorous fish on periphyton have been observed in subtropical (e.g. Schneck et al., 2013) and tropical streams (e.g. Power et al., 1989; Wootton and Oemke, 1992). Fish grazing was suggested to be more important in tropical streams than in temperate streams because herbivorous fish species represent a larger proportion of the total fish community in the tropics (Wootton and Oemke, 1992). Nevertheless, top-down effects of herbivorous fish on periphyton have also been observed in North American streams, both in experimental stream mesocosms (e.g. Martin et al., 2016; Veach et al., 2018) and on a mesohabitat scale in stream pools (e.g. Gelwick and Matthews, 1992; Stewart, 1987). Indirect top-down control of periphyton in running waters has also been described, via a three-level trophic cascade consisting of small zoobenthivorous fish, invertebrate grazers and periphyton (e.g. Dahl, 1998; Winkelmann et al., 2014). Moreover, the introduction of a fourth trophic level, by the inclusion of large omnivorous fish, was shown to induce a trophic cascade down to periphyton via the reduction of predation pressure on invertebrate grazers (Power, 1990; Wootton and Power, 1993). Nonetheless, the reduction of predation pressure on invertebrate grazers alone is unlikely to provide an effective management tool for enhancing benthic grazing (Winkelmann et al., 2014), for two reasons.

First, benthic invertebrate grazers have much longer generation times (~1 year) than zooplankton grazers (e.g., < 1 month in cladocerans and < 7 days in rotifers at 15 C; Gillooly, 2000). Thus, benthic invertebrate grazers cannot quickly increase their population density to the level needed to substantially reduce algal blooms (Winkelmann et al., 2014). Second, a phase of intense periphyton in response to the high light supply in unshaded shallow rivers in summer coincides with the natural reduction of grazer biomass due to the emergence of mayflies (Winkelmann et al., 2014). However, in contrast to benthic invertebrates, herbivorous fish are not subject to a seasonal reduction and are thus able to exert high grazing pressure throughout the vegetation period, regardless of the periphyton growth period. The enhancement of fish grazing by stocking herbivorous fish may therefore be an effective strategy for mitigating the effects of eutrophication in shallow rivers, with additional indirect effects induced by an increase in invertebrate grazing through trophic cascades.

European rivers are home to two fish species with a high potential for mitigating the effects of eutrophication: the herbivorous cypriniform common nase (*Chondrostoma nasus*) and the omnivorous cypriniform European chub (*Squalius cephalus*) (Leuciscidae: Leuciscinae). Both species are typical members of the fish communities in the hyporhithral and epipotamal zones of European rivers and have wide distribution ranges. The rheophilic common nase is naturally distributed in Central and Eastern Europe, e.g. in the Rhine and Danube river systems (Kottelat and Freyhof, 2007). In Central Europe, common nase was once one of the most abundant and productive fish species in hyporhithral and epipotamal river zones, but it has suffered large-scale population declines due to multiple anthropogenic stressors such as damming and water pollution (Peňáz, 1996). Nevertheless, the nase remains abundant in many European rivers (Reckendorfer et al., 2001) and its local abundance is gradually increasing in response to restoration measures (Ramler and Keckeis, 2019; Schmutz et al., 2014). Moreover, it is the only fish species in European rivers that is specialised for feeding on periphyton, preferentially on benthic diatoms (Corse et al., 2010; Vater, 1997). Nase scrape periphyton from coarse substrates, leaving characteristic grazing scars (Freyhof, 1995). Adult fish typically form large single-species shoals, ranging in size from a dozen up to several hundred individuals, that move actively within defined home ranges (Huber and Kirchhofer, 1998; Lusk, 1967). The large size (total length up to 46 cm; Kottelat and

Freyhof, 2007) and the shoaling behaviour of nase result in a high natural biomass per area. These features could be used to reduce biological clogging in the hyporheic zone, via the direct top-down control of periphyton.

European chub naturally occurs throughout most of Europe (see Kottelat and Freyhof, 2007). In contrast to the highly specialised nase, chub is a generalist, able to feed on periphyton, especially filamentous algae, and on benthic invertebrates (Balestrieri et al., 2006; Hellawell, 1971). As the fish ages and grows (total length up to 60 cm, Kottelat and Freyhof, 2007), it increasingly preys on small zoobenthivorous fish such as common minnows (*Phoxinus phoxinus*) and bullheads (*Cottus gobio*) (Hellawell, 1971; Mann, 1976). In addition to potential direct top-down effects on periphyton, by feeding on filamentous algae, large chub may indirectly reduce periphyton biomass via a four-level trophic cascade (chub, small zoobenthivorous fish, herbivorous invertebrates, periphyton). Moreover, benthic foraging by chub was found to cause localised substrate coarsening in the upper layer of the hyporheic zone (e.g. Pledger et al., 2017). Thus, in addition to trophic interactions, the bioturbation resulting from the activity of benthic foraging by chub could increase the water exchange between surface water and the hyporheic zone due to a reduction of clogging and thus contribute to mitigating eutrophication effects in rivers.

The research described in this thesis examined the potential for top-down control by herbivorous nase and/or omnivorous chub to mitigate the effects of eutrophication in medium-sized rivers. I therefore conducted three studies on different spatial and temporal scales in the hyporhithral zone of a medium-sized eutrophic gravel-bed river. In **study 1**, I aimed at assessing whether herbivorous nase is able to reduce periphyton biomass. For this, I quantified the effects of fish exclusion on periphyton biomass in three consecutive short-term experiments (18–19 days) at different densities of nase in the river using standard tiles on the river bottom naturally covered with periphyton that were accessible to fish and tiles that excluded fish foraging with electric exclosures. In **study 2**, I examined whether the top-down effects of herbivorous nase and omnivorous chub can reduce eutrophication effects in the hyporheic zone, with a focus on the underlying mechanisms. In a 4-week mesocosm-based field experiment, cage enclosures stocked with nase or chub were placed in the river and their effects compared with those of unstocked control cages. In **study 3**, I focused on the question whether enhancing stocks of

nase and chub offers an effective ecosystem-scale strategy for mitigating the effects of eutrophication in medium-sized rivers, similar to the biomanipulation successfully applied in lakes. To answer this question, I conducted a reach-scale experiment over 4 years along a 1.4-km long river stretch. In **studies 2 and 3**, the top-down control of periphyton by nase and chub was expected to reduce biological clogging, ultimately resulting in increases in both water exchange and the oxygen supply in the hyporheic zone. Successful biomanipulation by enhancing herbivorous and omnivorous fish stocks would offer a cost-efficient measure to mitigate the effects of eutrophication in medium-sized rivers, thereby contributing to the preservation of aquatic biodiversity and to achieving the goals set by the European Water Framework Directive (WFD).

5 Study 1

Benthic grazing in a eutrophic river: cascading effects of zoobenthivorous fish mask direct effects of herbivorous fish

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Benthic grazing in a eutrophic river: cascading effects of zoobenthivorous fish mask direct effects of herbivorous fish

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ABSTRACT

Benthic grazing strongly controls periphyton biomass. The question therefore arises whether benthic grazing could be used as a tool to reduce excessive growth of periphyton in nutrient-enriched rivers. Although benthic invertebrate grazers reduce the growth of periphyton, this is highly context dependent. Here we assessed whether the only obligate herbivorous fish in European rivers, the common nase (*Chondrostoma nasus* L.), is able to reduce periphyton biomass in a eutrophic river. We conducted three consecutive *in situ* experiments at low, intermediate and high densities of nase in the river using standard tiles on the river bottom naturally covered with periphyton that were accessible to fish and tiles that excluded fish foraging with electric enclosures. The biomass of benthic invertebrate grazers was very low relative to nase. We hypothesised that nase would reduce periphyton biomass on accessible tiles and therefore expected higher periphyton biomass on the enclosure tiles, at least at intermediate and high densities of nase in the river. Contrary to our expectation, the impact of fish grazing was low even at high fish density, as judged by the significantly lower chlorophyll *a* concentration on enclosure tiles even though the ash-free dry mass on accessible and enclosure tiles did not differ. The lower chlorophyll *a* concentrations on enclosure tiles might be explained by a higher biomass of invertebrate grazers on the enclosure tiles, which would indicate that the effect of invertebrate grazers was stronger than that of herbivorous fish grazers. The high biomass of invertebrate grazers on enclosure tiles likely arose from the exclusion of zoobenthivorous fish, which occur in the river at high densities. The results of our small-scale experiments suggested that cascading top-down effects of zoobenthivorous fish have a higher impact on periphyton biomass than direct effects of herbivorous nase.

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INTRODUCTION

Benthic grazing in running waters strongly affects periphyton biomass (*Feminella & Hawkins, 1995; Hillebrand, 2009; Holomuzki, Feminella & Power, 2010*). Invertebrates across different taxonomic groups are able to reduce standing crops of stream periphyton

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(e.g., Gastropoda: [Rosemond, Mulholland & Elwood, 1993](#); [Rosemond, Mulholland & Brawley, 2000](#), Ephemeroptera: [Hill & Knight, 1987](#); [Moulton et al., 2004](#); Trichoptera: [Lamberti & Resh, 1983](#); [Katano et al., 2007](#)), and benthic grazing limits periphyton biomass accrual in nutrient-enriched rivers ([Peterson et al., 1993](#); [Sturt, Jansen & Harrison, 2011](#)). Herbivorous fish might also affect periphyton accumulation in streams and shallow rivers. Strong top-down effects of herbivorous fish have been found in subtropical ([Schneck, Schwarzbald & Melo, 2013](#)) and tropical streams ([Power, Dudley & Cooper, 1989](#); [Wootton & Oemke, 1992](#); [Flecker et al., 2002](#)). However, it has been suggested that fish grazing is more important in tropical streams than in temperate streams due to the higher density and species richness of herbivorous fish in the tropics ([Wootton & Oemke, 1992](#)). Nevertheless, field experiments have shown that the highly abundant small herbivorous cyprinid *Camptostoma anomalum* reduces periphyton biomass in North American streams ([Power, Matthews & Stewart, 1985](#); [Stewart, 1987](#); [Gelwick & Matthews, 1992](#)). In mesocosms simulating small headwater prairie streams, the presence of one or two herbivorous fish species (*Chrosomus erythrogaster*, *C. anomalum*) results in a reduction of algal filament lengths and periphyton biomass ([Martin et al., 2016](#)).

In the light of the potentially high top-down impact of grazers in streams and rivers, the question arises whether enhancement of benthic grazing could be used as a tool for the mitigation of eutrophication effects in stream conservation approaches, similar to biomanipulation in lakes ([Shapiro & Wright, 1984](#); [Hansson et al., 1998](#)). In streams and shallow rivers, nutrient enrichment promotes excessive growth of periphyton, which in turn can cause high diurnal fluctuations of oxygen and pH ([Dodds & Welch, 2000](#); [Hilton et al., 2006](#)) and biological clogging of the hyporheic zone ([Ibisch, Seydell & Borchardt, 2009](#)). This reduces the habitat quality for fish and invertebrates ([Welch, Quinn & Hickey, 1992](#); [Hübner, Borchardt & Fischer, 2009](#)).

However, although the effects of invertebrate grazers on stream periphyton are generally strong ([Hillebrand, 2009](#)), they do not seem to be sufficient to prevent algal blooms owing to a temporal mismatch of algal and invertebrate generation times ([Rosemond, Mulholland & Brawley, 2000](#); [Winkelmann et al., 2014](#)). This mismatch has the highest effect in small streams in forested catchments, when canopy cover strongly limits periphyton growth during the vegetation season and therefore algal blooms occur usually only in spring prior to tree foliation ([Rosemond, Mulholland & Brawley, 2000](#); [Winkelmann et al., 2014](#)). In rivers, however, a full canopy cover is rarely reached; therefore, periphyton are not greatly light limited during the vegetation season. Hence, the seasonal offset of algal production and grazing pressure observed in narrow streams might be not as strong in wider rivers. Furthermore, rivers also accommodate larger and often more diverse fish communities, including herbivorous or facultative herbivorous fish ([Oberdorff, Guilbert & Lucchetta, 1993](#)). Consequently, in shallow rivers, a top-down control of periphyton might be facilitated by the promotion of fish grazing, possibly in combination with the enhancement of invertebrate grazing.

In European rivers, the large cyprinid common nase (*Chondrostoma nasus* L.) is the only obligate herbivorous fish species ([Vater, 1997](#)). It feeds exclusively on periphyton, and preferentially on benthic diatoms ([Freyhof, 1995](#); [Corse et al., 2010](#)). Despite large-scale

population declines, nase is still abundant in many European rivers (Reckendorfer et al., 2001; Melcher, Lautsch & Schmutz, 2012). The home ranges of nase are well defined, with an average daily activity range of 120 m (Huber & Kirchhofer, 1998); therefore, a continuous impact of nase on periphyton in these home ranges might be expected. However, to our knowledge, the quantitative impact of herbivorous nase on periphyton biomass has not yet been investigated. Therefore, our study aimed at assessing whether nase is able to reduce periphyton biomass in a eutrophic river. In three *in situ* experiments using standardized concrete tiles covered with periphyton and electrical exclusion, we quantified the effects of fish exclusion on periphyton biomass at different densities of nase. We expected that nase, as the only herbivorous fish in the river, would control periphyton biomass, i.e., that periphyton biomass would be lower on tiles accessible to fish than on enclosure tiles, at least at intermediate and high densities of nase.

METHODS

Experimental site

Experiments were conducted in the hyporhithral zone of the river Nister (Rhineland-Palatinate, Germany, 50°43'N, 7°44'E), a small gravel-bed river with a drainage area of 246 km². The average mean discharge is 6.4 m³ s⁻¹ in winter and 2.4 m³ s⁻¹ in summer (measured at Heimborn, ID 2724030100; data supplied by State Office for Environment of Rhineland-Palatinate). At the experimental sites, the river is about 10 m wide and never completely shaded during the vegetation season. The river bed mainly consists of cobbles (6.3–20 cm) and boulders (20–63 cm). Land use in the catchment is dominated by forestry, pasture and agriculture. Due to phosphate emissions from several minor municipal wastewater treatment plants and diffuse emissions from agriculture, nutrient levels in the river are high (mean ± SD: 106 ± 62 µg PO₄-P L⁻¹, 5.3 ± 1.2 mg NO₃-N L⁻¹; n = 18; monthly measurements between June 2015 and July 2017, except during winter flood). Eutrophication effects, such as oxygen oversaturation and extreme pH, have been observed, especially during spring algal bloom (maximum in April 2016: 182.3% O₂, pH 10.2).

The benthic algal and cyanobacterial community in the river is largely composed of adnate and loosely attached diatoms. During summer, filamentous cyanobacteria or filamentous green algae, especially *Cladophora* spp., can become dominant. We conducted the experiments after the spring peak and breakdown of periphyton biomass in early summer.

Common nase is the only herbivorous fish in the river. The fish scrape periphyton from coarse substrate, typically swim in shoals and have defined home ranges. Fish grazing pressure in the river can be expected to be highest in run segments with coarse substrate, which are the preferred feeding habitats of nase (Huber & Kirchhofer, 1998). The invertebrate community is dominated by scraping grazers, especially mayfly larvae (*Baetis* spp., *Ephemerella ignita*), chironomid larvae and the snail *Ancylus fluviatilis*.

To quantify effects of fish exclusion, we conducted three consecutive experiments at three different densities of nase (Table 1). The first two experiments were performed in summer 2013 at two different sites (sites A and B) representing typical nase feeding habitats (20 m in

Table 1 Fish densities in the river in the three experiments. Experiment I = low nose density; experiment II = intermediate nose density, and experiment III = high nose density. Values are the total number of individuals caught per m² and calculated stock per m² (large fish: *Ricker, 1975*; small fish: *De Lury, 1951*, given only in case of significant regression coefficients).

Fish	Experiment I		Experiment II		Experiment III	
	Site A		Site B		Site B	
	July 2013		June 2013		July 2016	
	Catch (ind m ⁻²)	Stock (ind m ⁻²)	Catch (ind m ⁻²)	Stock (ind m ⁻²)	Catch (ind m ⁻²)	Stock (ind m ⁻²)
>15 cm						
Nase	0.005	0.004	0.019	0.033	0.198	0.276
Chub	0.003	0.005	0.003	0.005	0.032	0.062
Dace	0.004	0.005	0.003	0.010	0.016	0.082
Other	0.005	0.012	0.003	0.007	0.036	0.076
Total	0.016	0.026	0.027	0.055	0.281	0.495
<15 cm						
Bullhead	0.51		0.31	0.84	0.27	0.4
Minnow	0.48		0.68		0.25	0.4
Stone loach	0.85	1.23	0.52	1.49	0.91	2.0
Total	1.83		1.51		1.43	2.8

length) at low (experiment I) and intermediate (experiment II) densities. Site A (low nose density) is located 2.5 km upstream from site B (intermediate nose density), and nose density was eight times higher at site B than at site A (Table 1). In 2015, stocks of herbivorous nose and the omnivorous European chub (*Squalius cephalus*) were experimentally increased in a 500 m reach including site B for a long-term food web manipulation experiment; the experimental reach was defined by fish barriers to avoid fish emigration. In July 2016, we performed a third experiment (experiment III) at site B with a nose density more than 8-fold higher than in experiment II and 70-fold higher than in experiment I (Table 1). At the time of experiment III, nose biomass per area at site B was approximately 100-fold higher than the total benthic invertebrate biomass (nose: 111.5 g m⁻², total invertebrates: 1.2 g m⁻²). Aside from nose, the omnivorous European chub and common dace (*Leuciscus leuciscus*) were the most abundant large fish at the experimental sites (Table 1). The small zoobenthivorous fish species bullhead (*Cottus gobio*), common minnow (*Phoxinus phoxinus*) and stone loach (*Barbatula barbatula*) generally occurred at high abundances at both experimental sites and during all experiments (Table 1). Fish stocks were assessed by electrofishing campaigns (EFGI 650, Bretschneider Spezialelektronik, Chemnitz, Germany) in June 2013 (site A and B) and July 2016 (site B). Electrofishing was approved by the fisheries department of the local environmental agency SGD Nord (Rhineland-Palatinate, Germany). For each experiment, stocks of large fish (>15 cm) were estimated in a 500-m reach including the experimental site using the mark-recapture method. Stocks of small fish (<15 cm) were estimated in 60-m (experiments I and II) and 40-m (experiment III) long sections close to the experimental site by a three-pass removal method.

Electric enclosures

We used low-intensity electric pulses following the principle developed by [Pringle & Blake \(1994\)](#) to prevent fish but not benthic invertebrates from foraging on standardized concrete tiles (40 cm × 40 cm) exposed on the stream bottom. The strength of the electric field determines which organisms are affected by electrical exclusion because the sensitivity to electric fields increases with body size ([Moulton et al., 2004](#)). The electric field strength that we used in our experiments was comparable to that used in other field studies in which macroconsumers (approximately ≥1 cm, in this case fish and shrimps) were selectively excluded, while smaller invertebrates were not affected by the electric field ([Pringle & Blake, 1994](#); [Pringle & Hamazaki, 1997](#); [Rosemond, Pringle & Ramirez, 1998](#)). Electrical exclusion has the advantage that it avoids experimental artefacts associated with traditional enclosures or exclosures, such as reduced current velocity, increased sedimentation and shading effects.

Two protruding aluminium conductors were attached at opposite sides of each tile and were insulated at the bottom of the tile. Fish enclosure tiles were connected to commercially available electrical fence chargers (experiments I and II: compact B400, Electra Landtechnik GmbH, Vöhl, Germany; experiment III: Voss.farming Aures 3, Elefant-Weidezaungeräte e.K., Ohrstedt, Germany; both approximately 0.3 J output energy) that emitted approximately 50 electrical pulses per minute and were powered by a 12-V battery. Sets of three enclosures were connected in parallel to a fence charger. Control tiles were constructed in the same manner as enclosures but were not connected to a fence charger. The effectiveness of exclusion was tested in two preliminary experiments with five individuals of nase in artificial indoor-stream channels (2.6 m × 0.9 m × 0.5 m) at the University of Koblenz–Landau, Koblenz. In both 24-h experiments, electric pulses effectively prevented fish from foraging on electrified tiles but did not significantly unsettle the animals (see [Article S1](#)). This is consistent with observations that we made during the field experiments.

Experimental setup and sampling

Experiments I (June 2013) and II (July 2013) ran 18 days, and experiment III (July 2016) ran 19 days. Each separate experiment had a total of 18 tiles; half the tiles were electrified to exclude fish, and the other half were non-electrified to allow fish access. Electrified and non-electrified tiles were placed at least 2 m apart from each other to avoid an electric field between enclosures and controls. To allow initial periphyton growth, tiles were exposed on the river bottom two weeks prior to the start of experiments. On at least three occasions during the experiments, we measured water depth (only in experiments I and II), photosynthetically active radiation (PAR) using a LI-250 light meter (LI-COR, Lincoln, NE, USA) with a spherical micro-quantum sensor (US-SWS/L; Heinz Walz GmbH, Effeltrich, Germany) and current velocity using a flow meter with a vane wheel flow sensor (HFA hand-held unit with FA sensor; Hoentzsch GmbH, Waiblingen, Germany) above each tile.

At the end of each experiment, we sampled periphyton and benthic invertebrates to control for possible effects of invertebrate grazing. Fence chargers were not turned off until immediately before sampling to ensure continuous fish exclusion. Periphyton and benthic

invertebrate samples were each taken from half of the area of each tile. First, half of the tile was covered with a metal frame (20 cm × 40 cm) to protect the area for periphyton sampling. Invertebrates were sampled from the uncovered area by scraping with a coarse brush. Animals and organic material were washed into a net (500- μm mesh), which was positioned at the downstream edge of the tile. Subsequently, the invertebrate samples were rinsed over a 500- μm sieve and stored in 70% ethanol. The tile was then carefully removed from the river bottom, and periphyton was removed from the other half of the tile by brushing the area carefully with a coarse brush and up to 500 mL river water. The resulting periphyton suspensions were transported in the dark to the laboratory.

In experiment III, additional periphyton and benthic invertebrate samples were taken approximately 10 m downstream of the experimental site to compare the colonization of tiles and natural substrates in the river. These samples were actually collected for another field study, and periphyton were sampled five days before the end of experiment III and invertebrates were sampled one day after the end of experiment III. Ten stones were randomly chosen over the entire width of the river in order to obtain one mixed sample; periphyton was removed by carefully brushing the stone surface with a coarse brush and river water. Benthic invertebrates were sampled with three Surber samplers (total area 0.24 m², 500- μm mesh).

Laboratory analyses

Nutrient concentrations were measured photometrically (nitrate: [DIN EN ISO 13395, 1996](#); phosphate: [DIN EN ISO 15681-2, 2005](#)) using a continuous flow analyser (CFA, AutoAnalyser 3; Seal Analytical GmbH, Norderstedt, Germany). Total periphyton biomass was estimated as ash-free dry mass (mg AFDM cm⁻²), and autotrophic periphyton biomass was estimated as chlorophyll *a* concentration ($\mu\text{g Chl } a \text{ cm}^{-2}$). Periphyton biomass was quantified considering the total volume of the obtained periphyton suspension and the sampled area of tiles (experiments I–III) and stones (additional samples in experiment III). The surface area of stones sampled close to the experimental site were determined by carefully wrapping the stone in aluminium foil; overlapping areas were cut off, and the foil was weighed. The total volume of each periphyton suspension was determined, and the suspension was then homogenized using a magnetic stirrer to ensure comparable aliquots. For quantification of AFDM, 10 mL aliquots were transferred to pre-weighed ceramic crucibles and dried at 60 °C for 24 h. Dried samples were weighed, ashed at 510 °C for 5 h in a muffle furnace and subsequently reweighed.

To determine Chl *a* concentrations, triplicate aliquots were centrifuged at 13,000 rpm for 3 min (16,060 $\times g$, Micro 200R; Hettich Zentrifugen, Tuttlingen, Germany). The aliquot volume was 2 mL in experiments I and II but 0.5 mL in experiment III because the periphyton suspensions were thicker. The supernatants were discarded, and pellets were stored at –80 °C. Chl *a* was extracted and spectrophotometrically analysed according to [Mewes, Spielvogel & Winkelmann \(2017\)](#). In short, pellets were homogenized in 500 μL of 96% ethanol buffered with 1 g MgCO₃ L⁻¹ using a disperser (Ultra Turrax T8; IKA, Staufen, Germany), except for samples in experiment III, which were homogenized in a mixing mill (MM 400; Retsch Technology GmbH, Haan, Germany). Another 0.5, 1.0 or 1.5 mL

of buffered 96% ethanol was added, depending on the intensity of green colouration, to prevent incomplete extraction in high-quantity samples. Chl *a* was extracted for at least 3 h at room temperature in the dark. Subsequently, the samples were centrifuged at 6,000 rpm ($3,421 \times g$, Micro 200R) for 3 min, and Chl *a* in the supernatant was measured spectrophotometrically (Specord 205; Analytic Jena, Jena, Germany) at 665 nm and corrected for turbidity at 750 nm. If the sample absorbance exceeded 1, the sample was appropriately diluted with buffered ethanol to give a reading of less than 1.

In experiment III, additional 2 mL aliquots were taken from the homogenized periphyton suspensions and stored at -80°C for later analysis of the benthic algal and cyanobacterial community composition. The mean percentage of each taxonomic group (diatoms, green algae and cyanobacteria) in the suspension was estimated microscopically ($400 \times$ magnification) relative to the total area covered by algae and cyanobacteria on the slide (which was set to 100% in each microscopic field of view). The mean percentage of each group was estimated from 50 fields of view per slide, and three slides were analysed per sample.

All individuals from each benthic macroinvertebrate sample were sorted under a dissecting microscope, identified to the lowest practicable taxonomic level and counted. For each taxon in each sample, at least 50 individuals were measured to the nearest 0.1 mm. If less than 50 individuals occurred for one taxon per sample, all individuals of the sample were measured. The individual body mass (dry mass) was calculated using length–weight regressions. For all taxa except Chironomidae, we used regression models from the literature (Meyer, 1989; Benke et al., 1999; Baumgärtner & Rothhaupt, 2003; Edwards et al., 2009). For Chironomidae, we used data from our own samples to obtain a power function relating body length (BL) and dry mass (DM): $\text{DM} = 0.0013 \cdot \text{BL}^{2.8024}$ ($r^2 = 0.96$, $n = 62$). We did this because Chironomidae were the dominant group in most of our samples, they consist of species different than those in samples reported in literature, and published regressions for Chironomidae are based on a relatively small sample size (e.g., 16 samples in Meyer, 1989). The samples used to determine the body length and dry mass of chironomid larvae were taken from the river Nister and had been stored in 70% ethanol for 6 months. The length of undamaged individuals was measured to the nearest 0.1 mm. Afterwards, each individual was transferred to a pre-weighed reaction tube and dried for 24 h at 60°C . After cooling in a desiccator, dry mass was determined to the nearest 0.01 mg using a microbalance (XS205 Dual Range; Mettler-Toledo, Columbus, OH, USA). To reduce measurement error, the dry mass of smaller specimens (<5 mm) was determined by weighing 2–15 individuals of a similar length together and calculating a mean individual body mass. To correct the individual dry mass for mass loss owing to preservation, we used a conversion factor of 1.26 (Mährlein et al., 2016).

