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## Impacts of piscicide-induced fish removal on resource use and trophic diversity of lake invertebrates

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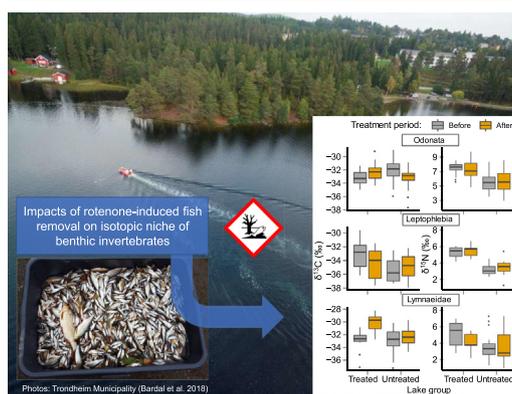
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### HIGHLIGHTS

- Lake benthic invertebrate taxa showed contrasting responses to rotenone treatment.
- Predatory invertebrates increased while grazers and collectors decreased in abundance.
- A partial niche expansion by benthic invertebrates in two out of three treated lakes.
- Holistic understanding of ecosystem impacts of chemical treatments are urgently needed.

### GRAPHICAL ABSTRACT



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### ABSTRACT

Chemical eradication of non-native species has become a widely used method to mitigate the potential negative impacts of altered competitive or predatory dynamics on biodiversity and natural ecosystem processes. However, the responses of non-target species can vary from rapid full recovery to delayed or absent recolonization, and little is known about the potential shifts in resource use and trophic diversity of native species following chemical treatments. We used a before-after-control-impact approach to study the effects of rotenone piscicide treatment on abundance and trophic niche of benthic invertebrates in three untreated and three treated lakes in central Norway, the latter group hosting non-native roach (*Rutilus rutilus*) and pike (*Esox lucius*) prior to rotenone treatment. Based on community composition data, the relative abundance of invertebrate grazers and collectors decreased while that of predators increased following fish removal in the treated lakes. The stable isotope data indicated minor shifts in resource use of, and trophic diversity among, benthic invertebrate communities. While the predatory dragonfly larvae (Odonata) and grazer snails (Lymnaeidae) showed increased  $\delta^{13}\text{C}$  values indicating increased reliance on littoral benthic algae, the collector mayfly larvae (*Leptophlebia*) showed decreased  $\delta^{13}\text{C}$  values following fish removal in treated lakes. Grazer snails also showed a shift to a lower trophic position, while the predatory dragonflies and collector mayflies showed no changes in  $\delta^{15}\text{N}$  values following fish removal. The community-level isotopic niches of benthic invertebrates showed no consistent changes, although the sample-size corrected and Bayesian estimates of standard ellipse areas ( $SEA_C$  and  $SEA_B$ )

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slightly increased in two of the three treated lakes due to an increased range in  $\delta^{15}\text{N}$ . In conclusion, our study findings indicate some changes in species assemblages but minor shifts in the resource use and trophic diversity of benthic invertebrate communities following fish removal in rotenone treated lakes.

## 1. Introduction

Freshwaters are considered among the most threatened ecosystems in the world due to the direct and indirect negative impacts of human activities ranging from local to global stressors (Carpenter et al., 2011; Reid et al., 2019; Albert et al., 2021). One of the major threats to freshwater biodiversity loss is the human-induced spread of non-native species (Rahel, 2002; Reid et al., 2019). Measures that are taken to reduce or eliminate the spread of non-native species include: increasing the public awareness of negative impacts, early detection monitoring, new legislation, and emergency funding supporting rapid-response activities, various eradication barriers, and chemical treatment of invaded waterbodies (Lodge et al., 2006; Vander Zanden and Olden, 2008; Jones et al., 2021). Chemical treatment for eradication is often the selected solution when a non-native species has established in a recipient freshwater ecosystem (Rytwinski et al., 2018). A widely used eradication method is rotenone, which is a natural plant-derived compound but a non-selective toxin that also affects non-target organisms, including native invertebrates that can be critical to local ecosystem structure and function (Relyea and Hoverman, 2006; Donnelly, 2018). While the short-term impacts of rotenone treatment on species' assemblages and abundances are well-documented (Vinson et al., 2010 and references therein), the potential impacts on food-web dynamics and resource use of native freshwater consumers are poorly documented.

Rotenone is the piscicide most often used to eradicate invasive, unwanted fish species in lakes and rivers (Vinson et al., 2010; Woodford et al., 2013; Beaulieu et al., 2021). Previous studies have indicated rapid declines in several taxonomic groups soon after rotenone treatment, followed by partial or full recovery of invertebrate communities typically within one year (Vinson et al., 2010; Woods et al., 2016; Beaulieu et al., 2021). Zooplankton and gill-breathing benthic invertebrates (e.g., Ephemeroptera, Plecoptera and Trichoptera; hereafter EPT) are considered among the most sensitive taxa, whereas hard-bodied mollusks and large dragonfly larvae seem to be more tolerant (Melaas et al., 2001; Kjørstad and Arnekleiv, 2011; Woodford et al., 2013; Beaulieu et al., 2021). The rapid recovery of invertebrate communities seems to be related to high recolonization rates from connected, untreated waterbodies (Woods et al., 2016; Pham et al., 2018). In contrast, stronger negative impacts with delayed recolonization rates may occur in isolated cold-water systems such as mountain lakes, where the lack of upstream waterbodies and the short ice-free period reduce dispersal probabilities (Beaulieu et al., 2021) and the low water temperature prolongs the half-life of rotenone (Gilderhus et al., 1986). The negative short- and long-term impacts on non-target organisms can be lessened with careful planning regarding the concentration, duration, and selection of sites for rotenone treatments, or by using alternative, less lethal piscicides such as antimycin (Hamilton et al., 2009; Finlayson et al., 2010; Vinson et al., 2010). Nevertheless, improved understanding of the toxic and trophic effects of the selected piscicides are needed to avoid offsetting the benefits of non-native fish removal with significant damage to the non-target aquatic and riparian communities (Donnelly, 2018). Moreover, studies of rotenone impacts on resource use of different functional feeding groups and on community-level trophic diversity are lacking despite the known importance of ecological networks for ecosystem function, resilience, and services (Thompson et al., 2012; Harvey et al., 2017; Kovalenko, 2019).

In Norway, rotenone has been used for decades to eradicate non-native salmon fluke (*Gyrodactylus salaris*) ectoparasites in rivers where the socio-economically high value Atlantic salmon (*Salmo salar*) stocks have declined (Kjørstad and Arnekleiv, 2011; Kjørstad et al., 2016; Bardal, 2018). Recent studies of rotenone effects on Norwegian riverine ecosystems indicate marked declines of certain EPT species (e.g., *Baetis rhodani*, *Diura nanseni* and *Rhyacophila nubila*), whereas other species of Ephemeroptera

(e.g., *Ephemerella*), Trichoptera (e.g., Limnephilidae) and Coleoptera (elmid beetles) seem to be more tolerant (Kjørstad and Arnekleiv, 2011). Rotenone has also been used in Norwegian lakes to eradicate unwanted, non-native fish species such as roach (*Rutilus rutilus*), pike (*Esox lucius*) and European minnow (*Phoxinus phoxinus*) (Bardal, 2018; Sandodden et al., 2022). Roach, which was introduced to central Norway in the 1880s (Huitfeldt-Kaas, 1918), has been shown to have negative competitive impacts on native salmonid populations in Norway (Langeland and Nøst, 1994) and elsewhere (Winfield et al., 2008; Corrigan et al., 2011). In contrast to roach, non-native pike can have direct predatory impacts on native salmonid species, with potential cascading effects on invertebrates and food webs especially in shallow waterbodies (Byström et al., 2007; Haught and von Hippel, 2011; Sepulveda et al., 2015).

Fish often play an important role in lake food webs by feeding selectively on large, slow-moving and/or highly nutritious prey items, leading to shifts in the size structure and dominance hierarchies of invertebrate communities (Jeppesen et al., 2010, 2017; Ruppert et al., 2017). In addition to the direct impacts on abundance and community composition, fish predation risk may have indirect impacts by restricting the habitat and resource use of invertebrates (Bernot and Turner, 2001). Therefore, rotenone-induced removal of fish and sensitive invertebrate taxa has the potential to restructure freshwater food webs by altering direct and indirect competitive and predatory interactions (e.g., Power, 1990; Donnelly, 2018). Despite the lack of existing research focusing on rotenone impacts on freshwater food webs, the observed early-phase shifts in community assembly and the absence of fish predation pressure can be expected to provide previously inaccessible food and habitat resources to benthic invertebrates. This, in turn, may lead to a community-level niche expansion whereby predatory invertebrates might partly take over the trophic position of removed fishes as top predators.

