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Long-term responses of benthic invertebrates to rotenone treatment

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Abstract

Biological invasions are regarded as one of the largest threats to native biodiversity. The eradication of alien invasive fish or parasites by culling of hosts is a controversial conservation strategy, particularly when using indiscriminate methods involving whole ecosystem collateral damage. While short-term effects are abundantly documented, long-term surveys needed to detect potential wider ecosystem effects are scarce. Here, we report a five-year study on effects of the piscicide rotenone on invertebrate communities from a Norwegian watercourse using a Before-After-Control-Impact design. Kick-net samples of benthic invertebrates were collected from three lentic sampling stations and two lotic stations two to four times per year in both a treated and nearby control catchment. In general, only relatively minor short-term effects, measured as temporal beta-diversity of benthic invertebrates, were observed in lentic and lotic locations following rotenone treatment. However, the lotic fauna was severely negatively affected following a period of long-term rotenone exposure from a late autumn treated upstream lake where rotenone degradation was slow, likely due to low temperatures. Species turnover co-varied markedly between control and treatment locations, indicating that natural environmental variation override effects of rotenone treatment. Likewise, the abundance of invertebrate taxa varied considerably both over time and between control and treatment locations. Our study indicates minor short-term (i.e., <1 month) or long-term (i.e., 4 years) effects of rotenone treatment on benthic invertebrates, but severe effects on the lotic fauna 8 months after treatment following long-term low-dose exposure. This suggests that exposure time is of high importance. The findings from the current study therefore highlight both the importance of long-term monitoring including control sites for the study of rotenone treatment effects on benthic invertebrates as well as the importance of minimizing exposure time.

KEYWORDS
alien invasive species, benthic invertebrates, beta diversity, piscicide, recolonization

1 | INTRODUCTION

Biological invasions are regarded as one of the largest threats to native biodiversity (Mack et al., 2000), also in freshwater (Caffrey et al., 2014; Sala et al., 2000). Alien species may display invasive properties inducing serious ecological consequences through both direct and indirect ecological interactions (Kumschick et al., 2015), as well as major economic impacts (Dittel & Epifanio, 2009). Such alien invasive species may spread rapidly and cause problems that escalate from local to regional scale following establishment. Early targeted eradication actions are often therefore the only effective management method. This approach may be particularly effective when species are required to move through environmental corridors, such as in freshwater ecosystems (Hulme, 2009). However, options for cost-effective and reliable measures are often limited since it may become virtually impossible to eradicate invasive aquatic species in anything but small, enclosed waterbodies (Holdich, Reynolds, Souty-Grosset, & Sibley, 2009).

In aquatic ecosystems, one of the most widely used measures for eradication of invasive species is rotenone. This is a piscicide which is highly toxic to fish and to certain invertebrates but has low toxicity to birds and mammals (Ling, 2003). Rotenone is a compound originating from plant species in the Leguminosae family and has been used for centuries as a mean of capturing fish in areas where these plants are naturally occurring (Brooks & Price, 1961; Meadows, 1973). It has also been used as a fish management tool for almost a century (Finlayson et al., 2010a), either with the purpose of quantifying fish abundances, manipulating fish populations to maintain sport fisheries, or treatment of rearing ponds (McClay, 2000, 2005). It is one of the most successful measures for eradicating populations of invasive fish (Rytwinski et al., 2018). In addition, rotenone has been widely used for eradication of fish parasites through culling of host populations.

Use of rotenone is controversial largely due to its indiscriminate impacts on the wider aquatic fauna. Short-term impacts on nontarget organisms like benthic invertebrates is well documented (e.g., Dalu, Wasserman, Jordaan, Froneman, & Weyl, 2015; Koksvik & Aagaard, 1984; Melaas, Zimmer, Butler, & Hanson, 2001). The observed effects often include reduction in the total abundance of benthic invertebrates comparted to pretreatment levels. However, there are large variations ranging from little or no reduction (Bellingan et al., 2019; Cook, & jr., & Moore, R.L., 1969; Dudgeon, 1990) to up to 95% reduction (Hamilton, Moore, Williams, Darby, & Vinson, 2009). Part of the variation may be explained by taxa-specific tolerance to rotenone. Ephemeroptera, Plecoptera, and Trichoptera, which are abundant in oxygen rich lotic habitats, are generally reported to be rotenone sensitive (Arnekleiv, Dolmen, & Rønning, 2001; Eriksen, Arnekleiv, & Kjærstad, 2009; Mangum & Madrigal, 1999), whereas Coleoptera, Odonata, and Gastropoda, which are often abundant in lentic habitats, are considered rotenone tolerant (Arnekleiv, Dolmen, Aagaard, Bongard, & Hanssen, 1997; Chandler & Marking, 1982; Holocombe, Phipps, Sulaiman, & Hoffman, 1987; Kjærstad & Arnekleiv, 2011). There are also species-specific differences in tolerance among closely related taxa. For example, species of gill breathing Ephemeroptera are particularly sensitive to rotenone (Arnekleiv et al., 2001). Recovery of the total benthic invertebrate density can occur within a year (Kjærstad & Arnekleiv, 2004; Pham, Jarvis, West, & Closs, 2018). However, recovery of single taxa may potentially take several years (Arnekleiv et al., 1997; Mangum & Madrigal, 1999).

Long-term consequences of failing to act toward continuous spread of invasive fish or parasites may be severe to the ecosystem and ecosystem services (Crowl, Crist, Parmenter, Belovsky, & Lugo, 2008; Pyšek et al., 2020). Short-term disturbances to the ecosystem are expected and, to certain degree accepted, as appropriate collateral damage. However, long-term consequences of rotenone treatment may be less accepted. There is currently a lack of studies assessing long-term ecosystem effects caused by rotenone treatments (Vinson, Dinger, & Vinson, 2010). Therefore, this study focused on the long-term consequences of an intensive rotenone treatment on lotic and lentic benthic invertebrate communities. Specifically, we tested for the impact of rotenone treatment on the long-term abundance and temporal turnover of key taxa by comparing time series from a rotenone-treated watercourse and a nearby control watercourse.

2 | METHODS

2.1 | Study sites

The Fusta (rotenone treated) and Drevja (control) water courses are situated in Northern Norway (Figure 1). The Fusta watercourse has a catchment area of 538 km^2. The largest and lowermost lake of the catchment, Lake Fustvatnet (38 m a.s.l.), has a surface area of 11 km^2 and a maximum depth of 68 m. Concentrations of total nitrogen and total phosphorus from water samples taken in August 2014 of 70 and <2 μg/L, respectively, indicate oligotrophic conditions. River Fusta, which is the outlet of Lake Fustvatnet, is 8.5 km long and drains to the sea. The river has a mean annual discharge of 34 $m^3\ s^{-1}$.

The Drevja watercourse (untreated), which is bordering the Fusta watercourse, has a catchment area of 178 km^2. Lake Drevvatn (47 m a.s.l.), the largest and lowermost lake in the catchment, has a surface area of 5 km^2 and a maximum depth of 40 m. Concentrations of total nitrogen and phosphorus from water samples taken in August 2014 of 55 and <2 μg/L, respectively, indicate oligotrophic conditions. River Drevjaelva, which is the outlet of Lake Drevvatn, is 17.7 km long and drains to the sea. The river has a mean annual discharge of 12 $m^3\ s^{-1}$.