Data analysis

Stocks of large fish (>15 cm) were calculated using Chapman's modified Petersen estimator (Ricker, 1975). Stocks of small fish (<15 cm) were calculated using the De Lury (1951) regression method. In the case of non-significant regression coefficients ($R^2 < 0.88$), only the total number of caught individuals per m^2 is given because it represents a minimum

estimation for the population density of small fish. Chl *a* concentration was calculated per area of the tile surface (as in *Mewes, Spielvogel & Winkelmann, 2017*). Means of periphyton biomass (Chl *a* and AFDM) on enclosure and accessible tiles were compared using independent *t*-tests.

We assessed differences in the invertebrate community composition between enclosure and accessible tiles using analysis of similarities (ANOSIM) based on benthic invertebrate biomass. If ANOSIM results were significant, a similarity percentage (SIMPER) analysis was used to identify the taxa that were mainly responsible for the differences between enclosure and accessible tiles. In experiments I and III, nine enclosure and nine accessible tiles were analysed; in experiment II, only eight tiles of each type were analysed due to desiccation of one sample. To calculate the total biomass of invertebrate grazers in each sample, we weighted the biomass of each herbivorous and omnivorous species according to the average proportion of plant food in their diet (*Schmedtje & Colling, 1996*). The mean total grazer biomass of benthic invertebrates and the mean biomass of several dominant grazer taxa on enclosure and accessible tiles were compared using *t*-tests and adjusted for multiple comparisons using the Bonferroni-Holm correction.

To check whether environmental factors distorted the effects of fish exclusion on periphyton biomass, we used *t*-tests of differences in mean water depth, PAR and current velocity (averaged over the experimental period for each tile) between enclosure and accessible tiles. In addition, Pearson correlations were calculated to assess any influence of environmental factors on periphyton and grazer biomass. Non-normally distributed data were log-transformed for Pearson's correlation analysis. For all comparisons of means, Welch's test was used instead of the *t*-test when the assumption of homogeneity of variance was not met. Statistical analyses were performed and graphs were plotted using R version 3.3.3 (*R Development Core Team, 2016*).

RESULTS

When fish were excluded from the tiles, autotrophic periphyton biomass decreased, as shown by the significantly lower Chl *a* levels on tiles inaccessible to fish (enclosure) than on the tiles to which fish had access (control) at intermediate (experiment II) and high (experiment III) densities of nase (II: $p < 0.01$; III: $p = 0.02$; $n = 9$; *t*-test; *Figs. 1B* and *1C*). At a low nase density (experiment I), Chl *a* concentrations on the fish enclosure tiles were not significantly lower (Chl *a*: $p = 0.06$, $n = 9$, *t*-test, *Fig. 1A*). By contrast, total periphyton biomass measured as AFDM did not differ between accessible and enclosure tiles (I: $p = 0.33$, Welch test; II: $p = 0.19$, *t*-test; III: $p = 0.93$, Welch test; $n = 9$, *Figs. 2A–2C*). At a high nase density (experiment III), the variance of AFDM was significantly higher on the enclosure tiles than on the accessible tiles ($p < 0.01$, $n = 9$, *F*-test, *Fig. 2C*). Overall, both the Chl *a* concentration and AFDM were lowest on both sets of tiles at a low nase density and highest at a high nase density.

The algal and cyanobacterial communities on the tiles at a high nase density were dominated by diatoms (mean \pm SD, $n = 9$: enclosures: $63\% \pm 9\%$, controls: $59\% \pm 6\%$), followed by green algae (enclosures: $33\% \pm 9\%$, controls: $27\% \pm 8\%$) and cyanobacteria

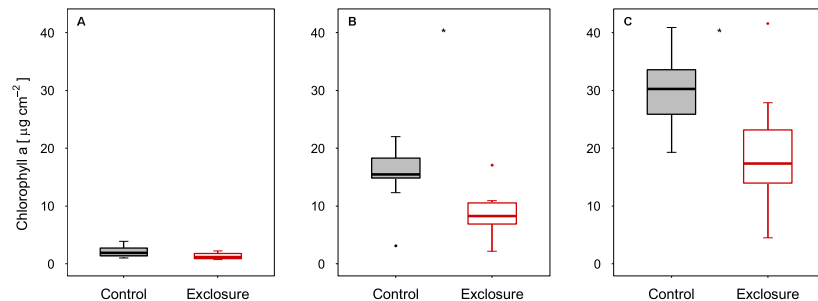


Figure 1 Chlorophyll *a* concentration for controls and exclosures at the end of the experiments.

Chlorophyll *a* per area on accessible control tiles and fish exclosure tiles ($n = 9$) at the end of the three experiments performed at (A) low, (B) intermediate and (C) high densities of nase. Boxes: 75 and 25%, whiskers: 95 and 5%, dots: outliers. * Significant ($p < 0.05$).

Full-size DOI: 10.7717/peerj.4381/fig-1

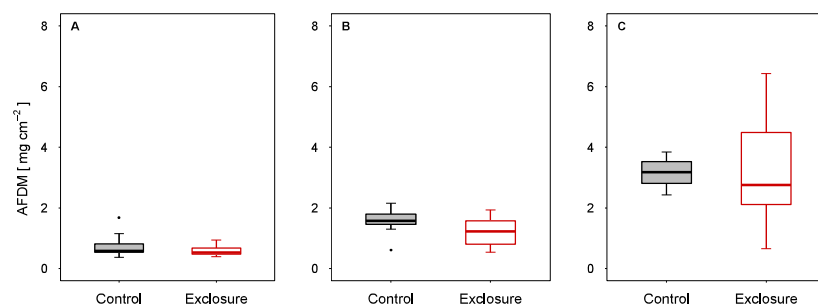


Figure 2 Ash-free dry mass for controls and exclosures at the end of the experiments. Ash-free dry mass per area on accessible control tiles and fish exclosure tiles ($n = 9$) at the end of the three experiments performed at (A) low, (B) intermediate and (C) high densities of nase. Boxes: 75 and 25%, whiskers: 95 and 5%, dots: outliers.

Full-size DOI: 10.7717/peerj.4381/fig-2

(exclosures: $3\% \pm 4\%$, controls: $14\% \pm 10\%$). Green algae were mostly filamentous, and included the taxa *Cladophora* spp. and *Microspora* spp. Benthic diatoms included loosely attached taxa such as *Navicula* spp., and stalked taxa such as *Gomphonema* spp., which were often found epiphytic on *Cladophora* spp. Cyanobacteria included mostly filamentous taxa, especially *Phormidium* spp. and bundles of *Homoeothrix* spp. The estimated proportion of cyanobacteria was higher on the accessible control tiles than on the exclosure tiles ($p < 0.01$, $n = 9$, t -test), which might point to effects of grazing on the periphyton community structure. On natural river stones, the algal and cyanobacterial communities differed from those on the tiles (mixed sample obtained from ten stones: 60% filamentous cyanobacteria, 38% diatoms, 2% filamentous green algae). The difference between tiles and natural substrates might have been even more intense owing to the time delay between samplings especially because there was a sunny period between the sampling dates. However, the communities on natural river stones and on the tiles generally consisted of similar taxa

(e.g., *Homoeothrix* spp., *Phormidium* spp., *Navicula* spp., *Gomphonema* spp., *Microspora* spp.). Periphyton biomass on the river stones was in the same order of magnitude as on the accessible tiles (mixed sample obtained from ten stones: $23.7 \mu\text{g cm}^{-2}$ Chl *a*, 2.4 mg cm^{-2} AFDM; accessible tiles of experiment III (mean \pm SD, $n = 9$): $30.0 \pm 6.5 \mu\text{g cm}^{-2}$ Chl *a*, $3.1 \pm 0.5 \text{ mg cm}^{-2}$ AFDM).

The composition of the benthic invertebrate community differed between enclosure and accessible tiles at high and intermediate nase densities, but not at a low nase density (I: $R = 0.08$, $p = 0.13$; II: $R = 0.18$, $p = 0.03$; III: $R = 0.64$, $p = 0.001$, ANOSIM). At a high nase density (experiment III), Chironomidae and *Baetis* spp. contributed most to the dissimilarity between enclosure and accessible tiles (34% and 28%, respectively; SIMPER). At an intermediate nase density (experiment II), Chironomidae and *Ephemerella ignita* contributed most to the dissimilarity between enclosure and accessible tiles (31% and 18%, respectively, SIMPER). The biomasses of the taxa responsible for the differences in SIMPER were consistently higher on the fish enclosure tiles. In all three experiments, Chironomidae and mayfly grazers (*Baetis* spp., *Ephemerella ignita* and occasionally *Ecdyonurus* spp.) contributed most to grazer biomass.

Grazer biomass was affected by the presence of fish. At a high nase density (experiment III), total grazer biomass and biomass of mayfly grazers were significantly higher on the fish enclosure tiles than on the accessible control tiles (total: $p < 0.001$, mayfly: $p = 0.001$; chironomid: $p = 0.16$, $n = 9$; Welch test; Fig. 3C). At an intermediate nase density, at least the Chironomidae biomass showed a tendency to increase (II: total: $p = 0.16$, mayfly: $p = 0.16$, chironomid: $p = 0.051$; $n = 8$; Welch test; Fig. 3B). At a low nase density, grazer biomass did not differ between enclosure and accessible tiles (I: total: $p = 0.30$, mayfly: $p = 0.24$, chironomid: $p = 0.36$; $n = 9$; t -test, Fig. 3A), and grazer biomass was overall lowest in this experiment.

Invertebrate colonization of the tiles was comparable but not identical to that of the natural substrates close to the experimental site in experiment III. Some taxa, such as *Ancylus fluviatilis* and *Elmis* sp., occurred more often on natural substrates. However, Chironomidae, *Baetis* spp. and *Ephemerella ignita* were among the most important invertebrate grazers (45% of grazer biomass) in the river, and had densities comparable to those on the tiles (see Table S2).

There was no evidence that the environmental factors water depth, light and current velocity affected the experimental results. The water depths at exclusion and control tile sites were similar (I: $p = 0.82$; II: $p = 0.75$; III not measured; t -test; $n = 9$; Table 2). The light supply was also similar at exclusion and control tile sites in all three experiments (I: $p = 0.72$, Welch test; II: $p = 0.64$, t -test; III: $p = 0.45$, Welch test; $n = 9$, Table 2). Current velocities were similar at exclusion and control tile sites in experiments I and II (I: $p = 0.88$, II: $p = 0.63$, $n = 9$, t -test) but was significantly higher around the fish enclosure tiles than around the control tiles in experiment III ($p = 0.03$, $n = 9$, t -test, Table 2). However, although some significant correlations were found between environmental factors and biotic response variables in experiments I and II (Table 3), there was no significant

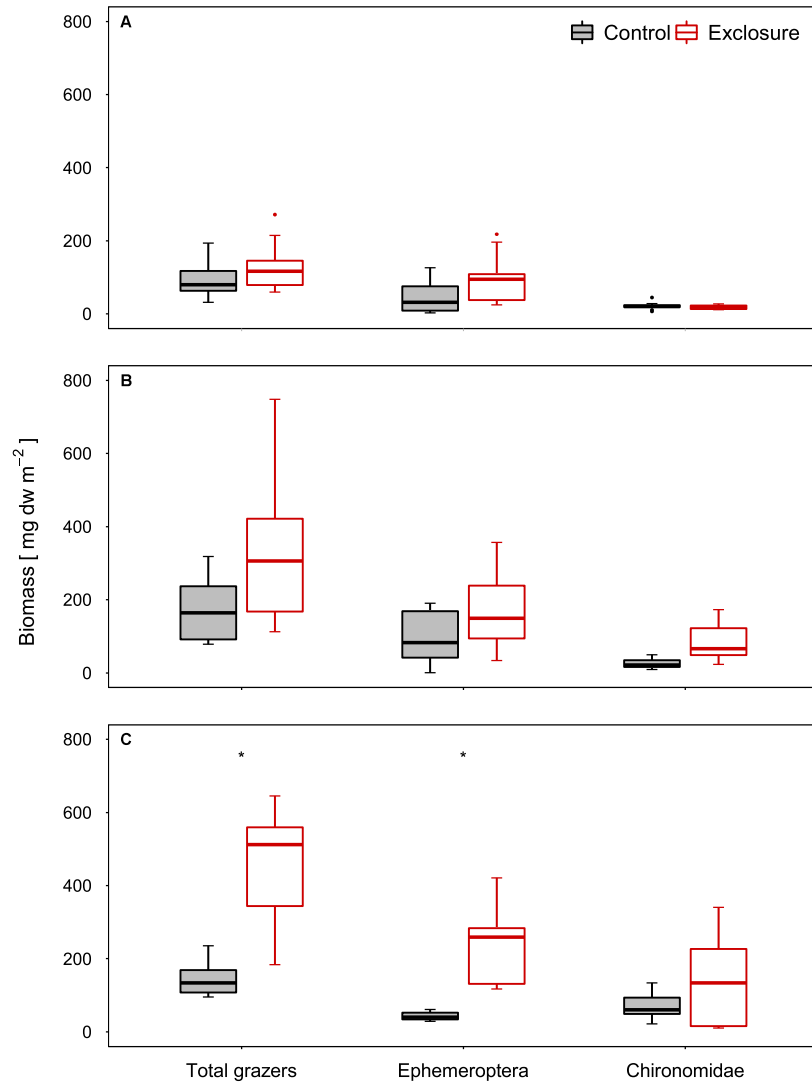


Figure 3 Biomass of invertebrate grazers for controls and exclusions at the end of the experiments. Biomass of invertebrate grazers on accessible control tiles and fish exclusion tiles ($n \geq 8$) at the end of the three experiments performed at (A) low, (B) intermediate and (C) high densities of nase. Boxes: 75 and 25%, whiskers: 95 and 5%, dots: outliers. * Significant ($p < 0.05$).

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Table 2 Mean values of environmental parameters for controls and exclusions in the three experiments. Mean values (\pm SD) of water depth, light supply (PAR) and current velocity on accessible control tiles and fish exclusion tiles ($n = 9$) averaged over the experiment duration (number of measurements, $n \geq 3$).

Nase density	Water depth (cm)		PAR ($\mu\text{mol m}^{-2} \text{s}^{-1}$)		Current velocity (m s^{-1})	
	Exclusion	Control	Exclusion	Control	Exclusion	Control
Low (I)	21.9 \pm 10.3	20.7 \pm 11.5	846.1 \pm 696.2	979.1 \pm 849.1	0.19 \pm 0.04	0.20 \pm 0.08
Intermediate (II)	29.3 \pm 13.5	27.5 \pm 9.8	153.6 \pm 61.7	169.4 \pm 78.0	0.27 \pm 0.12	0.29 \pm 0.08
High (III)			127.4 \pm 45.3	111.8 \pm 41.4	0.33 \pm 0.06	0.27 \pm 0.06

Table 3 Pearson correlation coefficients between biotic response variables and environmental factors (water depth, light supply PAR and current velocity).

	Low nase density (I)			Intermediate nase density (II)			High nase density (III)	
	Water depth	PAR	Current velocity	Water depth	PAR	Current velocity	PAR	Current velocity
Exclusion								
Chl <i>a</i>	0.89*	-0.31	0.58	0.02	-0.36	0.75*	0.60	0.24
AFDM	0.62	0.03	0.60	-0.07	-0.12	0.72*	0.62	0.23
Grazer biomass	-0.36	0.75*	0.44	-0.57	0.48	0.58	0.57	0.18
Control								
Chl <i>a</i>	0.72*	-0.15	0.50	-0.23	0.44	-0.22	0.45	0.51
AFDM	0.64	0.03	0.51	-0.03	0.21	-0.36	0.44	0.44
Grazer biomass	-0.83*	0.68*	0.73*	-0.32	0.41	0.40	-0.02	0.48

Notes.

*Significant ($p < 0.05$).

relationship between current velocity and Chl *a* (exclusions: $p = 0.45$; controls: $p = 0.18$; $n = 9$), AFDM (exclusions: $p = 0.47$; controls: $p = 0.22$; $n = 9$) or between current velocity and grazer biomass (exclusions: $p = 0.65$; controls: $p = 0.15$; $n = 9$; Table 3) in experiment III, where current velocity differed between exclusion and control tile sites.

DISCUSSION

As grazers are able to strongly control periphyton biomass (*Feminella & Hawkins, 1995; Hillebrand, 2009*) and eutrophication-driven algal blooms detrimentally affect the ecological quality of running waters (*Dodds & Welch, 2000; Biggs, 2000; Hilton et al., 2006*), the active promotion of benthic grazing might be a future tool for improving the quality of shallow rivers or unshaded streams. However, in contrast to the known top-down effects of benthic invertebrate grazers, there is a considerable gap of knowledge concerning the top-down effects of herbivorous fish. To assess whether top-down effects of herbivorous fish are able to control periphyton biomass, we conducted *in situ* exclusion experiments in which fish were not allowed to gain access to tiles colonized by periphyton and compared the results to those obtained with accessible control tiles. The unspecific exclusion of fish in our study was expected to be sufficient to reduce grazing intensity, because the common nase (*C. nasus*) is a specialized periphyton feeder (*Freyhof, 1995; Corse et al., 2010*) and the only herbivorous fish in the river. We expected strong top-down effects by nase on periphyton biomass, especially because we frequently observed visible traces of feeding

of nase in the river during summer. Therefore we hypothesized that nase can reduce periphyton biomass, which in our experiments would result in higher periphyton biomass on enclosure tiles compared to accessible tiles in a eutrophic river with intermediate and high densities of nase.

In contrast to our hypothesis, autotrophic periphyton biomass (measured as Chl *a*) was lower on the enclosure tiles at intermediate and high densities of nase. This possibly indicates a low impact of fish grazing at this experimental scale. This result was surprising because it had been observed in other small-scale experiments that herbivorous fish exert strong top-down effects on periphyton (e.g., [Wootton & Oemke, 1992](#); [Flecker et al., 2002](#); [Schneck, Schwarzbald & Melo, 2013](#); [Martin et al., 2016](#)), and it was even shown that the small cyprinid species central stoneroller (*C. anomalum*) reduces periphyton on the mesohabitat scale ([Power, Matthews & Stewart, 1985](#); [Stewart, 1987](#); [Gelwick & Matthews, 1992](#)).

A possible explanation for the lower autotrophic periphyton biomass observed on fish enclosure tiles might be a strong indirect top-down effect of zoobenthivorous fish at the experimental site. This is unexpected because we assumed that direct top-down control by nase would be stronger than indirect top-down control over two trophic levels, especially as the biomass of nase was more than 100-fold higher than that of invertebrates at the experimental site. Unfortunately, there is uncertainty whether zoobenthivorous fish were completely excluded by the electrical fences on the tiles because we did not test the effectiveness of the electrical exclusion for small fish. However, other studies that used electrical enclosures at a similar intensity reported the exclusion of animals ≥ 1 cm ([Pringle & Blake, 1994](#); [Pringle & Hamazaki, 1997](#); [Rosemond, Pringle & Ramirez, 1998](#)), which would have excluded all zoobenthivorous fish in our experiments. In addition, our results strongly indicate that benthic invertebrates might have been released from predation pressure, thereby increasing invertebrate grazing. This assumption is supported by our observation of a higher invertebrate grazer biomass in general and mayfly grazers in particular on enclosure tiles than on control tiles. If indeed invertebrate grazing was responsible for the observed results, then the indirect control of periphyton biomass by zoobenthivorous fish via invertebrate grazers was stronger than the direct top-down effect of herbivorous nase in our small-scale experiments.

In principle, the occurrence of a trophic cascade from fish over invertebrate grazers on periphyton seems likely because comparable effects have been observed under near-natural conditions in stream ecosystems for both zoobenthivorous fish ([Winkelman et al., 2014](#)) and drift-feeding fish ([Huryn, 1998](#); [Buria et al., 2010](#); [Pagnucco, Remmal & Ricciardi, 2016](#)) and in many small-scale experiments (e.g., [Power, 1990b](#); [Flecker & Townsend, 1994](#); [Dahl, 1998](#); [Kurle & Cardinale, 2011](#)). Moreover, effects of zoobenthivorous fish on the composition of the benthic invertebrate community have been observed on different experimental scales (large scale: [Winkelman et al., 2007](#); [Winkelman et al., 2011](#); [Worischka et al., 2014](#); small scale: [Dahl, 1998](#); [Shelton et al., 2016](#)). Grazing mayfly larvae and chironomids are important prey species for zoobenthivorous fish ([Copp, Spathari & Turmel, 2005](#); [Ureche et al., 2010](#); [Worischka et al., 2015](#)), which is in agreement with the strongest effects of fish exclusion on mayflies and chironomids in our experiments.

However, the strength of the observed effects did not correspond to the density of small zoobenthivorous fish (catch per m²). Based only on the total density, the strongest effects would have been expected in experiment I with the highest density of zoobenthivorous fish (low nase density). However, our results showed the strongest effect in experiment III (significant decrease in Chl *a* concentrations and significant increase in benthic grazers), in which the total density of zoobenthivorous fish was lowest. One explanation for these results is that the strength of the predation pressure on the tiles might have been affected by a species shift within the zoobenthivorous fish community, owing to differences in their foraging behaviour. Stone loach (*B. barbatula*), whose stock was highest at high nase densities (experiment III), feeds in habitats with higher current velocities than other zoobenthivorous fish species (Worischka et al., 2012) and can therefore be expected to feed frequently on the experimental tiles. Another explanation for these results is that the strong effects are due to the relatively high density of the large omnivorous fish European chub (*S. cephalus*) and dace (*L. leuciscus*). Both species feed to a considerable proportion on benthic prey (Vlach, Švátora & Dušek, 2013) and might therefore have reduced benthic grazers on the tiles accessible to fish.

While the explanations stated above focused on top-down regulation, the higher Chl *a* concentration on accessible control tiles might also be the result of a stimulation of periphyton growth due to fish grazing, which represents an overcompensation of top-down regulation. During the first two weeks of experiment III, we frequently observed nase foraging on the control tiles and found highly visible traces of feeding. Such newly grazed patches, free from senescent algae and detritus, offer optimal growth conditions for new algae (Lamberti & Resh, 1983; McCormick & Stevenson, 1989), thus yielding comparatively more Chl *a*. This explanation is supported by the observation that while autotrophic biomass was significantly higher in the controls in experiments II and III, total biomass (AFDM), including heterotrophs and detritus other than living algae, was similar in all experiments. The presence of herbivorous fish on the accessible tiles might have caused increased removal of detritus (Power, 1990a; Flecker, 1996), allowing algae to increase their growth rate, thereby overcompensating fish grazing. The significantly lower variance of AFDM in the controls at high nase density (experiment III) could indicate that grazing by nase had a homogenizing effect on the spatial scale of the experimental site, leading to more similar total periphyton biomass in the controls, thereby supporting the second explanation.

Several methodical issues possibly led to an increase in variability and a lower observed effect of fish. First, annual and seasonal differences in the abundance and developmental stages of benthic invertebrates between the experiments cannot be ruled out completely. Unfortunately, we were not able to run the experiments in parallel and had to use a consecutive experimental design. An underestimation of indirect effects of zoobenthivorous fish might have resulted from the conservative estimation of the total grazer biomass. Especially for calculating the grazer biomass of Chironomidae, we used a small average proportion of plant food in their diet (20%; Schmedtje & Colling, 1996). Nevertheless, Chironomidae larvae are a diverse group that vary greatly in their feeding habits. Several taxa feed predominantly on algae (Cummins, 1973; Pinder, 1992; Tarkowska-Kukuryk, 2013) and

are able to exert strong top-down effects on periphyton (Power, 1990b; Tarkowska-Kukuryk, 2013). Assuming that mostly grazing chironomids settled on the tiles, it seems likely that grazing by chironomid larvae caused the lower Chl *a* concentration on the enclosure tiles in experiment II even though chironomid biomass did not significantly increase.

In addition, we suspect that especially in experiment I, bottom-up effects were likely more important than top-down effects because light intensity was highest and periphyton biomass was lowest for accessible and enclosure tiles of this experiment, while invertebrate grazer biomass was low. Higher light availability could have promoted fast periphyton growth, which leads to self-shading and ultimately to detachment of periphyton and thereby masks potential effects of fish exclusion (Higgins, Hecky & Guildford, 2008).

The use of artificial substrates, which might result in a different community structure of periphyton and invertebrates owing to uniform surface texture, size and colonization time (Cattaneo & Amireault, 1992), means our experimental results cannot be directly transferred to the situation in a real ecosystem. However, the relatively high proportion of filamentous green algae and cyanobacteria, especially the occurrence of attached taxa such as *Cladophora* spp., shows that the concrete tiles were a reasonably good facsimile and that colonization time was long enough to allow the development of mid- to late-successional stages of periphyton. Therefore, we think that although the periphyton assemblage on the tiles was not identical to that of natural substrates, it sufficiently represents the natural colonization of the river bed during summer.

Furthermore, the exact fish densities at the experimental site (20 m in length) were not known because fish moved within their natural home range. This uncertainty might have been overcome by using an enclosure design. However, such a design seems undesirable because it would have affected both abiotic conditions and fish behaviour. Because of the different habitat use of zoobenthivorous fish and nase, we chose different spatial scales for fish stock estimations. Zoobenthivorous fish are mostly stationary and small; we estimated their density in 60-m sections very close to the experimental sites. Nase, on the other hand, usually swim in shoals that move actively within defined home ranges (Huber & Kirchhofer, 1998). Therefore, we found it necessary to estimate nase stocks on a large scale (500 m) to reflect the potential grazing impact of nase at the experimental sites. Although we cannot rule out that nase changed their feeding places from time to time over the experimental periods, we expect that within the experimental period of more than two weeks, differences in day-to-day feeding areas were reasonably integrated over time.

Finally, the small spatial and temporal scale of our experiments does not allow us to draw a general conclusion from our experimental results on the possible top-down control of periphyton by nase in eutrophic rivers. Especially the effect of invertebrate grazing seems likely to be particularly strong on a small spatial scale but less relevant at larger scales (Englund, 1997; Gil, Jiao & Osenberg, 2016).

CONCLUSIONS

Our results indicate that the active promotion of benthic grazing might be a possible tool to reduce eutrophication effects in rivers, but also highlight the complexity of top-down control in river food webs. In our small-scale experiments, cascading effects of

zoobenthivorous fish via invertebrate grazers might have been stronger than direct top-down effects of herbivorous nase. However, we cannot determine whether fish grazing or invertebrate grazing is more important on the ecosystem scale because the potential impact of herbivorous nase remained unclear, likely owing to the unspecific exclusion of fish in our experiment. To assess the top-down effects of herbivorous nase in eutrophic rivers, large-scale and long-term experiments that consider the impact of spatial and seasonal variability are needed.

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Competing Interests

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Author Contributions

- Madlen Gerke performed the experiments, analyzed the data, wrote the paper, prepared figures and/or tables, reviewed drafts of the paper.
- Daniel Cob Chaves conceived and designed the experiments, performed the experiments, reviewed drafts of the paper.
- Marc Richter performed the experiments, reviewed drafts of the paper.
- Daniela Mewes performed the experiments, contributed reagents/materials/analysis tools, reviewed drafts of the paper.
- Jörg Schneider performed the experiments, contributed reagents/materials/analysis tools, reviewed drafts of the paper, organised and performed electrofishing-campaigns, contributed fish density data.

- Dirk Hübner contributed reagents/materials/analysis tools, reviewed drafts of the paper, organised and performed electrofishing-campaigns, contributed fish density data.
- Carola Winkelmann conceived and designed the experiments, analyzed the data, wrote the paper, reviewed drafts of the paper.

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Electrofishing permit was obtained from the fisheries department of the local environmental agency SGD Nord (Rhineland-Palatinate, Germany).