Stable isotope analysis has become a popular method to elucidate the structure and function of aquatic food webs and the responses of biotic communities to human stressors (Layman et al., 2012; Glibert et al., 2019). The ratios of stable carbon ( $^{13}\text{C}$ : $^{12}\text{C}$ ; hereafter denoted as  $\delta^{13}\text{C}$ ) and nitrogen ( $^{15}\text{N}$ : $^{14}\text{N}$ ; hereafter  $\delta^{15}\text{N}$ ) isotopes are often utilized to evaluate consumers' reliance on littoral benthic versus pelagic planktonic carbon sources and their relative trophic position in the food web (Layman et al., 2012; Glibert et al., 2019). In addition, stable isotopes are frequently used to quantify population- and community-level niche width or niche overlap (Layman et al., 2007, 2012; Jackson et al., 2011). Hence, stable isotope analysis is a potentially powerful tool for revealing the impacts of rotenone-induced fish removal on the structure and function of treated aquatic ecosystems, including changes in the littoral–pelagic resource use or community-level trophic diversity of benthic macroinvertebrates. However, to the best of our knowledge, no previous stable isotope studies have investigated rotenone-treatment impacts on trophic dynamics within freshwater aquatic communities.

Here we utilized a before-after-control-impact design (BACI; Smith, 2014) to study the impacts of rotenone treatment on the resource use and trophic diversity of lake benthic invertebrate communities. Specifically, we used benthic invertebrate abundance and stable isotope data collected one year before and after rotenone treatment in three treated lakes and compared the results with data from three untreated control lakes. All treated lakes hosted non-native roach and pike populations prior to rotenone treatments in 2016. While the high-resolution community composition data reflects the relative abundance of different functional feeding groups, the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of benthic invertebrates were expected to reveal rotenone-induced fish removal impacts on the resource use and community-level trophic diversity (i.e., isotopic niche area occupied by benthic invertebrates in  $\delta^{13}\text{C}$ – $\delta^{15}\text{N}$  bi-plot space; Layman et al., 2007; Jackson et al., 2011). Our BACI design allowed us to test the following

**Table 1**

Abiotic characteristics of the six study lakes. Water samples were taken in July 2019 at the surface (<1 m) over the deepest part of the lake and analyzed in an accredited laboratory (Analysesenteret, Trondheim, Norway). See Bardal et al. (2018; Table 7) for water quality data from the treated lakes in August 2015 and 2017. Abbreviations for observed fish species (prior to rotenone application in treated lakes): RO = roach, PI = pike, BT = brown trout, SB = three-spined stickleback, AC = Arctic charr, WF = whitefish, EE = European eel. † The species has disappeared after the roach introduction.

Lake group	Altitude (m a.s.l.)	Surface area (ha)	Maximum depth (m)	Color (mg Pt L <sup>-1</sup> )	Tot. nitrogen (μg L <sup>-1</sup> )	Tot. phosphorus (μg L <sup>-1</sup> )	Rotenone applied (L)	Initial CFT-Legumine concentr. (mean ± SD ppm)	Fish species
<b>Treated</b>									
Lianvatnet	222	11.1	15	21	230	6.7	563.5	1.23 ± 0.71	BT, RO, PI, WF <sup>†</sup>
Theisendammen	156	8.7	9	41	230	7.9	530.5	1.42 ± 0.59	BT, RO, PI, AC, SB
Haukvatnet	189	10.2	16	21	190	4.3	833	1.17 ± 0.39	BT, RO, PI, WF <sup>†</sup> , EE
<b>Untreated</b>									
Holstdammen	204	4.4	14	37	270	5	0	0 ± 0	BT, SB
Store-Leirsjøen	196	29	27	35	170	2.9	0	0 ± 0	BT, SB, AC
Lauglovatnet	185	8.8	15	70	240	5.8	0	0 ± 0	BT, SB

hypotheses: (1) The community composition would change following fish removal, with large predatory invertebrates becoming more abundant. (2) At the taxon level, invertebrates would shift their trophic niche following removal of fish predation pressure, with the responses depending on the functional feeding group or initial degree of fish predation pressure on the taxon. (3) At the community level, trophic diversity would increase as invertebrate taxa utilize new resources, including prey resources at higher trophic levels that were inaccessible to invertebrates prior to fish removal due to direct and indirect predatory effects.

## 2. Materials and methods

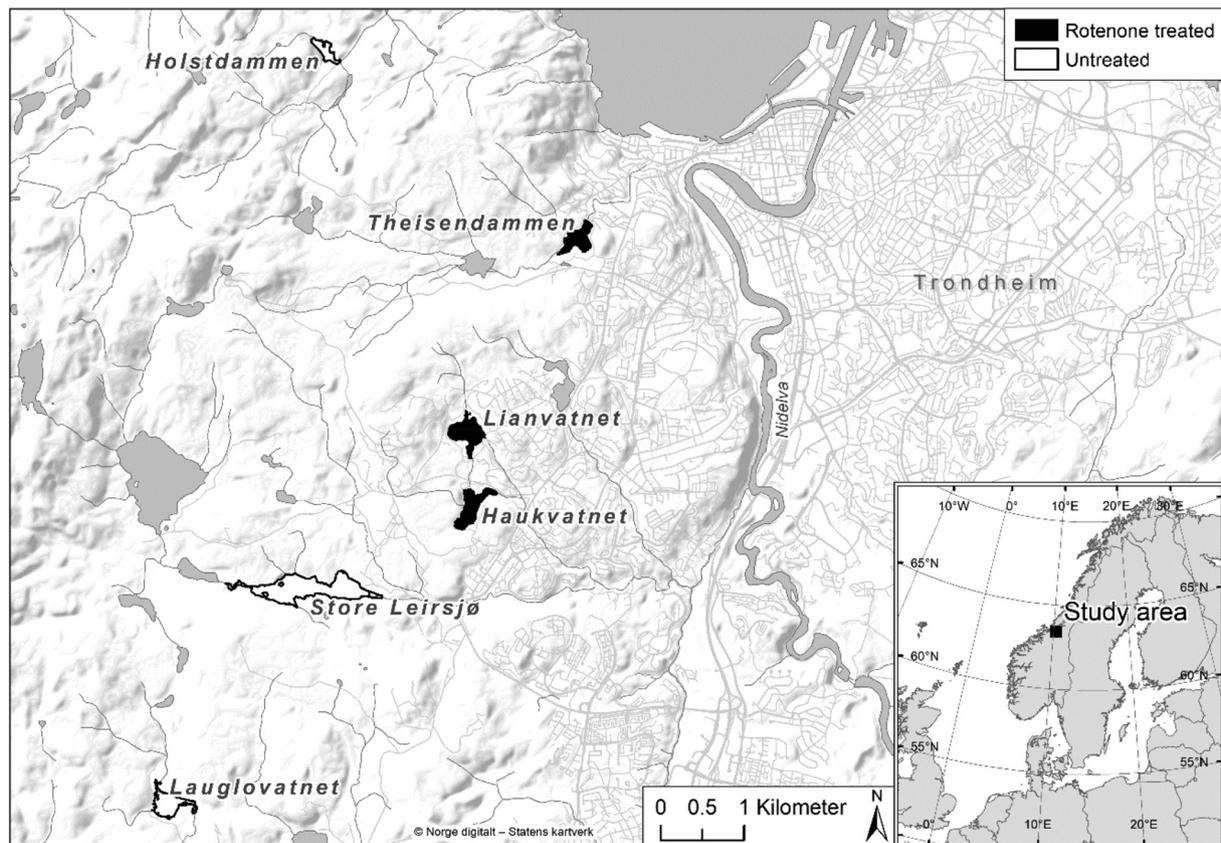
### 2.1. Study lakes and rotenone treatments

The six study lakes, including the rotenone-treated Lianvatnet, Haukvatnet and Theisendammen and the untreated control lakes Holstdammen, Store-

Leirsjøen and Lauglovatnet (Table 1), are situated in the Bymarka nature park, close to the city of Trondheim, in central Norway (Fig. 1). The Bymarka nature park is a very popular area for recreational activities such as fishing, cross-country skiing, hiking, and mountain biking.