Both watercourses are surrounded by sparsely vegetated mountain areas, spruce dominated forests and scattered farmlands.

2.2 | Study design

We performed the study using a Before-After-Control-Impact design (Josefsson et al., 2020). Rotenone was used in the Fusta watercourse to eradicate the invasive parasite *Gyrodactylus salaris* through exterminating its host populations of salmonid fish. Three rotenone treatments were conducted in the watercourse, with two treatments of the River Fusta (August 2011 and 2012) and one subsequent treatment of the upstream Lake Fustvatnet (October 2012), causing the downstream River Fusta to be treated a third time. The neighboring

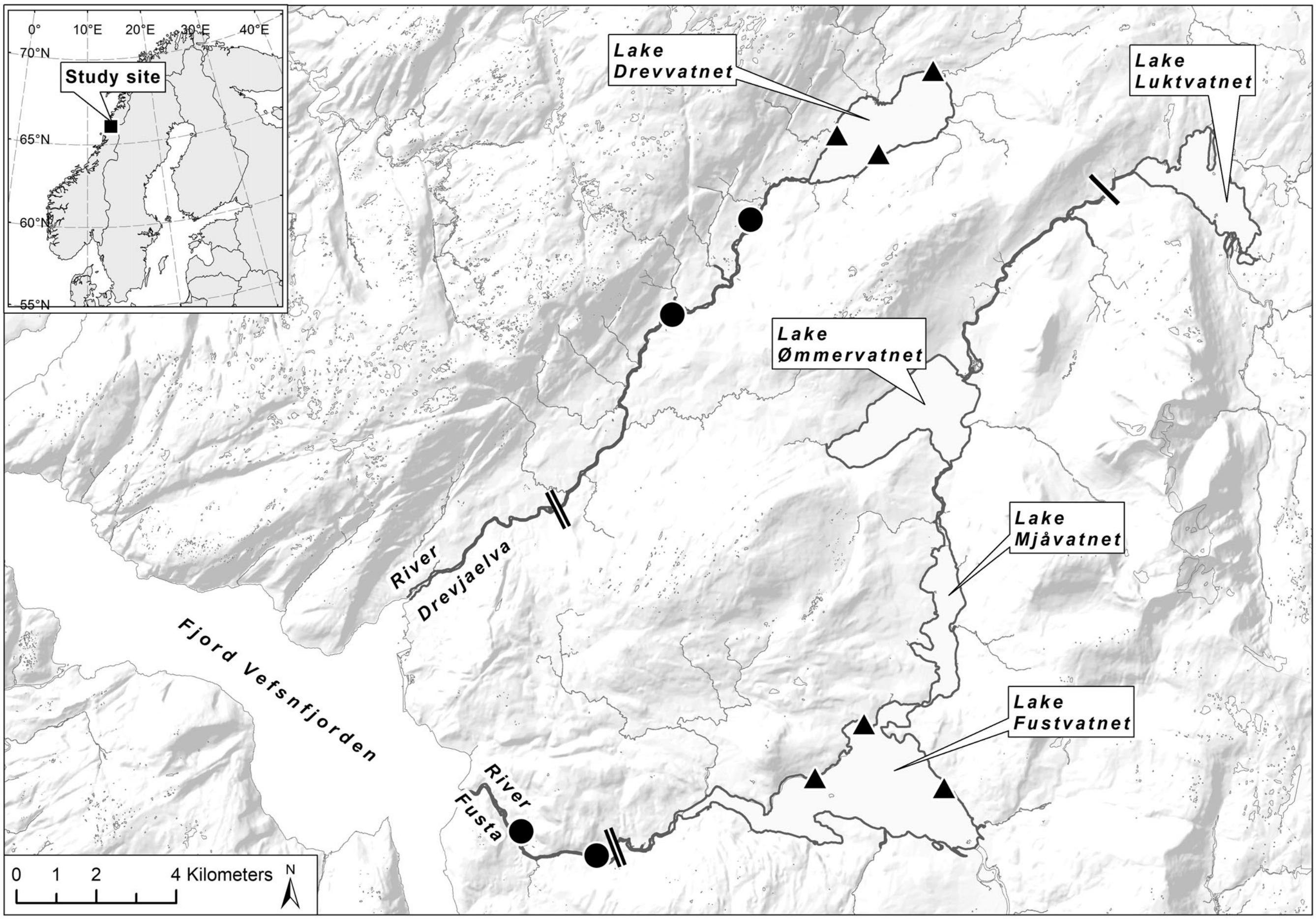

FIGURE 1 Overview of the two studied watercourses Fusta and Drevja. Triangles indicate the position of the lentic sampling stations and dots of the lotic sampling stations. A double black bar indicates the upstream limit of the treatment of River Fusta and River Drevjaelva in August 2011 and August 2012, and a single black bar indicates the upstream limit of the treatment of the Fusta watercourse in October 2012

Drevja watercourse was used as a control site (Figure 1). Sampling was conducted at two lotic and three lentic sampling stations in each watercourse during the autumn from 2011 to 2016, as well as one spring sampling in June 2013, 8 months after the lake treatment. Sampling started in August 2011 before the first rotenone treatment and ended in Autumn 2016. An overview of times of sampling and rotenone treatments is given in Table 1.

Riverine stations were situated in riffle areas dominated by cobbles (particle size of 64–256 mm) and pebbles (particle size 4–64 mm) and with scattered patches of river moss and alga. Two of the stations in each lake were situated in exposed areas where pebbles (particle size of 4–64 mm) dominated the substrate. One of the stations in each lake was situated in sheltered areas with a substrate dominated by finer inorganic and organic substrate (particle size <2 mm) and with little or no aquatic vegetation.

2.3 | Sampling and sample processing

Benthic invertebrates were collected using a kick net with a 25 × 25 cm frame and a mesh size of 0.25 mm. For each sample, an area of approximately 0.5 m^2 was covered with a sampling duration of 1 min. At each combination of sampling occasion and sampling station, five replicate samples were taken. The samples were preserved in 96% ethanol before subsequent subsampling of each sample (1/10) in the laboratory. All invertebrates in the subsample were sorted and identified to the lowest possible taxonomic unit using a stereo microscope and following Engblom (1996) for Ephemeroptera, Lillehammer (1988) for Plecoptera, Rinne and Wiberg-Larsen (2017) for Trichoptera, Holmen (1987) and Nilsson and Holmen (1995) for Coleoptera and Glöer (2002) for Gastropoda. The number of specimens from the sub sample was then multiplied by 10. All specimens of the remaining sample that differed morphologically from the specimens in the sub sample were picked out, identified to lowest possible taxon and counted.