Data Availability

The following information was supplied regarding data availability:

The raw data is available as [Supplemental Files](#), and also at OwnCloud—University of Koblenz-Landau: <https://owncloud.uni-koblenz-landau.de/owncloud/s/ASfpVRpiXvD3q1M>.

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The following supplement information accompanies the article

**Benthic grazing in a eutrophic river: cascading effects of
zoobenthivorous fish mask direct effects of herbivorous fish**

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Supplemental description of the preliminary experiments

We performed two consecutive 24-h experiments with five adult specimens of nase taken from the river Nister in an artificial indoor stream channel (2.6 m × 0.9 m × 0.5 m) filled with water also taken from the river Nister (520 L, conductivity 347 $\mu\text{s cm}^{-1}$). The photoperiod during the experiments was 15:9 (light:dark) h. Fish were not fed for three days prior to the experiments.

In the first experiment, we exposed two different electrified enclosure tiles in the stream channel to find the optimal construction: one with two aluminium insulators (variant A) and the other with two aluminium insulators and two additional steel insulators (variant B). The second experiment aimed to test whether a) electric exclusion effectively prevents fish from foraging and b) fish feed on the periphyton growing on the non-electrified control tile. Thus, in the second experiment, we exposed one electrified enclosure tile and one non-electrified control tile in the stream channel. Prior to the experiments, the tiles were preconditioned in the river Nister for two weeks to grow periphyton, which was used as bait during the experiments. The enclosure tiles were connected to a fence charger (compact B400, Electra Landtechnik GmbH, Vöhl, Germany, 0.3 J output energy) that emitted electric pulses every 1.22 s for 2.04 ms. Electric pulses were measured using an oscilloscope (TEK 2245A, American Tektronix, Beaverton, US). During the experiments, fish contacts with tiles were filmed with a webcam (320 × 320 pixel resolution) that was installed above the stream channel. The number of fish contacts with tiles and contact durations were analysed using VLC media player (version 2.2.1). This analysis was only possible for the day period.

In the first experiment, we observed one fish contact of 8 s with the variant A tile and three contacts with a total duration of 17 s with the variant B tile. As both constructions of electric enclosures seemed to be similarly effective in preventing fish from foraging, we used the more simple construction (variant A) in the second experiment. In the second experiment, there were considerably more fish contacts with the non-electrified control tile than with the electrified enclosure tile (control: 86 contacts, 45.3 min total duration; enclosure: 5 contacts, 1.4 min total duration). During the experiment, the fish intensively grazed periphyton on the non-electrified control tile and almost completely removed the periphyton from the tile, whereas there were no traces of feeding on the electrified enclosure tile at the end of the experiment.

6 Study 2

Cypriniform fish in running waters reduce hyporheic oxygen depletion in a eutrophic river

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Cypriniform fish in running waters reduce hyporheic oxygen depletion in a eutrophic river

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Abstract

1. The hyporheic zone is an important habitat for benthic invertebrates and early-developmental stages of gravel spawning fish. However, the eutrophication of running waters and, in turn, the excessive periphyton biomass leads to its biological clogging. The result of these processes is oxygen depletion and a reduction in the habitat quality of the hyporheic zone.
2. This study assessed whether top-down effects of two important European river fish species, the large herbivorous cypriniform common nase (*Chondrostoma nasus*, L.) and the large omnivorous cypriniform European chub (*Squalius cephalus*, L.), can reduce eutrophication effects in the hyporheic zone. A 4-week mesocosm-based field experiment in a eutrophic river was conducted using cage enclosures stocked or not with either nase or chub.
3. The top-down control of periphyton was expected to reduce biological clogging and thereby increase oxygen availability in the hyporheic zone. Accordingly, we hypothesised that in enclosures stocked with either fish the concentrations of dissolved oxygen in the hyporheic zone would be higher and the periphyton biomass would be lower than in enclosures without fish stocking.
4. Hyporheic oxygen concentrations were significantly higher in enclosures stocked with either nase or chub than in enclosures without fish stocking. However, periphyton ash-free dry mass was significantly reduced only in enclosures stocked with nase, not in those stocked with chub. Thus, the positive effects of nase and chub on hyporheic oxygen availability were caused by different mechanisms.
5. Our results demonstrate that nase and chub can reduce eutrophication effects in the hyporheic zone of running waters. Hence, protecting and enhancing stocks of herbivorous and omnivorous fish will contribute to restoring the hyporheic zone in efforts to preserve biodiversity in eutrophic rivers.

KEYWORDS

benthic grazing, biological clogging, biomanipulation, *Chondrostoma nasus*, *Squalius cephalus*

Dirk Hübner and Madlen Gerke should be considered joint first author.

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1 | INTRODUCTION

The hyporheic zone is the connecting ecotone between river and groundwater ecosystems (Brunke, 1999). An intact hyporheic zone provides key ecological functions: it acts as a filter that mediates the exchange of water, nutrients, organic matter, and contaminants; it plays a crucial role in biogeochemical cycling and buffers against physical and chemical influences (Brunke & Gonser, 1997; Findlay, 1995; Stanford & Ward, 1988). It also serves as an important habitat and refuge for benthic invertebrates and the early developmental stages of gravel-spawning fish (Baxter & Hauer, 2000; Brunke & Gonser, 1997; Findlay, 1995; Williams & Hynes, 1974). Due to the importance of the hyporheic zone for ecosystem functioning and biodiversity, efforts aimed at stream restoration must also include restoration of the hyporheic zone (Boulton, 2007; Hester & Gooseff, 2010).

The ecological functionality of the hyporheic zone strongly depends on its permeability, with negative effects induced by the clogging of riverbed sediments (Brunke & Gonser, 1997). Regulated rivers within agricultural catchments, common in Central Europe, are especially prone to a reduced permeability of their hyporheic zones by two different clogging processes, physical and biological (Brunke, 1999; Brunke & Gonser, 1997; Hartwig & Borchardt, 2015; Ibisch, Seydell, & Borchardt, 2009). Physical clogging by anorganic fine sediments (e.g. as a result of erosion from agricultural fields) reduces the pore space and therefore the seepage rate, which in turn impairs the hydrological connectivity of surface water and groundwater (Brunke & Gonser, 1997; Hartwig & Borchardt, 2015). Biological clogging of the hyporheic zone is due to the excessive periphyton growth in nutrient-enriched streams and shallow rivers that is induced by eutrophication (Hartwig & Borchardt, 2015; Ibisch et al., 2009). The resulting oxygen depletion (Hartwig & Borchardt, 2015; Ibisch et al., 2009) strongly reduces habitat quality for gravel-spawning fish and sensitive invertebrates, such as juvenile freshwater mussels (Geist & Auerswald, 2007; Hübner, Borchardt, & Fischer, 2009; Keckeis, Bauer-Nemeschkal, & Kamler, 1996; Malcolm, Youngson, & Soulsby, 2003). Extensive clogging also impairs invertebrate communities (Jones, Growns, Arnold, McCall, & Bowes, 2015) and decreases biodiversity (Descloux, Datry, & Marmonier, 2013).

Biological clogging is subject to temporal variation but its overall extent is related to the nutrient-driven accrual of periphyton biomass (Ibisch et al., 2009). The accumulation of periphyton mats on substrates that leads to biological clogging is counteracted by the removal of periphyton by floods or grazing. Grazing by fish and invertebrates controls periphyton biomass (Feminella & Hawkins, 1995; Hillebrand, 2009; Holomuzki, Feminella, & Power, 2010), decreases periphyton biomass accumulation in nutrient-enriched rivers (Gerke et al., 2018; Peterson et al., 1993; Sturt, Jansen, & Harrison, 2011), and may alter periphyton community structure (e.g. Abe, Uchida, Nagumo, & Tanaka, 2007; Gelwick & Matthews, 1992; Lamberti & Resh, 1983; Pringle & Hamazaki, 1997). Clogging is also influenced by algal morphology, as the filamentous branches of green algae (e.g. the abundant *Cladophora* sp.) tend to trap sediments from the water

(Berger, Henriksson, Kautsky, & Malm, 2003; Dodds, 1991), which may increase external clogging of the hyporheic zone.

In standing waters, the enhancement of zooplankton grazing by biomanipulation is a well-established technique to control eutrophication effects (Benndorf, 1990; Hansson et al., 1998; Shapiro & Wright, 1984). However, whether the active enhancement of grazing might be similarly used to control the effects of eutrophication in running waters is unknown. Given the potentially strong impact of the top-down control of periphyton by grazing in rivers, we expected that enhancing stocks of herbivorous and omnivorous fish would lead to increased indirect control of the eutrophication effects in rivers, especially those in which a drastic reduction in nutrient input and the restoration of flood dynamics are not feasible.

In North and South American streams, strong direct effects of herbivorous fish on periphyton have been observed both in small-scale experiments (Flecker et al., 2002; Martin, Gido, Bello, Dodds, & Veach, 2016; Schneck, Schwarzbald, & Melo, 2013; Veach, Troia, Jumpponen, & Dodds, 2018; Wootton & Oemke, 1992) and on a mesohabitat scale (Gelwick & Matthews, 1992; Power, Dudley, & Cooper, 1989; Power, Matthews, & Stewart, 1985; Stewart, 1987). Grazing fish were also shown to reduce the filament lengths of filamentous green algae (e.g. Bertrand & Gido, 2007; Martin et al., 2016; Murdock, Dodds, Gido, & Whiles, 2011). In Central European rivers, the large cypriniform common nase *Chondrostoma nasus* (Linnaeus, 1758) (Leuciscidae: Leuciscinae) is the only obligate herbivorous fish species (Vater, 1997) and it is specialised for feeding on periphyton (Corse et al., 2010; Freyhof, 1995). Nase typically swim in shoals and scrape periphyton from coarse substrates. Despite large-scale population declines, the common nase remains one of the most abundant fish species in many European rivers (Reckendorfer, Keckeis, Tiitu, Winkler, & Zornig, 2001) and its potential for reducing eutrophication effects in rivers may be accordingly significant.

Indirect top-down effects cascading through three trophic levels, from zoobenthivorous fish to invertebrate grazers and then to periphyton, have been demonstrated in several small-scale experiments (e.g. Dahl, 1998; Gerke et al., 2018; Pagnucco, Remmal, & Ricciardi, 2016) and under near-natural conditions in stream ecosystems (Winkelmann et al., 2014). In two mesocosm experiments conducted in a Northern California river, predatory and omnivorous fish were shown to induce a four-level trophic cascade down to periphyton, by releasing grazing invertebrates from predation pressure (Power, 1990; Wootton & Power, 1993). In Central European rivers, one of the most widespread, common and at least partially piscivorous fish species is the large omnivorous cypriniform European chub *Squalius cephalus* (Linnaeus, 1758) (Leuciscidae: Leuciscinae). While it partially feeds on periphyton, especially on filamentous algae (Balestrieri, Prigioni, Remonti, Sgrosso, & Priore, 2006; Hellawell, 1971), and benthic invertebrates, with progressing age and size it increasingly preys on small zoobenthivorous fish such as minnows, *Phoxinus phoxinus* (Linnaeus, 1758) and bullheads, *Cottus gobio* (Linnaeus, 1758) (Hellawell, 1971; Mann, 1976). Thus, we predicted that, in addition to its potential direct top-down effects on periphyton by feeding on filamentous algae, large chub would indirectly

reduce periphyton biomass, and therefore eutrophication effects as well, via a four-level trophic cascade. Specifically, in response to a reduction in the local density of zoobenthivorous fish through either chub predation or displacement, benthic invertebrates would be released from predation pressure, thus increasing invertebrate grazing and reducing periphyton biomass.

To assess whether the top-down effects of herbivorous nase and omnivorous chub lead to a reduction in eutrophication effects in the hyporheic zone, we conducted a mesocosm-based field experiment using cage enclosures in a eutrophic river. The top-down control of periphyton by nase and chub was expected to reduce biological clogging, ultimately resulting in an increased oxygen supply in the hyporheic zone. Accordingly, we hypothesised that enclosures stocked with either nase or chub would show (1) higher oxygen concentrations within the hyporheic zone and (2) a lower periphyton biomass than in enclosures without fish stocking. Along with a reduction of periphyton biomass, we expected grazing-induced changes in the structure of the periphyton community, especially regarding the proportion of green algae within the periphyton. The feeding activity of nase was expected to directly reduce periphyton biomass and that of omnivorous chub to directly and/or indirectly reduce periphyton biomass via the above-described four-level trophic cascade.

2 | MATERIAL AND METHODS

2.1 | Experimental site

The experiment was conducted in the hyporhithral zone of the river Nister (Rhineland-Palatinate, Germany, 50°43'24 N, 7°44'24 E), a small gravel-bed river with a catchment area of 246 km². The average mean discharge is 6.3 m³/s in winter and 2.4 m³/s in summer (measured at Heimborn, ID 2724030100; data supplied by the State Office for Environment of Rhineland-Palatinate). The land use type in the catchment area is dominated by forestry, pasture and agriculture. At the experimental site, the Nister flows from east to west and the land use type is forest on its right bank (north) and pasture on its left bank (south). Therefore, the river is only partly shaded, on the right bank side. The river's width here averages 15 m. The river bed is relatively homogenous and mainly consists of cobbles (6.3–20 cm). Due to phosphate emissions in the catchment area from several minor municipal wastewater treatment plants and diffuse emissions from local agriculture, the river is highly eutrophic, with the effects including oxygen oversaturation and an extremely high pH during the daytime, especially during the spring algal bloom (Gerke et al., 2018). The hyporheic zone in the river is impaired by clogging especially during summer, as indicated by high proportions of fine sediments in the substrate and low hyporheic oxygen concentrations (unpublished data, see Appendix S1 for details). Moreover, high differences of electrical conductivity and pH-values between surface water and hyporheic water suggest reduced subsurface-surface exchange (Geist & Auerswald, 2007).

The benthic algal and cyanobacterial community in the river is dominated by adnate and loosely attached diatoms, but filamentous cyanobacteria (e.g. *Homoeothrix* sp.) and filamentous green algae (*Ulothrix* sp., *Cladophora* sp.) can become dominant during late spring and summer. The experiment described herein was conducted in early summer, after both the spring peak of the algal bloom and the breakdown of periphyton biomass. The invertebrate community is dominated by chironomid larvae and scraping grazers, such as the mayfly *Ephemerella ignita* (Poda, 1761) and the snail *Ancylus fluviatilis* (Müller, 1774).

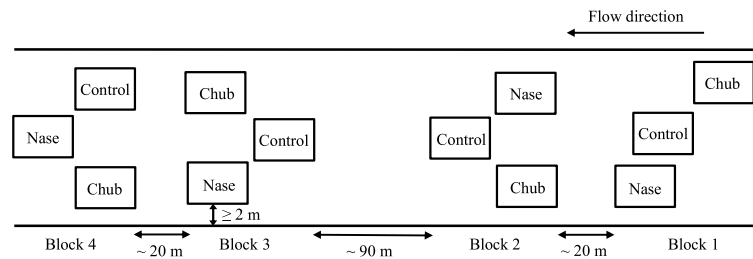
The obligate herbivorous common nase dominates the community of large fish, followed by the omnivorous European chub (estimated local biomass densities of fish >15 cm assessed in a 550 m river stretch directly upstream of the experimental site: common nase: 75 g/m², European chub: 18 g/m²). On a mesohabitat scale, much higher natural fish densities can be expected in run sections, as they are the preferred feeding habitats of nase (Huber & Kirchhofer, 1998). In contrast, due to high predation risk in the river from cormorants, *Phalacrocorax carbo sinensis* (Linnaeus, 1758), shallow riffles are usually avoided by large fish. Among the small zoobenthivorous fish, bullhead, common minnow, and stone loach, *Barbatula barbatula* (Linnaeus, 1758), occur in high densities (Gerke et al., 2018).

2.2 | Experimental setup

The enclosure experiment was conducted using in situ fish cages (8 m²) and three treatment groups: (1) enclosures stocked with common nase; (2) enclosures stocked with European chub; and (3) control enclosures without fish stocking. The fish biomass density (mean ± standard deviation) was 308.6 ± 0.3 g/m² for enclosures stocked with nase and 312.5 ± 1.7 g/m² for enclosures stocked with chub, achieved by stocking each enclosure with six to nine nase individuals (total length of 28.0–34.5 cm, mean total length 30.8 cm) or four chub individuals (total length of 28.5–43.0 cm, mean total length 37.3 cm). The fish used for the experiment were caught in the vicinity of the cage locations by electrofishing (using EFGI 650, Bretschneider Spezialelektronik). Electrofishing was approved by the fisheries department of the local environmental agency, the SGD Nord (Rhineland-Palatinate, Germany).

Overall, 12 enclosures were arranged in four experimental blocks over a river section of c. 150 m, with one enclosure representing each treatment within each block. All enclosures were positioned in a run section with moderate water depth and current velocity (see Appendix S2 for details), representing a typical feeding habitat of adult nase and chub. Current velocity and water depth were similar among the three treatments (see Appendix S2). The three different treatment enclosures within a block were staggered, with the central enclosure shifted, so as to reduce mutual hydrological interference (Figure 1). Thus, control enclosures in the three blocks were positioned upstream of the fish enclosures and/or in a shifted position in the middle of the river, while the fish enclosures stocked with nase

FIGURE 1 Experimental setup showing the arrangement of the experimental blocks and of the treatments (enclosures stocked with nase or with chub and control enclosures without fish stocking) within each block.



or chub were positioned in an alternating sequence (see Figure 1). This arrangement was chosen to ensure that water flow through the control enclosures was optimal and never impaired by the enclosures stocked with fish. This excluded the possibility that any positive effects of the fish treatments were due to a higher water flow in the respective enclosure. The distance between experimental blocks was at least 20 m; the second and third experimental blocks were separated by c. 90 m because the water depth in the intermediate section was insufficient for large fish.

Each cage enclosure (length \times width \times height: 4 \times 2 \times 0.5 m) was constructed using 18 robust vertical PVC pipes (46 mm in diameter) as a core frame. Flexible horizontal PVC pipes (19 mm in diameter) were attached on all sides with a spacing of 20 mm. This spacing distance minimised the flow resistance of the enclosures and allowed small fish to pass through the cage interstices. The enclosure walls were regularly cleaned to remove deposited litter. The top openings of the enclosures were covered with removable fishing net (mesh size 25 mm) to prevent both the fish from jumping over the enclosure walls and the entry of bird predators while the side walls of the enclosures were not additionally equipped with net. The bottom of the enclosures remained open and allowed the fish to access the natural river bed. The transition zone between the enclosure walls and the river bed was secured against digging fish with a strip of fishing net (mesh size 25 mm) that was attached to the lowest part of the enclosure walls and buried in the substratum along their edge. The enclosures were fixed within the river bed using iron bars. Overall, the enclosures proved sufficiently stable also at high water levels. Two weeks prior to the installation of the enclosures, a multilevel probe (manufactured by the central workshop of the University Kassel, Kassel, Germany) for the extraction of hyporheic water, following Lenk et al. (1999), was buried at the planned location of each enclosure. The enclosures were installed on 31 May 2017 and the experiment was started by stocking the enclosures with fish one week later, on 7 June 2017. The fish remained within the enclosures for 4 weeks, until 5 July 2017.

2.3 | Sampling

The concentration of hyporheic dissolved oxygen was sampled weekly beginning 1 week prior to the start of the experiment (directly before the installation of the enclosures on 31 May 2017) and continuing until the end of the experiment (5 July 2017). The

multilevel probes (Lenk et al., 1999) allowed the collection of hyporheic water samples from three different depths (8, 13, and 23 cm) within the riverbed. Water samples from each depth horizon were obtained using a polypropylene syringe (B. Braun Melsungen AG, Melsungen, Germany) and their dissolved oxygen content was immediately measured (WTW, Multi 3430 with FDO 925 probe, Wissenschaftlich Technische Werkstätten, Weilheim; Germany).

Periphyton was sampled according to the same weekly schedule (except on 7 June 2017) as the water samples. For periphyton sampling, six stones were randomly chosen from the area of each enclosure and their periphyton pooled to yield one sample per enclosure. Periphyton was removed by carefully brushing the stone surface with a coarse brush and river water. The resulting periphyton suspensions were transported in the dark to the laboratory.

Benthic invertebrates were sampled one week prior to the start of the experiment (directly before installation of the enclosures on 31 May 2017) and at the end of the experiment (5 July 2017). Sampling was performed using a Surber sampler (0.08 m², mesh size 500 μ m) positioned at the upstream end of each enclosure. The invertebrate samples were rinsed over a 500 μ m-sieve and stored in 70% ethanol.

2.4 | Laboratory analyses

Periphyton biomass was characterised based on the total periphyton biomass (estimated as ash-free dry mass, in mg AFDM/cm²), as a measure of total organic matter, and the autotrophic periphyton biomass (estimated as the chlorophyll *a* concentration in μ g Chl *a*/cm²), as a measure of the mass of photosynthetically active algae. Both were quantified based on the total volume of the obtained periphyton suspension and the sampled area of the stones. The surface area of the stones was estimated by carefully wrapping each stone in aluminium foil; overlapping areas were cut off, and the foil was then weighed. After their total volumes were determined, the periphyton suspensions were homogenised using a magnetic stirrer to ensure comparable aliquots.

Ash-free dry mass was quantified by transferring 10-ml aliquots to pre-weighed ceramic crucibles and then drying the samples at 105°C for 24 hr. The dried samples were weighed, ashed in a muffle furnace at 510°C for 5 hr and then reweighed. Chlorophyll *a* concentrations were measured by centrifuging triplicate 2-ml aliquots at 16,060 g for 3 min (Micro 200R; Hettich Zentrifugen).

The supernatants were discarded, and the pellets stored at -80°C . Chlorophyll *a* was extracted and then analysed spectrophotometrically according to Mewes, Spielvogel, and Winkelmann (2017). In short, four 3-mm glass beads and 1.8 ml of 96% ethanol buffered with 1 g MgCO_3/L was added to each pellet and the mixtures were then homogenised using a mixing mill (MM 400, Retsch Technology GmbH). Chl *a* was extracted for at least 3 hr at room temperature in the dark. Subsequently, the samples were centrifuged for 3 min at 3,421 g (Micro 200R), and Chl *a* in the supernatant was measured spectrophotometrically (Specord 205; Analytic Jena, Jena, Germany) at 665 nm, correcting for turbidity at 750 nm. If the sample absorbance exceeded 1, the sample was appropriately diluted with buffered ethanol to obtain a reading <1 . The Chl *a* concentration was calculated per area as described in Mewes et al. (2017).

For later analyses of benthic algal and cyanobacterial community composition, additional 2-mL aliquots were taken from the homogenised periphyton suspensions and stored at -80°C . The mean percentage of each taxonomic group (diatoms, green algae and cyanobacteria) in the suspension was estimated microscopically ($400\times$ magnification) and expressed relative to the total area covered by algae and cyanobacteria on the slide (set to 100% in each microscopic field of view). The mean percentage of each group was estimated from 50 fields of view per slide, and three slides were analysed per sample.

All individuals from each benthic macroinvertebrate sample were sorted under a dissecting microscope, identified to the lowest practicable taxonomic level and counted. For each taxon in each sample, at least 50 undamaged individuals were measured to the nearest 0.1 mm. If <50 individuals occurred for one taxon per sample, all individuals of the sample were measured. Invertebrate biomass was estimated by calculating the individual body mass (dry mass) using length–weight regressions (Baumgärtner & Rothhaupt, 2003; Benke, Huryn, Smock, & Wallace, 1999; Burgherr & Meyer, 1997; Gerke et al., 2018; Mährlein, Pätzig, Brauns, & Dolman, 2016; Meyer, 1989; Smock, 1980). To calculate the total biomass of invertebrate grazers in each sample, the biomass of each herbivorous and omnivorous species was weighted according to the average proportion of plant food in the diet of that species (Schmedje & Colling, 1996).

2.5 | Data analyses

To assess the effects of stocking the enclosures with nase or chub on the extent of biological clogging, we tested the effects of the fish treatments on hyporheic oxygen concentrations. The effect on periphyton biomass of stocking the enclosures with nase or chub was examined by measuring the effects of the fish treatments on AFDM and the Chl *a* concentration. Whether the fish treatments changed the proportion of green algae within periphyton was also determined. Potential effects of the fish treatments on invertebrate grazing were investigated by measuring the effects of the fish treatments on overall invertebrate biomass and specifically on invertebrate grazer biomass. In all cases, with the exception of the effect

of chub on benthic invertebrates, the effects of the fish treatments were tested against the control treatment without fish stocking. The effect of chub on invertebrate biomass (and specifically on grazer biomass) was tested against the other two treatments combined by applying orthogonal contrasts (contrast I: chub versus nase and control; contrast II: nase versus control). In general, all univariate statistical analyses were calculated by applying a generalised linear mixed model (GLMM) in which the treatment was a fixed factor and the experimental block a random factor, in order to account for differences in the localities of the enclosures. In the analyses of the hyporheic oxygen concentration, the depth horizon was included as a fixed factor and the interaction treatment \times depth was entered into the model. Parameters were estimated using the maximum likelihood method. Differences in invertebrate community composition were assessed using permutational multivariate analysis of variance (PERMANOVA) based on the Bray–Curtis dissimilarities calculated from the invertebrate biomass (fourth-root transformed). A PERMANOVA was performed with 999 permutations stratified within the experimental blocks. For a detailed assessment of the potential effect of chub treatment on invertebrate community composition, in addition to the full model, the orthogonal contrasts chub versus nase and control (contrast I) and nase versus control (contrast II) were analysed as well.

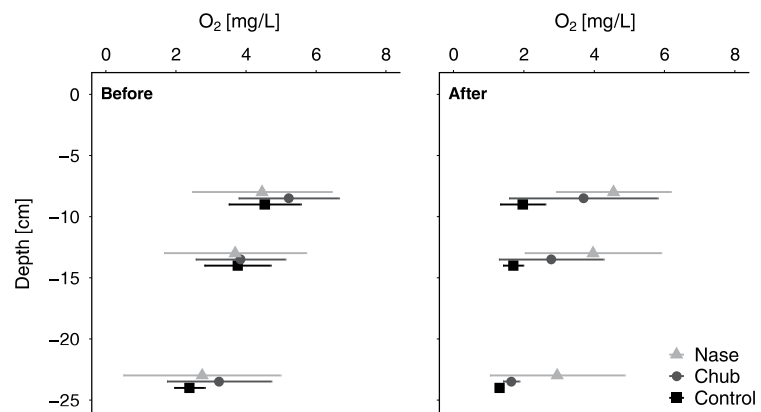
Prior to the tests of the hypotheses, we examined whether the habitat quality at the different enclosure sites differed so as to cause a bias in the results, by testing for potential differences between the enclosures in the initial values (before installation of the enclosures and fish stocking) of the dependent variables used for hypothesis testing according to the future fish stocking treatment. The same statistical methods as described above were applied, except that, rather than the control treatment, the overall model was used as the testing framework. There were no significant effects of future treatment on the dependent variables prior to the experiment (Table B1 in Appendix S2). Consequently, the measurements obtained after termination of the experiment were used for hypothesis testing without a correction for the initial values.

Both the statistical analyses and graph plotting were performed using R version 3.6.1 (R Development Core Team, 2019). Generalised linear mixed models were applied using the *lme* function included in the R-package *nml* (Pinheiro et al., 2018). PERMANOVA was performed using the *adonis* function from the R-package *vegan* (Oksanen et al., 2018).