Rotenone treatments in Lianvatnet, Haukvatnet and Theisendammen were conducted by the Norwegian Veterinary Institute in September 2016 to remove roach and pike. Both are non-native fish species in the Trondheim area and have had negative effects on the native brown trout that were restocked after the rotenone treatments (Hesthagen et al., 2020; Sandodden et al., 2022). All six study lakes are relatively small (surface area = 4.4–11.1 ha), shallow (maximum depth = 9–27 m), oligotrophic (total phosphorus = 2.9–7.9 μg L<sup>-1</sup>), surrounded by mainly coniferous forests and thus have relatively comparable abiotic characteristics, although the treated lakes are closer to populated areas (Table 1, Fig. 1).

Brown trout used to be the most abundant and valued fish species in all six study lakes. Brown trout is still the dominant fish species coexisting with



**Fig. 1.** Map of the study region showing the location of the three rotenone treated (black) and three untreated (white) lakes in the Bymarka nature park on the western side of Trondheim city in central Norway.

three-spined stickleback in the untreated Holstdammen and Laugvatnet, and also with Arctic charr in Store-Leirsjøen (Table 1). In contrast, roach and pike, which were introduced several decades ago (Hesthagen et al., 2020; Sandodden et al., 2022), dominated the fish communities in the treated lakes (Bardal et al., 2018) but do not occur in the untreated control lakes. European whitefish (*Coregonus lavaretus*) were present in Lianvatnet and Haukvatnet in the 1970s, but probably disappeared a few years after the roach introduction. In addition to salmonids and sticklebacks, a single European eel (*Anguilla anguilla*) of 0.5 kg was recorded in Haukvatnet while collecting dead fish after the rotenone treatment in 2016 (Bardal et al., 2018). European crayfish (*Astacus astacus*) was also present in Lianvatnet and Haukvatnet before and after the rotenone treatment. Eurasian beaver (*Castor fiber*) was reintroduced to Theisendammen in 1975 and has since spread to nearby lakes (Halley and Rosell, 2002), including all the present study lakes.

Rotenone treatments were conducted between 19 and 22 September 2016. A total of 1927 L of the rotenone solution CFT-Legumine piscicide were applied in Lianvatnet, Theisendammen, Haukvatnet and their small tributaries (Table 1) (Bardal, 2018; Sandodden et al., 2022). Immediately after the treatment, the concentrations of CFT-Legumine were on average 1.2–1.4 ppm (Table 1), and the concentrations subsequently decreased to non-detectable values by February 2017, i.e., five months after the treatment (Bardal et al., 2018; Sandodden et al., 2022). Therefore, potential changes in the  $\delta^{13}\text{C}$  values of organisms collected during the following open-water season is expected to reflect changes in their resource use and trophic diversity and not per se the carbon compound in rotenone (formula  $\text{C}_{23}\text{H}_{22}\text{O}_6$ ), particularly as elimination from organism whole body tissues has been shown to be quick (Gingerich, 1986) and non-accumulative (Vasquez et al., 2012).

## 2.2. Sample collection

We collected semiquantitative samples of benthic invertebrates (i.e., total counts of different taxa) and qualitative stable isotope samples of different functional feeding groups to evaluate rotenone-treatment impacts on community composition and resource use of lake benthic invertebrates, respectively, following a BACI design (Smith, 2014) in three treated and three untreated control lakes. Littoral benthic macroinvertebrates and pelagic zooplankton were collected from each study lake in early June and at the end of July and October in 2015 and 2017, i.e., one year before and after the rotenone treatment in September 2016. For community composition analyses (hereafter CCA; Fig. S1, Appendix A and B), benthic invertebrates were collected from the shallow littoral areas using a 250- $\mu\text{m}$  mesh kick net, with three replicate samples taken at each station. To reflect the habitat variation in each lake, three stations were sampled before and after the rotenone treatment: two stations located in sheltered areas with aquatic vegetation and a soft bottom substrate, and the third station located in a wind exposed area with a gravel substrate and little or no aquatic vegetation. The samples were collected following a z-sweep method: the kick net was hauled swiftly 2–3 cm over the bottom in three sweeps (forward, back, and forward again) for 1 s and 1 m each way (sweep) at <1 m depth approximately 1.5 m offshore. Samples for CCA were stored in 96% ethanol for further laboratory processing.

The benthic invertebrates were identified to the lowest possible taxonomic level and counted (Appendix B), using a stereo microscope and the following identification keys: Engblom (1996) for Ephemeroptera, Lillehammer (1988) for Plecoptera, Rinne and Wiberg-Larsen (2017) for Trichoptera, Norling and Sahlén (1997) for Odonata, Jansson (1996) for Heteroptera, Holmen (1987) and Nilsson and Holmen (1995) for Coleoptera, Elliott et al. (2015) for Hirudinea, and Glöer (2002) for Gastropoda. The full CCA dataset included 4085 observations (i.e., individual counts) belonging to 142 taxa, with the rank ranging from species to class level (Appendix A and B).

Samples of basal resources, benthic and pelagic invertebrates, crayfish, and fish were collected for stable carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) isotope analyses (hereafter SIA) to study the overall food-web structure in each

study lake (Fig. S2–S3; Layman et al., 2012). Benthic invertebrates were collected for SIA simultaneously with the sampling for CCA, using a kick net (250- $\mu\text{m}$  mesh) and a bucket sieve (1000- $\mu\text{m}$  mesh). Pelagic zooplankton were collected for SIA by taking several vertical hauls with a 90- $\mu\text{m}$  plankton net through the uppermost 5 m of the water column. All benthic and pelagic invertebrates were sorted, cleaned of detritus and other unwanted material with water, and identified to genus, family or species level using a stereo microscope. Only soft body tissue was dissected from mollusks and trichopterans with cases.

For SIA samples of basal resources, three replicate samples of particulate organic matter (POM, divided to <90- $\mu\text{m}$  and >90- $\mu\text{m}$  size fractions), detritus, periphyton and leaves were collected from the shallow littoral areas (depth < 0.5 m) of each lake. To obtain POM, 10 L of lake water were filtered through a 90- $\mu\text{m}$  mesh in the field, followed by filtration of <90- $\mu\text{m}$  POM on pre-combusted glass-fiber filters and scraping of >90- $\mu\text{m}$  POM from the mesh into Eppendorf tubes in the laboratory. Fallen leaves (mainly birch *Betula* sp., rowan *Sorbus* sp. and some small willow *Salix* sp.) and detritus (including birch seeds, conifer needles, bark and remains of dead roots, grasses, macrophytes and mosses) were collected from <20 cm depth along the lake shorelines. Periphyton were scraped from the surfaces of submerged stones, wood, and plants. Fish were collected with benthic and pelagic Nordic multi-mesh survey gillnets (Appelberg et al., 1995) in October 2015 and crayfish were collected with crayfish funnel traps in June 2015 and 2017. Muscle tissue samples used here for SIA were dissected posterior to the fish dorsal fin and from the crayfish tail. All basal resource, invertebrate, fish and crayfish SIA samples were kept frozen at  $-20\text{ }^\circ\text{C}$  until drying, homogenization and weighing for final SIA.

## 2.3. Stable isotope analyses

All frozen SIA samples were later freeze-dried for 48 h and homogenized using a metallic pestle. Because many invertebrates store energy as glycogen, and variations in the glycogen content can lead to large differences in C:N ratios not necessarily reflective of variation in lipid content (DeNiro and Epstein, 1978; Kiljunen et al., 2006), lipids were not extracted or corrected based on the measured C:N ratios. All SIA were performed at the Environmental Isotope Laboratory, University of Waterloo, on a Delta Plus Continuous Flow Stable Isotope Ratio Mass Spectrometer (Thermo Finnigan, Bremen, Germany) coupled to a 4010 Elemental Analyzer (Costech International S. p. A., Milan Italy). All resulting measurements were expressed using standard delta notation as parts per thousand differences (‰) with respect to the international reference standards, Vienna Pee Dee Belemnite for  $\delta^{13}\text{C}$  (Craig, 1957) and atmospheric nitrogen gas for  $\delta^{15}\text{N}$  (Mariotti, 1983). Analytical accuracy was validated against internal laboratory standards (EIL-72 cellulose and EIL-32 graphite) cross-calibrated against the International Atomic Energy Agency standards CH3 and CH6 for carbon and N1 and N2 for nitrogen. Internal laboratory standards inserted at the beginning, middle, and end of sample runs were used in data normalization to ensure measurement precision and accuracy, with quality control/assurance checks indicating an error for reportable data of no more than 0.2‰ and 0.3‰ for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , respectively.