2.4 | Rotenone treatment and fish re-establishment

The rotenone treatments of River Fusta were performed on August 18th 2011 and August 17th 2012 using a rotenone formulation CFT-Legumine with a 2.5% active gradient. In both treatments, the release of rotenone took place over a 7-hr period from two stations in the

TABLE 1 Time of sampling (grey) and rotenone treatments (black) in River Fusta, River Drevjaelva, Lake Fustvatnet, and Lake Drevvatnet

	1.-4. Aug. 2011	18. Aug 2011	24.-25. Aug. 2011	3.-8. Aug. 2012	17. Aug. 2012	13. Sep. 2012	3.-4. Oct. 2012	16.-20. Oct. 2012	24.-25. Oct. 2012	Jun. 2013	Aug. 2013	Oct. 2013	Aug. 2014	Oct. 2014	Aug. 2015	Oct. 2015	Aug. 2016	Oct. 2016
River Fusta	grey	black	grey	grey	black	grey			grey	grey	grey	grey	grey	grey	grey	grey	grey	grey
River Drevja	grey		grey	grey		grey			grey	grey	grey	grey	grey	grey	grey	grey	grey	grey
Lake Fustvatnet	grey			grey			grey	black	grey	grey	grey	grey	grey	grey	grey	grey	grey	grey
Lake Drevvatnet	grey			grey			grey		grey	grey	grey	grey	grey	grey	grey	grey	grey	grey

river, in the upper and in the middle part (Stensli & Wist, 2014). The upper release was situated approximately 1 km upstream of our uppermost sampling station, and the release in the middle part was just downstream of our lowermost sampling station. In addition, riverbanks of slow flowing areas, adjacent ponds, oxbow lakes, and streams were rotenone treated with local point releases. The total use of CFT-Legumine in River Fusta was 754 and 573 L in 2011 and 2012, respectively. The water temperature was approximately 15°C during both treatments and the water flow was 12 $m^3\ s^{-1}$ in 2011 and 11 $m^3\ s^{-1}$ in 2012. In the lower part of River Fusta, treatment concentration of CFT-Legumine was higher than 0.5 ppm over a period of several hours, which was the desired minimum concentration, periodically reaching up to 7 ppm (Adolfsen, Sandvik, & Waaler, 2014).

The rotenone treatment of Lake Fustvatnet took place between October 16th and October 20th 2012. The total use of CFT-Legumine in Lake Fustvatnet was 139,200 L, of which 96,600 L were used for the surface waters and 42,600 L were used for deeper areas. A CFT-Legumine formula of 3.3% rotenone was used, except for the surface layer where the treatment included 25,800 L with 2.5% rotenone (Moen & Bardal, 2014). The 3.3% formula did not contain the synergist piperonylbutoxid and the solvent N-metylpyrrolidone since the former increases the toxicity to invertebrates and not to fish (Finlayson et al., 2010b). Measurements of rotenone concentration were performed over scattered sites in the surface water and at different depths during and after the treatment. On October 21, 2012, no measurements were below 0.5 ppm CFT-Legumine, and the average concentration was 0.68 ppm. Due to low water temperatures, the breakdown of rotenone was slow. In April 2013, the average concentration of CFT-Legumine was 0.1 and 0.3–0.4 ppm in the surface layers and in the deeper parts (>10 m), respectively. In June 2013, the concentration was below 0.1 ppm at all depths. No rotenone was detected in mid-October 2013 (Adolfsen et al., 2014).

Fish was reestablished by stocking of Atlantic salmon (*Salmo salar*) in River Fusta and Arctic charr (*Salvelinus alpinus*) and brown trout (*Salmo trutta*) in Lake Fustvatnet. Three thousand Atlantic salmon smolts were released in the river in 2013 (Lo & Holthe, 2014) followed by planting of 187,000 Atlantic salmon eggs in 2014. In both 2015 and 2016, 12,000 Atlantic salmon smolts were released, as well as 384,000 young of the year (YOY) Atlantic salmon in 2015 and 120,000 YOY in 2016 (Holthe, Bjørnå, & Lo, 2018). In Lake Fustvatnet 35,000 Arctic charr, mainly 2-year-old, were released during the spring of 2014, as well as 93,000 brown trout YOY (Holthe, Bjørnå, & Lo, 2015; Lo & Holthe, 2014).

The two lakes upstream of Lake Fustvatnet, Lake Ømmervatnet and Lake Mjåvatnet, were rotenone treated on October 14th–16th and October 19, 2012, respectively.

The lowest 5 km of River Drevjaelva, from a fish upstream migration barrier and downstream to the fjord-outlet of the river, was rotenone treated in August 2011 and August 2012. Our control area was situated 6–10 km upstream of this fish barrier and as such without influence of this treatment. Fish were also stocked in River

Drevjaelva after the treatment but only in the lower treated part below the barrier. We lack information of the abundance or natural variation in fish populations size in the untreated part of River Drevjaelva (brown trout) or in Lake Drevvatnet (brown trout and Arctic charr).

2.5 | Data analyses

Changes in taxa composition between each subsequent sampling period was measured as temporal beta-diversity index (TBI). We calculated two different TBI indices, using the percentage difference from abundance data and the Sorensen index based on presence–absence data. The percentage difference ($D_{\%\mathrm{diff}}$) between two sampling periods (t_i and t_{i+1}) was defined as $D_{\%\mathrm{diff}} = (\mathrm{B} + \mathrm{C})/(2\mathrm{A} + \mathrm{B} + \mathrm{C})$, where A is the sum of the abundance of all taxa found in both sampling period t_i and t_{i+1}. This represents the unscaled similarity between the two sampling periods. B is the part of abundance of the common taxa that is higher in samples at t_i than at t_{i+1} and is the unscaled sum of taxa *losses* between sampling periods. C is the part of the abundance that is higher t_{i+1} than at t_i and is the unscaled sum of taxa *gains* between the two sampling periods. The equation for the Sorensen index, using presence–absence data, is the same as for the $D\%_{\mathrm{diff}}$ outlined above (see Legendre, 2019 for more details). Differences in abundances and presence–absence data among subsequent sampling occasions were tested for significance with a paired *t* test. The *p*-values (based upon 9,999 random permutations) were controlled for the family-wise error rate using Holm correction. The calculations of the TBI indices and significance tests were done in the R package "adespatial" (Dray et al., 2019).

We analyzed data from riverine and lake habitat separately. Responses to rotenone treatment is expected to vary among habitats. The abundance of each invertebrate taxon is expressed as average number of specimens per kick-sample for each sampling period at each site.

A permutational multivariate analysis of variance (PERMANOVA) with 9,999 permutations was performed (see Anderson, 2001) with the "adonis" function of the R package "vegan" (Oksanen et al., 2020). We tested for differences in community composition (Bray–Curtis dissimilarity) over time, between treated and untreated localities and the interaction between time and treatment.

3 | RESULTS

3.1 | Rivers

The temporal species turnover appeared to co-vary in the treated and untreated river, especially for abundance data (Figure 2). The exception was the period after the lake rotenone treatment in October 2012 to summer 2013 where benthic invertebrate abundances of the rotenone treated River Fusta changed significantly (Figure 2a, Appendix S1), mainly due to losses (Figure 2b). For presence–absence data, there was a significant dissimilarity of species occurrences in the treated river from immediately after the lake treatment in October 2012 until August 2015 (Figure 2d, Appendix S2). The species loss-rate of the treated river was at its highest from October 2012 to June 2013 (Figure 2e) and gain-rate at its highest from June 2013 to August 2013 (Figure 2f, Appendix S2). No significant changes in abundance or species occurrence were found in the untreated river (Figure 2a,b, Appendices S1 and S2).

The total abundance of benthic invertebrates was generally higher in the untreated river compared to the treated river both before and after the treatments (Figure 3a), and this was especially prominent for Ephemeroptera and Oligochaeta (Figure 3b,f, respectively).