3 | RESULTS

In accordance with our first hypothesis, in which higher oxygen concentrations were predicted for fish-stocked enclosures within the hyporheic zone, at the end of the experiment the hyporheic oxygen concentration was significantly higher in enclosures stocked with either nase ($p = 0.001$) or chub ($p = 0.02$) than in control enclosures without fish stocking (GLMM, $n = 4$; Figure 2). Prior to the experiment,

FIGURE 2 Mean oxygen concentration in the hyporheic zone (8, 13, and 23 cm depth) in enclosures stocked with nase or with chub and in control enclosures without fish stocking ($n = 4$). Left: before the installation of the enclosures (1 week prior to the start of the experiment), Right: at the end of the experiment. Error bars represent the standard deviations.



the hyporheic oxygen concentrations did not differ among the future treatments ($p = 0.59$, see Table B1 in Appendix S2 for details; Figure 2). While the oxygen concentration decreased with increasing hyporheic depth ($p = 0.007$), the effects of the fish treatments were independent of the depth horizon (overall treatment \times depth: $p = 0.73$). Water samples from enclosures stocked with fish had a 1.6 mg/L higher average oxygen concentration than samples taken from control enclosures. However, despite the positive effect of fish stocking, hyporheic oxygen concentrations generally decreased during the course of the experiment across all treatments (Table 1, Figure 2) and water temperature did not increase in a way that would explain this decrease (see Appendix S3 for details). Therefore, the process of biological clogging was not averted by the presence of large fish but only attenuated or decelerated, especially within the upper two depth horizons.

The second hypothesis, that top-down control of fish would reduce periphyton biomass, was only partly supported. Total periphyton biomass at the end of the experiment was significantly lower in enclosures stocked with nase than in control enclosures ($p = 0.048$), with 30.5% less AFDM on average in the former (GLMM, $n = 4$; Figure 3a). By contrast, total periphyton biomass did not significantly differ between enclosures stocked with chub and control enclosures ($p = 0.98$, GLMM, $n = 4$; Figure 3a). Autotrophic periphyton biomass, measured as Chl *a*, was not reduced compared to the control enclosures in either the nase- or the chub-stocked enclosures ($p = 0.26$ and $p = 0.49$ respectively; GLMM, $n = 4$; Figure 3b). Despite the significant difference in total periphyton biomass at the end of the

experiment, the top-down pressure of nase varied over time (see Appendix S3 for details).

The nase and chub treatments also differed in their effect on the general composition of the algal community (Table 2). The estimated proportion of green algae was significantly lower in enclosures stocked with nase than in control enclosures ($p = 0.02$) while there was no significant effect of chub ($p = 0.27$; GLMM, $n = 4$). At the end of the experiment, green algae were largely dominated by *Cladophora* sp.

As predicted, indirect top-down effects of European chub on benthic macroinvertebrates were observed, including an overall effect of the treatments on invertebrate biomass ($p = 0.048$, $n = 4$, ANOVA of GLMM). At the end of the experiment, total invertebrate biomass in the enclosures stocked with chub differed significantly from that in the other two treatments (contrast I: $p = 0.02$, GLMM, Figure 4), whereas there was no difference in the total invertebrate biomass between the enclosures stocked with nase and the control enclosures (contrast II: $p = 0.33$, GLMM, Figure 4). The invertebrate biomass in the chub-stocked enclosures exceeded, on average, that in the other treatments, by 65.1%. However, there was no overall treatment effect on grazer biomass ($p = 0.44$, $n = 4$, ANOVA of GLMM) and grazer biomass did not differ between the enclosures stocked with chub and the other two treatments (contrast I: $p = 0.30$; contrast II: $p = 0.49$; GLMM; Figure 4). This would also explain the lack of effects of the chub treatment on periphyton biomass.

Generally, benthic invertebrate community composition was affected by the fish treatments (PERMANOVA full model: $p = 0.04$, $R^2 = 0.17$), although this effect was weaker than the effect of the experimental blocks, which accounted for the majority of the differences in the community composition ($p = 0.004$, $R^2 = 0.51$). A comparison of the benthic invertebrate community composition in the enclosures stocked with chub versus the other treatments revealed statistically significant but weak effects of chub on the community composition (PERMANOVA contrast I: $p = 0.02$, $R^2 = 0.11$). There were no significant effects of the enclosures stocked with nase compared to the control enclosures (PERMANOVA contrast II: $p = 0.41$, $R^2 = 0.06$).

TABLE 1 Average difference in the oxygen concentration in the hyporheic zone at the end of the experiment compared to the initial values

Depth [cm]	Enclosures without fish stocking [mg/L O ₂]	Enclosures stocked with fish [mg/L O ₂]
8	-2.57	-0.72
13	-2.07	-0.40
23	-1.08	-0.84

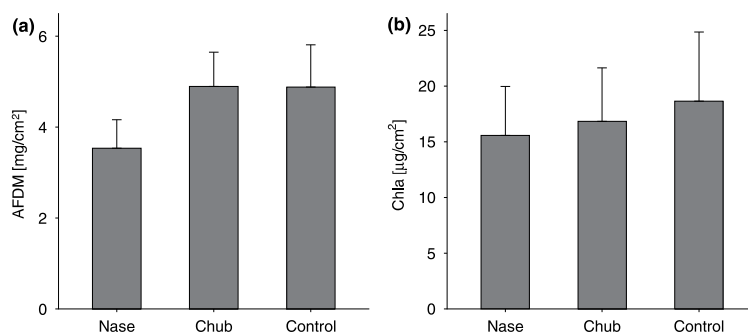


FIGURE 3 (a) Mean ash-free dry mass (AFDM) and (b) mean chlorophyll *a* (Chla) concentration in enclosures stocked with nase or with chub and in control enclosures without fish stocking ($n = 4$) at the end of the experiment. Error bars represent the standard deviations.

TABLE 2 Estimated proportion (mean \pm standard deviation) of higher periphyton taxa contributing to the algal and cyanobacterial communities in the three treatments ($n = 4$) at the end of the experiment

	Diatoms [%]	Green algae [%]	Cyanobacteria [%]
Nase	64 \pm 9	17 \pm 6	20 \pm 10
Chub	63 \pm 8	30 \pm 7	7 \pm 7
Control	57 \pm 5	34 \pm 9	9 \pm 11

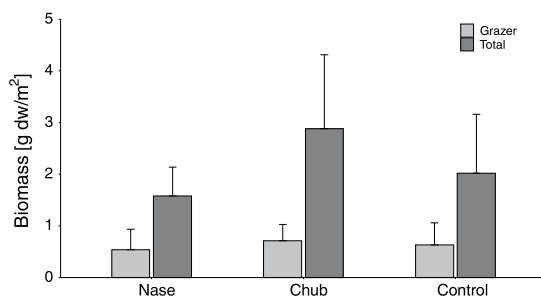


FIGURE 4 Mean total invertebrate biomass and mean biomass of invertebrate grazers in enclosures stocked with nase or with chub and in control enclosures without fish stocking ($n = 4$) at the end of the experiment. Error bars represent the standard deviations.

4 | DISCUSSION

Biological clogging of the hyporheic zone is a severe consequence of eutrophication in running waters, as it causes oxygen depletion (Hartwig & Borchardt, 2015; Ibisch et al., 2009), which in turn strongly reduces habitat quality in this important ecotone (Geist & Auerswald, 2007; Hübner et al., 2009; Malcolm et al., 2003). Our mesocosm experiment showed positive effects of both herbivorous and omnivorous fish on oxygen availability in the hyporheic zone of a eutrophic river, suggesting a reduction of biological clogging. To our knowledge, this is the first study demonstrating that top-down effects by fish can reduce the impact of eutrophication in the hyporheic zone of running waters.

During the experiment, hyporheic oxygen concentrations decreased across all treatments. A similar decrease regularly occurred

in the late spring and early summer in nearby stretches of the river (own unpublished data 2015–2019). Clogging might have been more pronounced during the experiment due to the decelerated water flow caused by the enclosure cages and the subsequent increase in local sedimentation. Nevertheless, oxygen concentrations were less significantly decreased in enclosures stocked with fish than in control enclosures without fish stocking. This indicates either a reduced or a considerably slowed clogging in the presence of these fish species.

The positive effects of herbivorous nase and omnivorous chub on hyporheic oxygen availability were caused by different mechanisms. Initially, we expected that nase, as a specialised periphyton feeder (Corse et al., 2010; Freyhof, 1995), would directly reduce periphyton biomass accrual, thereby reducing biological clogging. Our results support this expectation, as judged by the significantly lower total periphyton biomass (AFDM) measured at the end of the experiment in enclosures stocked with nase. Several other studies have similarly shown that herbivorous fish can reduce periphyton, both on a small scale (e.g. Flecker et al., 2002; Martin et al., 2016; Schneck et al., 2013; Veach et al., 2018; Wootton & Oemke, 1992) and on a mesohabitat scale (Gelwick & Matthews, 1992; Power et al., 1985, 1989; Stewart, 1987). However, compared to the mean hyporheic oxygen concentration, which was more than twice as high in enclosures stocked with nase than in those without fish stocking, the reduction in the mean AFDM was rather small. Thus, even a small reduction of periphyton biomass is sufficient to cause a large increase in hyporheic oxygen availability. Because hyporheic oxygen availability is mainly controlled by water transport and heterotrophic respiration rates (Brunke & Gonser, 1997), it might be affected by two different processes associated with biological clogging: (1) the reduced permeability of the hyporheic zone as a consequence of both external clogging by benthic algae (Battin & Sengschmitt, 1999; Ibisch et al., 2009) and internal clogging by infiltrated algal cells (Kloep & Röske, 2004); and (2) an increase in biological oxygen demand in the hyporheic zone due to the enhanced decomposition of decaying algae (Rode, Hartwig, Wagenschein, Kebede, & Borchardt, 2015). In this respect, the relatively large effect of herbivorous nase on hyporheic oxygen availability may have reflected the reduced input of organic matter into the hyporheic zone by fish grazing, resulting in a decreased biological oxygen demand but only a slight reduction in the extent of external clogging.

In contrast to total periphyton biomass, autotrophic periphyton biomass (measured as Chl *a*) was not lower in the enclosures stocked with nase. One explanation for this result is the masking of grazing effects on Chl *a* by compensatory growth due to high rates of algal productivity. The autotrophic growth rate at the experimental site was presumably high, due to high nutrient and light availability (Lamberti, Gregory, Ashkenas, Steinman, & McIntire, 1989; Sturt et al., 2011). Moreover, fish grazing might even have stimulated algal growth. Benthic grazing reduces competition for nutrients and light by removing the upper layers of algae, thereby offering optimal growth conditions for new algae (Lamberti & Resh, 1983; McCormick & Stevenson, 1989, 1991).

In this respect, as an indicator of periphyton biomass accumulation over time, AFDM may be more appropriate than Chl *a* because it includes live, dead, and senescent algae (Lamberti et al., 1989). Support for a stimulation of algal growth by fish grazing comes from our finding of a significantly lower proportion of green algae, especially filamentous *Cladophora*, in the nase-stocked enclosures, which indicated grazing-induced changes in periphyton community structure. The lower proportion of filamentous green algae in the nase-stocked enclosures may have additionally contributed to reducing the extent of clogging, given the potential of the enhanced trapping of sediments in the filamentous branches of *Cladophora* (Berger et al., 2003; Dodds, 1991) to increase external clogging of the hyporheic zone.

Among the possible mechanisms underlying the increased oxygen availability in the chub-stocked enclosures was the ability of these fish to reduce biological clogging by exerting indirect top-down effects on periphyton. We expected that chub negatively affect the densities of small zoobenthivorous fish either by predation (Hellawell, 1971; Mann, 1976) or by displacement (Magurran, 1989). The subsequent release of benthic invertebrates was expected to promote invertebrate biomass and, in turn, grazer biomass, eventually leading to increased grazing. The results showed that the presence of chub positively affected total benthic invertebrate biomass, thus indicating a negative effect of the fish on zoobenthivorous fish densities in the enclosures. However, the biomass of invertebrate grazers was not promoted by the presence of chub, which would explain why periphyton biomass in enclosures stocked with chub was not reduced. Hence, the positive effects of chub on benthic invertebrate biomass did not cascade down to periphyton. This result was surprising, because the results of a small-scale experiment conducted close to the experimental site indicated the strong impact of the cascading effects of zoobenthivorous fish on periphyton biomass in the Nister (Gerke et al., 2018). Moreover, four-level trophic cascades induced by predatory and omnivorous fish were found in two mesocosm experiments in a Northern California river (Power, 1990; Wootton & Power, 1993). The absence of a similar cascade down to periphyton in our experiment can be explained by the fact that chub is an opportunistic feeder (Balestrieri et al., 2006) that also feeds on large invertebrate grazers such as mayfly larvae. These are generally highly susceptible to predation while grazing on the stone surface (Kohler & McPeck, 1989).

The observed positive effect of chub on the oxygen availability in the hyporheic zone can be best explained by the enhancement of bioturbation. Benthic foraging by chub might have disturbed the river bed, thereby increasing permeability in the upper layer of the hyporheic zone. This scenario seems likely because chub and other benthic-feeding fish can act as *zoogeomorphic agents*, by increasing the mobility of sediments and thereby causing substrate coarsening (Pledger, Rice, & Millett, 2016, 2017; Statzner, Sagnes, Champagne, & Viboud, 2003).

On larger spatial scales, the top-down effects of herbivorous nase that lead to an increased oxygen availability in the hyporheic zone might be more important than those of chub, due to the shoaling behaviour of nase. Specifically, adult nase typically form large single-species shoals of a dozen up to several hundred individuals that move actively within defined home ranges (Huber & Kirchhofer, 1998; Lusk, 1967). Within the feeding habitats of their home range, the densities and consequently the top-down effects of nase are presumably similar to those observed in our mesocosm experiment. The effects of chub might be less intense at larger scales because biomass in the enclosures exceeded those found in the river. Nonetheless, natural assemblages of river fish accommodate several species and rheophilic cypriniform fish such as nase and chub are important species in the hyporhithral and epipotamal zones of European rivers (Aarts & Nienhuis, 2003). As in our experiment both nase and chub positively affected hyporheic oxygen availability, under natural conditions the sympatric occurrence of the two species is likely to have synergistic effects. Hence, protecting and enhancing the stocks of both herbivorous and omnivorous fish may contribute to a reduction of eutrophication effects and a restoration of the hyporheic zone in running waters.

Although the effects of nase and chub on hyporheic oxygen concentration were not strong enough to impede or even reverse clogging, these fish may play an important role in the river's local biodiversity. In Central European rivers, the period during which fish eggs and larvae of several fish species develop within the hyporheic zone (intragravel period) coincides with the spring algal peak. Among these species are the salmonid grayling, *Thymallus thymallus* (Linnaeus, 1758) and the cypriniforms common barbel, *Barbus barbus* (Linnaeus, 1758), and nase (Britton & Pegg, 2011; Hübner et al., 2009; Kamler, Keckeis, & Bauer-Nemeschkal, 1998). A low oxygen supply due to the reduced permeability of spawning gravels has been identified as a critical factor compromising the survival of salmonid eggs and larvae (e.g. Malcolm et al., 2003; Soulsby, Youngson, Moir, & Malcolm, 2001; Suttle, Power, Levine, & McNeely, 2004), and the survival of grayling eggs and larvae is negatively affected by eutrophication (Hübner et al., 2009). Lithophilic cypriniforms such as nase also require a permeable and well-oxygenated hyporheic zone for successful development (Duerregger et al., 2018; Keckeis et al., 1996; Nagel, Pander, Mueller, & Geist, 2019). High densities of large herbivorous and omnivorous fish are therefore likely to substantially improve habitat quality in the short intra-gravel period of spring spawning fish such as grayling, barbel, and nase.

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CONFLICTS OF INTEREST

The authors have no conflict of interest to declare.

DATA AVAILABILITY STATEMENT

Data supporting the findings of this study are available in the open cloud repository (Hübner, Gerke, Fricke, Schneider, & Winkelmann, 2020; <https://cloud.uni-koblenz-landau.de/s/yjPsJAp57mmQTZL>).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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The following supplement information accompanies the article

**Cypriniform fish in running waters reduce hyporheic oxygen
depletion in a eutrophic river**

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Appendix S1: Characteristics of the hyporheic zone in the river

Hyporheic water samples and sediment samples were taken in a river section (~ 90 m) approximately 250 m upstream of the experimental site (sampling dates: water samples: June 13, 2017; sediment samples: May 16, 2017). Samples were taken in the middle of the river and near the river margins (Table A1).

Hyporheic water samples were taken from multilevel probes that were similar to those used in the experiment described in this paper and that had been installed into the river bed in autumn 2016. Water samples were obtained using a polypropylene syringe and their dissolved oxygen content, electrical conductivity and pH-value was immediately measured (WTW, Multi 3430 with FDO 925, TetraCon 925 and SenTix 940 probe, Wissenschaftlich Technische Werkstätten, Weilheim; Germany).

Freeze core samples were taken according to the technique by Humpesch & Niederreiter (1993). In short, a hollow steel lance (Umwelt und Wissenschaftstechnik, Mondsee, Austria) was driven into the riverbed, and approximately 15 L of liquid nitrogen was poured into the lance, freezing the sediments to the lance. Afterwards, the core was immediately pulled out of the riverbed using a tackle. Each core was divided into 10 cm layers. The samples were fractioned by wet sieving using a vibratory sieve shaker (Retsch AS 200 basic, Haan, Germany) equipped with wet sieves of decreasing mesh sizes (20 mm, 6.3 mm, 2 mm, 0.63 mm, 0.2 mm, 0.063 mm; Retsch, Test Sieve ISO 3310-1); large stones (cobbles > 63 mm) were sorted out manually and excluded from further analyses. The clay and silt fraction (< 0.063 mm), which passed the smallest sieve size, was washed into a 20 L bucket, and the sieving was finished once the water leaving the sieve tower was clear. The total volume of the retained washing water containing the clay and silt fraction was determined, and two 50-ml aliquots were taken from the homogenised sample. The fractions retained on each sieve and the aliquots of the washing water were dried at 105°C and weighed.

Table A1: Mean values (\pm standard deviation) of hyporheic oxygen concentration in 8 cm depth, absolute differences of electrical conductivity and pH between 8 cm depth and surface water ($\Delta 8 \text{ cm} - 0 \text{ cm}$) and proportions of dry weight of fine sediments (total fines $< 2 \text{ mm}$, clay and silt $< 0.063 \text{ mm}$) in different layers of freeze core samples. N = sample size.

Variable	Sampling location			
	Middle of the river	N	Near river margins	N
Dissolved oxygen (8 cm) [mg L^{-1}]	2.0 ± 0.2	3	1.8 ± 0.5	6
Electrical conductivity ($\Delta 8 \text{ cm} - 0 \text{ cm}$) [$\mu\text{s cm}^{-1}$]	11.7 ± 10.7	3	23.4 ± 16.0	5
pH ($\Delta 8 \text{ cm} - 0 \text{ cm}$)	1.5 ± 0.4	3	1.3 ± 0.2	6
Proportion of fines $< 2 \text{ mm}$ (0-10 cm) [w%]	8.4 ± 2.6	3	13.7 ± 8.3	3
Proportion of fines $< 2 \text{ mm}$ (10-20 cm) [w%]	15.2 ± 14.4	3	23.8 ± 5.7	3
Proportion of fines $< 0.063 \text{ mm}$ (0-10 cm) [w%]	1.4 ± 1.2	3	3.7 ± 0.3	3
Proportion of fines $< 0.063 \text{ mm}$ (10-20 cm) [w%]	2.0 ± 1.1	3	2.9 ± 0.3	3

Humpesch, U.H., Niederreiter, R., 1993. Freeze-core method for sampling the vertical distribution of the macrozoobenthos in the main channel of a large deep river, the River Danube at river kilometre 1889. *Large Rivers* 87–90. <https://doi.org/10.1127/lr/9/1993/87>

Appendix S2: Results of response variables and environmental parameters at the enclosure sites prior to the experiment

Table B1: Mean values (\pm standard deviation) of the response variables and the environmental parameters current velocity and water depth at the enclosure sites directly before the installation of enclosures on May 31, 2017 (one week prior to the start of the experiment). P-values refer to the results of ANOVAs of General Mixed Effects Models (fixed factor: future treatment, random factor: experimental block, $n = 4$) and PERMANOVA (invertebrate community composition). For current velocity, values after the installation of enclosures are additionally shown (measured on June 2, 2017).

Variable	Nase	Chub	Control	p-value
Hyporheic oxygen [mg L^{-1}]				0.59
8 cm	4.46 ± 1.99	5.22 ± 1.43	4.54 ± 1.03	
13 cm	3.69 ± 2.03	3.85 ± 1.28	3.76 ± 0.95	
23 cm	3.05 ± 2.36	3.23 ± 1.49	2.39 ± 0.43	
Ash-free dry mass [mg cm^{-2}]	1.26 ± 0.04	1.28 ± 0.19	1.35 ± 0.16	0.58
Chlorophyll <i>a</i> [$\mu\text{g cm}^{-2}$]	7.72 ± 1.50	8.62 ± 2.54	9.03 ± 3.01	0.24
Proportion of green algae [%]	9.4 ± 6.3	8.8 ± 4.8	7.8 ± 1.6	0.89
Total invertebrate biomass [g dw m^{-2}]	2.16 ± 0.51	1.75 ± 0.85	1.81 ± 0.54	0.62
Grazer biomass [g dw m^{-2}]	0.77 ± 0.21	0.55 ± 0.15	0.60 ± 0.24	0.36
Invertebrate community composition				0.32
Current velocity [m s^{-1}]				
before enclosure installation	0.36 ± 0.11	0.35 ± 0.12	0.34 ± 0.13	0.89
after enclosure installation	0.26 ± 0.09	0.22 ± 0.08	0.25 ± 0.13	0.32
Water depth [cm]	24.0 ± 2.3	25.4 ± 5.3	26.8 ± 4.3	0.65

Current velocity and water depth were measured above the multilevel probe installed at the planned location of each enclosure. Current velocity was measured approximately 5 cm below the water surface using a flow meter with a vane wheel flow sensor (HFA hand-held unit with FA sensor; Hoentzsch GmbH, Waiblingen, Germany). The measurements were conducted under low-flow conditions (daily mean discharge at the stream gauge Heimborn approximately 4 km upstream of the experimental site: May 31: $0.89 \text{ m}^3\text{s}^{-1}$, June 2: $0.94 \text{ m}^3\text{s}^{-1}$; average mean discharge in summer: $2.37 \text{ m}^3\text{s}^{-1}$).

Appendix S3: Dynamics of the surrounding conditions in the river during the experiment

To characterise the surrounding conditions in the river, water temperature, oxygen concentration, pH-value and turbidity of the surface water were recorded hourly during the experiment using a multi parameter sensor (Qualilog 8, Seba Hydrometrie, Kaufbeuren, Germany) that was installed approximately 250 m upstream of the experimental site.

The surrounding conditions in the river changed considerably during the experiment. Most importantly, periphyton biomass dynamics were not entirely characterised by a steadily increase of periphyton biomass over time, but were most probably characterised by two periphyton detachment events (June 4 and 23, 2017). Both detachments were characterised by a short increase of turbidity at exactly the same time as the daily variations of oxygen concentration and pH value decreased (Fig. C1). This strongly suggests that the detached algal particles increased turbidity while the reduced periphyton biomass resulted in a lower daily variation of oxygen saturation and pH.

The first detachment was triggered by a medium sized flood event (Fig. C2). However, no such reason could be identified for the second detachment, indicating that it was probably caused by senescence of periphyton. The first detachment occurred three days before the start of the experiment and the second approximately two weeks before the end of the experiment.

The dynamics of the surrounding conditions in the river during the experiment, especially the detachment of periphyton, likely affected periphyton development within the enclosures. Periphyton biomass in the control enclosures without fish stocking increased from the first sampling (one week prior to the start of the experiment) on May 31 to the sampling on June 21. The second detachment of periphyton occurred two days later on June 23 and the following sampling on June 28 showed a reduced periphyton biomass. The very high periphyton biomass found at the end of the experiment on July 5 indicates a period of high biomass accrual during the last week of the experiment. This is also supported by the temporal dynamic of the ratio of ash free dry mass to chlorophyll *a* concentration of the periphyton, which reflects the proportion of the periphyton community composed of autotrophic organisms (autotrophic index, Fig. C3). The autotrophic index decreased from the sampling on June 21 to the sampling after the

second detachment on June 28. It then strongly increased from June 28 to the end of the experiment on July 5 parallel to the high increase of periphyton biomass, indicating the accumulation of heterotrophic and non-living periphyton components such as bacteria and detritus.

Despite the significant difference in total periphyton biomass (AFDM) at the end of the experiment, top-down pressure of nase varied over time. The significant reduction of AFDM in enclosures stocked with nase was found only at the end of the experiment on July 5, thus following the above-described period of high biomass accrual after the second detachment (Fig. C4). Interestingly, enclosures stocked with chub seemed to have a lower total periphyton biomass before the detachment but did not differ from control enclosures at the end of the experiment (Fig. C4)

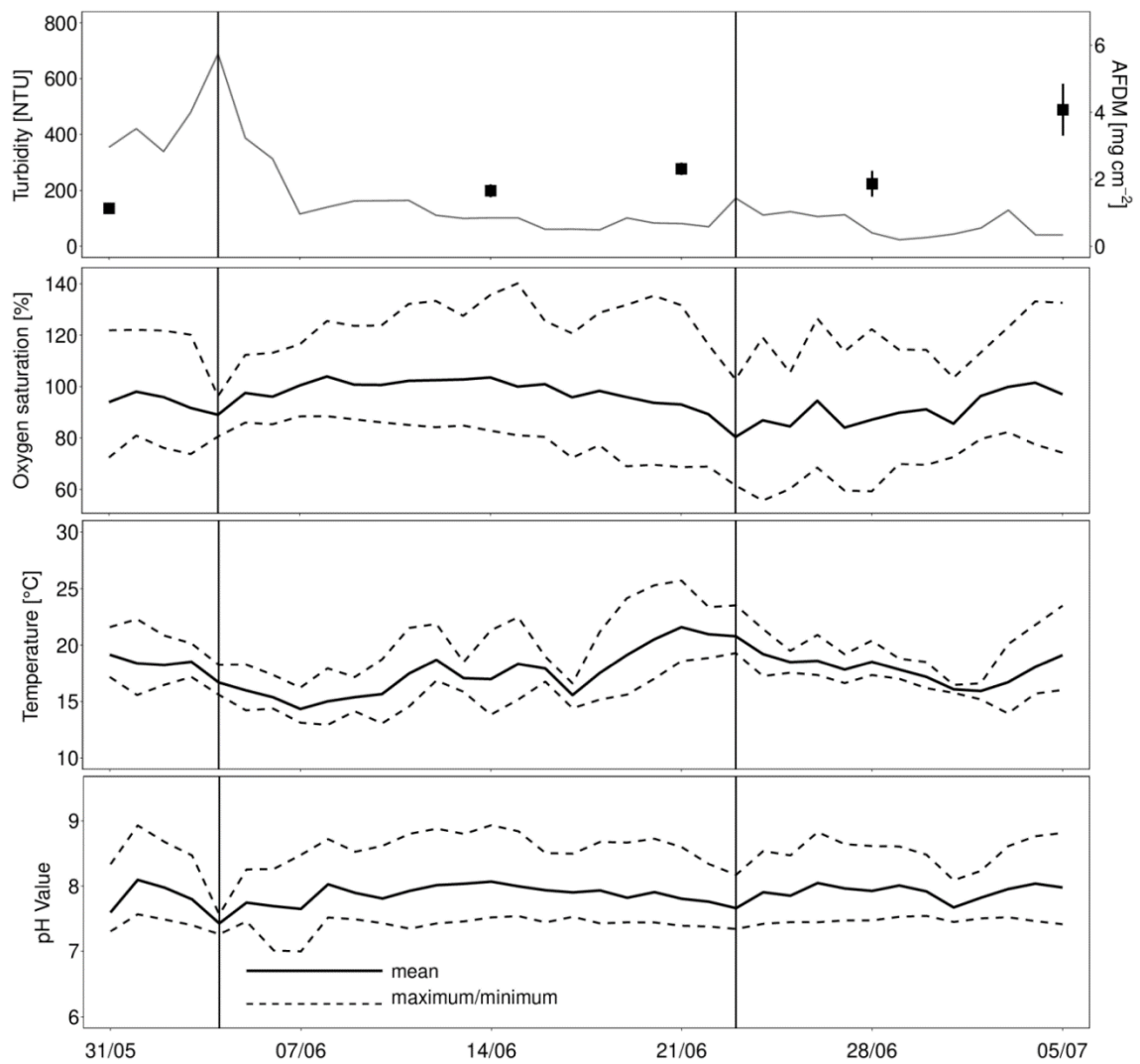


Fig. C1: Daily maximum values of water turbidity and daily mean, minimum and maximum values of oxygen saturation, water temperature and pH measured close to the experimental site as well as mean (\pm standard deviation, $n = 4$) ash free dry mass of periphyton in the control enclosures between May 31 – July 5, 2017 (enclosure installation: May 31, experiment duration: June 7 – July 5, 2017). The vertical lines mark the two periphyton detachments observed during the course of the experiment.