We used SIA to study the overall food-web structure in each study lake (Fig. S2–S3; Layman et al., 2012). Specifically, we tested the effect of rotenone treatment on (1) resource use of the entire benthic invertebrate community and of different functional feeding groups, and on (2) food-web structure (i.e., “trophic diversity” sensu Layman et al., 2007) using SIA data of benthic invertebrates sampled from the treated and untreated study lakes before and after rotenone treatment. In all SIA computations, only benthic invertebrate taxa with a sample size of  $n \geq 2$  in each sampling event were included to ensure the datasets were comparable across the study lakes and to avoid biases arising from single influential observations. Based on Rosner's test, two outliers with exceptional  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values were omitted from subsequent analyses (a Nematode sample from Haukvatnet in 2017 with  $\delta^{13}\text{C} = -42.7\text{‰}$  and  $\delta^{15}\text{N} = 10.9\text{‰}$ , and a *Caenis* sp. sample from Theisendammen in 2017 with  $\delta^{13}\text{C} = -17.7\text{‰}$  and  $\delta^{15}\text{N} = 11.4\text{‰}$ ). The resulting SIA data included a total of  $n = 479$

samples of the dominant benthic invertebrate taxa and showed normal distribution for both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  (Shapiro-Wilk test  $p > 0.05$ ).

## 2.4. Statistical analyses

To investigate rotenone treatment impacts on relative abundance (CCA) and resource use (SIA) of benthic invertebrates, we used generalized mixed effects models (GLMM) where the log-transformed individual counts and the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of benthic invertebrate taxa were used as the response variables and *Lake group*, *Year*, and a two-way interaction between the two (*Lake group\*Year*) were used as explanatory variables, with *Lake* set as a random factor (1 | *Lake*) to account for temporal replication within each lake. By including the *Lake group\*Year* interactions, we expected significant temporal shifts (*Year*) in CCA and SIA data to occur only in treated lakes (*Lake group*).

For the CCA data, the GLMMs were run separately for the different functional feeding groups, including predators ( $n = 898$  observations), collectors ( $n = 763$ ), grazers ( $n = 450$ ), filter feeders ( $n = 152$ ), and shredders ( $n = 226$ ). For the SIA data, the GLMMs were run separately for (1) the entire benthic invertebrate community ( $n = 479$ ), and (2) for specific taxa belonging to different functional feeding groups observed in the treated and untreated lakes before and after rotenone treatment: predatory Odonata dragonfly larvae ( $n = 140$ ), collector *Leptophlebia* mayfly larvae ( $n = 50$ ), and grazer Lymnaeidae snails ( $n = 59$ ). The GLMMs were run using the lmer function in the lme4 v.1.1-26 package (Bates et al., 2015).

To test for impacts on community-level trophic diversity, we computed Standard Ellipse Areas (SEA) based on the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of the dominant benthic invertebrates ( $n \geq 2$  in each lake and sampling event) sampled from the treated and untreated lakes before and after rotenone treatment. Both sample-size corrected ( $SEA_C$ ) and Bayesian ( $SEA_B$ ) estimates of SEA were computed using the SIBER (Stable Isotope Bayesian Ellipses in R; Jackson et al., 2011) method in the SIAR v.4.2 package (Parnell et al., 2010) in R (R Core Team, 2020). The lake-specific  $SEA_B$  estimates (Fig. 4) and SEA ellipses (Fig. 5) were computed and visualized for the before and after periods to detect statistically significant differences (i.e., non-overlapping 95% credibility intervals of  $SEA_B$ ) and potential changes in the orientation of the community-level isotopic niches, such as changes in  $\delta^{15}\text{N}$  or  $\delta^{13}\text{C}$  range reflecting vertical structure within and niche diversification at the base of a food web, respectively (Layman et al., 2007). All statistical analyses were performed using R computing program v. 4.0.3 (R Core Team, 2020).

## 3. Results

### 3.1. Impacts on community composition

The CCA data indicated consistent declines for EPT taxa (Ephemeroptera, Plecoptera, Trichoptera) and Basommatophora snails, whereas Diptera (mainly Chironomidae larvae), Odonata and Amphipoda showed increased abundances following rotenone application in the treated lakes (Fig. S1, Appendix B). In the untreated lakes, no corresponding changes in benthic invertebrate community composition were observed, with EPT taxa showing increased abundance in some cases (Fig. S1).

The GLMM analyses of CCA data indicated differing responses of the benthic invertebrate functional feeding groups to rotenone treatment. As indicated by significant *Lake group\*Year* interactions, the relative abundances of grazers and collectors decreased following rotenone treatment in treated lakes, whereas no corresponding shifts were observed in the untreated lakes (Table 2, Fig. 2). As indicated by the significant positive main effect of *Year*, predators were more abundant during the post-treatment period (Table 2, Fig. 2). In contrast, *Lake group* and *Year* had no significant main or two-way interaction effects on abundance of filter feeders or shredders.

### 3.2. Food web structures

The stable isotope biplots indicated that fish, crayfish and predatory benthic invertebrates (e.g., Odonata) were typically the top predators

**Table 2**

Generalized mixed effects models predicting relative abundance (log-transformed counts) of different functional groups of benthic invertebrates as a function of *Lake group* (treated versus untreated lakes), *Year* (before versus after rotenone treatment), and their two-way interaction (*Lake group\*Year*). Parameter estimates and their standard errors (SE), t- and p-values, and lower and upper confidence limits (95% CI) are shown, with statistically significant parameters highlighted in bold (i.e., 95% CIs not overlapping zero).

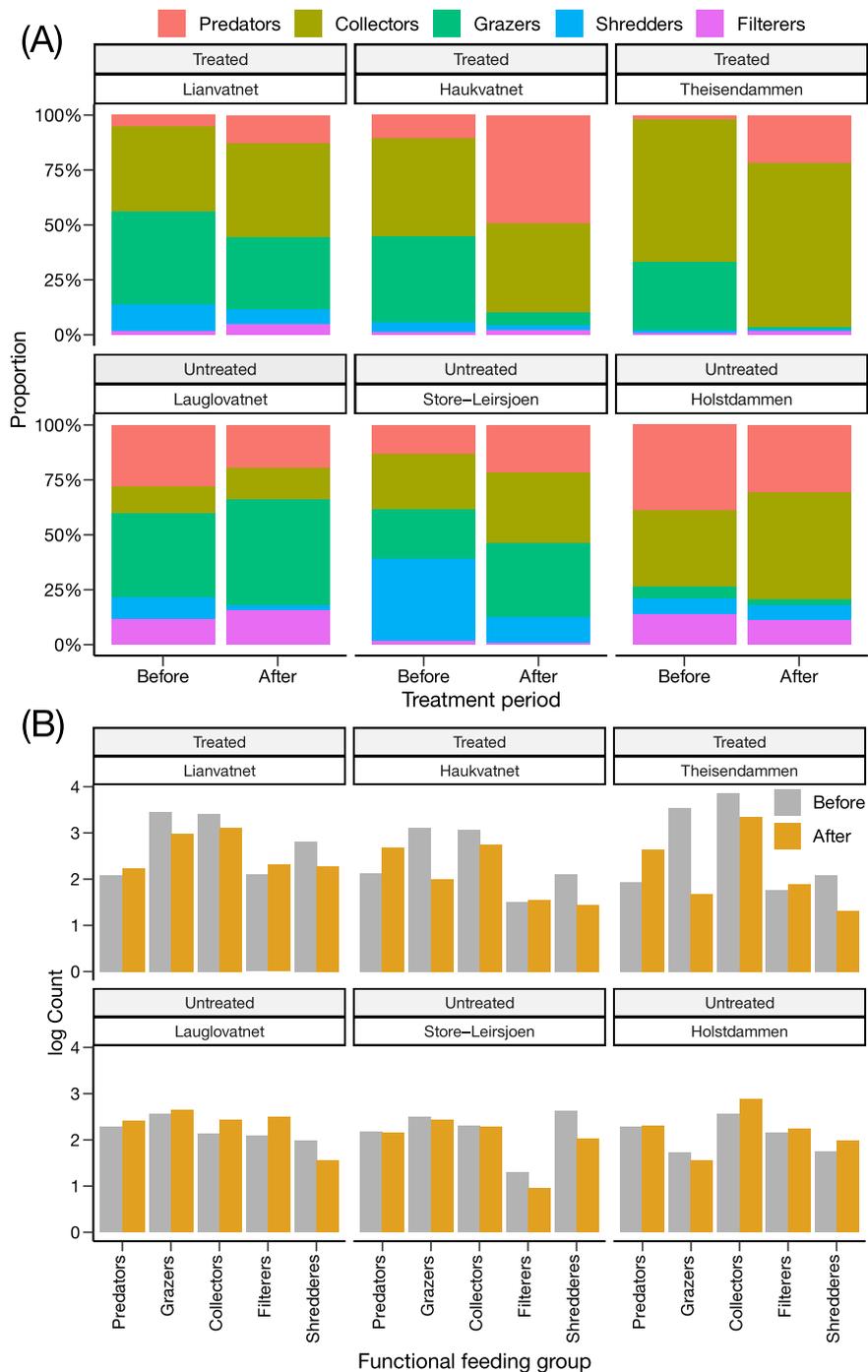
Rotenone impacts in Bymarka lakes						
GLMM results for community composition analyses ("count data"): all count data included ( $n > 0$ ), log10-transformed counts						
Functional feeding group					95% CI	
Predictor	Estimate	SE	t	p	Lower	Upper
<b>Predators</b>						
Intercept	0.36	0.03	10.99	<0.001	<b>0.30</b>	<b>0.43</b>
Lake group	0.01	0.05	0.11	0.913	-0.09	0.10
Year	0.19	0.04	4.40	<0.001	<b>0.11</b>	<b>0.28</b>
Lake group*Year	-0.11	0.07	-1.59	0.113	-0.24	0.03
<b>Collectors</b>						
Intercept	0.85	0.06	13.94	<0.001	<b>0.71</b>	<b>0.98</b>
Lake group	-0.38	0.10	-3.78	<b>0.003</b>	-0.59	-0.16
Year	-0.03	0.06	-0.47	0.642	-0.14	0.09
Lake group*Year	0.28	0.11	2.62	<b>0.009</b>	<b>0.07</b>	<b>0.50</b>
<b>Grazers</b>						
Intercept	1.01	0.10	10.25	<0.001	<b>0.78</b>	<b>1.24</b>
Lake group	-0.56	0.14	-3.91	<b>0.006</b>	-0.89	-0.24
Year	-0.15	0.09	-1.63	0.104	-0.32	0.03
Lake group*Year	0.33	0.13	2.61	<b>0.009</b>	<b>0.08</b>	<b>0.58</b>
<b>Filter feeders</b>						
Intercept	0.56	0.14	3.98	<b>0.004</b>	<b>0.24</b>	<b>0.87</b>
Lake group	0.05	0.20	0.27	0.791	-0.40	0.49
Year	0.11	0.12	0.91	0.365	-0.13	0.34
Lake group*Year	-0.10	0.16	-0.61	0.541	-0.40	0.21
<b>Shredders</b>						
Intercept	0.60	0.09	6.68	<0.001	<b>0.40</b>	<b>0.80</b>
Lake group	0.03	0.14	0.21	0.841	-0.27	0.33
Year	-0.07	0.12	-0.63	0.531	-0.30	0.16
Lake group*Year	-0.05	0.17	-0.31	0.761	-0.38	0.28