The total abundance of benthic invertebrates reflects the temporal turnover indices (Figure 3a). There were no clear short-term effects on the total abundance or the abundance on most taxa following the two rotenone treatments of River Fusta (Figure 3). As an exception, the abundance of Plecoptera decreased strongly in the treated river immediately after the first and second river treatment (89% and 92%, respectively), whereas the changes were smaller in the untreated river (+17% and −37%, respectively) (Figure 3c).

In contrast, there was a marked increase in the abundance of taxa observed in samples from both the treated and the untreated river in October 2012, followed by a pronounced decline in the treated river in June 2013 (Figure 3a). However, the observed response in total abundance masks distinct differences in response to the lake rotenone treatment of riverine benthic invertebrate taxa.

The abundance of Ephemeroptera in the treated river decreased shortly after the two river treatments (Figure 3b). This was also found in the untreated river after the first river treatment. The Ephemeroptera *Baetis rhodani and Baetis* sp. decreased in the treated river after both treatments and in the untreated river after the first treatment. Abundances of all taxa from the rivers not presented in the figures are listed in Appendix S3. For other Ephemeroptera, like *Heptagenia dalecarlica* and *Ephemerella* sp., the abundance in both rivers was relatively high shortly after the river treatments. Immediately after the lake treatment in October 2012, *B. rhodani* was the only Ephemeroptera with reduced abundance and was not recorded in the treated river. In June 2013, 8 months after the lake treatment, there was a dramatic decline in the Ephemeroptera abundance in the treated river (Figure 3b) with only a few specimens present of *Siphlonurus* sp., *B. rhodani*, and *H. dalecarlica*. However, a decline was also found in the untreated river, but here most taxa were present in June 2013. In the subsequent years, the Ephemeroptera abundances in the treated river increased, mainly *B. rhodani* and *H. dalecarlica*. A similar increase occurred in untreated river, in addition to an increase in *B. muticus*.

There was a strong decrease in the abundance of Plecoptera in the treated river after both river treatments, whereas there was an increase in the untreated river. Taxa with reduced abundances included *Diura* sp., *Isoperla* sp. and *Leuctra* sp. Shortly after the lake treatment in October 2012, the abundance of Plecoptera in the treated river decreased with 95% compared to September 2012

(Figure 3c), and only three specimens of the genus *Nemoura* were recorded. Most taxa were present in the untreated river and with a relatively high abundance of *Amphinemura borealis*, *Diura* sp., and *Isoperla* sp. In June 2013, 8 months after the lake treatment, no Plecoptera were detected in the treated river, while at the same time, there was an average of more than 200 specimens of Plecoptera in the untreated river (Figure 3c).

Contrary to the river treatments, where the negative effect was minor, Trichoptera abundances decreased in the treated river immediately after the lake treatment but did not change in the untreated river (Figure 3d). Eight months after the lake treatment in June 2013, there was a dramatic decrease (99%) in the abundance of Trichoptera in the treated river, and only a few specimens of *Rhyacophila nubila*, *Hydropsychidae* sp., and *Micrasema* sp. were present. A corresponding but smaller decline (41%) was observed in the untreated river.

Both rivers had high abundance of Chironomidae shortly after the lake treatment (Figure 3e). The abundance of Chironomidae co-varied considerably among rivers. The abundance of Oligochaeta in the treated river decreased shortly after the first river treatment and increased subsequent to the second river treatment and the lake treatment (Figure 3f). In June 2013, 8 months after the lake treatment, the abundance of Oligochaeta was at its minimum in the treated river.

There was a low abundance of Hydrachnidia in the treated river, and the taxon was absent in 2013 (Appendix S3). In the untreated river, Hydrachnidia was recorded in all sampling occasions and with the highest abundances after the treatments. The abundance of the riffle beetles (Elmidae) decreased in the treated river following the treatment. In the untreated River Drevjaelva, the taxon also decreased in abundance, albeit at a much lower rate than in the treated River Fusta.

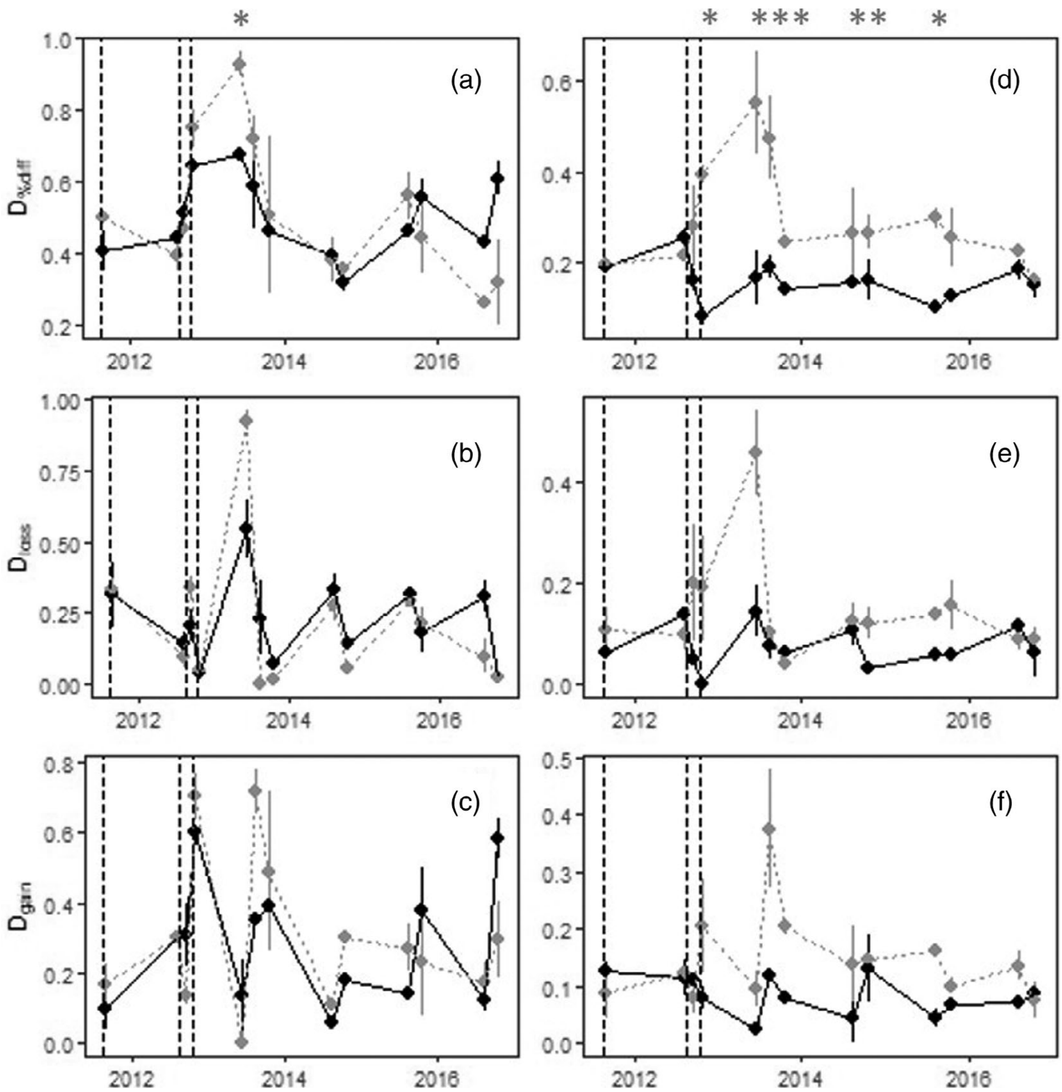

FIGURE 2 Rivers: Mean changes in dissimilarity (% difference) for abundance data (a–c) and for presence–absence data (d–f) between sampling occasions (a and d) and its components; losses (b and e) and gains (c and f). Grey: River Fusta (rotenone treated), black: River Drevjaelva (untreated). Dashed vertical lines indicate the timing of the rotenone treatments. Error bars indicate maximum and minimum values of the temporal biodiversity indices at individual sampling stations. Significant differences in diversity indices between sampling periods ($p < 0.05$) in River Fusta are marked with grey asterisk (no significant differences detected in River Drevjaelva)