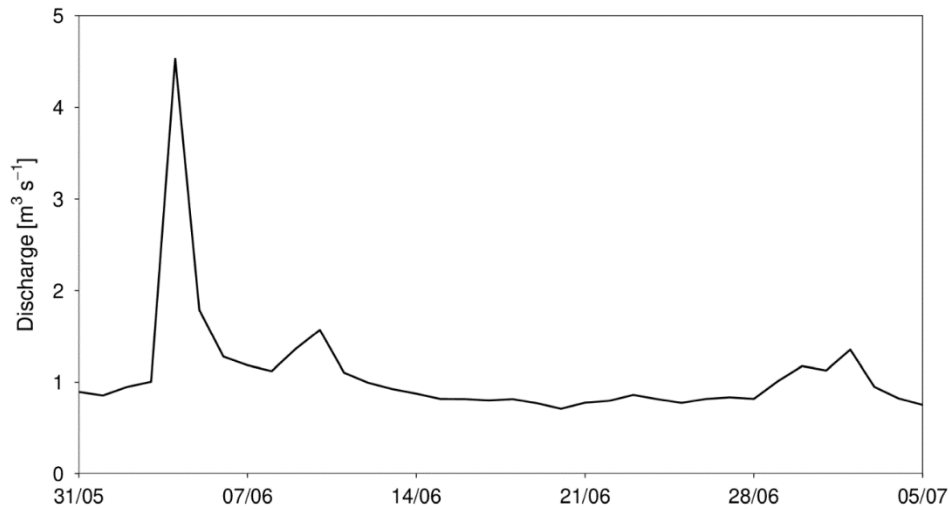


Fig. C2: Daily mean values of discharge at the stream gauge located in Heimborn (approximately 4 km upstream of the experimental site) between May 31 – July 5, 2017 (enclosure installation: May 31, experiment duration: June 7 – July 5, 2017).

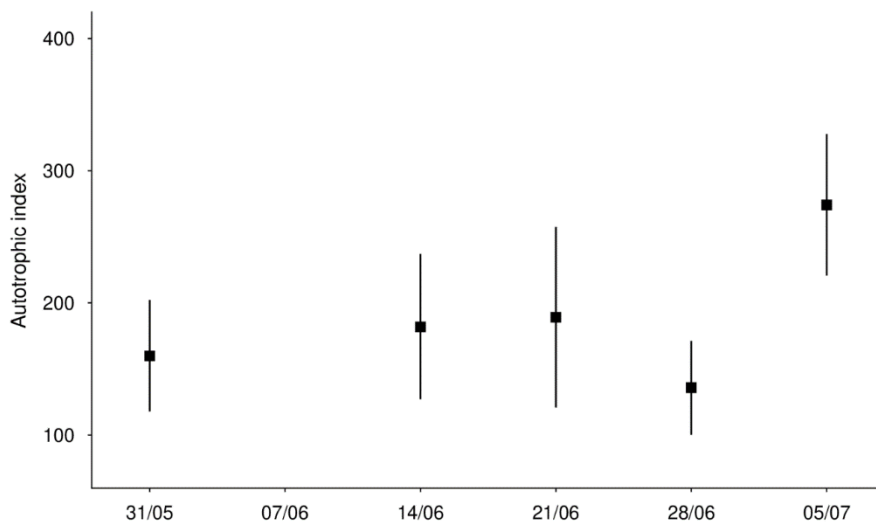


Fig. C3: Mean autotrophic index (ratio of ash free dry mass to chlorophyll *a* concentration) of periphyton in the control enclosures without fish stocking. Error bars represent the standard deviations.

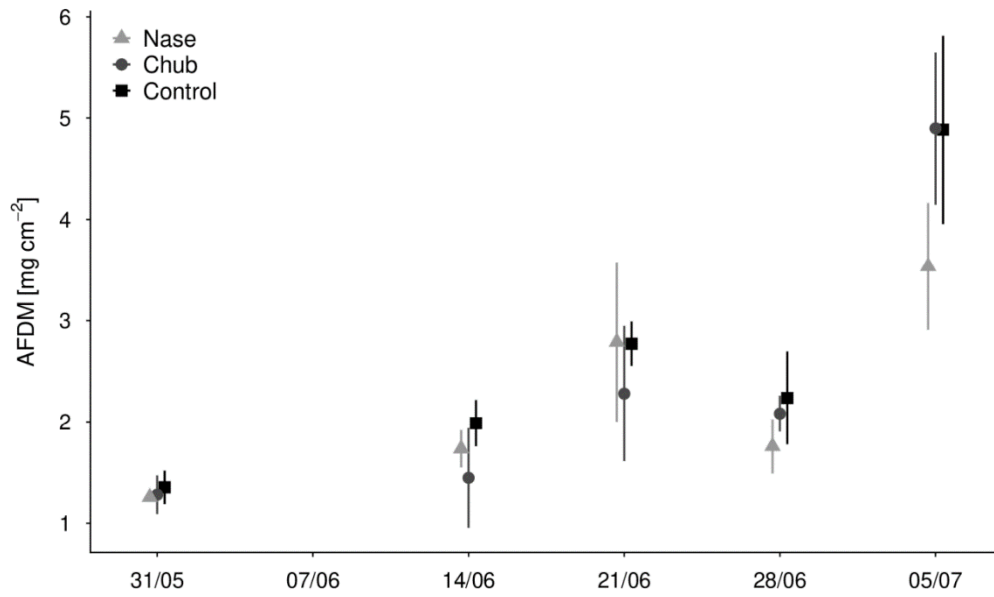


Fig. C4: Mean ash-free dry mass in enclosures stocked with nase or with chub and in control enclosures without fish stocking ($n = 4$) over the course of the experiment. Error bars represent the standard deviations.

7 Study 3

Can top-down effects of cypriniform fish be used to mitigate eutrophication effects in medium-sized European rivers?

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Can top-down effects of cypriniform fish be used to mitigate eutrophication effects in medium-sized European rivers?



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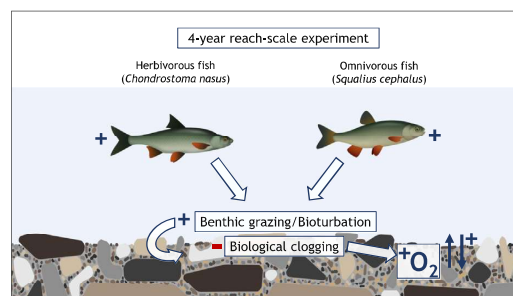
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HIGHLIGHTS

- Eutrophication in running waters causes oxygen depletion in the hyporheic zone.
- Fish stock enhancement increased hyporheic oxygen supply and water exchange.
- Fish stocking did not necessarily decrease periphyton biomass on the river bed.
- Biomanipulation has the potential to mitigate eutrophication effects in rivers.

GRAPHICAL ABSTRACT



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ABSTRACT

Eutrophication seriously threatens the ecological quality and biodiversity of running waters. In nutrient-enriched streams and shallow rivers, eutrophication leads to excessive periphyton growth and, in turn, biological clogging, oxygen depletion in the hyporheic zone and finally a reduction in the hyporheic habitat quality. Top-down control of the food-web by manipulating fish stocks, similar to the biomanipulation successfully applied in lakes, offers a promising approach to mitigating the effects of eutrophication in shallow rivers, especially those in which major reductions in nutrient input are not feasible. We conducted a reach-scale experiment over 4 years in a medium-sized eutrophic river to assess whether the top-down effects of two important large European cypriniform fish species, herbivorous common nase (*Chondrostoma nasus*) and omnivorous European chub (*Squalius cephalus*), would mitigate the effects of eutrophication. The enhancement of fish stocks was expected to reduce biological clogging, via the top-down control of periphyton by benthic grazing and enhanced bioturbation, thus increasing oxygen availability in the hyporheic zone as well as water exchange between the surface water and the hyporheic zone. As expected, enhancing the stocks of nase and chub increased both oxygen availability and vertical exchange flux of water in the upper layer of the hyporheic zone. However, periphyton biomass (chlorophyll *a*) was significantly reduced only in deeper pool habitat. Thus, while experimental biomanipulation in a shallow river significantly mitigated the effects of eutrophication in the hyporheic zone, top-down effects on periphyton biomass were rather small. Overall, to our knowledge, our results provide first evidence that the biomanipulation achieved by enhancing herbivorous and omnivorous fish stocks has the potential to mitigate the effects of eutrophication in medium-sized European rivers.

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1. Introduction

Eutrophication is a world-wide problem that seriously threatens the ecological quality of surface water bodies by promoting the excessive growth of algae (Smith et al., 1999). Nutrient enrichment has been identified as one of the major stressors on aquatic biota in European streams and rivers (Dahm et al., 2013; Hering et al., 2006). In Europe, 70% of the land area is predicted to contain river catchments with a high potential to exhibit undesirable periphyton growth due to nutrient enrichment (McDowell et al., 2020). In such nutrient-enriched streams and shallow rivers, intense periphyton growth negatively affects physical-chemical surface water quality by causing large diel fluctuations in oxygen concentrations and pH (Smith et al., 1999). Moreover, eutrophication-driven periphyton blooms cause biological clogging of the hyporheic zone (Hartwig and Borchardt, 2015; Ibisch et al., 2009). External clogging by benthic algal mats on the riverbed reduces the water exchange between surface water and the hyporheic zone (Ibisch et al., 2009). Following the detachment of algal mats, internal clogging by infiltrated dead algal cells both reduces streambed permeability and increases biological oxygen demand in the hyporheic zone, by enhancing the decomposition of decaying algae (Hartwig and Borchardt, 2015; Ibisch and Borchardt, 2002). The result is oxygen depletion in the hyporheic zone, which in turn strongly reduces the habitat quality for gravel-spawning fish and sensitive invertebrates, such as juvenile freshwater mussels (Geist and Auerswald, 2007; Keckeis et al., 1996).

There are two principal approaches to controlling eutrophication: a reduction of its sources and a mitigation of its effects. The reduction of phosphorus input, being the limiting nutrient in most European rivers (McDowell et al., 2020), is generally regarded as a key tool for controlling eutrophication. In the past four decades, there have been successes in reducing phosphorus inputs from point sources (e.g. through enhanced wastewater treatment) and diffuse sources (e.g. through riparian buffer strips) (Jarvie et al., 2013). However, in many cases no reduction in nuisance algal growth occurred after reductions in river water phosphorus concentrations (Bowes et al., 2012; Jarvie et al., 2013). One difficulty in this context is that the reduction of nutrient input is less likely to substantially decrease periphyton biomass accrual unless nutrient concentrations in the river are reduced below algal growth-limiting threshold concentrations (e.g. threshold of total phosphorus in temperate streams and rivers derived from regression analysis: $43 \mu\text{g L}^{-1}$; Dodds et al., 2006, 2002). Reducing nutrient concentrations to limiting levels is expensive and difficult to achieve, especially in catchment areas which are densely populated and include a high level of agricultural activity (Bowes et al., 2012). In streams, light limitation by riparian canopy may be an effective tool to control eutrophication (Burrell et al., 2014; Ghermandi et al., 2009). However, a full canopy cover can rarely be reached in wider rivers and periphyton growth is therefore not exceedingly light-limited during the vegetation season. Another possibility is to mitigate the effects of eutrophication through top-down control of the food web by manipulating fish stocks. Specifically for standing waters, the most common biomanipulation method is the active removal of zooplanktivorous fish or the stocking of piscivorous fish in order to reduce predation pressure on zooplankton communities and thereby enhance their grazing on phytoplankton (Benndorf, 1990; Hansson et al., 1998). However, the same approach cannot be applied to running waters, because their food web structure differs from that of standing waters. Unlike in standing waters, the main consumers of autotrophic biomass in streams and shallow rivers are benthic grazers such as macroinvertebrates and herbivorous fish (e.g. Power, 1990; Power et al., 1985). An increase in benthic grazing therefore requires either an increase in the stock of herbivorous fish or a reduction of predation pressure on benthic invertebrate grazers.

Benthic grazing generally exerts strong top-down effects on periphyton in running waters (Feminella and Hawkins, 1995; Hillebrand, 2009). Herbivorous fish in North American streams were found to reduce periphyton biomass (e.g. Gelwick and Matthews, 1992; Stewart,

1987). There is also evidence for the indirect top-down control of periphyton in running waters via three- or even four-level trophic cascades, with the latter consisting of omnivorous predatory fish, predatory insects, invertebrate grazers and periphyton (three-level cascades: e.g. Winkelmann et al., 2014; four-level cascades: e.g. Power, 1990). The enhancement of fish grazing by stocking herbivorous fish may therefore mitigate the effects of eutrophication in shallow rivers, with additional indirect effects induced by an increase in invertebrate grazing through trophic cascades (Gerke et al., 2018). In addition to trophic interactions, the bioturbation resulting from the activity of benthic foraging fish could increase the water exchange between surface water and the hyporheic zone due to a reduction of clogging and thus contribute to mitigating eutrophication effects in rivers. Benthic-foraging cypriniform fish were shown to increase the mobility of sediments, resulting in substrate coarsening in the upper layer of the hyporheic zone (e.g. Pledger et al., 2017, 2016).

European rivers are home to two fish species with a high potential for mitigating the effects of eutrophication: the large herbivorous cypriniform common nase (*Chondrostoma nasus*) and the large omnivorous cypriniform European chub (*Squalius cephalus*) (Leuciscidae: Leuciscinae). Both species are typical members of the fish communities in the hyporhithral and epipotamal zones of European rivers, specifically in Central and Eastern Europe where the distribution ranges of both species overlap. In Central Europe, the rheophilic common nase was once one of the most abundant and productive fish species in these river zones (Peñáz, 1996). Despite large-scale population declines, the nase remains one of the most abundant fish species in many European rivers (Reckendorfer et al., 2001). It is the only fish species in European rivers that is specialised for feeding on periphyton, preferentially on benthic diatoms (Corse et al., 2010; Vater, 1997). Nase scrape periphyton from coarse substrates, leaving characteristic grazing scars (Freyhof, 1995). Adult fish typically form large single-species shoals ranging in size from a dozen up to several hundred individuals that move actively within defined home ranges (Huber and Kirchhofer, 1998; Lusk, 1967). In contrast to the highly specialised nase, European chub is a generalist, able to feed on periphyton, especially filamentous algae, and on benthic invertebrates (Balestrieri et al., 2006; Hellawell, 1971). Chub of larger size classes prey on small fish such as common minnows (*Phoxinus phoxinus*) and bullheads (*Cottus gobio*) (Mann, 1976; Hellawell, 1971).

In a previous short-term field mesocosm experiment using 8-m² enclosures, the top-down effects of both fish reduced hyporheic oxygen depletion, suggesting a reduction of biological clogging attributable to benthic grazing (nase) and bioturbation (chub) (Hübner et al., 2020). This result suggests that the simultaneous enhancement of nase and chub stocks can induce synergistic top-down effects (Hübner et al., 2020). However, the top-down effects observed in mesocosm experiments, where foraging by fish is concentrated in a small area, might be less relevant at larger, realistic scales (Gil et al., 2016). In the present study, we conducted a 4-year reach-scale experiment in the hyporhithral zone of a medium-sized eutrophic gravel-bed river to assess whether enhancing nase and chub stocks offers an effective ecosystem-scale strategy for mitigating the effects of eutrophication in the hyporheic zone. Based on the results of the mesocosm experiment, we expected that the enhancement of fish stocks would reduce biological clogging of the hyporheic zone via the top-down control of periphyton and by enhancing bioturbation. Specifically, we hypothesised that the enhancement of fish stocks would increase both the dissolved oxygen concentration and the vertical exchange flux of water in the hyporheic zone in addition to reducing periphyton biomass on the riverbed.

2. Material & methods

2.1. Experimental site

The reach-scale experiment was conducted along a 1.4-km long stretch in the hyporhithral zone of the Nister River (Rhineland-Palatinate,

Germany, 50°43'24 N, 7°44'24 E), a medium-sized gravel-bed river with a catchment area of 246 km² (catchment area supplied by the State Office of Survey and Geobasis Information of Rhineland-Palatinate). Following the broad typology of European rivers developed by Lyche Solheim et al. (2019), the Nister River is assigned to type R-08 (Mid altitude, siliceous, medium-large rivers). Mid-altitude siliceous or calcareous rivers in this size range are generally common in Europe (river types R-08 and R-10: 6652 water bodies; 8.6% of all river water bodies assigned to a broad river type according to the typology of Lyche Solheim et al., 2019).

The river's hydrological regime is strongly rainfall-dependent and generally follows a seasonal pattern, with maximum discharge in winter and minimum discharge in summer (average mean discharge winter: 6.3 m³ s⁻¹, summer: 2.4 m³ s⁻¹; measured approximately 4 km upstream of the experimental site, at gauge Heimbörn, ID 2724030100; data supplied by the State Office for Environment of Rhineland-Palatinate). Land-use in the catchment area is dominated by forestry (48%), pasture (26%) and agriculture (16%) (Local Environmental Agency, SGD SGD Nord, 2020). Due to phosphate emissions in the catchment area from several small municipal wastewater treatment plants and diffuse emissions from the different types of land-use, the Nister is highly eutrophic, with the effects including oxygen oversaturation and an extremely high daytime pH, especially during the spring algal bloom (e.g. maximum oxygen saturation in April 2016: 182%, pH: 10.2; Gerke et al., 2018). At the experimental site, forest covered the right bank and pasture the left bank of the river (see pictures in Appendix A). Under flow conditions of approximately 1 m³ s⁻¹, the width of the experimental reach averages 14 m and the water depth 0.28 m. The riverbed at the experimental site mainly consists of cobbles (6.3–20 cm) and boulders (20–63 cm).

The benthic algal and cyanobacterial community in the Nister is largely composed of diatoms, but during the summer filamentous cyanobacteria and filamentous green algae (mostly *Cladophora* sp.) may become dominant (Gerke et al., 2018). The community of benthic invertebrates is largely composed of chironomid larvae and scraping grazers (e.g. the mayfly *Ephemera ignita* and the snail *Ancylus fluviatilis*) (Gerke et al., 2018). The fish species used in the experiment, common nase and European chub, are the most common large fish species in the river, with nase occurring at high natural densities in some sections. According to reports of locals, the natural densities of large fish in the Nister may have been considerably higher around 25 years ago. The densities of large fish in the river may have decreased over the past two decades due to predation by cormorants (*Phalacrocorax carbo sinensis*), which have been observed to forage in the catchment area since the late 1990s. Among the small zoobenthivorous fish, bullhead (*Cottus gobio*), common minnow (*Phoxinus phoxinus*) and stone loach (*Barbatula barbatula*) occur in high densities (Gerke et al., 2018).

2.2. Experimental design and fish stock manipulation

Our reach-scale experiment was conducted in two stretches of the experimental reach of the Nister River in two 2-year experimental phases, following a modified BACI design (Before-After-Control-Impact, Stewart-Oaten et al., 1986). Stocks of nase and chub differed between the two stretches in the first experimental phase (dissimilarity phase, June 2015 – August 2017) but were similar in the second phase (similarity phase, August 2017 – July 2019). The lower stretch (length 550 m) served as the reference stretch, with high fish stocks throughout the experiment, and the upper stretch as the impact stretch (length 595 m), with very low fish stocks during the dissimilarity phase and high fish stocks during the similarity phase (Fig. 1).

This two-phase design allowed us to detect the effects of an increased fish stock regardless of the temporal variability or natural differences in other factors between the experimental stretches. To minimise possible edge effects in the upper part of the reference stretch, the two stretches were separated by a buffer stretch (length 220 m). The design of the experiment differed somewhat from a classical BACI design. First,

there was no reference phase comprising natural, undisturbed conditions. Instead, fish stocks were manipulated in both phases to ensure the desired fish densities. Second, the experiment started with the dissimilarity phase and was followed by the similarity phase because the initial natural biomasses per area of nase and chub (> 15 cm) were already higher in the reference stretch than in the impact stretch (reference: 88.5 g m⁻²; impact: 18.3 g m⁻²). Consequently, a large adjustment of the fish stock in the impact stretch was necessary only during the similarity phase.

The upper and lower ends of the reference, buffer and impact stretches were closed off by dynamic fish weirs constructed according to Mühlbauer et al. (2003) to facilitate manipulation of the fish stock. Prior to the experiment, the dynamic fish weirs were installed over the entire cross-profile of the river (see Fig. A.3 in Appendix A). Each weir consisted of a set of linked panels made of PVC pipes (19 mm in diameter) connected to each other at 20-mm intervals, which effectively prevented the passage of large fish (body size >20 cm). The weirs were installed perpendicularly, as shown in Fig. 2, with the lower rim placed more upstream than the floating upper rim. The lower rim of each panel was fixed at the river bottom, while the upper rim was kept afloat on the water surface by floating devices. During low- and mid-flow conditions, the fish weirs were cleaned at 3-day to 2-week intervals, depending on the season, by moving leaf litter and debris from the upstream side to the downstream side of the barrier. The dynamic fish weir is highly resistant to floods because during high water levels and logging both water and debris are able to pass over the submerged weir without damaging it (Mühlbauer et al., 2003). This implies that the dynamic fish weir does not function as a fish barrier during high-flow conditions (see Fig. A.4 in Appendix A). It was therefore necessary to regularly control and adjust the fish stocks during the experiment, especially in spring following the long winter flood periods.

Stocks of nase and chub (> 15 cm) were regulated at the beginning of each experimental phase and additionally controlled and adjusted three times per year (spring, summer, autumn) by electrofishing (EFGI 650, Bretschneider Spezialelektronik, Chemnitz, Germany). Electrofishing was approved by the fisheries department of the local environmental agency (SGD Nord, Rhineland-Palatinate, Germany). The fish stocks were typically assessed by continuous sampling from the downstream to the upstream weir over the entire length of each experimental stretch in two passes using the mark-recapture method. Using

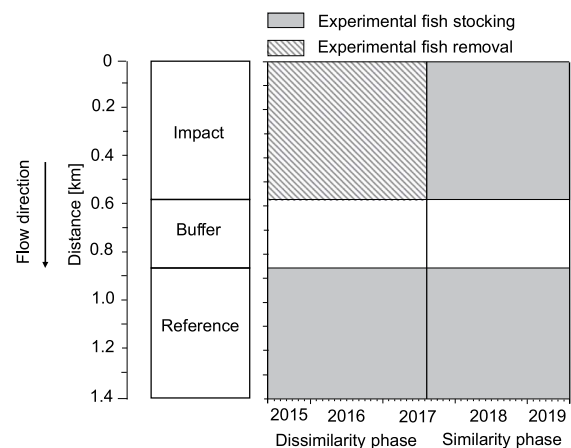


Fig. 1. Timetable and spatial set-up of the reach-scale experiment. During the dissimilarity phase, nase and chub were stocked in the reference stretch and removed from the impact stretch. During the subsequent similarity phase, nase and chub were stocked in both experimental stretches in order to achieve similar fish stocks in the reference and impact stretches.

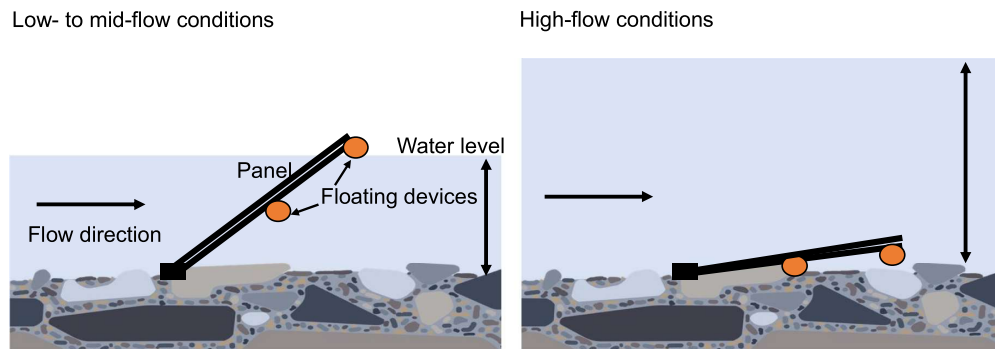


Fig. 2. Schematic side view of a dynamic fish weir under different flow conditions.

this method allowed the best possible stock estimation of the mobile shoaling species nase. Each fish (> 15 cm) caught in the first pass was colour-marked using a needleless injector (Dermojet, Akra Dermojet, Pau, France). The total length of each caught fish was measured to the nearest centimetre. Mortality due to electrofishing was negligible (< 0.1%).

During the dissimilarity phase (June 2015 – August 2017), fish caught in the second pass were removed from the impact stretch and transferred to the reference stretch. After the stocks of nase and chub in the reference stretch reached at least a total of 1700 individuals for both species combined, additional fish were transferred to the buffer stretch. In the impact stretch, depending on the recapture rate in the second pass, a third or fourth electrofishing pass was conducted during the dissimilarity phase to remove as much fish as possible with reasonable effort (maximum one working day). From July 2016 until the end of the dissimilarity phase, in August 2017, removal rather than mark-recapture was used in the impact stretch to ensure maximally efficient fish removal.

During the similarity phase (August 2017 – July 2019), stocks of nase and chub were assessed in both experimental stretches using the mark-recapture method and then boosted in the impact stretch by stocking fish that had been caught directly downstream of the reference stretch. Because the natural fish density downstream of the reference stretch was relatively low, it was necessary to additionally transfer fish from the reference to the impact stretch in order to ensure similar fish stocks in the two stretches. A single electrofishing pass was additionally conducted in the buffer stretch during each electrofishing session in order to obtain a raw estimate of fish densities.