(high  $\delta^{15}\text{N}$ ) in the treated (Fig. S2) and untreated (Fig. S3) lakes. Before treatment, the non-native roach and pike occupied the highest trophic position in the food webs of treated lakes. The intermediate and highly variable  $\delta^{13}\text{C}$  values of roach indicate a mixed diet consisting of prey items that relied on littoral (e.g., periphyton with high  $\delta^{13}\text{C}$ ), terrestrial (e.g., detritus with intermediate  $\delta^{13}\text{C}$ ) or pelagic (plankton with low  $\delta^{13}\text{C}$ ) basal resources (Fig. S2). The high  $\delta^{15}\text{N}$  values of two pike individuals caught from Haukvatnet indicated piscivorous foraging on roach with corresponding  $\delta^{13}\text{C}$  values (Fig. S2).

In untreated lakes, the native brown trout and three-spine stickleback populations occupied the highest trophic positions ( $\delta^{15}\text{N}$ ), with three-spine stickleback utilizing a slightly narrower and more pelagic niche (low  $\delta^{13}\text{C}$ ) as compared to brown trout (Fig. S3). In some study lakes, the basal resources (i.e., periphyton, particulate organic matter POM, detritus, and leaves) showed largely overlapping  $\delta^{13}\text{C}$  or  $\delta^{15}\text{N}$  values (Fig. S2 and S3), which prevented the use of isotopic mixing models to estimate the relative prey proportions of benthic invertebrates (cf. Layman et al., 2012).

### 3.3. Rotenone impacts on resource use

We found no strong evidence for altered resource use by the benthic invertebrate communities following rotenone treatment. The GLMM analyses indicated no significant *Lake group\*Year* two-way interactions for either  $\delta^{13}\text{C}$  or  $\delta^{15}\text{N}$  values (Fig. 3, Table 3). Although rotenone treatment had no significant effect on  $\delta^{13}\text{C}$  or  $\delta^{15}\text{N}$  values, the benthic invertebrates in treated lakes had significantly (on average 1.9‰) higher  $\delta^{15}\text{N}$  values compared to untreated lakes (Fig. 3, Table 3).



**Fig. 2.** (A) Relative proportions and (B) individual counts (log-transformed) of the different functional feeding groups of benthic macroinvertebrates in the treated and untreated lakes before and after the rotenone treatment.

The dominant invertebrate taxa observed in both lake types and study periods included three taxa belonging to different functional feeding groups: (1) predatory Odonata dragonfly larvae, (2) collector *Leptophlebia* mayfly larvae and (3) grazer Lymnaeidae snails. GLMM analyses run separately for each taxa indicated some significant *Lake group*\**Year* two-way interactions for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values (Fig. 4, Table 4). Specifically, the  $\delta^{13}\text{C}$  values of Odonata slightly increased in treated lakes but decreased in untreated lakes following rotenone treatment (Fig. 4). In contrast, the  $\delta^{13}\text{C}$  values of *Leptophlebia* slightly decreased in treated lakes but increased in untreated lakes (Fig. 4). No significant shifts were evident in the  $\delta^{15}\text{N}$  values of Odonata and *Leptophlebia* following rotenone treatment (Fig. 4, Table 4). Lymnaeidae snails in treated lakes had higher  $\delta^{13}\text{C}$  and lower

$\delta^{15}\text{N}$  values following rotenone treatment, while no evident shifts were observed in untreated lakes (Fig. 4, Table 4). On average, all three taxa had higher  $\delta^{15}\text{N}$  values in treated lakes that corresponded with the elevated  $\delta^{15}\text{N}$  values of benthic and pelagic basal resources (Fig. S2–S3).

#### 3.4. Impacts on community-level trophic diversity

We found no consistent changes in the community niche size or trophic positioning of benthic invertebrate communities following rotenone treatment. Based on the 95% Bayesian credibility intervals for  $SEA_B$  estimates, the community-level isotopic niche area estimates overlapped between the study periods in each lake (Table 5, Fig. 5). However, the isotopic

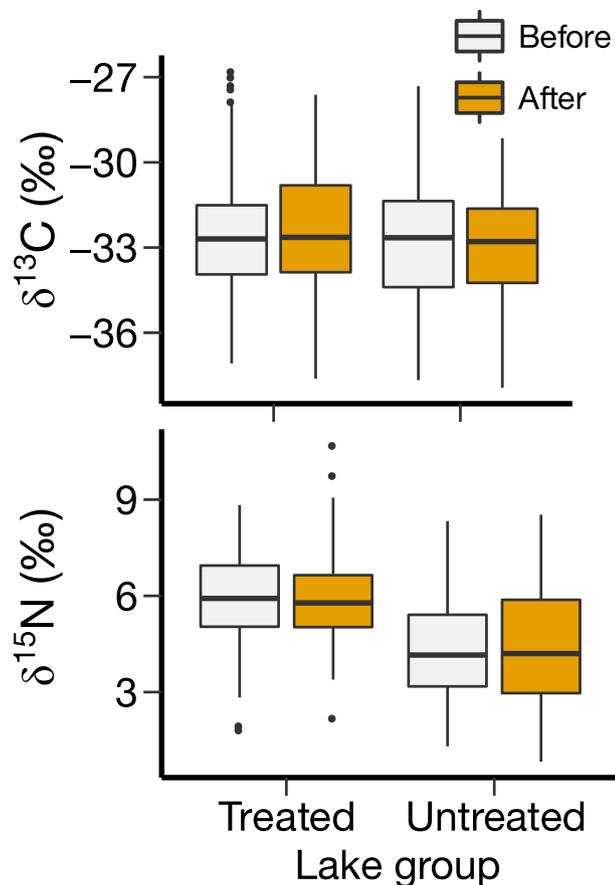


Fig. 3. Boxplots showing the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of benthic invertebrates collected from the treated (black) and untreated study lakes before and after the rotenone treatment.

