



## Road effects on benthic macroinvertebrate assemblages in boreal headwater streams

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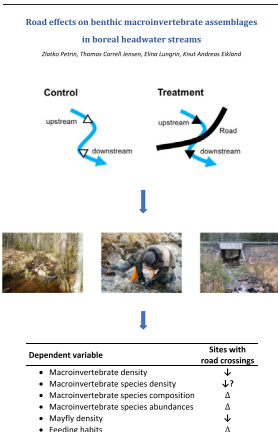
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### HIGHLIGHTS

- Macroinvertebrate communities in streams crossed by roads are compared to control streams.
- Road crossings impact structure and feeding habits of macroinvertebrate communities.
- Road effects driving freshwater biodiversity loss may be highly underestimated especially in smaller headwater streams.

### GRAPHICAL ABSTRACT



### ARTICLE INFO

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### ABSTRACT

Roads constitute a worldwide network of ecological barriers traversing countless streams and rivers. A large fraction of the land area lies in close proximity to roads. Ecological effects of roads likely extend well beyond the road network, suggesting wide ranging impacts on lotic ecosystems. Road impacts are multifaceted including fragmentation, changing hydrology, sedimentation and pollution. Yet, the ecological impacts are incompletely documented. We examined the effects of roads traversing lotic ecosystems on the structure and function of benthic macroinvertebrates in small temperate headwater streams. Ecological effects differed between reaches upstream and downstream of roads compared to upstream and downstream reaches in control streams. Total macroinvertebrate density and mayfly density were lower downstream of roads than upstream of roads, but they were similar at downstream and upstream reaches of control streams. Species density, but not species richness, tended to be lower downstream than upstream of roads, likely due to the lower macroinvertebrate densities at downstream sites. There were no comparable effects in control streams. Species composition and species abundances differed between road impacted streams and control streams likely because streams that were traversed by roads selected for a different set of species compared to control streams as indicated by checkerboard distribution of species in streams that were traversed by roads. Functional impacts included a greater prevalence of predators in control streams, and a higher proportion of grazers and shredders in streams that were traversed by roads. The study is inconclusive regarding the mechanisms mediating the ecological impact of roads. The ecological effects are likely caused by a combination of factors including fragmentation, pollution

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and hydrological change among others. Given the vast global road network, the quantitative significance of road effects driving freshwater biodiversity loss may be highly underestimated especially in smaller headwater streams comprising the major part of fluvial ecosystems.

## 1. Introduction

A huge human-made experiment is currently being performed worldwide: streams and rivers are intersected by a wide range of infrastructures including the most widely ranging type of infrastructure, roads. In Norway alone, the public and private roads network amounts to ca. 97,700 km and 100,000 km respectively, enough to circle the equator 4.9 times (Norwegian Road Federation, 2022). Globally, the total network of roads has been reported to reach 64,000,000 km covering 190,000 km<sup>2</sup> of the earth's land surface, comparable to almost half of the surface area of Norway (Haddad, 2015). The ecological effects of roads likely extend hundreds of meters away from the road and hence well beyond the strips of land that form a reticulate network routing the traffic (Forman and Alexander, 1998). Reflecting the reticulate nature of road networks, a large proportion of the land area lies in relative proximity to roads (Haddad et al., 2015; Lister et al., 2015). For instance, less than one fifth of the land area in the United States lies >1 km from roads, and half of the land area is closer than 400 m, but roads often follow streams and rivers suggesting that the average distance between roads and fluvial ecosystems may be even smaller (Riitters and Wickham, 2003). Norway's network of perennial streams and rivers amounts to 479,995 km (Vann-Nett, 2022). The density of the road network as well as the ratio between the total length of Norway's public roads network and the total length of its fluvial network, suggest numerous intersections with concomitant ecological effects.