Due to a high abundance of cormorants in the catchment area, the impact of their predation had to be controlled in order to sustain the manipulated fish stocks. Therefore, cormorant management measures (deterrence and lethal shooting) were applied throughout the experiment. Deterrence measures were conducted during daily patrols by volunteers of the local river protection association. Lethal shooting was applied as an aid to deterrence only between August 15th and February 15th outside of the cormorant breeding season in accordance with state regulations of Rhineland-Palatinate.

2.3. Field sampling

In both experimental stretches, field samples were taken in a subsection along a riffle-pool-riffle sequence (length: reference stretch: 90 m, impact stretch: 100 m). Equipment for sampling the hyporheic zone was installed at three different sampling sites within the subsections (A, B and C; Fig. 3). To represent different hyporheic flow conditions, the sampling sites consisted of: (A) a riffle crest, (B) a riffle tail (potential upwelling zone) and (C) a riffle head (potential downwelling zone).

In April 2015, nine multi-level probes (manufactured by the central workshop of the University Kassel, Germany) following Lenk et al. (1999) were installed in the riverbed. They allowed the collection of hyporheic water samples from four different depths (8 cm, 13 cm, 23 cm and 33 cm within the riverbed). The probes were evenly distributed across the three sampling sites in each stretch (Fig. 3). In August 2015, five temperature lances (Hartmann GmbH, Neuwied, Germany) were installed in the riverbed (one at site A, two each at sites B and C) to record the vertical temperature profiles that were used to calculate the vertical flux of water in the hyporheic zone, as a measure of hydrologic exchange. Four temperature loggers (Tidbit v2, Onset Computer Corp., Bourne, Massachusetts, USA) attached to each lance recorded the temperature at depths of 5, 10, 20 and 30 cm within the riverbed at 15-min intervals. The temperature lances were retrieved at the end of each sampling season in October to read the data from the loggers and immediately thereafter reinstalled in the riverbed. Periphyton samples were taken from two sites in each stretch (Fig. 3), one in a moderate- to fast-flowing shallow area (riffle site) and the other in a slow-flowing, relatively deep area (pool site) (see Appendix B for details on water depth and current velocity at the sampling sites).

During the dissimilarity phase, hyporheic water and periphyton sampling started two weeks after the initial fish stock manipulation, in June 2015. Because the initial fish densities in the reference stretch were already considerably high, a longer adaptation period between fish stock manipulation and the first sampling was not necessary. During the similarity phase, sampling started after a 2-month adaptation period following fish stock manipulation. Consequently, samples were collected from October 2017 until the end of the experiment, in July 2019. Hyporheic water and periphyton samples were always collected on the same date, with an interval of one month during the dissimilarity phase and two weeks during the similarity phase. All samples were collected at low- to mid-flow conditions between spring and autumn.

On each sampling date, the multi-level probes were sampled simultaneously in the two experimental stretches by two teams. Water samples (35 mL) from each depth horizon were obtained using a polypropylene syringe to apply a slight negative pressure. The obtained samples were carefully transferred to a 100 mL plastic vessel without shaking to minimise the entry of atmospheric oxygen. Immediately thereafter the dissolved oxygen concentration (DO) and electrical conductivity of each sample were measured (WTW, Multi 3430 with FDO 925 and TetraCon 925 probe, Wissenschaftlich Technische Werkstätten, Weilheim; Germany). In addition to hyporheic water sampling, the physical and chemical characteristics of the surface water (temperature, pH, DO, electrical conductivity) were measured directly in the river at each probe site. At the end of the sampling season in 2016, many of the multi-level probes were clogged at several depths, necessitating their retrieval in autumn 2016 to exchange their clogged porous filters. Moreover, we found that in the impact stretch, the water depth at some

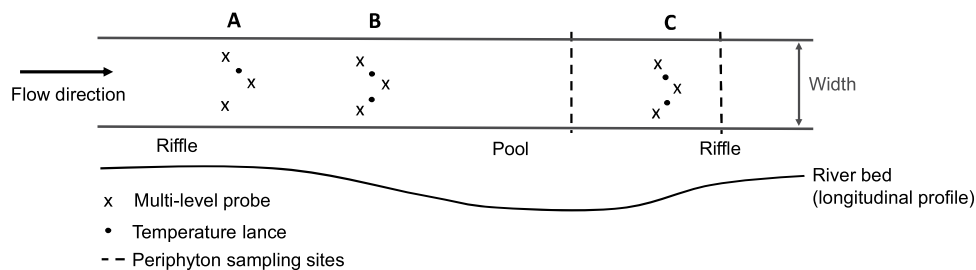


Fig. 3. Arrangement of the sampling sites (A, B and C) and measurement equipment in the experimental stretches.

of the sampling sites, originally selected during mid-flow conditions, was insufficient for large fish during the low-flow conditions in summer. This necessitated shifting the sampling sites within the impact stretch to deeper sites. The sampling sites within the reference stretch were shifted as well to ensure equal treatment in both experimental stretches.

For periphyton sampling, ten flat stones of similar texture were randomly chosen over the entire width of the river at each sampling site and pooled to obtain one sample per sampling site. Periphyton was removed from the stones by carefully brushing their surfaces with a coarse brush and collecting the periphyton with river water. The resulting suspensions were kept in the dark by placing them in a cooling box until further processing in the laboratory later the same day.

The hyporheic zone at the experimental site was further characterised by analysing the nitrate concentration in water samples, estimating the fine sediment accumulation rates using sediment traps and assessing sediment composition using the freeze-core sampling technique of Humpesch and Niederreiter (1993) (see Appendix C for details).

2.4. Laboratory analyses

Periphyton was characterised with respect to its total biomass (measured as ash-free dry mass, in mg AFDM cm^{-2}) and its autotrophic biomass (measured as the chlorophyll *a* concentration in $\mu\text{g Chl } a \text{ cm}^{-2}$). Both were quantified based on the total volume of the obtained periphyton suspension and the sampled area of the stone. The surface area of the stones was estimated by carefully wrapping each stone in aluminium foil, cutting off overlapping areas, and weighing the foil. After their total volumes had been determined, the periphyton suspensions were homogenised using a magnetic stirrer. AFDM was quantified by drying duplicate 10-mL aliquots at 105°C for 24 h. The dried samples were weighed, ashed in a muffle furnace at 510°C for 5 h and then reweighed using a microbalance (XS205 Dual Range, Mettler-Toledo, Columbus, Ohio, USA). The Chl *a* concentration was determined in triplicate 2-mL aliquots centrifuged for 3 min at 13,000 rpm ($16,060 \times g$, Micro 200R; Hettich Zentrifugen, Tuttlingen, Germany). The supernatants were discarded, and the pellets stored at -80°C . Chl *a* was extracted by resuspending the pellets in 96% ethanol containing $1 \text{ g MgCO}_3 \text{ L}^{-1}$ buffer and then incubating the samples at room temperature in the dark for at least 3 h. They were then analysed spectrophotometrically (Specord 205; Analytic Jena, Jena, Germany) according to Mewes et al. (2017).

2.5. Data analysis

The sizes of nase and chub stocks were estimated using Chapman's modified Petersen estimator (Ricker, 1975). The biomass of individual fish was calculated using the length-weight regressions obtained from our data, based on the weights and lengths (measured to the nearest cm) of nase and chub individuals caught on three different electrofishing dates (autumn 2016, summer 2017, spring 2018). The data were used to obtain a power function relating the total length (*L*) and wet

weight (*W*) for nase ($W = 0.0073 * L^{3.0945}$, $r^2 = 0.96$, $n = 799$) and chub ($W = 0.0098 * L^{3.041}$, $r^2 = 0.97$, $n = 297$). For each electrofishing date and each stretch, the total biomass of the caught fish was calculated by summing the individual biomasses of all fish as estimated using the power functions. To estimate the stock biomass, the mean individual biomass of nase and chub was calculated by dividing the total biomass of the caught fish of one or the other species by the number of the caught fish of that species. The total biomass of a fish stock was calculated by multiplying the mean individual biomass of nase and chub by the respective estimated stock size.

Vertical flux of water was calculated from the temperature time series data according to the amplitude attenuation method of Keery et al. (2007). This method assumes that the vertical flux of water in the hyporheic zone is predominantly vertical, that the physical and thermal properties of the sediment and fluid remain constant over time, and neglects thermal dispersivity. The Matlab program VFLUX 2 (Gordon et al., 2012; Irvine et al., 2015) was used to automatically calculate the vertical flux of water between sensors located at different depths. The values of the physical properties used in the calculations were those suggested in the documentation included with the program: dispersivity, 0.001 m ; saturated-bed thermal conductivity, $0.0045 \text{ cal s}^{-1} \text{ cm}^{-1} \text{ }^\circ\text{C}$; volumetric heat capacity of the sediment, $0.5 \text{ cal cm}^{-3} \text{ }^\circ\text{C}$ and volumetric heat capacity of the water, $1.0 \text{ cal cm}^{-3} \text{ }^\circ\text{C}$. Sediment porosity was estimated from the weight loss of the dried freeze-core samples (mean porosity = 0.19 , $n = 61$), assuming a grain density of 2650 kg m^{-3} as suggested by Keery et al. (2007). Data subsets whose diurnal signals were below the sensor precision of 0.2°C as well as data for which the VFLUX2 program calculated a thermal diffusivity outside a plausible range for streambed materials (Irvine et al., 2015) were excluded from further analyses. Amplitudes below the sensor precision usually occurred during winter (December – March) and at the 30-cm depth horizon.

To minimise problems associated with multiple testing, only the response variables most likely to be affected by fish stock enhancement were selected to test our hypothesis: DO (mg L^{-1}) at different depth horizons of the hyporheic zone, absolute values of the vertical flux of water between 5- and 10-cm depth (m day^{-1}), Chl *a* concentration ($\mu\text{g cm}^{-2}$) and AFDM (mg cm^{-2}). The other environmental variables assessed in the monitoring program were only used to additionally characterise the hyporheic zone at the experimental site (see Appendix C for details). Hyporheic DO data were averaged over all probes and sampling sites because the relatively large number of 9 probes allowed us to calculate a robust mean over different hyporheic flow conditions. The difference of the mean values (averaged over all probes) between the impact and the reference stretch on each sampling date was calculated to account for seasonal changes and autocorrelation of the variables. For periphyton biomass, we did not average the data over the sampling sites because we obtained pooled samples from only two different sites. The differences between the impact and the reference stretch on each sampling date were therefore calculated separately for the riffle and the pool sampling sites. As the vertical flux of water was calculated from continuous time series data, the differences between the experimental stretches were calculated based on weekly mean values

(averaged over all temperature lances). To test the hypothesis, the mean difference in each variable between the experimental stretches during the dissimilarity phase and the similarity phase was compared. Analysing the differences between the stretches minimised any temporal variability as well as the effects of factors similarly influencing the two stretches (Smith et al., 1993). The effects of the experimental manipulation were registered as a change in the mean difference between the stretches in the similarity phase vs. the dissimilarity phase, tested by randomised intervention analysis (RIA; 10,000 iterations; Carpenter et al., 1989).

The data of hyporheic DO from 2015 showed a clear effect of probe installation (Fig. D.1 in Appendix D). Therefore, only data of hyporheic DO measured starting from spring 2016 were included in the statistical analyses, as they represented undisturbed sediment conditions. This reduced the sample size for hyporheic DO in the dissimilarity phase from 18 to 12. In addition, only vertical flux data obtained starting in spring 2017 could be used in the analysis, because shifting the sampling sites in the two stretches at the end of the season in 2016 apparently resulted in distinct changes in the vertical flux of water (Fig. D.2 in Appendix D). This reduced the sample size for vertical flux data in the dissimilarity phase from 47 to 20. By contrast, there was no distinct change in the hyporheic DO following the shifting of the sampling sites (Fig. D.2 in Appendix D) and the 2016 data could therefore be included in the statistical analyses. At one of the three sampling sites in the impact stretch, extremely high nitrate concentrations and electric conductivity values were measured on each sampling date in 2016 (i.e. before the sampling sites were shifted), suggesting a lateral influx of nitrate-rich groundwater (Fig. D.3 in Appendix D). Therefore, data of hyporheic DO obtained at this sampling site (3 of 9 probes) were also omitted from the statistical analyses.

Statistical analyses and graph plotting were performed using R version 3.6.2 (R Development Core Team, 2019).

3. Results

3.1. Fish stock manipulation

Overall, fish stock manipulation was successful throughout the experiment. During the dissimilarity phase, mean fish density was ~17-fold higher and mean fish biomass per area ~51-fold higher in the reference stretch than in the impact stretch (Fig. 4, Table E.1 in Appendix E). Although the fish stock in the impact stretch was assessed by the mark-recapture method only on three dates, the numbers of captured fish

determined on the other three dates were also very low (mean \pm SD: 0.011 ± 0.003 individuals [Ind] m^{-2} ; see Table E.2 in Appendix E for details). Taken together, these results demonstrated that the fish stock in the impact stretch was very low throughout the dissimilarity phase. During the similarity phase, the differences in fish biomass per area and fish density between the two experimental stretches were much smaller. However, fish biomass per area and fish density were still somewhat higher in the reference stretch than in the impact stretch. During the similarity phase, fish biomass per area and fish density in both stretches were generally lower than in the reference stretch during the dissimilarity phase, due to the difficulties in obtaining enough fish from the natural fish stock of the river. However, fish biomass per area and fish density were considerably enhanced in the impact stretch throughout the similarity phase (20-fold increase in mean biomass per area and 8-fold increase in mean density compared to the dissimilarity phase; Fig. 4, Table E.1 in Appendix E).

3.2. Dissolved oxygen concentration and vertical flux of water in the hyporheic zone

Our hypothesis that fish stock enhancement increases hyporheic oxygen concentrations was supported by the experimental results. The mean between-stretch difference in DO at the uppermost depth horizon (8 cm) was significantly higher following fish stock enhancement in the impact stretch during the similarity phase ($p = 0.01$, RIA, dissimilarity phase: $n = 12$, similarity phase: $n = 19$; Fig. 5), indicating a relative increase in the DO in the presence of nase and chub. The increase in the mean between-stretch difference was even more pronounced when the analysis included only the hyporheic DO measured at probe sites located in the middle of the river ($p < 0.01$, RIA, dissimilarity phase: $n = 12$, similarity phase: $n = 19$; Fig. 5).

In deeper horizons of the hyporheic zone (13, 23 and 33 cm), the mean difference in the DO between the two stretches was similar during the two experimental phases ($p > 0.05$, RIA, dissimilarity phase: $n = 12$, similarity phase: $n = 19$; Fig. 5), suggesting that the effect of fish stock manipulation was limited to the upper layer of the hyporheic zone.

Despite the positive effect of fish stocking, the mean hyporheic DO was generally lower during the similarity phase than during the dissimilarity phase, especially in the case of the exceptionally dry summer of 2018, when discharge was consistently low (Fig. 6, Table 1). However, the decrease in the mean hyporheic DO during the similarity phase was smaller in the impact stretch than in the reference stretch (12%

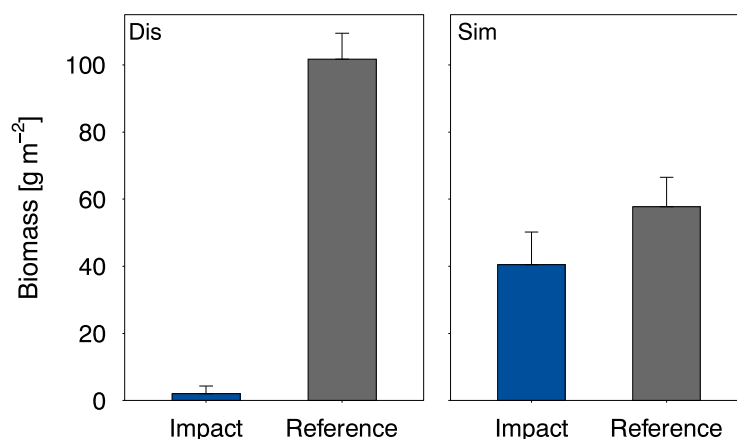


Fig. 4. Mean total biomass per area of nase and chub (> 15 cm) in the impact and reference stretches. Left: dissimilarity phase (Dis), impact stretch: $n = 3$, reference stretch: $n = 6$. Right: similarity phase (Sim), $n = 5$. Error bars represent the standard deviations.

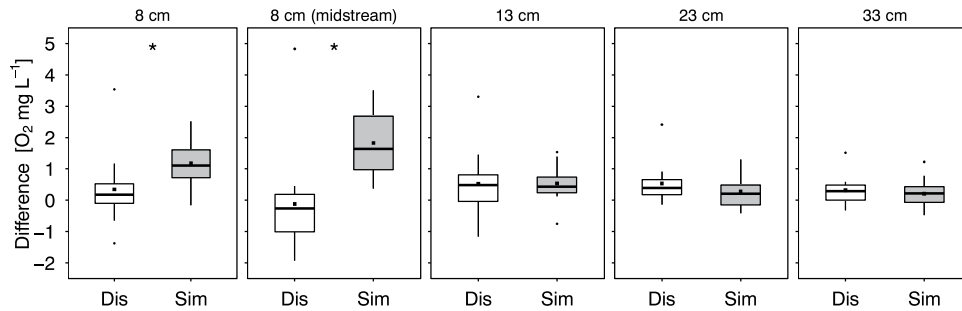


Fig. 5. Differences in the oxygen concentrations in the hyporheic zone (8-, 13-, 23- and 33-cm depth; midstream: including only sampling sites in the middle of the river) between the impact stretch and the reference stretch during the dissimilarity phase (Dis; 12 sampling dates) and the similarity phase (Sim; 19 sampling dates). Boxes: 75% and 25%, whiskers: 95% and 5%, squares: mean differences, dots: outliers. *Significant ($p < 0.05$).

vs. 37% reduction at 8 cm depth compared to the dissimilarity phase, Table 1).

During the 4-year experiment, the mean hyporheic DO was generally higher in spring than in summer, indicating a large effect of biological clogging following the spring breakdown of algal biomass (Fig. 6). Fish stocking seemed to have the strongest positive effect on hyporheic oxygen availability during spring, as indicated by the largest between-stretch difference in the DO between May and mid-June, i.e. during the similarity phase (mean difference vs. reference stretch: 2.6 mg L^{-1} ; Fig. 6, Table 1).

In accordance with the positive effect of fish stocking on hyporheic oxygen availability, the mean between-stretch difference in the vertical flux of water between 5 and 10 cm depth increased significantly following fish stocking in the impact stretch during the similarity phase ($p < 0.001$, RIA, dissimilarity phase: $n = 20$, similarity phase: $n = 37$; Fig. 7), suggesting a relative improvement in riverbed permeability.

3.3. Periphyton biomass

Our hypothesis that fish stock enhancement would reduce periphyton biomass on the riverbed was only partly supported. Periphyton

biomass in the impact stretch was already lower during the dissimilarity phase (Table 1), resulting in a negative difference compared to the reference stretch. The mean between-stretch difference in autotrophic periphyton biomass (Chl *a*) at the pool site became significantly more negative during the similarity phase ($p < 0.01$, RIA, dissimilarity phase: $n = 17$, similarity phase: $n = 20$; Fig. 8), indicating a reduction of periphyton biomass due to enhanced benthic grazing following fish stock enhancement. The between-stretch difference in total periphyton biomass (estimated as AFDM) at the pool site showed a similar tendency, although the magnitude of the change between the dissimilarity phase and the similarity phase was not significant ($p = 0.06$, RIA, dissimilarity phase: $n = 17$, similarity phase: $n = 20$; Fig. 8). At the riffle site, the between-stretch differences in Chl *a* and AFDM did not change significantly from the dissimilarity phase to the similarity phase (Chl *a*: $p = 0.6$, AFDM: $p = 0.1$, RIA, dissimilarity phase: $n \geq 17$, similarity phase: $n = 20$, Fig. 8).

4. Discussion

In standing waters, the enhancement of grazing pressure by biomanipulation is a well-established technique to mitigate the effects

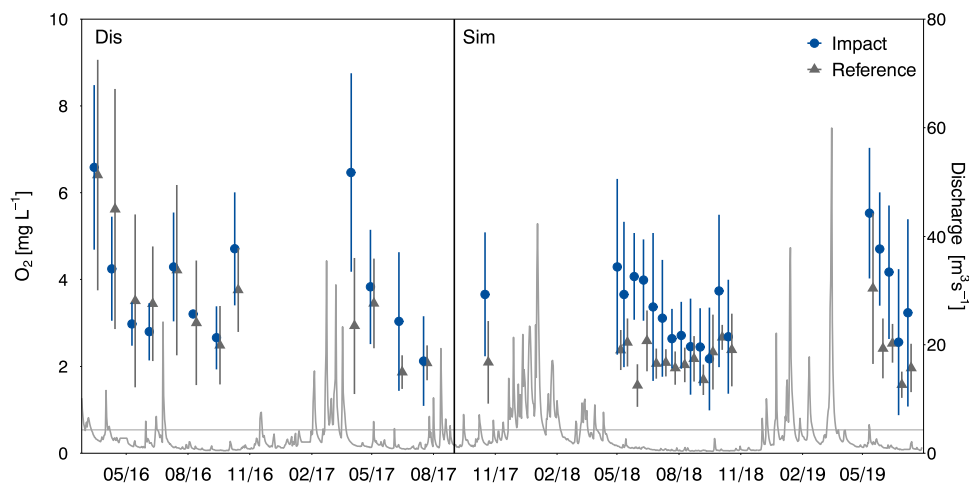


Fig. 6. Mean oxygen concentration (left y-axis) in the upper hyporheic zone (8-cm depth) of the experimental stretches during the dissimilarity phase (Dis) and the similarity phase (Sim). The transition between the phases is indicated by the black vertical line between 07/17 and 10/17. Error bars represent the standard deviations; $n \geq 4$ samples except for one sampling date in August 2016 (impact stretch: $n = 2$, reference stretch: $n = 3$). The grey trace shows the daily mean discharge (right y-axis) during the experiment. The average annual mean discharge at the nearby upstream gauge Heimborn is indicated by the grey horizontal line.

Table 1

Mean values (\pm SD) of the response variables in the impact stretch and the reference stretch during the dissimilarity phase and the similarity phase. Midstream: sampling sites limited to the middle of the river. Spring: sampling dates between May and mid-June. Sampling size (n) refers to the number of sampling dates.

Response variable	Dissimilarity phase			Similarity phase		
	Impact	Reference	n	Impact	Reference	n
DO [mg L^{-1}]						
8 cm	3.91 \pm 1.43	3.57 \pm 1.34	12	3.43 \pm 0.41	2.25 \pm 0.49	19
8 cm, midstream	3.61 \pm 1.44	3.72 \pm 1.39	12	4.19 \pm 1.21	2.36 \pm 0.48	19
8 cm, midstream, spring	3.05 \pm 0.64	3.40 \pm 0.64	4	5.34 \pm 0.91	2.69 \pm 0.57	7
13 cm	3.01 \pm 1.25	2.48 \pm 0.89	12	2.37 \pm 0.54	1.84 \pm 0.35	19
23 cm	2.57 \pm 1.05	1.90 \pm 0.64	12	2.03 \pm 0.75	1.62 \pm 0.31	19
33 cm	2.19 \pm 0.74	1.87 \pm 0.64	12	1.77 \pm 0.37	1.56 \pm 0.30	19
Vertical flux [m day^{-1}]	0.093 \pm 0.017	0.099 \pm 0.087	20	0.101 \pm 0.011	0.085 \pm 0.007	37
Chl a (riffle) [$\mu\text{g cm}^{-2}$]	14.0 \pm 10.1	17.4 \pm 10.7	17	22.5 \pm 12.0	24.9 \pm 12.4	20
Chl a (pool) [$\mu\text{g cm}^{-2}$]	13.3 \pm 10.4	13.8 \pm 9.3	17	10.4 \pm 7.0	16.3 \pm 13.3	20
AFDM (riffle) [mg cm^{-2}]	1.59 \pm 0.99	2.02 \pm 1.04	18	2.08 \pm 0.70	2.62 \pm 1.06	20
AFDM (pool) [mg cm^{-2}]	1.41 \pm 0.92	1.40 \pm 0.48	17	1.66 \pm 1.00	1.83 \pm 0.83	20

of eutrophication (Benndorf, 1990; Hansson et al., 1998). However, whether top-down control of the food web might be similarly used to mitigate eutrophication effects in running waters is unknown. Our large-scale field experiment clearly showed the high potential of fish stock manipulation to mitigate the effects of eutrophication in running waters, similar to biomanipulation in lakes. In accordance with our hypothesis the enhancement of herbivorous and omnivorous fish stocks mitigated eutrophication effects, evidenced by the significantly higher oxygen availability and higher rate of vertical water exchange in the hyporheic zone at relatively large spatial and temporal scales. These results are in line with those of a short-term mesocosm experiment conducted in the same river, which showed the positive effects of herbivorous nase and omnivorous chub on oxygen availability in the hyporheic zone (Hübner et al., 2020).

In our study, the effects on oxygen availability were restricted to the uppermost horizon of the hyporheic zone. The upper 10 cm layer of the hyporheic zone contains the highest faunal density, biomass and species richness and is therefore biologically most important (Palmer, 1997; Williams and Hynes, 1974; Winkelmann et al., 2003). Consequently, improving oxygen availability in the upper part of the hyporheic zone is a crucial indicator of the effectiveness of any biomanipulation. In addition, the restriction of the experimental effects to the upper reaches strongly supports the conclusion that fish stock manipulation was responsible for the positive effects in the hyporheic zone. Benthic foraging by chub

has a depth-limited impact on hyporheic oxygen availability because these fish primarily disturb the surface layer of a gravel bed, as has been demonstrated before (e.g. Pledger et al., 2017, 2016). The fact that the increase in hyporheic oxygen availability was most pronounced in the middle of the river, where large fish and the grazing scars of nase on coarse substrates were most frequently observed, further supports the conclusion that the effects observed in our study were mediated by fish. Rheophilic nase likely preferred the middle of the river because of its higher current velocity and water depth (Huber and Kirchhofer, 1998). Especially during low-flow conditions, the water depth at some of the sampling sites near the river margin was insufficient for large fish. However, the depth restriction of the effects can also be explained by physical factors. For example, fine particles, such as detached algal cells, usually accumulate directly beneath the coarse armour layer of the hyporheic zone, resulting in the development of a relatively thin clogging layer during low-discharge periods (Brunke, 1999; Schälchli, 1992). Consequently, an abrupt reduction of particle input might have resulted in the same effects. Nonetheless, the occurrence of an abrupt change only in the impact stretch and at exactly the time of the experimental manipulation is not only very unlikely, it is also not supported by our data on sediment accumulation and fine sediment content.

The extent of external clogging can be affected by grazers via a reduction of periphyton biomass and via declogging processes such as bioturbation by chub. The amount of decaying algal biomass in the hyporheic zone (i.e. the extent of internal clogging) can be affected by reducing periphyton directly (nase) and by facilitating the removal of decaying biomass via bioturbation (chub). Consequently, a combined reduction in external and internal clogging as well as the biological oxygen demand would explain the significant positive effects on hyporheic oxygen availability observed in our study despite only small reduction in periphyton biomass following fish stock enhancement.

This line of thought is further supported by our observation of significant positive effects of fish stock enhancement on vertical water flux in the upper layer of the hyporheic zone. This finding suggests that benthic foraging by chub disturbed the riverbed, which in turn reduced clogging in the upper layer of the hyporheic zone. Support for this explanation comes from an earlier mesocosm experiment, in which the presence of chub alone positively affected hyporheic oxygen availability even though it did not reduce periphyton biomass (Hübner et al., 2020). Moreover, evidence for bioturbation in natural riverbeds also comes from a previous field experiment in which chub and other benthic-feeding fish were shown to increase the mobility of sediments and thereby cause substrate coarsening (Pledger et al., 2017).