niche areas ( $SEA_C$  and  $SEA_B$ ) slightly increased following rotenone treatment in Lianvatnet and Theisendammen (18% and 34% increase in  $SEA_C$ , respectively) due to increased  $\delta^{15}\text{N}$  range (Table 5, Figs. 5–6). In contrast, a slight niche reduction (20% decrease in  $SEA_C$ ) was observed in the third treated lake Haukvatnet (Table 5, Figs. 5–6). Some insignificant changes in isotopic niche areas also occurred in the untreated lakes, with the benthic invertebrate community in Holstdammen showing the most drastic niche reduction (33% decrease in  $SEA_C$ ) as opposed to slight niche expansion in

Table 3

Generalized mixed effects models predicting  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of benthic invertebrates as a function of *Lake group* (treated versus untreated lakes), *Year* (before versus after rotenone treatment), and their two-way interaction (*Lake group*\**Year*). Parameter estimates and their standard errors (SE), *t*- and *p*-values, and lower and upper confidence limits (95% CI) are shown, with statistically significant parameters highlighted in bold (i.e., 95% CIs not overlapping zero).

Response	95% CI					
Predictor	Estimate	SE	t	p	Lower	Upper
$\delta^{13}\text{C}$						
Intercept	-32.49	0.63	-51.87	<b>&lt;0.001</b>	<b>-33.67</b>	<b>-31.31</b>
Lake group	-0.55	0.89	-0.62	0.568	-2.22	1.12
Year	-0.02	0.25	-0.08	0.939	-0.51	0.47
Lake group*Year	-0.27	0.38	-0.71	0.477	-1.01	0.48
$\delta^{15}\text{N}$						
Intercept	5.93	0.38	15.80	<b>&lt;0.001</b>	<b>5.22</b>	<b>6.64</b>
Lake group	-1.63	0.53	-3.07	<b>0.033</b>	<b>-2.63</b>	<b>-0.63</b>
Year	0.03	0.18	0.18	0.854	-0.31	0.38
Lake group*Year	0.00	0.27	-0.01	0.993	-0.52	0.52

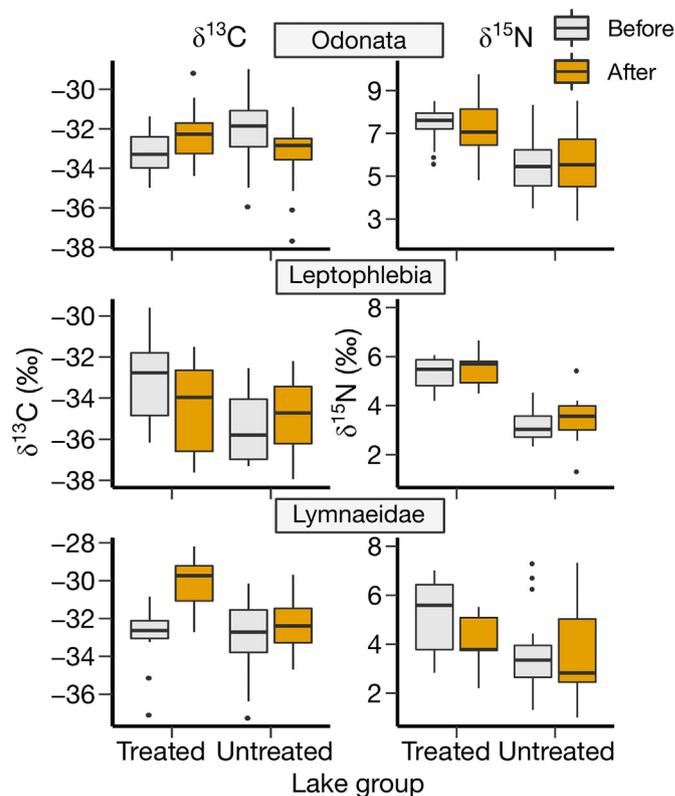


Fig. 4. Boxplots showing the  $\delta^{13}\text{C}$  (left) and  $\delta^{15}\text{N}$  (right) values of Odonata predators, *Leptophlebia* collectors and Lymnaeidae grazers sampled from the treated and untreated lakes before and after the rotenone treatment.

Lauglovatnet and Store-Leirsjøen (23% and 10% increase in  $SEA_C$ , respectively; Table 5, Figs. 5–6).

#### 4. Discussion

In general, our results indicated only minor changes in the community composition and resource use of benthic invertebrates following rotenone-induced fish removal. The CCA data (Fig. 2, Fig. S1, Appendix B) supported our first expectation as the relative abundance of grazers and collectors decreased while that of predators increased following rotenone-induced fish removal. Regardless of shifts in the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of some functional feeding groups, the community-level isotopic niche areas of benthic invertebrates showed no consistent changes following fish removal, although the  $SEA_C$  and  $SEA_B$  areas slightly increased in two of the three treated lakes evidently due to the increased  $\delta^{15}\text{N}$  range. Our study findings indicate a partial recovery and minor shifts in the resource use and trophic diversity of lake benthic invertebrate communities following rotenone-induced fish removal. However, more research is needed to elucidate potential impacts of rotenone treatments on biota and fundamental ecological processes in highly connected and altered freshwater ecosystems (Carpenter et al., 2011; Reid et al., 2019).

##### 4.1. Rotenone impacts on community composition

Previous research of rotenone impacts on freshwater invertebrate communities has indicated that gill-breathing insect larvae (e.g., Trichoptera and Plecoptera) are typically eliminated first and replaced by more tolerant taxa such as chironomids and occasionally by amphipods (Mangum and Madrigal, 1999; Beaulieu et al., 2021; Schnee et al., 2021). Correspondingly, our CCA results showed declines in EPT taxa and an increased abundance of chironomids and amphipods in the treated lakes (Fig. S1). Moreover, our GLMM analyses demonstrated reduced abundance of grazers (snails) and collectors (e.g., Ephemeroptera and Trichoptera) at lower

**Table 4**

Generalized mixed effects models predicting  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of different functional feeding groups of benthic invertebrates as a function of *Lake group* (treated versus untreated lakes), *Year* (before versus after rotenone treatment), and their two-way interaction (*Lake group*\**Year*). Parameter estimates and their standard errors (SE), t- and p-values, and lower and upper confidence limits (95% CI) are shown, with statistically significant parameters highlighted in bold (i.e., 95% CIs not overlapping zero).

Response					95% CI		
	Predictor	Estimate	SE	t	p	Lower	Upper
$\delta^{13}\text{C}$ : Odonata (predators)							
Intercept	-33.089	0.513	-64.471	<0.001		-34.051	-32.124
Lake group	0.895	0.721	1.242	0.273		-0.463	2.247
Year	0.708	0.273	2.590	<b>0.011</b>		<b>0.175</b>	<b>1.246</b>
Lake group*Year	-1.742	0.384	-4.540	<0.001		-2.495	-0.992
$\delta^{15}\text{N}$ : Odonata (predators)							
Intercept	5.499	0.448	12.276	<0.001		6.688	8.395
Lake group	2.041	0.639	3.194	<b>0.026</b>		-3.241	-0.845
Year	-0.088	0.259	-0.340	0.694		-0.618	0.415
Lake group*Year	-0.016	0.370	-0.042	0.966		-0.708	0.741
$\delta^{13}\text{C}$ : Leptophlebia (collectors)							
Intercept	-33.112	0.833	-39.771	<0.001		-34.670	-31.552
Lake group	-2.613	1.241	-2.107	<b>0.083</b>		-4.931	-0.324
Year	-1.334	0.615	-2.170	<b>0.036</b>		-2.528	-0.121
Lake group*Year	1.969	1.006	1.957	<b>0.057</b>		<b>0.010</b>	<b>3.944</b>
$\delta^{15}\text{N}$ : Leptophlebia (collectors)							
Intercept	5.352	0.340	15.741	<0.001		4.723	5.982
Lake group	-2.301	0.509	-4.522	<b>0.006</b>		-3.246	-1.376
Year	0.133	0.263	0.506	0.615		-0.385	0.648
Lake group*Year	-0.139	0.430	-0.323	0.748		-0.965	0.725
$\delta^{13}\text{C}$ : Lymnaeidae (grazers)							
Intercept	-32.788	0.751	-43.652	<0.001		-34.181	-31.378
Lake group	-0.173	1.018	-0.170	0.872		-2.098	1.726
Year	2.626	0.744	3.530	<0.001		<b>1.192</b>	<b>4.102</b>
Lake group*Year	-2.402	0.871	-2.757	<b>0.008</b>		-4.102	-0.699
$\delta^{15}\text{N}$ : Lymnaeidae (grazers)							
Intercept	4.943	0.884	5.589	<0.001		3.276	6.603
Lake group	-1.477	1.233	-1.198	0.295		-3.800	0.852
Year	-1.191	0.514	-2.317	<b>0.025</b>		-2.190	-0.177
Lake group*Year	1.363	0.603	2.263	<b>0.028</b>		<b>0.181</b>	<b>2.539</b>