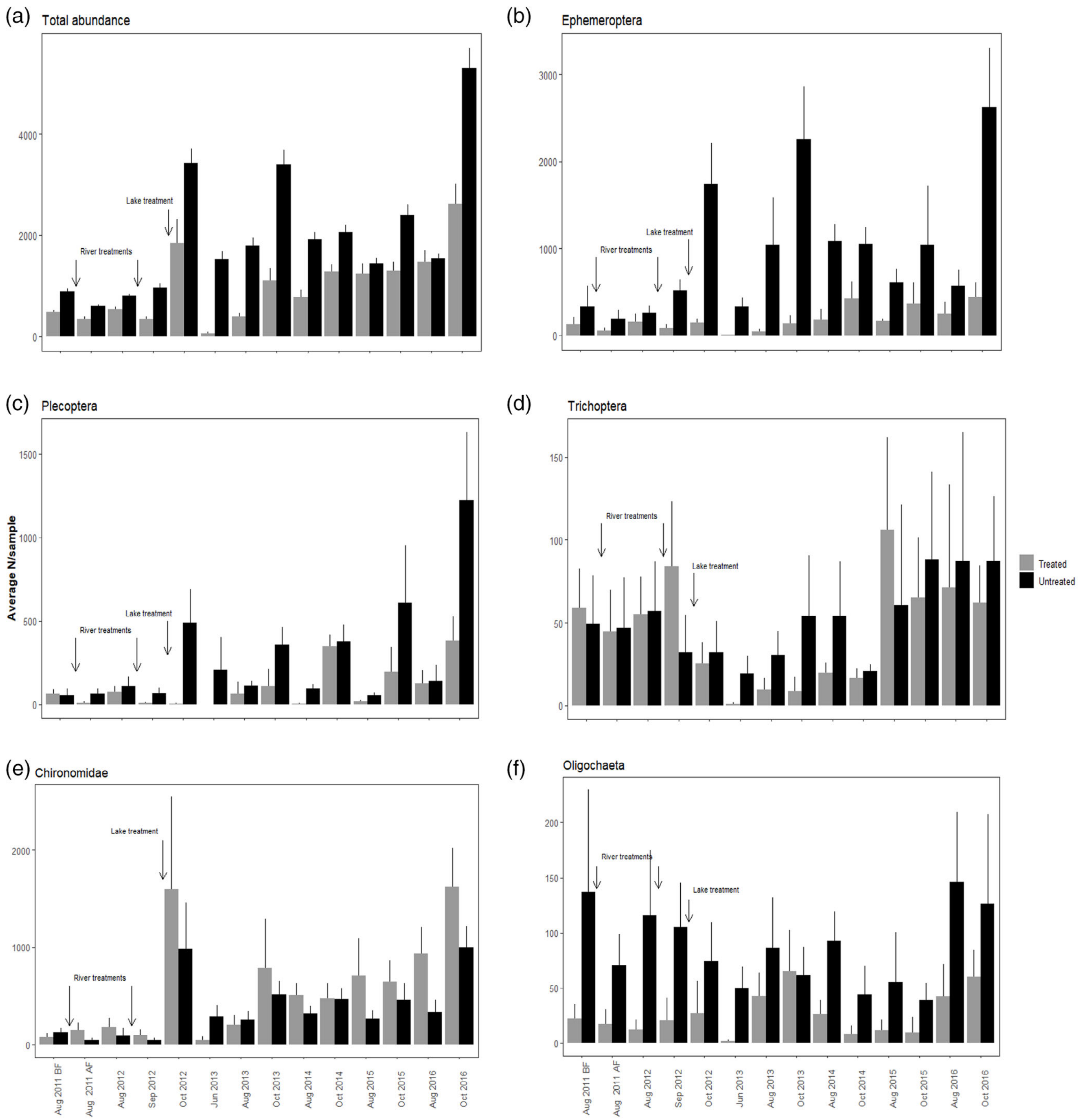

FIGURE 3 Rivers: Average abundance (with standard deviation) of benthic invertebrates of River Fusta (rotenone treated) and River Drevjaelva (untreated) in the period 2011–2016. (a) Total abundance, (b) Epemeroptera, (c) Plecoptera, (d) Trichoptera, (e) Chironomidae, and (f) Oligochaeta. Timing of rotenone treatments are marked with arrows

Permutation tests showed that the taxa composition expressed as Bray–Curtis dissimilarity, differed significantly over time irrespective of rotenone treatment status ($p < 0.001$) and between the treated and untreated river ($p < 0.001$). However, the difference was nonsignificant between the two rivers before the rotenone treatments ($p = 0.333$). The interaction between treatment (treated and untreated) and time (before and after the first river treatment) were nonsignificant ($p = 0.125$). However, when testing for effects of the lake treatment, this interaction was significant ($p < 0.001$). Overview of the full test results are given in Appendices S7–S8 and S10.

3.2 | Lakes

Temporal species turnover appeared to co-vary in the treated and untreated lakes (Figure 4). The untreated lake tended to have a