However, the apparent lack of a strong reduction of periphyton biomass might also be explained by the high spatial and temporal variability of periphyton biomass on the riverbed, which might have prevented the detection of grazing effects on a large scale (Ibisch et al., 2009). Compared to periphyton biomass, the extent of biological clogging in the hyporheic zone can be expected to change more slowly, because organic

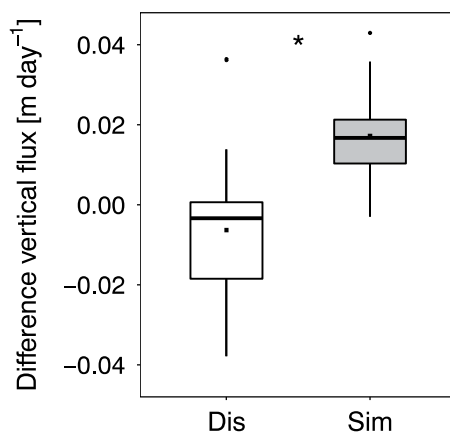


Fig. 7. Differences in the vertical flux of water in the hyporheic zone (5–10 cm depth) between the impact and reference stretches during the dissimilarity phase (Dis; $n = 20$, weekly mean values between April – August 2017) and the similarity phase (Sim; $n = 37$, weekly mean values between April – October 2018 and between April – July 2019). Boxes: 75% and 25%, whiskers: 95% and 5%, squares: mean differences, dots: outliers. *Significant ($p < 0.05$).

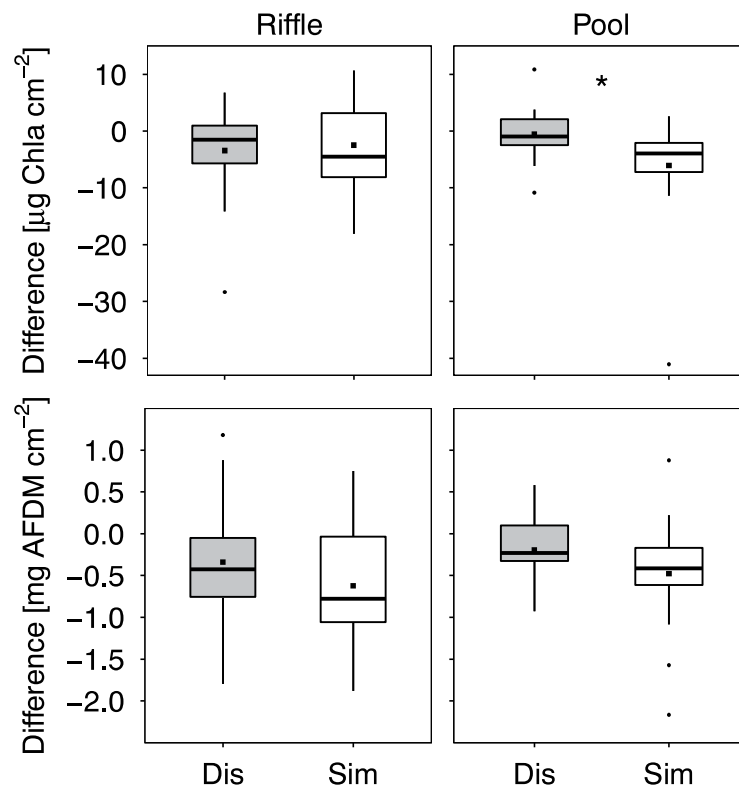


Fig. 8. Differences in periphyton biomass between the impact and reference stretches during the dissimilarity phase (Dis; $n \geq 17$) and the similarity phase (Sim; $n = 20$) at the riffle and the pool site. Upper row: differences in chlorophyll *a* concentrations per area, bottom row: differences in ash-free dry mass per area. Boxes: 75% and 25%, whiskers: 95% and 5%, squares: mean differences, dots: outliers. *Significant ($p < 0.05$).

matter accumulates in the hyporheic zone during low discharge and is only flushed out by the next flood (Brunke, 1999). This temporal and spatial integration may have facilitated the observation of the effects on hyporheic oxygen availability due to a much lower variability of hyporheic DO compared to periphyton biomass. Due to the methodological constraints inherent to any large-scale field experiment, we were unable to distinguish between the different mechanisms.

Nevertheless, our data showed a reduction of periphyton biomass at the pool site, which was likely caused by nase, because grazing effects of nase and smaller grazing fish species have been observed before (e.g. Hübner et al., 2020; Power et al., 1985). However, enhancing the density of large omnivorous chub might also have reduced periphyton biomass due to the indirect promotion of grazing by benthic invertebrates as a consequence of reduced predation by highly abundant small zoobenthivorous fish. The results of an earlier small-scale experiment conducted in the reference stretch of the same river indeed indicated a strong impact of the cascading effects of zoobenthivorous fish on periphyton biomass (Gerke et al., 2018). Indirect top-down control of periphyton through a three-level trophic cascade was also observed on a large scale under near-natural conditions (Winkelman et al., 2014). In mesocosm experiments in a Northern California river, omnivorous fish were found to induce a four-level trophic cascade, down to periphyton, by reducing the abundance of primary predators and therefore predation pressure on grazing invertebrates (Power, 1990; Wootton and Power, 1993).

Although fish stock manipulation led to a relative improvement in hyporheic oxygen availability and vertical water flux, the absolute oxygen concentrations and vertical fluxes of water in the hyporheic zone at

the experimental site were still rather low, especially under the prolonged low-flow conditions that occurred during the dry summer of 2018. The estimated vertical fluxes of water were at the lower end of the range determined in a similar gravel-bed river ($0.11\text{--}1.08\text{ m day}^{-1}$; Ingendahl et al., 2009). Low vertical water fluxes strongly indicate that the sediment permeability of a riverbed is affected not only by biological clogging due to eutrophication but also by physical clogging with inorganic fine sediments. The relatively high mean proportions of clay and silt-sized particles in our freeze-core samples are consistent with this observation. For comparison, they were close to the proportions of clay and silt-sized particles in freeze-core samples taken from a site in the transition zone of hyporhithral to epipotamal of a Mongolian gravel-bed river that is impaired by physical clogging due to intense pasture use (Hartwig and Borchardt, 2015). In addition to fine sediment loads, physical clogging in the Nister could arise from a bedload deficit resulting from erosion-control structures, such as bank reinforcements, in the upper reaches of the catchment. Consequently, the overall extent of physical clogging will not be considerably reduced by fish stock enhancement alone.

Furthermore, given the seasonal trend in both experimental stretches of declining hyporheic oxygen concentrations from spring until the end of the summer, it seems that the top-down effects of fish cannot avert but only attenuate or decelerate biological clogging. Consequently, in eutrophic rivers, mitigation measures limited to fish stock enhancement will not ensure good habitat quality for sensitive organisms, such as freshwater mussels, that require the sufficient, long-term availability of oxygen in the hyporheic zone (Geist and Auerswald, 2007). The measures could, however, support biodiversity conservation by enhancing habitat

conditions during sensitive periods. In Central European rivers, the eggs and larvae of different spring-spawning fish, such as the salmonid grayling (*Thymallus thymallus*) and the cypriniform common nase, develop over a period of only a few weeks (Hübner et al., 2009; Kamler et al., 1998). During this intra-gravel period, they require a permeable and well-oxygenated hyporheic zone (salmonids: e.g. Malcolm et al., 2003; nase: e.g. Nagel et al., 2020). The positive effects of biomanipulation in our experiment were most pronounced during spring. Accordingly, the enhancement of herbivorous and omnivorous fish stocks may substantially improve habitat quality for the eggs and larvae of spring-spawning fish such as grayling and nase, especially when conducted in combination with the bioturbation resulting from spawning activity (Gutmann Roberts et al., 2020).

The relatively large spatial and temporal scales of the field experiment were a major strength of this study but also resulted in several of the difficulties common to field studies. For example, large fish could move freely within the long (> 500 m) river stretches but they could also move out of the experimental stretches when the dynamic fish weirs became submerged during high-flow conditions. This naturally impaired an exact adjustment of the fish stocks in both experimental stretches throughout the 4-year experiment. However, because we observed that both fish species are closely bound to their home ranges in the river, variability in fish densities is not expected to weaken the conclusions drawn from the experimental results. Furthermore, two experimental stretches in the field can never be identical. The main difference between the stretches in our study was that the total surface area of the river channel in the impact stretch was approximately 36% larger than in the reference stretch, mainly due to a wide shallow section in the upper part of the impact stretch. Because we estimated the fish stocks based on the length of the stretches during field work, fish density and biomass per area were similar but not identical in the reference and impact stretches during the similarity phase. However, the shallow upper part of the impact stretch was hardly used as a habitat by large fish, especially during the low-flow conditions in summer, because shallow non-turbulent areas have a higher predation risk and are thus usually avoided by adult fish (Allouche and Gaudin, 2001; Huber and Kirchhofer, 1998). The sampling sites in both experimental stretches included suitable resting and feeding habitats for large nase and chub, with a variety of depth and flow conditions as well as favourable structures such as tree roots and overhanging vegetation. It was therefore likely that the area of usable habitat for large fish and therefore the real distribution of fish densities during the similarity phase were similar in the two experimental stretches. However, adequate enhancement of fish stocks under non-experimental conditions, as would be necessary when biomanipulation is used as management tool, will no doubt be even more difficult than was the case under the conditions of our field study. As re-establishing large self-sustaining populations of the rheophilic specialist species common nase in rivers where population densities have strongly declined over the past decades might pose a major challenge in the establishment of biomanipulation as a management tool in running waters, the feasibility of this approach needs to be further investigated.

We expected positive effects of fish stocking on benthic invertebrate grazers due to a reduction in the abundance of small zoobenthivorous fish by the large omnivorous chub. Nevertheless, high densities of herbivorous and omnivorous fish may also exert negative effects on the benthic invertebrate community due to intense bioturbation or consumption of small benthic grazers inhabiting periphyton such as chironomids. In fact, grazing and detritivorous fishes have been shown to reduce the abundance of benthic invertebrate taxa in a tropical stream, either through resource depression, bioturbation or intimidation by fish (Flecker, 1992). However, in a previous mesocosm experiment, the presence of nase did not affect benthic invertebrates and the presence of chub positively affected benthic invertebrate biomass at fish densities that were even higher than in our reach-scale experiment (Hübner et al., 2020). Nevertheless, future studies should also examine potential

ecosystem-scale effects of fish stock enhancement on the benthic invertebrate community.

5. Conclusion

Our study is the first to demonstrate that the use of biomanipulation, via the enhancement of large herbivorous and omnivorous fish stocks, has the potential to mitigate the effects of eutrophication in medium-sized European rivers. High fish densities increased hyporheic oxygen availability and can thus be expected to substantially improve habitat quality, especially during the short intra-gravel period of spring-spawning fish species. However, the top-down effects of fish on periphyton biomass were rather small and their effects on the hyporheic zone did not seem to be strong enough to reduce the extent of physical clogging or to avoid biological clogging during prolonged low-flow conditions. While biomanipulation can contribute to mitigating the effects of eutrophication, sustainable restoration of the hyporheic zone in eutrophic rivers, and in turn the preservation of biodiversity, might only be achieved when it is used in combination with a reduction of nutrient and fine sediment inputs.

CRedit authorship contribution statement

Madlen Gerke: Investigation, Formal analysis, Visualization, Writing - original draft. **Dirk Hübner:** Funding acquisition, Resources, Investigation, Writing - review & editing. **Jörg Schneider:** Funding acquisition, Resources, Investigation, Writing - review & editing. **Carola Winkelmann:** Conceptualization, Methodology, Funding acquisition, Project administration, Supervision, Resources, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2020.142547>.

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The following supplement information accompanies the article

**Can top-down effects of cypriniform fish be used to mitigate
eutrophication effects in medium-sized European rivers?**

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Appendix A: Pictures of the experimental site and the dynamic fish weirs



Figure A.1: Sampling subsection in the reference stretch. Picture taken looking in upstream direction on May 29, 2018.



Figure A.2: Sampling subsection in the impact stretch. Picture taken looking in upstream direction on October 27, 2016.



Figure A.3: Dynamic fish weir during mid-flow conditions. Picture taken on March 23, 2016. The discharge at the gauge Heimborn approximately 4 km upstream of the experimental site was $2.2 \text{ m}^3\text{s}^{-1}$ around the time the photograph was taken.



Figure A.4: Partly submerged dynamic fish weir during high-flow conditions. Picture taken on June 23, 2015. The discharge at the gauge Heimborn was $4.1 \text{ m}^3\text{s}^{-1}$ around the time the photograph was taken.

Appendix B: Water depth and current velocity at the sampling sites

On each sampling date, current velocity and water depth were measured above each multilevel probe at the probe sampling sites. Current velocity was measured at the river bottom and approximately 5 cm below the water surface using a flow meter with a vane wheel flow sensor (HFA hand-held unit with FA sensor; Hoentzsch GmbH, Waiblingen, Germany). At each algae sampling site, water depth was measured in the middle of the river and near both river margins; current velocity was measured at the river bottom above ten stones across a transect over the entire width of the river.

Table B.1: Mean values (\pm SD) of water depth and current velocity (bottom and surface velocity) at the multi-level probe sampling sites and the algae sampling sites in the reference stretch and the impact stretch during the experiment. Sampling size (n) refers to the number of sampling dates (probe sampling sites: n = 29, periphyton sampling sites: n = 34).

	Reference			Impact		
	Water depth [cm]	Bottom velocity [m s^{-1}]	Surface velocity [m s^{-1}]	Water depth [cm]	Bottom velocity [m s^{-1}]	Surface velocity [m s^{-1}]
Probe sampling site						
A	26.9 \pm 8.8	0.27 \pm 0.09	0.47 \pm 0.19	25.9 \pm 8.0	0.28 \pm 0.14	0.44 \pm 0.14
B	35.7 \pm 10.2	0.28 \pm 0.19	0.46 \pm 0.18	38.9 \pm 12.1	0.17 \pm 0.11	0.33 \pm 0.15
C	37.2 \pm 10.8	0.27 \pm 0.13	0.45 \pm 0.15	23.7 \pm 9.5	0.34 \pm 0.11	0.52 \pm 0.17
Periphyton sampling site						
Riffle	23.9 \pm 6.7	0.31 \pm 0.14		22.5 \pm 6.9	0.31 \pm 0.13	
Pool	38.7 \pm 9.5	0.15 \pm 0.09		37.7 \pm 8.1	0.18 \pm 0.13	

Appendix C: Additional environmental variables to characterise the hyporheic zone in the river*Field sampling and laboratory analyses*

On each probe sampling date, additional water samples for nitrate analysis (13 mL) were obtained from each depth horizon, filtered through syringe filters (0.2- μ m cellulose acetate membrane) and stored in 15-mL Falcon tubes. Water samples were transported cooled to the laboratory and stored at -20 C° until analysis. All samples were analysed within five months after sampling. The nitrate concentrations of the water samples were measured photometrically (DIN EN ISO 13395, 1996) using a continuous-flow analyser (CFA, AutoAnalyser 3; Seal Analytical GmbH, Norderstedt, Germany).

To analyse fine sediment accumulation rates, three sediment traps (one at each sampling site) were installed in each stretch in spring 2016. The traps consisted of a cylindrical wire-mesh basket (diameter 14 cm, height 21 cm, surface area 154 cm^2) filled with pre-screened gravel (grain size 18 – 36 mm) enclosed in a fine-meshed gauze bag (mesh size 15 μ m), thereby allowing fine sediment input only from the surface. The traps were placed in larger outer baskets (diameter 21 cm), which were buried in the riverbed and not lifted during sampling. These sediment traps were sampled every 2 weeks from May 2016 to September 2018. The exposure duration was 1 – 2 weeks, depending on the season and the expected amount of sediment accumulation (e.g. by floods). At each sampling date, the bag containing the inner basket was lifted and its contents (gravel and fine sediment) were washed into a 10-L bucket. Sediment trap samples were kept in a cool room and analysed for dry weight and organic matter within the following 2 days.

The sediment composition was assessed using the freeze-core sampling technique of Humpesch and Niederreiter (1993). Freeze-core samples were taken once in November 2015 and three times per year (spring, summer and autumn) between May 2016 and October 2018. At each sampling date, six freeze-cores (two per sampling site) were collected from each experimental stretch at a depth of 30 cm. This was accomplished by pouring $\sim 15\text{ L}$ of liquid nitrogen into a hollow steel lance (Umwelt und Wissenschaftstechnik, Mondsee, Austria) driven into the riverbed, thus freezing the sediments to the lance. Immediately thereafter, the core was pulled out of the riverbed

using a tackle. Each core was divided into 10-cm layers. Freeze-core samples were stored either frozen or dried to minimise the degradation of organic carbon prior to the analyses. Sediment trap samples and the dried freeze-core samples were fractionated by wet sieving using a vibratory sieve shaker (Retsch AS 200 Basic, Haan, Germany) equipped with wet sieves of decreasing mesh sizes (20 mm, 6.3 mm, 2 mm, 0.63 mm, 0.2 mm, 0.063 mm; Retsch, Test Sieve ISO 3310-1). Only fine sediment (< 2 mm) was included from the sediment trap samples, and large stones (cobbles > 63 mm) were removed manually from the freeze-core samples. For all sediment samples, the clay and silt fraction (< 0.063 mm), defined as the fraction passing through the smallest sieve size, was washed into a 20-L bucket; sieving was considered to be completed once the water leaving the sieve tower was clear. The total volume of the retained washing water containing the clay and silt fraction was determined and two 50-ml aliquots were taken from the homogenised sample. The fractions retained on each sieve and the aliquots of the washing water were dried at 105°C and then weighed. To assess the organic carbon content in the different fine sediment fractions (< 2 mm), the dried samples were weighed, ashed in a muffle furnace at 510°C for 5 h and then reweighed using a microbalance (XS205 Dual Range, Mettler-Toledo).

Results

In accordance with the generally lower oxygen concentrations during the similarity phase, the mean nitrate concentrations in the hyporheic zone were also lower during this phase than during the dissimilarity phase (Table C.1), indicating enhanced denitrification under low-oxygen conditions. During both experimental phases and in both stretches, the mean absolute difference in electrical conductivity between the surface water and the hyporheic zone at 8 cm depth was > 10 $\mu\text{s cm}^{-1}$ and was only slightly higher at 13 cm depth (Table C.1), suggesting a physical barrier between the surface water and the hyporheic zone within the first 8 cm. The difference in electrical conductivity between the surface water and the hyporheic zone increased during the similarity phase, especially in the reference stretch, indicative of a further reduction in riverbed permeability. This finding is in line with the low DO and vertical flux found in the reference stretch during the similarity phase. The accumulation rates of organic and inorganic clay and silt (particles < 63 μm) accumulation followed a similar pattern and were generally higher

during the similarity phase, especially in the reference stretch (Table C.1). Overall, according to these results the extent of physical and biological clogging was more pronounced during the similarity phase than during the dissimilarity phase, likely due to the prolonged period of low flow in 2018. This was not reflected by the weight proportion of clay and silt in the freeze-core samples, which did not show a striking increase during the similarity phase (Table C.1).

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Table C.1: Mean values (\pm SD) of the environmental variables used to characterise the hyporheic zone in the impact and reference stretches during experiment. ‘Diff hz-sw’: absolute difference in electrical conductivity between the upper hyporheic zone (8-cm depth) and surface water. Content of fines ($< 63 \mu\text{m}$) [w%]: total dry weight proportion of particles $< 63 \mu\text{m}$ (clay and silt) in different layers of the freeze-core samples. Organic carbon content in fines ($< 63 \mu\text{m}$) [w%]: dry weight proportion of organic carbon in the clay and silt fraction. Sampling size (n) refers to the number of sampling dates.

Variable	Dissimilarity phase			Similarity phase		
	Impact	Reference	n	Impact	Reference	n
Nitrate [mg L^{-1}]						
8 cm	3.47 ± 2.02	2.40 ± 1.76	11	1.51 ± 1.47	0.31 ± 0.44	14
13 cm	3.24 ± 2.15	1.78 ± 1.76	11	1.06 ± 0.75	0.24 ± 0.15	14
23 cm	3.11 ± 2.55	1.89 ± 1.78	11	0.67 ± 0.53	0.15 ± 0.09	14
33 cm	3.39 ± 2.81	1.98 ± 1.96	11	0.48 ± 0.43	0.13 ± 0.08	14
Electrical conductivity (diff. hz-sw) [$\mu\text{s cm}^{-1}$]						
8 cm	10.5 ± 8.5	13.3 ± 6.9	12	15.3 ± 8.1	25.3 ± 12.1	19
13 cm	11.5 ± 7.6	15.0 ± 11.0	12	16.9 ± 8.0	26.7 ± 11.2	19
23 cm	11.7 ± 5.9	22.0 ± 14.2	12	19.6 ± 5.6	31.4 ± 9.8	19
33 cm	16.3 ± 10.4	32.0 ± 20.6	12	19.8 ± 7.0	42.9 ± 12.6	19
Accumulation of inorganic fines ($< 63 \mu\text{m}$) [$\text{g m}^{-2} \text{d}^{-1}$]	153 ± 94	195 ± 120	18	349 ± 194	426 ± 223	12
Accumulation of organic fines ($< 63 \mu\text{m}$) [$\text{g m}^{-2} \text{d}^{-1}$]	41 ± 27	53 ± 36	18	79 ± 34	96 ± 40	12
Content of fines ($< 63 \mu\text{m}$) (0–10 cm) [w%]	3.2 ± 1.0	2.9 ± 0.6	5	4.2 ± 0.5	2.9 ± 1.0	3
Content of fines ($< 63 \mu\text{m}$) (10–20 cm) [w%]	3.5 ± 1.0	3.0 ± 0.7	5	4.6 ± 0.3	3.1 ± 1.0	3
Content of fines ($< 63 \mu\text{m}$) (20–30 cm) [w%]	3.5 ± 1.1	2.8 ± 0.4	5	4.6 ± 0.2	3.1 ± 1.0	3
Organic carbon content in fines ($< 63 \mu\text{m}$) (0–10 cm) [w%]	7.0 ± 0.3	8.2 ± 1.3	5	7.3 ± 0.6	7.2 ± 0.6	3
Organic carbon content in fines ($< 63 \mu\text{m}$) (10–20 cm) [w%]	6.8 ± 0.4	8.0 ± 1.2	5	7.1 ± 0.7	6.9 ± 0.5	3
Organic carbon content in fines ($< 63 \mu\text{m}$) (20–30 cm) [w%]	6.7 ± 0.6	8.0 ± 1.3	5	7.2 ± 0.6	7.0 ± 0.5	3

Appendix D: Supplementary information on data analysis

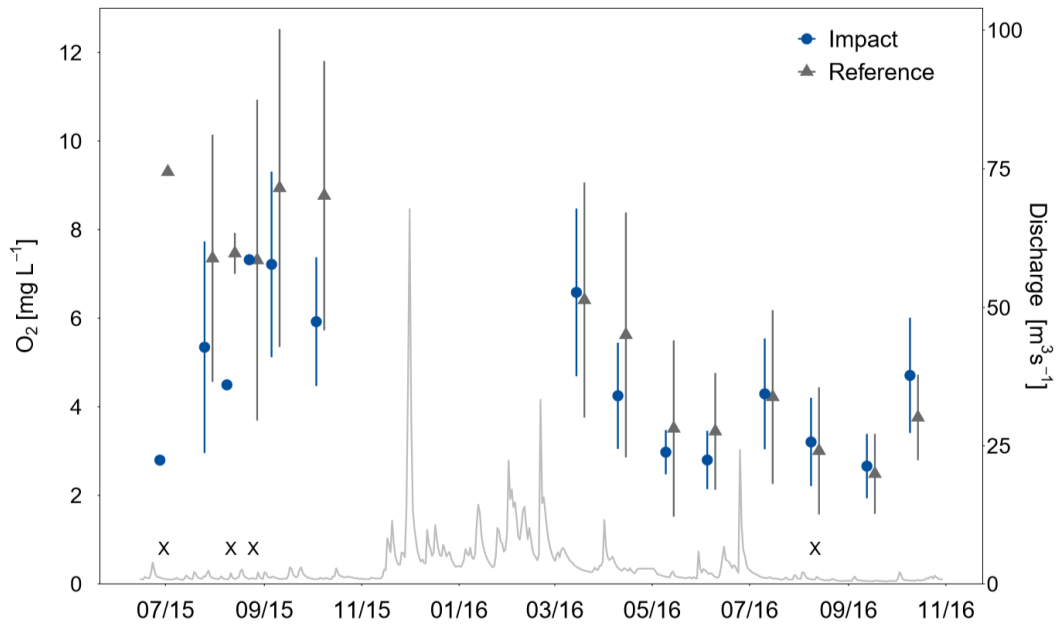


Figure D.1: Mean oxygen concentration (left y-axis) in the upper hyporheic zone (8-cm depth) of the experimental stretches during the dissimilarity phase in 2015 and 2016. Measurements in 2015 started two months after the installation of the multilevel probes. Error bars represent the standard deviations; $n \geq 7$ samples except for sampling dates marked with X ($n = 3$ or $n = 2$, if no standard deviation is shown). The grey trace shows the daily mean discharge (right y-axis).

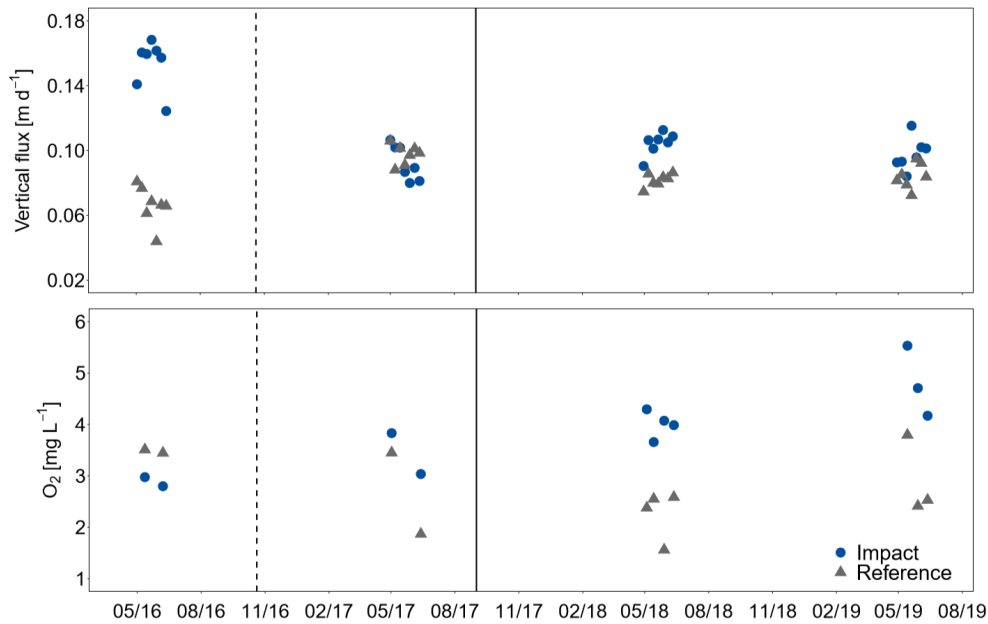


Figure D.2: Mean absolute values of the vertical flux (5 – 10 cm depth) and mean oxygen concentration in the upper hyporheic zone (8-cm) of the experimental stretches during spring (sampling dates between May and mid-June) in the four years of the experiment. The transition between the phases is indicated by the vertical line between 08/17 and 11/17. The shifting of the sampling sites in the two stretches at the end of the season in 2016 is indicated by the dashed line.