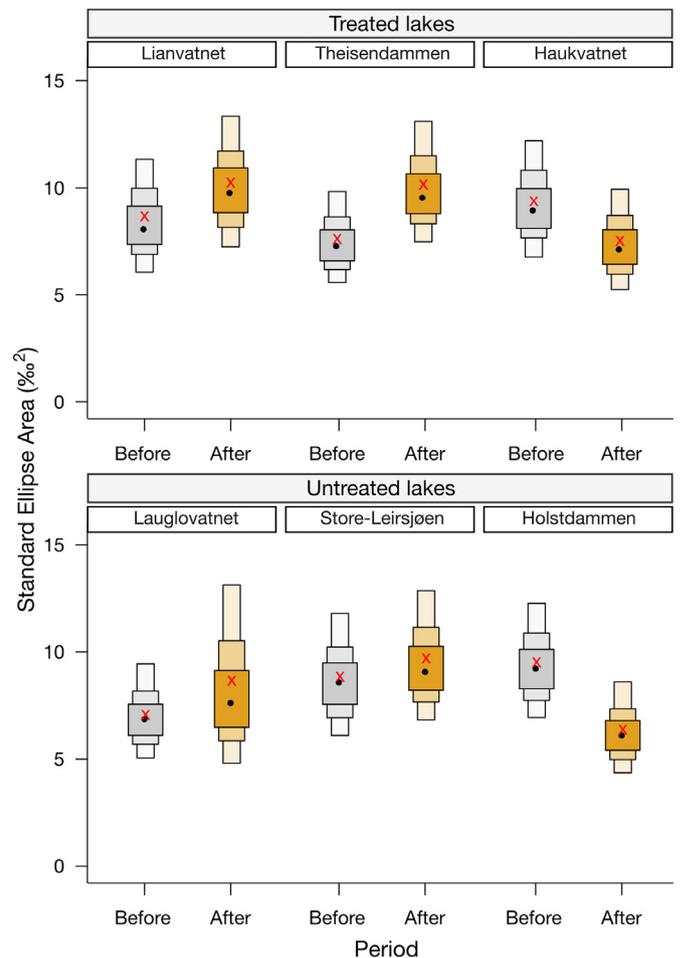
trophic levels (Fig. 2 & S1, Appendix B). However, some collector taxa (e.g., Gammaridae amphipods) showed increased abundances following rotenone treatment (Fig. S1, Appendix B), likely due to the absence of fish predation (Beaulieu et al., 2021).

The contrasting responses of functional feeding groups likely arise from their vulnerability to the direct and indirect impacts of rotenone toxicity

**Table 5**

Sample sizes (n), means and ranges of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values (in ‰), as well as isotopic niche areas based on sample-size corrected ( $SEA_C$ ) and Bayesian estimates (modes, upper and lower 95% credibility intervals) of Standard Ellipse Areas ( $SEA_B$ ) estimated using SIBER package in R (Layman et al., 2007) for benthic invertebrates sampled from treated and untreated lakes before and after the rotenone treatment.

Lake	Period	n	Mean		Range		$SEA_C$	$SEA_B$		
			$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$		Mode	L 95%	U 95%
Treated										
Lianvatnet	Before	41	-32.98	6.31	8.26	4.98	8.65	8.21	6.03	11.32
	After	41	-32.48	6.42	9.58	6.37	10.23	9.81	7.22	13.28
Haukvatnet	Before	44	-32.65	5.25	8.25	6.68	9.36	9.01	6.74	12.17
	After	40	-32.91	5.63	7.95	5.94	7.51	7.21	5.21	9.89
Theisendammen	Before	47	-31.87	6.22	8.48	3.41	7.60	7.33	5.48	9.79
	After	50	-32.12	5.88	8.10	6.78	10.15	9.61	7.43	13.09
Untreated										
Lauglovatnet	Before	40	-34.48	3.75	7.38	5.75	7.05	6.87	4.96	9.37
	After	17	-34.88	3.49	5.21	5.04	8.65	7.67	4.73	13.05
Store-Leirsjøen	Before	36	-32.97	4.15	8.56	5.38	8.83	8.55	5.99	11.68
	After	39	-33.36	4.03	8.70	7.32	9.69	9.19	6.77	12.76
Holstdammen	Before	48	-31.66	5.01	9.99	5.46	9.51	9.23	6.83	12.17
	After	36	-31.83	5.34	5.40	7.03	6.37	6.13	4.22	8.52



**Fig. 5.** Bayesian estimates of Standard Ellipse Areas ( $SEA_B$ ) measuring the isotopic niche areas of the benthic invertebrate communities in the treated and untreated lakes before (grey) and after (orange) the rotenone treatment. The boxes depict the 95, 75 and 50% credibility intervals and the black dots denote the modes of  $SEA_B$  estimates, whereas the red crosses indicate the sample-size corrected  $SEA_C$  estimates.

and altered fish predation. Large-sized invertebrates, including predatory insect larvae, amphipods and algae-grazing snails, are typically most heavily predated by fishes that prefer feeding on large-sized prey items

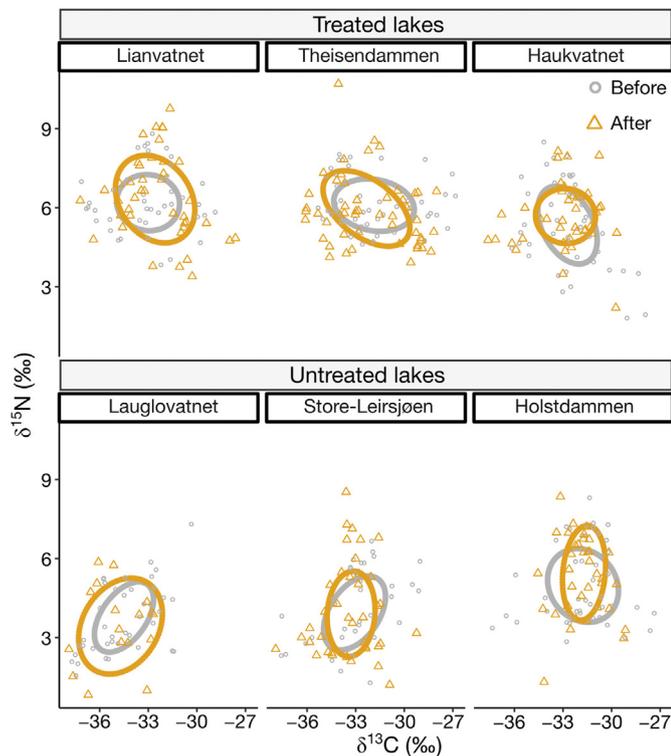


Fig. 6. Standard Ellipse Areas (SEA) encompassing the core isotopic niches of the benthic invertebrate communities (based on the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of taxa with  $n \geq 2$ ) in the treated and untreated lakes before and after the rotenone treatment.

(e.g., Jeppesen et al., 2017). The reduced fish predation and high recolonization rates of large, mobile benthic invertebrates from refugia (i.e., connected untreated lakes and streams; Pham et al., 2018; Bellingan et al., 2019) likely explains the rapid increase in abundance of predatory invertebrates and some large collectors (e.g., Gammaridae) in our treated lakes. Compared to small-sized pelagic zooplankton, these large benthic invertebrates are also less sensitive and thus recover faster from rotenone treatments (Chandler and Marking, 1982; Vinson et al., 2010; Beaulieu et al., 2021). For example, based on 24-hour LC50 experiments, benthic dragonfly larvae and snails can tolerate two to three orders of magnitude greater rotenone concentrations than *Daphnia* zooplankton (Chandler and Marking, 1982).