The direct and indirect effects of roads on fluvial ecosystems are multifaceted including fragmentation, hydrological change and altered rates of sedimentation and debris flow (Haddad, 2015; Jones et al., 2000; Wemple et al., 1996). Roads also add to the pollution of streams and rivers, for instance through the combustion of fossil fuels by motor vehicles, or salting of roads during winter at high latitudes (Blasius and Merritt, 2002; Gjessing et al., 1984; Hintz and Relyea, 2019; Hintz et al., 2022; Maltby et al., 1995). The ecological consequences of the intersections between roads and streams and rivers have been only incompletely documented despite their quantitative significance (Forman and Alexander, 1998; Haddad, 2015). Research on the varied effects of roads also is likely biased in favour of their effects on fish that are more charismatic than the more diverse and functionally significant benthic macroinvertebrates (McKay et al., 2013; Perkin and Gido, 2012; Wallace and Webster, 1996).

Land use change has caused increased rates of habitat fragmentation affecting species diversity (Haddad et al., 2015). In streams and rivers, due to their dendritic nature, fragmentation – or connectivity – likely is of particular importance for community assembly and hence the maintenance of ecological continuity (Brown et al., 2011; Carrara et al., 2012; Vannote et al., 1980). Reflecting the directional nature of lotic ecosystems, species diversity may systematically vary in response to fragmentation if benthic macroinvertebrates tend to exhibit net downstream migration, for instance by drifting, that is compensated for by upstream movement of benthic macroinvertebrates and upstream migration of the aerial stages of aquatic insects (Bishop and Hynes, 1969; Müller, 1954; Turner and Williams, 2000). The natural colonization cycle, directional movements and the continuous redistribution of stream organisms may contribute to the establishment of similar benthic macroinvertebrate assemblages along streams and rivers, at least at the spatial scale of the colonization cycle (Elliott, 1971; Müller, 1954; Townsend and Hildrew, 1976). However, if fragmentation interfered with the natural colonization cycle, directional movements, or omnidirectional dispersal of benthic macroinvertebrates, then different species assemblages might establish and persist in the reaches upstream and downstream from the site of interruption in biotic connectivity

depending on the changed environmental conditions (Müller, 1954; Speirs and Gurney, 2001; Townsend and Hildrew, 1976). Changed environmental conditions facilitating the establishment of different benthic macroinvertebrate assemblages may for instance include altered habitat quality (Larsen et al., 2011; Monaghan et al., 2005; Niemi et al., 1990). Pollution constitutes another significant cause of structural change in benthic macroinvertebrate assemblages and may reflect the deposition of harmful substances across large scales, as for instance the deposition of acid substances, or it may reflect the local release of detrimental substances, as in point source pollution (Bott et al., 2012; Rosenberg and Resh, 1993; Stoddard et al., 1999). Pollution may cause the establishment of a depauperate benthic fauna, yet the consequences for benthic macroinvertebrate density may depend on the exact type of pollutant (Hellawell, 1988; Larsen and Ormerod, 2010; Rosenberg and Resh, 1993).