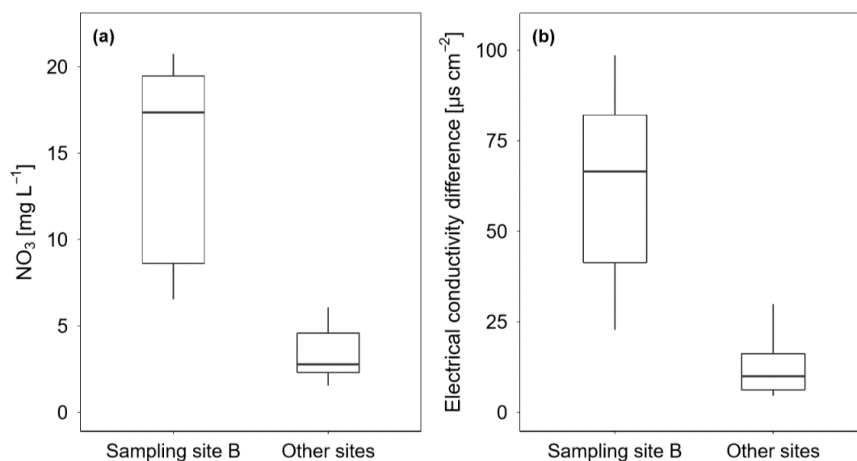


Figure D.3: (a) Mean nitrate concentration in the upper hyporheic zone (8-cm depth) and (b) mean absolute difference in electrical conductivity between the upper hyporheic zone and surface water in the impact stretch in 2016 (nitrate: $n = 7$, electrical conductivity: $n = 8$). Sampling site B (three multi-level probes) is shown compared to all other sampling sites (six multi-level probes). Boxes: 75 % and 25 %, whiskers: 95 % and 5 %.

Appendix E: Fish densities during the experiment

Table E.1: Mean (\pm SD) biomass per area and density of nase and chub (> 15 cm) during the two phases in the reference and impact stretches (n refers to the number of sampling dates).

	Dissimilarity phase		Similarity phase	
	Reference stretch (n = 6)	Impact stretch (n = 3)	Reference stretch (n = 5)	Impact stretch (n = 5)
Biomass [g m⁻²]				
Nase	81.82 \pm 7.09	0.82 \pm 1.41	42.60 \pm 7.58	30.45 \pm 7.37
Chub	19.84 \pm 2.66	1.17 \pm 1.08	15.18 \pm 3.43	10.00 \pm 3.68
Total	101.66 \pm 7.74	1.99 \pm 2.35	57.78 \pm 8.68	40.45 \pm 9.76
Density [Ind m⁻²]				
Nase	0.186 \pm 0.036	0.010 \pm 0.017	0.118 \pm 0.068	0.090 \pm 0.014
Chub	0.052 \pm 0.012	0.004 \pm 0.005	0.066 \pm 0.011	0.023 \pm 0.009
Total	0.238 \pm 0.043	0.014 \pm 0.022	0.184 \pm 0.071	0.114 \pm 0.023

Table E.2: Nase and chub densities in the impact, reference and buffer stretches during the experiment (white background: dissimilarity phase, grey background: similarity phase). Values are the total number of fish captured per m² (catch) and calculated stock per m² following stock manipulation.

Date	Species	Catch [Ind m ⁻²]			Stock [Ind m ⁻²]		Number of electrofishing passes		
		Impact	Reference	Buffer	Impact	Reference	Impact	Reference	Buffer
Jul 2015	Nase	0.026	0.108	0.044	0.000	0.190	4	2	1
	Chub	0.017	0.028	0.011	0.000	0.055			
Oct 2015	Nase	0.019	0.166	0.065	0.029	0.247	2	2	1
	Chub	0.005	0.024	0.036	0.010	0.060			
Apr 2016	Nase	0.000	0.098	0.025	0.000	0.154	2	2	1
	Chub	0.003	0.016	0.014	0.002	0.029			
Jul 2016	Nase	0.002	0.118	0.033		0.202	3	2	1
	Chub	0.008	0.020	0.018		0.055			
Oct 2016	Nase	0.001	0.133	0.028		0.171	2	2	1
	Chub	0.006	0.024	0.014		0.052			
Apr 2017	Nase	0.011	0.061	0.027		0.149	2	2	1
	Chub	0.004	0.031	0.006		0.063			
Aug 2017	Nase	0.004	0.106	0.023	0.113	0.232	2	2	1
	Chub	0.006	0.018	0.008	0.036	0.068			
Apr 2018	Nase	0.001	0.041	0.009	0.082	0.058	2	2	1
	Chub	0.002	0.021	0.003	0.014	0.052			
Sep 2018	Nase	0.071	0.116	0.031	0.096	0.122	2	2	1
	Chub	0.019	0.034	0.027	0.028	0.074			
Apr 2019	Nase	0.006	0.069	0.003	0.082	0.080	2	2	1
	Chub	0.007	0.037	0.016	0.015	0.079			

8 General discussion

Eutrophication of streams and shallow rivers results in excessive periphyton growth, which causes biological clogging and thus oxygen depletion in the hyporheic zone (Hartwig and Borchardt, 2015; Ibsch et al., 2009). The end result is a serious degradation of habitat quality for gravel-spawning fish and benthic invertebrates (Geist and Auerswald, 2007; Hübner et al., 2009). A variety of management tools have been established for eutrophication control in standing waters, which have been the major focus of eutrophication research over the past several decades (Smith et al., 1999). However, in running waters efficient tools for controlling eutrophication are still lacking. Controlling eutrophication by reducing P input, which is doubtless effective (Schindler et al., 2016), is particularly difficult to achieve in running waters with densely populated and/or agricultural catchments due to their tight connection with the surrounding landscape. Specifically, nutrient inputs from non-point sources remain difficult to control, despite the implementation of best management practices such as riparian buffer strips (Dodds, 2006). In forested streams, riparian shading can effectively control eutrophication during summer (Burrell et al., 2014; Ghermandi et al., 2009) but reducing the light availability to periphyton also reduces the efficiency of P retention (Sabater et al., 2000). Thus, higher P loads are transported downstream and may exacerbate nutrient enrichment in wider river sections, where riparian shading is less effective and periphyton growth is not exceedingly light limited. Top-down control of the food-web by manipulating fish stocks, similar to the biomanipulation successfully applied in lakes (Benndorf, 1990; Hansson et al., 1998), offers an alternative approach to mitigating the effects of eutrophication in medium-sized shallow rivers. With this background, I aimed at assessing the potential for top-down control by common nase, the only obligate herbivorous fish species in European rivers, and omnivorous European chub as a tool to mitigate the effects of eutrophication in medium-sized rivers. I therefore performed three experimental studies, using three different temporal and spatial scales, in a medium-sized eutrophic gravel-bed river.

The results of the fish enclosure experiments (study 1) showed that exclusion of the fish from the experimental tiles led to a reduction in autotrophic periphyton biomass, most likely due a higher biomass of invertebrate grazers (Figure 1). This was in contrast to the

initial expectation that periphyton biomass would be lower on accessible tiles due to grazing by nase. These observations suggested that, on small spatial and temporal scales, the cascading top-down effects of zoobenthivorous fish have a larger impact on periphyton biomass than do the direct effects of herbivorous nase. Thus, while this study provided indication that the active promotion of benthic grazing could mitigate the effects of eutrophication in rivers, it also highlighted the complexity of top-down control in river food webs. Specifically, given the unspecific exclusion of fish in study 1, the potential impact of herbivorous nase on periphyton biomass remained unclear, as fish belonging to three different trophic levels or functional feeding groups may have fed on the experimental tiles: primary consumers (grazers, nase), secondary consumers (zoobenthivorous, e.g. bullhead) or tertiary consumers (omnivorous, including some facultative-piscivorous species, e.g. chub). In addition, the cascading top-down effects of zoobenthivorous fish on periphyton biomass might have been particularly strong at the small scale of the enclosure experiments but less relevant at larger scales, as benthic invertebrates could completely avoid predation by fish on the enclosure tiles. Whether fish grazing or invertebrate grazing would predominate at an ecosystem scale could not be determined.

The results of the mesocosm experiment (study 2) demonstrated that herbivorous nase and omnivorous chub can reduce the impact of eutrophication effects in the hyporheic zone of running waters. The top-down effects of nase and chub reduced hyporheic oxygen depletion, suggesting a reduction of biological clogging. However, a significant decline in periphyton biomass occurred only in the enclosures stocked with nase, not in those stocked with chub. While the presence of chub had positive effects on total benthic invertebrate biomass, indicating a reduction in the local density of zoobenthivorous fish, it did not increase the biomass of invertebrate grazers. This would explain why the positive effects of chub on benthic invertebrate biomass did not cascade down to periphyton. The indirect top-down control of periphyton in the river may therefore have had less of an impact than suggested by the results of the enclosure experiments (study 1). The absence of a four-level cascade down to periphyton can be explained by the fact that chub is an opportunistic omnivore that also feeds on large invertebrate grazers such as mayfly larvae (Balestrieri et al., 2006). Although unable to reduce periphyton biomass, the presence of chub still had positive effects on hyporheic oxygen availability albeit by

a mechanism different from that of nase. While the positive effects of nase on hyporheic oxygen availability could be attributed to a reduction of periphyton biomass by benthic grazing, the reduction of hyporheic oxygen depletion in the presence of chub can be best explained by the enhancement of bioturbation due to intense benthic foraging by chub (Figure 1).

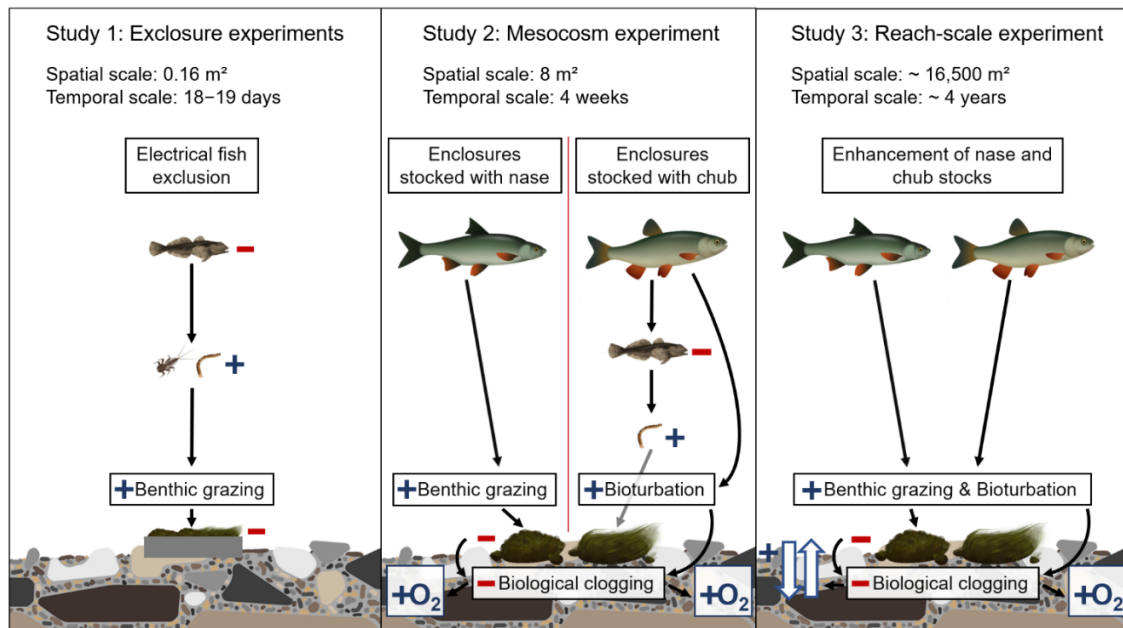


Figure 1: Graphical abstract summarising the main findings of the three experimental field studies. O₂: oxygen concentration in the hyporheic zone. White filled arrows: vertical exchange flux of water in the hyporheic zone (only determined in study 3). Hyporheic zone pictogram: Michael Götten. Fish, mayfly larva and periphyton pictograms: Janno Worischka. Chironomid larva pictogram: www.somethingfishy.ie, Inland Fisheries Ireland, 2010.

The results of the reach-scale experiment (study 3), the core contribution of my thesis, demonstrated that enhancing the stocks of nase and chub mitigated the effects of eutrophication on an ecosystem level under relatively realistic conditions. High fish densities significantly increased both oxygen availability and the vertical exchange of water in the upper layer of the hyporheic zone, even though the effects of fish stocking on periphyton biomass were relatively small (Figure 1). The positive effect of fish stocking on hyporheic oxygen availability was most pronounced in spring. This suggests that high fish densities can substantially improve habitat quality for the early-developmental stages of spring spawning fish species such as grayling (*Thymallus*

thymallus) and nase, which develop within the hyporheic zone over a period of only a few weeks. However, the effects of fish stocking did not seem to be strong enough to reduce the extent of physical clogging or to avoid biological clogging during prolonged low-flow conditions. Nevertheless, the results indicate that fish stock enhancement can contribute to controlling eutrophication in rivers and thus support biodiversity conservation, by improving habitat conditions during sensitive periods. To the best of my knowledge, I demonstrated for the first time that the biomanipulation achieved by enhancing herbivorous and omnivorous fish stocks can mitigate the effects of eutrophication in medium-sized European rivers.

Rivers are highly dynamic, open ecosystems that are strongly influenced by abiotic disturbances (e.g. floods), in contrast to lakes, which are more stable, more isolated and more controlled by biotic interactions (Dent et al., 2002). The trophic cascade concept (Carpenter et al., 1985), one of the key concepts in freshwater ecology, was developed in response to observations in whole-lake experiments. It provided the theoretical basis for the ‘classical’ biomanipulation approach commonly applied in lakes, i.e. enhancing the densities of piscivorous fish to decrease the densities of zooplanktivorous fish and thus increase the abundance and size of zooplankton grazers to ultimately decrease phytoplankton biomass (Carpenter et al., 1985). Transferring this approach to running waters would require reducing the predation pressure on invertebrate grazers (e.g. through the removal of zoobenthivorous fish) to increase their grazing on periphyton. While this trophic cascade has been demonstrated in the benthic food webs of streams (e.g. Huryn, 1998; Power, 1990; Winkelmann et al., 2014), its effect size tends to be smaller than in the pelagic food webs of lakes (Shurin et al., 2002). While the direct transfer of the ‘classical’ lake biomanipulation approach to benthic stream food webs relies exclusively on cascading top-down effects of benthivorous fish, the strength of the biomanipulation approach investigated in this thesis is that it relies on at least two pillars, the enhancement of direct grazing effects, by stocking herbivorous fish, and the enhancement of bioturbation, by stocking omnivorous fish. Although not observed in the studied river, an additional effect of omnivorous fish may be their ability to promote invertebrate grazing indirectly, by reducing predation by small zoobenthivorous fish.

Large herbivorous fish such as common nase reach a much higher biomass per area than invertebrate grazers. For example, following the fish stock enhancement in study 3 the

biomass per area of common nase was approximately 100-fold higher than the total invertebrate biomass per area in the reference stretch of the experimental reach. The advantage of this difference was previously demonstrated in field experiments showing that the effect size of benthic grazers on periphyton biomass increases with increasing biomass per area (Hillebrand, 2009). Furthermore, in contrast to invertebrate grazers, the biomass of herbivorous fish is not subject to seasonal reductions. Nevertheless, the effect of fish grazing on periphyton biomass was less pronounced than expected, especially compared to the positive effect on hyporheic oxygen availability. However, the results of studies 2 and 3 strongly suggest that benthic grazing by nase was one of the main mechanisms for mitigating the effects of eutrophication in the experiments. By contrast, in previous studies stocking with phytoplanktivorous filter-feeding fish—such as the non-native silver carp (*Hypophthalmichthys molitrix*), which has been shown to successfully control cyanobacterial blooms in Asian lakes that lack large zooplankton (Zhang et al., 2008)—did not lead to effective biomanipulation in European standing waters (Horn, 2003; Triest et al., 2016). This was likely due to the inability of these fish to filter small phytoplankton taxa ($< 10 \mu\text{m}$) and to the suppression of large cladoceran zooplankton (Radke and Kahl, 2002). The mesocosm experiment (study 2) showed no evidence of a similar negative effect of herbivorous nase on benthic invertebrate grazers that would weaken the top-down effects on periphyton biomass. Thus, a key difference between the potential of biomanipulation to mitigate eutrophication effects in European rivers vs. lakes is the added potential of enhanced stocks of herbivorous fish.

A second major mechanism likely contributing to the mitigation of eutrophication effects in the hyporheic zone was the increased bioturbation due to benthic foraging. Thus, the mesocosm experiment (study 2) pointed out another difference between rivers and lakes. In lakes, bioturbation by benthic foraging fish such as common carp (*Cyprinus carpio*) can have adverse effects by increasing sediment resuspension, and thereby internal nutrient loading and turbidity (Bonneau and Scarnecchia, 2014; Breukelaar et al., 1994). However, such strong biotic feedbacks, which can shift shallow lakes from the clear-water to the turbid water state, are unlikely to occur in rivers, where water retention times are shorter and flood regimes result in a higher relative importance of physical forcing (Dent et al., 2002; Johnson et al., 2014). Unlike in shallow eutrophic lakes, benthic foraging fish such as chub can positively affect the habitat quality in eutrophic rivers, by

causing localised substrate coarsening in the upper layer of the hyporheic zone (e.g. Pledger, 2017), thereby increasing its permeability, water exchange between surface water and the hyporheic zone and hyporheic oxygen availability. Although a reduction in periphyton biomass was not observed in enclosures stocked with chub in the mesocosm experiment (study 2), bioturbation activities by benthic-feeding riverine fish may even disturb periphyton assemblages and thus reduce periphyton biomass (Pringle and Hamazaki, 1998).

Besides grazing, the frequency of flood disturbance is a crucial determinant of periphyton dynamics in rivers. Provided that fish densities are not reduced by high discharge events, intermediate flood disturbances may support the effectiveness of biomanipulation in rivers, as illustrated by the following considerations. In eutrophic rivers, the nuisance growth of attached, filamentous, relatively grazing-resistant algae such as *Cladophora* sp. is promoted by the stable high light and temperature conditions that accompany the low water flow in summer (Dodds, 1991). Filamentous branches of attached green algae tend to trap sediments suspended in the water (Berger et al., 2003; Dodds, 1991), which may additionally increase external clogging of the hyporheic zone. Although in the mesocosms of study 2 the proportion of filamentous green algae was reduced in the presence of nase, indicating grazing-induced changes in periphyton community structure, the excessive development of filamentous algae was frequently observed in the river during summer and could thus apparently not be prevented by fish grazing. This would explain the weak positive effects of fish on hyporheic oxygen availability that were also observed in study 3 during prolonged low-flow conditions in summer. Flood disturbances, however, can abruptly remove periphyton (Biggs, 2000, 1995) and considerably decrease the biomass of *Cladophora* (Dodds and Gudder, 1992; Freeman, 1986), in turn reducing the extent of external biological clogging (Hartwig and Borchardt, 2015; Ibsch et al., 2009). In addition, the removal of algal biomass by flood disturbances causes a shift in the periphyton community towards early-successional stages, such as benthic diatoms, which can be easily grazed and are a preferred food of nase (Corse et al., 2010). Consequently, flood disturbances may restrict the persistence of grazing-resistant taxa and therefore the predominance of periphyton species that thrive in eutrophic waters (O'Hare et al., 2018). This line of thought is further supported by long-term field observations and field experiments in a California river, which showed

that flood disturbances can lengthen food chains and increase the impact of trophic cascades, by increasing the proportion of early-successional edible prey (Power et al., 2008, 1996). In eutrophic lakes, however, the shift from edible to inedible phytoplankton in response to intense grazing by daphnids is a negative feedback mechanism that strongly reduces the effectiveness of biomanipulation (Benndorf, 1990; Mehner et al., 2002). The dominance of grazer-resistant phytoplankton taxa during summer may reduce the food chain to one functional trophic level (Power et al., 1996). While flood disturbances in rivers limit this shift, the frequency of flood disturbances usually cannot be controlled and may vary considerably between years. This can result in considerable interannual variation in the strength of top-down control in river food webs (Power, 1992). In fact, interannual variations in the frequency of flood disturbances were observed during the 4 years of the reach-scale experiment in study 3 (e.g. 2016: large flood event in mid-June, 2018: no flood events in summer). Furthermore, extremely dry years such as in 2018 may become more frequent in most of Europe in response to climate change (Feyen and Dankers, 2009), which could increase eutrophication effects and hinder the long-term effectiveness of biomanipulation in rivers.

A further consideration is that hydromorphological degradation in rivers may reduce the frequency of periphyton removal by flood events. Whether a flood event causes sediment mobility leading to substantial removal of periphyton depends on the bed composition and sediment supply (Hoyle et al., 2017). Hydromorphological alterations such as damming and bank reinforcements result in a sediment deficit, e.g. by trapping gravel behind dams and reducing lateral gravel supply, which in turn restricts sediment mobility (Hauer et al., 2018; Rollet et al., 2014). For example, the relatively high proportions of fine sediment observed in the freeze core samples taken from the experimental reach (study 3) were likely the product of both fine sediment loads and the sediment deficit resulting from bank reinforcements in the upper reaches of the catchment. Due to the reduced sediment mobility, a higher level of flood disturbance is necessary to cause a substantial removal of periphyton. Conversely, measures that restore sediment dynamics, e.g. the removal of dams or bank reinforcements, can facilitate periphyton removal by flood events, thus increasing the long-term effectiveness of biomanipulation.

The results of my thesis further demonstrate that oxygen availability in the upper layer of the hyporheic zone in rivers is a valuable indicator of eutrophication and its mitigation;

it can therefore be used in environmental monitoring and to guide future studies. Since the mean reduction (~30%) in periphyton biomass on the riverbed following fish stocking (studies 2 and 3) was much smaller than the mean reduction in phytoplankton biomass following successful biomanipulations in lakes (e.g. up to 95 % in shallow lakes 5 years after the biomanipulation; Hansson et al., 1998), it is unlikely that biomanipulation alone will achieve reductions in periphyton biomass comparable to those restoring a 'clear-water state' in shallow lakes. This is largely due to the fundamental differences in the food web structure and the controlling factors in river vs. lake ecosystems. Nevertheless, studies 2 and 3 also showed that even a small reduction of periphyton biomass is sufficient to cause a comparatively large increase in hyporheic oxygen availability. Hence, the effectiveness of biomanipulation in mitigating eutrophication effects in rivers will probably be underestimated if only the reduction in periphyton biomass is measured. Human activities have resulted in multiple pressures that threaten the biodiversity and ecological functioning of river ecosystems, including nutrient enrichment and other land-use related impacts (e.g. fine sediment inputs), hydromorphological alterations that disrupt sediment dynamics and the flow regime, and the introduction of alien species (Dahm et al., 2013; Grizzetti et al., 2017). The magnitude and number of these pressures rule out a restoration of the 'pre-industrial' state of river ecosystems. For example, solely the reduction of nutrients to algal growth-limiting levels in intensively used catchments is extremely difficult and expensive. A more realistic approach is to mitigate the magnitude of adverse anthropogenic effects to a level under which habitat conditions are sufficient to preserve aquatic biodiversity and critical ecosystem functions, even if not all adverse effects can be considerably reduced.

As demonstrated in my thesis, protecting and enhancing stocks of herbivorous and omnivorous fish can significantly improve hyporheic habitat quality even if the reduction in periphyton biomass is relatively small. The biomanipulation of fish stocks proposed in this thesis thus offers a first step in the development of an effective supportive management tool to control eutrophication in running waters. However, it is also clear that, given the multiple anthropogenic impacts on rivers, sustainable restoration of the hyporheic zone, and in turn the preservation of biodiversity and ecosystem functions, will require other measures besides biomanipulation, especially those aimed at reducing nutrient and fine sediment inputs.

9 Perspectives

The biomanipulation approach investigated in this thesis has broad potential applicability in eutrophic small to medium-sized gravel-bed rivers in Europe. However, before biomanipulation can be used as a management tool in running waters, many challenges and questions need to be addressed in future large-scale and long-term field experiments. Sustaining high fish densities through continuous fish stocking is problematic in the long term, most importantly because stocking possibilities are limited to those that allow the preservation of genetic diversity, ideally by using spawners from the same water body (Wetjen et al., 2020). Thus, a prerequisite for the effectiveness and feasibility of biomanipulation in running waters is the establishment of large, self-sustaining fish populations. However, this may prove to be difficult; for example, the population densities of the rheophilic specialist species common nase have strongly declined during the past few decades (Peñáz, 1996). Although restoration measures are increasingly being conducted and local recovering of nase populations has been observed (e.g., Ramler and Keckeis, 2019; Schmutz et al., 2014), the population densities are far below historical levels. In the reach-scale experiment, high fish densities could be relatively easily sustained by transferring fish from nearby sections of the river to the experimental reaches, although cormorant management measures (deterrence and shooting) were required throughout the experiment in order to sustain the manipulated fish stocks. Future studies should investigate whether high fish stocks can be sustained under non-experimental conditions (i.e. without closing off river stretches by fish weirs and regular restocking measures), e.g. by habitat restoration measures and management measures limiting cormorant predation pressure, which can considerably reduce river fish populations (Jepsen et al., 2018). The latter would add another trophic level to the biomanipulation approach, and in itself may produce cascading effects that lead to the mitigation of eutrophication effects.

Successful biomanipulation in running waters using enhanced fish stocks will also benefit from reach-scale studies conducted in eutrophic rivers differing in their degree of nutrient loading or in their extent of physical clogging. In lakes, more effective biomanipulation can be achieved when it is accompanied by a reduction in P-loadings to a level resulting in a slightly eutrophic or mesotrophic state (Benndorf, 1990; Benndorf et al., 2002). Whether biomanipulation is more effective in running waters with nutrient

loads lower than in this study remains to be determined. Moreover, the results of study 3 indicated that the riverbed in the studied reach was affected not only by biological clogging due to eutrophication but also by physical clogging with inorganic fine sediments. Reach-scale studies in eutrophic rivers that are less impaired by physical clogging could reveal whether the positive effects of enhanced fish stocks are stronger if biological clogging is the primary stressor affecting hyporheic habitat quality.

Furthermore, my thesis mainly focused on the effects of high fish densities on hyporheic habitat quality but whether an improvement of habitat conditions through biomanipulation has a positive effect on aquatic biota, e.g., on the recruitment success of gravel-spawning fish, or results in long-term advantages for the community structure of fish and benthic invertebrate assemblages remains to be determined. The answer to this question has practical implications for the goal of the WFD to achieve the ‘good’ ecological status of surface waters, which requires that the aquatic community deviates only slightly from the one that would be expected under conditions of minimal anthropogenic impact (Kallis and Butler, 2001). Strategies for answering this question include short-term bioindication studies using fish eggs (e.g. such as in Hübner et al., 2009) as well as studies based on long-term monitoring methods such as those used in the ecological status assessments of the WFD. For example, evidence of the long-term population growth of common nase (without applying restocking measures) would also indicate self-reinforcing positive effects, as the improved hyporheic habitat conditions would support an increased number of young-of-the-year fish and thus further habitat improvement.

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