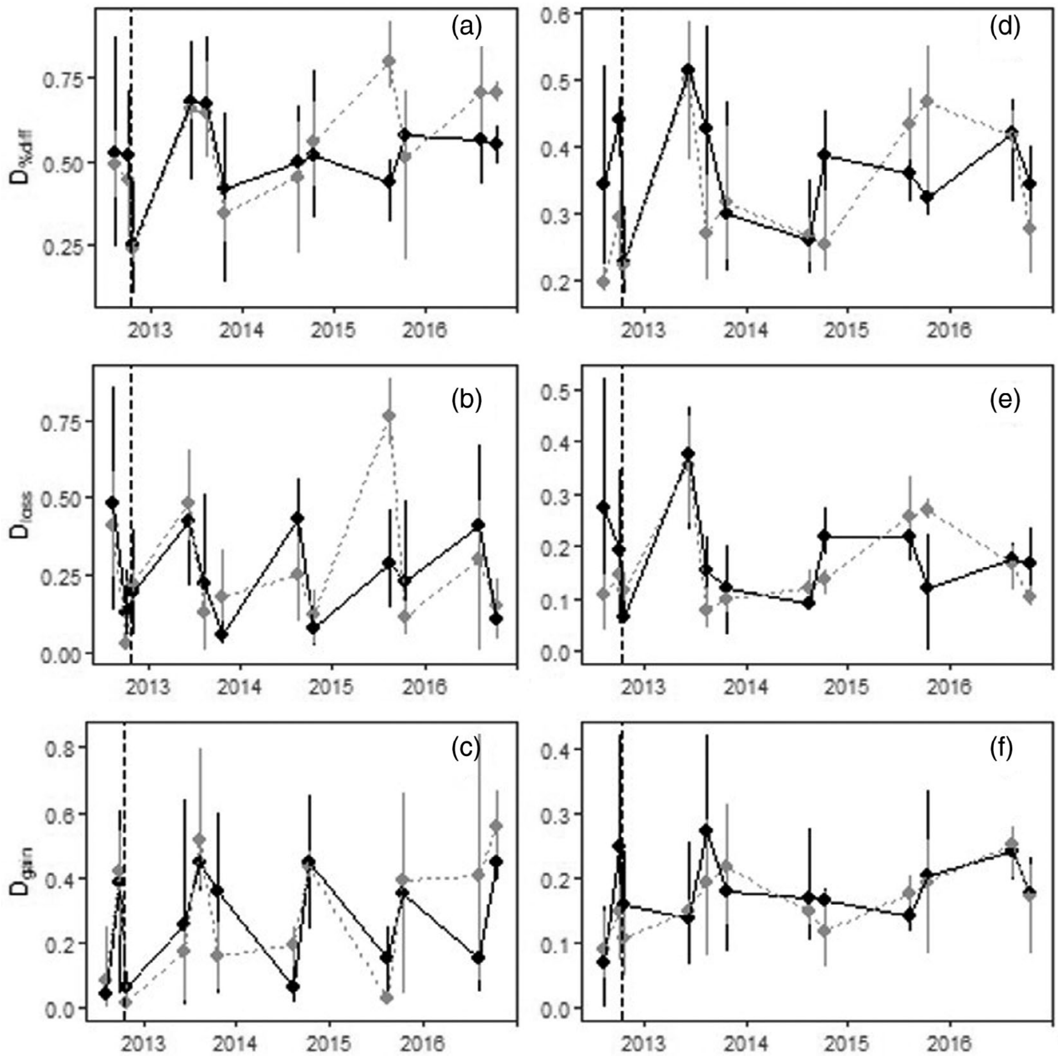

FIGURE 4 Lakes: Mean changes in dissimilarity (% difference) for abundance data (a–c) and for presence–absence data (d–f) between sampling occasions (a and d) and its components; losses (b and e) and gains (c and f). Grey: Lake Fustvatnet (rotenone treated), black: Lake Drevvatnet (untreated). Dashed vertical lines indicate the timing of the rotenone treatment of the lake. Error bars indicate maximum and minimum values of the temporal biodiversity indices at individual sampling stations

greater temporal turnover, both measured as dissimilarity in taxa abundances and species occurrences throughout most of the study period (Figure 4a,d). The highest losses in terms of species abundance occurred in the untreated lake in August 2012 and for the treated lake in August 2015 (Figure 4b). Gains were generally highest for the treated lake (Figure 4c,f), especially for abundance data. No significant changes in abundance or species occurrence indices between sampling periods were found in the treated or untreated lake (Appendices S4 and S5).

The total abundance of benthic invertebrates in the treated and untreated lake was similar or higher in the treated lake on most sampling occasions, both before and after the rotenone treatment (Figure 5a). However, abundances of invertebrate groups varied considerably between the two lakes, for example, with dominance of Plecoptera in the untreated lake and Chironomidae in the treated lake (Figures 7c and 5e, respectively).

The total abundances of benthic invertebrates in the lakes showed major variations even before the rotenone treatment, that is, the abundances decreased by about 50% from August 2011 to August 2012 in both lakes (Figure 5a). In October 2012, shortly after the treatment, the total abundance in both lakes decreased but stronger in the rotenone treated Lake Fustvatnet. In June 2013, 8 months after the treatment, a minor decrease occurred in both lakes. From August 2013 and onward the variations in abundance differed between the lakes, with higher values in the treated lake in August 2013, August and October 2014, October 2015, and August and October 2016.

The Ephemeroptera abundance varied over time both within and between lakes (Figure 5b). There was a high abundance in both lakes in June 2013, compared to pretreatment abundances mainly caused by the Ephemeroptera *Siphlonurus* sp. Abundances of all taxa from the lakes not presented in the figures are listed in Appendix S6. High abundances were also registered in the treated lake in August 2014