We studied the consequences of habitat fragmentation, pollution, and further factors, that means the combined effects of roads traversing streams, for the structure and function of benthic macroinvertebrate assemblages. Fragmentation and pollution should increase the structural dissimilarities between unaffected lotic ecosystems and those traversed by roads, as well as between those upstream and downstream reaches that are separated by roads, if the roads interfere with ecological continuity (Fagan, 2002; Perry and Schaeffer, 1987; Vannote et al., 1980). Whether only upstream, only downstream, or both upstream and downstream reaches are affected when the streams are traversed by roads will depend on the specific dynamic processes controlling community assembly in the affected streams. For instance, fragmentation may cause the isolation of upstream reaches from the remaining dendritic network increasing extinction rates in isolated habitats and limiting dispersal from downstream reaches and hence re-colonization of suitable habitat in upstream reaches (Economio and Keitt, 2010; Fagan, 2002; Perkin and Gido, 2012). Point source pollution may render downstream reaches uninhabitable at least for some benthic macroinvertebrate taxa (Hynes, 1960). If downstream and upstream reaches are linked through source-sink dynamics, then disruption of ecological continuity may affect both upstream and downstream reaches (Caudill, 2003; Müller, 1982; Pulliam, 1988). The complex causes and mechanisms of structural change in benthic macroinvertebrate assemblages in lotic ecosystems suggest that the distinction among the effects of habitat fragmentation, pollution, or any other effects may be no trivial task. We therefore preferred analysing the combined effects of habitat fragmentation, pollution and possibly further effects of roads traversing fluvial ecosystems on benthic macroinvertebrate structure. We expected differential effects on benthic macroinvertebrate density in upstream and downstream reaches in affected, but not unaffected control streams because isolation and altered habitat quality will frequently affect population size (Brooks et al., 2005; Caquet et al., 2007; Hart, 1978; Reice, 1980). We recognized the possibility of observing differential effects on benthic macroinvertebrate species density and species richness, as long as sensitive species were not merely replaced by more tolerant species potentially rescuing species density and species richness (Rosenberg and Resh, 1993). However, if roads traversing streams affected disturbance levels, then species density and species richness might in fact increase provided that disturbance shifted to intermediate levels (Connell, 1978; Resh et al., 1988; Townsend et al., 1997b). Due to the potentially opposite effects of the independent variables on the dependent variables, species density and species richness may change little. Nonetheless, we expected to detect differences in species composition and species abundance because fragmentation and pollution should allow for the establishment of, and potentially even select for, different species (Gibb and Hochuli, 2002; Hodkinson and Jackson, 2005; Rosenberg and

Resh, 1993). If dispersal limitation, sensitivity to pollution, or other factors reflecting disruptions in ecological continuity covaried with species traits, for instance feeding habits, then fragmentation and pollution might also affect the composition of species traits (Rawer-Jost et al., 2000; Townsend et al., 1997a; Vannote et al., 1980). Specifically, road crossings may cause a reduction in the forest cover in the vicinity of the physical structure traversing the stream. A reduced forest cover will result in lower rates of leaf litter input in the stream channel and decreased degrees of shading allowing for larger insulation and consequently higher rates of periphyton growth. Thus, we expected lower densities of shredders and higher densities of grazers at the road crossings and probably to some extent downstream from the road crossings (Vannote et al., 1980). If road crossings resulted in generally decreased macroinvertebrate densities, then we would also expect lower predator densities at impacted downstream sites reflecting lower availability of food resources. Effects on collectors would be expected, if the road crossings interfered with the hydrological regime and consequently affected sedimentation rates of fine particulate organic matter in addition to the changed rates of organic matter input through reductions in the forest cover. Likewise, if road crossings interfered with disturbance levels in addition to the hydrological regime in the streams, we may expect an increase in the proportion of opportunistic benthic macroinvertebrates, that means a larger proportion of r strategists, and we may expect changes in benthic macroinvertebrate current preferences (Townsend et al., 1997a).

## 2. Methods

We studied twelve small streams in the vicinity of Kongsberg in former county Buskerud, now Viken, South-eastern Norway (Fig. 1, Appendix, Table A.1). The study streams along the road E134 were located between Hokksund and the area west of Kongsberg. E134 is one of the main roads between Southeastern and Western Norway. The annual average daily traffic varies along this part of E134 from 10,950 vehicles at the eastern stretch to 5200 vehicles along the western stretch of the road.