Although grazer snails have hard shells that protect the animals from predation and environmental stress, their abundance declined in our treated study lakes. While the ultimate reason for snail decline cannot be solved with the available data, it might be associated with their low mobility and thus delayed recolonization, as well as with increased negative predatory and competitive impacts with other invertebrates that became dominant following rotenone treatment, such as odonates (Turner and Chislock, 2007) and amphipods (Wellborn, 2002). In general, our findings correspond with the numerous studies reviewed by Vinson et al. (2010) that showed a rapid return of invertebrate abundances to pre-treatment levels (i.e., from a few months up to one year). Although biodiversity and taxonomic composition measures may take up to a few years to return fully to pre-treatment levels (Vinson et al., 2010), the observed minor shifts in the functional community composition likely explains minor changes in the resource use and trophic diversity of benthic macroinvertebrates as discussed below.

#### 4.2. Impacts on resource use of benthic invertebrates

Based on SIA data, we found no major consistent shifts in the resource use of benthic invertebrate communities. The elevated  $\delta^{15}\text{N}$  values of benthic invertebrates in treated lakes were likely associated with higher

nitrogen loading from the more populated surrounding areas (Lake et al., 2001; Glibert et al., 2019) as compared to the more forested catchments of untreated lakes (Fig. 1). However, we found support for significant shifts in the resource use of some functional feeding groups, with the predatory dragonfly larvae (Odonata) and the grazer snails (Lymnaeidae) both showing higher  $\delta^{13}\text{C}$  values following rotenone treatment. Although algae are sensitive to rotenone treatments, the growth inhibition concentrations for freshwater algae generally exceed the LC50s of most invertebrates (Chandler and Marking, 1982; Van Ginkel et al., 2015), implying rotenone applications would initially favour algal biomass production through the removal of herbivorous invertebrates. As littoral benthic algae are known to have distinctively elevated  $\delta^{13}\text{C}$  values (Hecky and Hesslein, 1995), the observed increases in dragonfly larvae and the grazer snail  $\delta^{13}\text{C}$  were likely to have been related to, and consistent with, increased reliance on carbon produced by littoral benthic algae. The decreased  $\delta^{15}\text{N}$  values of grazer snails following rotenone treatment may also be associated with more specialized algal diets and reduced  $^{15}\text{N}$  fractionation due to increased periphyton quantity and quality (Li et al., 2018).

Overall, the absence of fish predation appears to have had both direct and indirect effects, with indirect effects resulting from the increased utilization of shallow littoral habitats and algal food resources associated with decreased predation risk (e.g., McCollum et al., 1998; Jones and Sayer, 2003). Increased littoral reliance of predatory invertebrates may also have resulted partly from decreased availability of pelagic crustacean zooplankton that are among the most sensitive taxa to negative rotenone impacts (Vinson et al., 2010; Beaulieu et al., 2021). In our treated study lakes, pelagic zooplankton likely showed prolonged recolonization rates due to the high rotenone concentration in the epilimnion and the absence of large, untreated lakes within the rotenone treated watersheds (Bardal et al., 2018; Sandodden et al., 2022). In contrast to predators and grazers, the collector mayfly larvae (*Leptophlebia*) showed decreased  $\delta^{13}\text{C}$  values, possibly arising from increased utilization of  $^{13}\text{C}$ -depleted methane-derived carbon (Jones and Grey, 2011) following rotenone-induced fish mass deaths and reduced sediment bioturbation (Oliveira Junior et al., 2019). Altogether, our SIA results indicate that the responses to rotenone treatment varied among the benthic invertebrate functional feeding groups, likely due to their contrasting sensitivity to the direct and indirect toxicity effects and to the altered competitive and predatory interactions following fish removal.

#### 4.3. Impacts on community-level trophic diversity

Our BACI study of six boreal lakes in central Norway indicate minor shifts in isotopic niche metrics estimated for benthic invertebrate communities. Our prediction of a niche expansion of benthic invertebrates following fish removal was only partly supported. The isotopic niche areas ( $SEA_c$  and  $SEA_B$ ) slightly increased following rotenone treatment in Lianvatnet and Theisendammen due to increased  $\delta^{15}\text{N}$  range, but not in the third treated lake Haukvatnet. Prior to rotenone treatment, roach likely preyed heavily on predatory odonates, as indicated by the corresponding  $\delta^{13}\text{C}$  values and circa 3–3.5‰ higher  $\delta^{15}\text{N}$  values of roach as compared to odonates (Fig. S2), which seemed to partly take over the trophic position of removed fishes as top predators. The observed lack of niche expansion in the relatively deep Haukvatnet may be associated with the limited shallow littoral areas and the higher initial rotenone concentration and prolonged exposure time in the cold profundal areas (Bardal et al., 2018; Sandodden et al., 2022), which may jointly limit the resource availability and potential for niche expansion by the benthic invertebrate community following rotenone-induced fish removal.

Some, although statistically non-significant, community-level niche shifts occurred in the untreated lakes, with the benthic invertebrate community in Holstdammen showing the most evident niche reduction as opposed to slight niche expansion in Lauglovatnet and Store-Leirsjøen. These results suggest that some temporal shifts might have arisen from other environmental factors rather than solely from the rotenone treatment. For example, the limited SIA data of basal resources indicates marked

temporal fluctuations, with for example the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of periphyton showing large between-year differences in mean values and standard deviations in treated and untreated lakes (Fig. S2–S3).

#### 4.4. Study limitations

Our study compared years just before and after rotenone treatment and, therefore, lack insight into potential co-occurring longer-term changes in the benthic invertebrate communities, especially following re-establishment of native brown trout populations. Whereas the lack of high frequency baseline sampling precluded us from detecting or commenting on short-term shifts in the isotopic baselines that may have confounded result interpretation, to our knowledge, no major changes in land use or other human activities influencing nutrient and carbon runoff occurred in the vicinity of our study lakes. Nevertheless, future studies of rotenone impacts should consider utilizing multiple isotopes (C, N, H and S; Solomon et al., 2011; Ofukany et al., 2014) and sampling of biota in the littoral, pelagic and profundal lake habitats to elucidate impacts on the entire community and on habitat linkages within lake ecosystems. Future studies should also consider impacts on connected, untreated downstream and upstream lotic and lentic ecosystems, which commonly act as important refugia for recolonizing taxa (Vinson et al., 2010; Pham et al., 2018; Beaulieu et al., 2021), but whose biodiversity might also be negatively impacted by disturbance in treated waterbodies within the watershed (Woods et al., 2016).

#### 4.5. Conclusions

Freshwater ecosystems are considered as biodiversity hotspots, but they are also among the most vulnerable ecosystems due to increasing local and global human disturbances (Carpenter et al., 2011; Reid et al., 2019; Albert et al., 2021). Non-native fish species can have cascading negative impacts on native communities and on the fundamental ecological processes that determine the function, services, and resilience of freshwater ecosystems (Simon and Townsend, 2003; Villéger et al., 2017). Rotenone treatment is a widely applied method for eradicating unwanted non-native fish species (Vinson et al., 2010), and the present and previous field studies indicate at least a partial recovery of the predominant native invertebrate taxa. Our study systems showed some changes in species assemblages but only minor shifts in the resource use and trophic diversity of benthic invertebrate communities following rotenone treatment. However, some freshwater ecosystems may still show unexpected, irreversible negative responses to rotenone treatment. For example, the disappearance of some non-target species might induce unexpected cascading impacts on the entire community, potentially altering biotic interactions and abiotic processes (e.g., Power, 1990; Oliveira Junior et al., 2019). Therefore, chemical treatments and other management actions likely to cause major disturbance to vulnerable freshwater ecosystems should be considered and conducted with caution, especially if endangered species occur within the watershed. Increased public attention and awareness of the potential negative impacts of non-native species, as well as other preventative measures, are urgently needed to conserve the biodiversity and resilience of freshwater ecosystems to ongoing and future anthropogenic stressors.

#### CRedit authorship contribution statement

**Antti P. Eloranta:** Writing – original draft, Conceptualization, Formal analysis, Visualization, Funding acquisition. **Gaute Kjærstad:** Writing – review & editing, Investigation, Conceptualization, Funding acquisition. **Michael Power:** Writing – review & editing, Conceptualization, Resources. **Hanna-Kaisa Lakka:** Writing – review & editing, Investigation, Data curation. **Jo Vegar Arnekleiv:** Writing – review & editing, Investigation, Funding acquisition. **Anders G. Finstad:** Writing – review & editing, Project administration, Funding acquisition, Conceptualization.

#### 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.2022.155364>.

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