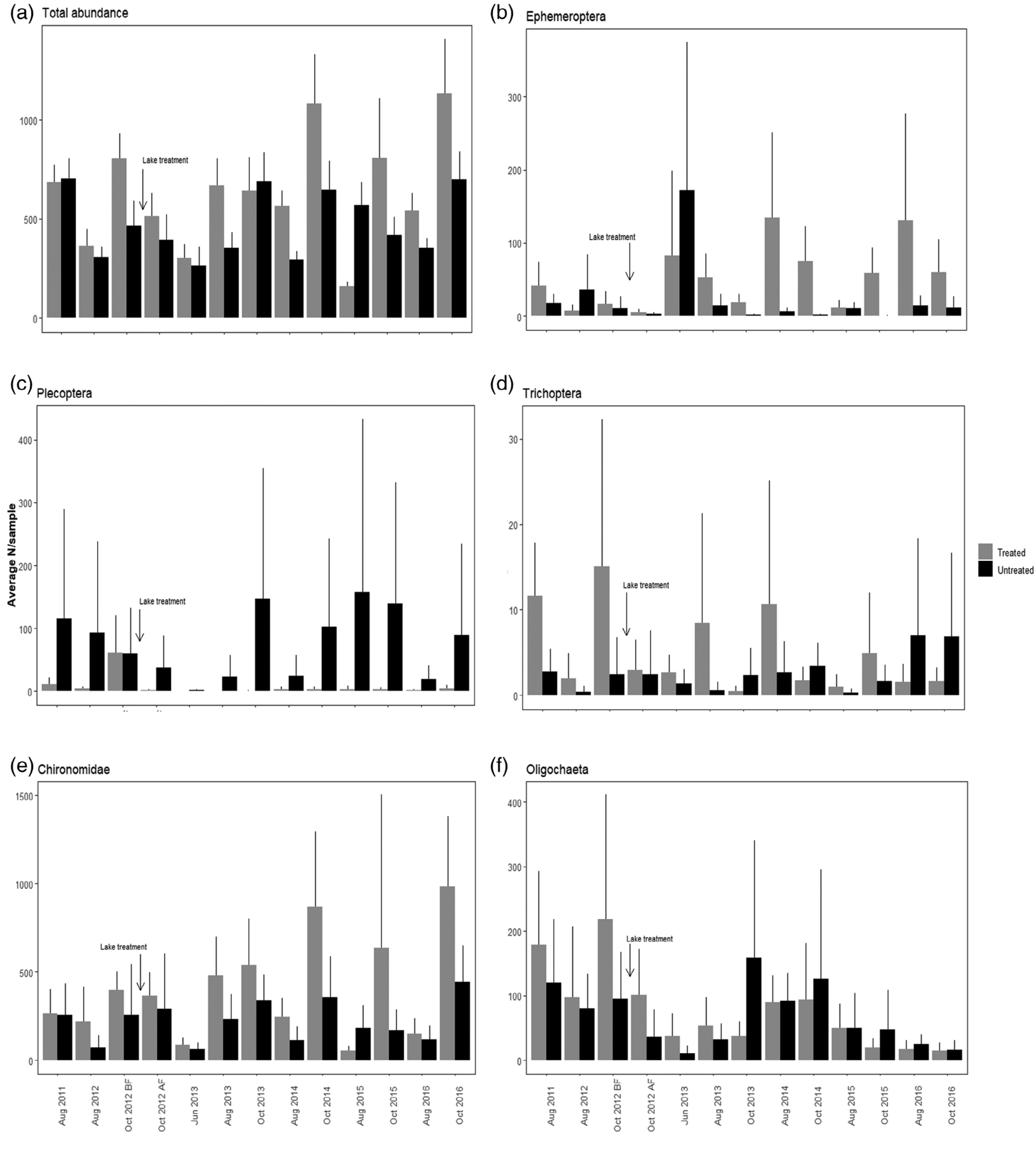

FIGURE 5 Lakes: Average abundance (with *SD*) of benthic invertebrates of Lake Fustvatnet (rotenone treated) and Lake Drevvatnet (untreated) in the period 2011–2016. (a) Total abundance, (b) Ephemeroptera, (c) Plecoptera, (d) Trichoptera, (e) Chironomidae, and (f) Oligochaeta. Timing of the rotenone treatment is marked with an arrow

and August 2016. In October 2012, shortly after the lake treatment the abundances was lower in both lakes, compared to samples taken just before the treatment. The abundance of Ephemeroptera taxa decreased immediately after the lake treatment, especially *Centroptilum luteolum* in the treated lake and Leptophlebiidae in both the treated and untreated lakes. Species with increased abundances in the treated lake after the treatment included *Centroptilum luteolum*, *Caenis horaria*, and *Ephemera vulgata*. However, the abundance of *E. vulgata* decreased strongly in August and October 2015 (73% and 71%, respectively), compared to 2014. Except from *Siphlonurus* sp., there was no marked temporal change in the abundances of Ephemeroptera in the untreated lake.

The abundance of Plecoptera in the treated lake was generally low except in October 2012 just before the rotenone treatment (Figure 5c). In the untreated lake, abundances varied but were generally much higher than in the treated lake. In the treated lake shortly after the treatment in October 2012, there was a strong decrease in Plecoptera abundances (Figure 5c), with only a few specimens of the genus *Nemoura* present. The decline was much smaller in the untreated lake. In June 2013, 8 months after the treatment, no Plecoptera were recorded in the treated lake, and only a few specimens were observed in the untreated lake. The genus *Capnia* had the most marked decrease in abundance in the treated lake following the treatment, whereas the abundance of *Capnia* peaked in some of the years after the treatment in the untreated lake.

For Trichoptera, the abundances in both lakes varied across sampling occasions (Figure 5d). In October 2012, shortly after the treatment, abundances decreased markedly in the treated lake but did not change in the untreated lake. However, a decreased abundance also occurred before the treatment in both lakes between August 2011 and August 2012. The highest abundances in the treated lake after the treatment were recorded in August 2013 and in August 2014. In the untreated lake, the abundances peaked in 2016. Polycentropodidae had the most pronounced decrease among Trichoptera after the treatment in the treated lake, whereas the family increased slightly during the same period in the untreated lake. A few Trichoptera taxa in the treated lake, all found at low abundances, were only recorded before or after the treatment.

The abundance of Chironomidae showed only minor changes after the treatment in both lakes (Figure 5e). In subsequent years, the abundances of Chironomidae in the treated lake varied compared to pretreatment levels, whereas it did not change or were higher in the untreated lake.

The abundance of Oligochaeta (Figure 5f) and Hydrachnidia generally varied to a similar extent in both lakes. For Corixidae and Coleoptera, and especially of the genus *Callicorixa* and dytiscid beetles, the abundances increased in the treated Lake Fustvatnet after the treatment. No Corixidae was recorded in untreated Lake Drevvatnet, while Dytiscidae occurred sporadically.

Permutation tests showed that the taxa composition expressed as Bray–Curtis dissimilarity, differed significantly over time irrespective of rotenone treatment status ($p < 0.001$) and between the treated and untreated lake ($p < 0.001$). However, the difference was nonsignificant between the two lakes before the rotenone treatment ($p = 0.255$) or over time before the treatment ($p = 0.120$). Additionally, there was no significant difference in taxa composition when testing the interaction between treatment and time (before and after the lake treatment) ($p = 0.371$). Overview of the full test results are given in Appendices S9 and S11.

4 | DISCUSSION

The immediate impact of rotenone treatments in August 2011 and 2012 was minor for benthic invertebrates in the rotenone-treated River Fusta. Only a few taxa showed a marked decrease in abundance in the treated river compared to the untreated River Drevjaelva, including the Ephemeroptera *Baetis rhodani* and the Plecoptera genera *Diura* and *Isoperla*. Because the upstream Lake Fustvatnet was treated 2 months after the last river treatment, River Fusta also received rotenone from the lake. Still, the immediate effect of the lake rotenone treatment on benthic invertebrate community in the downstream river was minor. One exception was the Trichoptera *Rhyacophila nubila*, which occurred at high abundances in both rivers, but which disappeared from the River Fusta subsequently to the treatment of the upstream lake. All the above-mentioned taxa are known to be rotenone sensitive (Arnekleiv et al., 2001; Engstrom-Heg, Colesante, & Silco, 1978; Gladsø & Raddum, 2002), and negative impacts were expected.

The rotenone treatment of Lake Fustvatnet took place in October, just before the lake ice formed. Breakdown of rotenone was thus slowed down due to low temperatures and limited light. Consequently, the treated river was exposed to rotenone from the lake for several months. Even though the toxic effect of rotenone decreases with decreasing water temperatures (Kjærstad, & Arnekleiv, & Speed, J.D.M., 2015; Meadows, 1973), the long exposure apparently had a pronounced negative effect. In June 2013, 8 months after the lake treatment, we recorded the lowest abundance of benthic invertebrates in the treated river. A similar decrease did not occur in the untreated river. The benthic invertebrate community in the treated river at this time was dominated by Chironomidae, Oligochaeta, Nematoda, and elmid beetles, the latter group adversely affected known to be rotenone tolerant (Kjærstad & Arnekleiv, 2011). Oligochaeta are reported to be sensitive (Arnekleiv et al., 1997; Mangum & Madrigal, 1999) or tolerant to rotenone (Fjellheim 2004, Kjærstad, & Arnekleiv, & Speed, J.D.M., 2015, Pham et al., 2018), suggesting that tolerance to rotenone is taxa specific within oligochaetes. Other taxa occurred at very low abundances, and several taxa were missing, including rotenone-tolerant species like the Ephemeroptera *Ephemerella* (Engstrom-Heg et al., 1978). Moreover, despite that the freshwater pearl mussel (*Margaritifera margaritifera*) is known to be rotenone tolerant (Dolmen, Arnekleiv, & Haukebø, 1995), all known specimens in the treated river died during the spring of 2013 (Larsen, 2015). Permutations tests showed a significant interaction effect of taxa composition (Bray–Curtis dissimilarity) between treatment and time (before versus after the lake treatment) for the rivers. The temporal beta diversity confirms that the changes in taxa composition were by far highest, and significant, in the treated river from October 2012 to June 2013. This decline in benthic invertebrates was mainly due to losses both in terms of taxa abundances and presence–absence. This indicates that rivers may receive rotenone for several months following treatment of upstream lakes, which can have major impacts. Most taxa in the treated river, including rotenone-tolerant taxa, decreased to very low abundances and some disappeared, like the freshwater pearl mussel.