We speculated that the hypothesized effects should be larger in smaller lotic ecosystems and therefore focused our research on small streams (Petrin et al., 2007). At each stream, we selected two approximately fifty-meter-long study reaches mainly comprising riffles and runs with small cascades. The average distance between the upstream and downstream reaches at each stream was 150 m. Six streams were located at and traversed by the major road E134 between the upstream and downstream reaches, whereas the remaining six streams served as controls. Given the reticulate nature of the road network, it was difficult to find close, adequate control streams that were comparable in size and structure to the impacted streams. Nonetheless, the impacted and control streams were located in the same catchments suggesting that the characterizing environmental variables were comparable as also indicated by the physical, morphological and chemical data that we collected when visiting the study sites (Tables A.1, A.2 and A.3). However, we abstained from formally testing

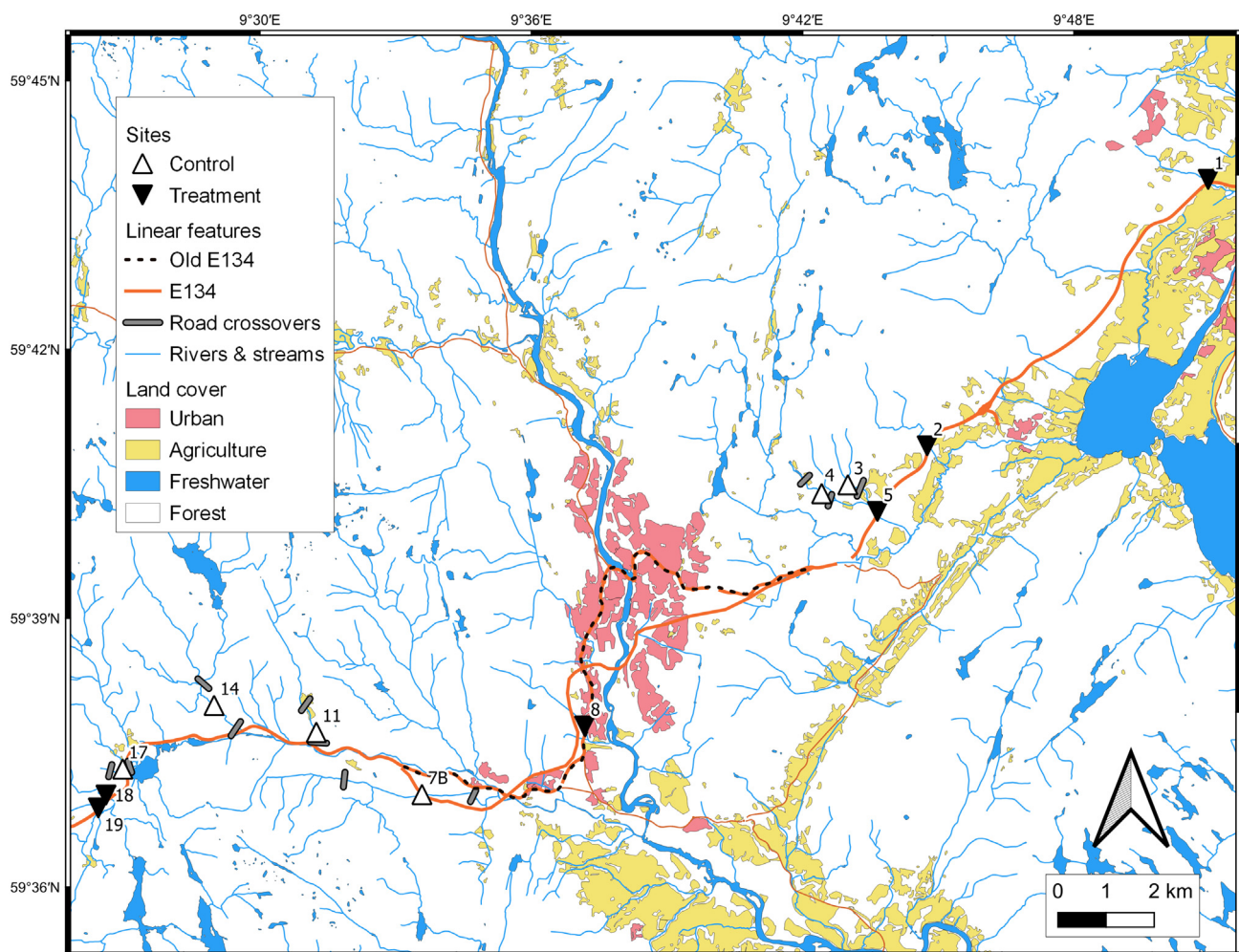


Fig. 1. Geographical distribution of study sites along the road E134 and land use in the study area. A part of the E134 route at Kongsberg was changed after the completion of the present study and opened in summer 2020. The locations of the old as well as of the new E134 are shown in the map. The route of the new E134 traverses one of the control sites (7B) between the upstream and downstream reaches. The old route of E134 is located approximately 400 m north of control site 7B.