Despite the strong negative impacts recorded in the treated river 8 months after the lake rotenone treatment, recolonization was relatively fast. The highest gains in terms of species occurrence in the treated river occurred in August 2013. Already in August 2014, the total abundances exceeded pretreatment levels. In August and October 2016, the change in taxa composition, in terms of abundance and presence–absence of taxa between sampling occasions, were rather similar for the treated and the untreated river. This indicates that the benthic fauna of the treated river had recovered to a great extent. Several taxa, like the Ephemeroptera *Heptagenia dalecarlica* and *Ephemerella*, the Plecoptera *Isoperla* and *Amphinemura borealis* and Oligochaeta reached much higher abundances in 2013, as compared to pretreatment levels. Ephemeroptera and Plecoptera are known to be among the most abundant groups in the drift of swift-flowing temperate streams (Brittain & Eikeland, 1988). These groups likely colonized rapidly from drift in untreated tributary streams, although some may have survived as eggs. Oligochaeta may have avoided rotenone exposure by digging deeper into the substratum. However, it took almost 4 years after the last rotenone treatment before most of the taxa had recolonized. The Plecoptera *Taeniopteryx nebulosa* were among the slowest to recolonize and appeared in 2016. Also, two rotenone tolerant taxa, the Ephemeroptera *Ephemerella* and elmid beetles, did not reached pretreatment abundances in the treated river 4 years after the treatments.

According to Vinson et al. (2010), the overall invertebrate abundances generally return to pretreatment levels quicker than the biodiversity and taxonomic composition measures. This is in accordance with our findings. However, the recolonization will greatly depend on the taxonomic resolution of the data. Biodiversity measured at family and genera level will recolonize fast, while it may take longer for all species to recolonize. We have identified some taxa to species level, such as Ephemeroptera, Plecoptera, and Trichoptera, and some taxa to a higher taxonomic level, such as the specious Chironomidae and Hydrachnidia. Thus, changes in abundance and recolonization patterns of many species in this study remain unknown.

A reduction of total benthic invertebrate abundance was observed both in the treated lake and untreated lake immediately after the lake treatment, but to a higher extent in the treated lake. This indicates both natural changes and a negative effect of rotenone. Taxa with clearly decreasing abundances shortly after the treatment in Lake Fustvatnet, but not in the untreated Lake Drevvatnet, included the Ephemeroptera *Centroptilum luteolum* and the Plecoptera *Capnia* sp. While *Capnia* is known to be rotenone sensitive, *C. luteolum* is quite tolerant (Kjærstad & Arnekleiv, 2011). The reason for the seemingly high sensitivity of *C. luteolum* to rotenone in the treated lake is unknown.

The recolonization in terms of abundance of benthic invertebrates in the treated lake was fast, reaching pretreatment levels 8 months after the treatment. A few increased in abundance after the treatment in the treated lake but not in the untreated lake. This was particularly evident for the diving beetles (Dytiscidae). Beetles are generally known to be very rotenone tolerant (Engstrom-Heg et al., 1978). Some will also benefit from increased prey availability because of the general increase in invertebrate abundance after the recolonization. Even though most taxa increased in abundance during the years following the treatments, the Plecoptera *Capnia*, the Trichoptera Polycentropodidae, and the Gastropoda *Gyraulus acronicus* decreased and had not reached their pretreatment levels in the treated lake 4 years after the treatment. Considering that Gastropoda are rotenone tolerant (Arnekleiv et al., 1997; Holocombe et al., 1987), the decline of *G. acronicus* in the treated lake was surprising. Mangun & Madrigal (1999) found that of several taxa missing 5 years after rotenone treatments, most belonged to rotenone sensitive groups, like Ephemeroptera, Plecoptera, and Trichoptera. Taxa that were tolerant to rotenone were identified as "nonmissing" or "briefly missing" (Mangum & Madrigal, 1999).

Fish may play an important role in structuring benthic invertebrate communities (e.g., Alexiades & Kraft, 2017; Weyl, de Moor, Hill, & Weyl, 2010). Stocking of Atlantic salmon (*S. salar*) took place in the treated river between 2013 and 2016, whereas no fish was stocked in the untreated river. Despite fish removal, the abundance of most benthic taxa in the treated river did not increase after the river treatments compared to pretreatment abundances, indicating that the benthic community not yet was re-established. Total abundances strongly increased immediately after the lake treatment, mainly due to an increase in Chironomidae. This occurred in both rivers, suggesting natural variation rather than absence of fish. After fish stocking from 2013 and onward, abundances generally increased in the treated and untreated rivers, and consequently fish stocking had a limited effect on the abundances of benthic invertebrates. However, for Trichoptera, which mostly constituted of large-bodied taxa like *Arctopsyche ladogensis*, Hydropsychidae, and *Rhyacophila nubila*, the abundances of the treated river did not reach pretreatment levels until 2015, probably due to fish predation.

The total invertebrate abundances of the treated and untreated lakes covaried, except for August 2015 when the total abundances decreased markedly in the treated lake. This was most likely due to predation from fish stocked during the previous year. The decrease was particularly high for Chironomidae and Ephemeroptera, including the large-sized and important fish prey, *Ephemera vulgata*. Contrary to the untreated lake, the abundances during some years after the treatment in the treated lake were higher than pretreatment levels, indicating that predation pressure from fish was lower after than before the treatment. The change in invertebrate abundances was more prominent in the impacted treated localities than in the untreated localities. This illustrates the importance of using control sites when performing time-series experiments.

It is striking to observe the large fluctuations in the abundance and in the presence–absence of benthic invertebrates over time of both treated and untreated localities. Even before the lake treatment, the invertebrate abundances in both lakes were reduced by approximately 50% from August 2011 to August 2012. According to Vinson et al. (2010), the accumulation curve for benthic invertebrate genera showed little inclination for flattening out during a 10-year monthly sampling of a Utah river. We did not see the same pattern in our data, perhaps due to fewer taxa present and low taxonomic resolution for several species-rich groups. Even though it will vary greatly with habitat type, latitude, etc., a rising taxa accumulating curve would certainly be found elsewhere and shows how random the community composition may be registered at any given time.

Our study found minor short-term (i.e., <1 month) effects of the river rotenone treatments, whereas the lake treatment caused major temporary negative effects on the lotic fauna 8 months after the treatment. This is likely caused by leaking of rotenone to the lotic system during the subsequent winter and spring. Accordingly, long-term low-dose exposure seem to have higher negative effects than high-dose short-term exposure, suggesting that it is important to minimize exposure time. For example, this could likely be accommodated by choosing an earlier date for the lake treatment in the River Fusta. No major short- or long-term (i.e., 4 years) effects on lake benthic invertebrate communities where detected. Recovery time of invertebrate abundance after the lake treatment was fast whereas the recovery in terms of presence–absence of species were comparably much longer, lasting for several years. The conclusion of this study would not have been possible with short-term monitoring without controls. The current study underpins the crucial importance of performing long-terms studies with before-after-control-impact during investigation of the effects of rotenone on benthic invertebrates.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Figshare at https://doi.org/10.6084/m9.figshare.16791889.v1.

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