for differences in physical, morphological and chemical variables between impacted and control sites given that a high number of statistical tests is likely to yield significant results by chance alone (type I error). One of the six control streams was completely untraversed by roads upstream from the study sites, four control streams were traversed by minor forest roads between 0.5 km and 1.7 km upstream from the study sites, and one control stream was traversed by a railway approximately 0.3 km upstream from the study site (Appendix, Table A.4).

Eighty-nine percent of the stream catchment area, on average, was covered by forest mainly comprising coniferous trees. Reflecting the generally small size of the streams, conifers typically dominated the riparian vegetation, yet alder (*Alnus* spp.) and birch (*Betula* spp.) trees regularly grew along the study reaches. Altitude at the study sites ranged from 38 to 381 m a.s.l. The study area exhibits a gradient in catchment geology. Firstly, a relatively large part of the north-eastern study area is characterized by marine deposits because the area is located below the marine limit, which is just below 200 m a.s.l. The south-western part of the study area is mainly located above the marine limit. Secondly, the south eastern part is also characterized by more calcareous bedrock, while the south western part consists of more acidic bedrock (Nilsen and Siedlecka, 2003). The gradient in catchment geology suggests systematic differences in water chemistry among streams (Appendix, Table A.3).

At each study reach, we collected ten replicate quantitative benthic samples using a Surber net (sampling area: 0.1 m<sup>2</sup>, mesh size: 500 µm). We therefore agitated the substrate down to a depth of 10 cm for 1 min. All samples were immediately preserved in 70 % ethanol for later analysis in the laboratory. The substrate at the sampled reaches mainly consisted of pebbles, cobbles and stones, but at some sites wood, twigs, cones, conifer needles, leaf fragments and aquatic mosses were also observed. Embeddedness and the size of the dominant mineral substrate particles were similar among stream categories and between reaches, although the size of the subdominant mineral substrate particles may have been slightly smaller at the streams that were traversed by roads between the upstream and downstream reaches (Appendix, Table A.2). In contrast, streams traversed by roads had a higher degree of fragmentation between upstream and downstream reaches as compared to control streams. This both applied to the continuity of the waterflow as well as the connectedness of the stream corridor between upstream and downstream reaches (Appendix, Table A.2). We collected all samples at low flow in mid-April 2012 following the first episode of the spring snowmelt, which peaked in March and ended by the beginning of April. In the laboratory, we sorted all benthic samples using a 500 µm sieve. All benthic macroinvertebrates were classified to the lowest possible taxonomic level, usually species, but some dipterans were classified to genus. However, we did not classify oligochaetes, water mites, ostracods, copepods and non-biting midges any further. We also collected water samples that were analysed for pH, conductivity, alkalinity, the levels of chloride, sulphate, nitrate, calcium, magnesium, sodium, total nitrogen, total organic carbon, total aluminium, organic monomeric aluminium, total monomeric aluminium, total phosphorous, ammonium, suspended particles, turbidity, cadmium, copper, nickel, zinc, lead, mercury, and hydrocarbons (C<sub>10</sub>–C<sub>40</sub>) according to standard procedures by an accredited water laboratory (Analysesenteret, 2022 <https://www.trondheim.kommune.no/analysesenteret/>).

We studied the effects of road-crossings on benthic macroinvertebrate density, species density, species richness, evenness, species composition, species abundances, nestedness, checkerboardedness, mayfly, stonefly, caddisfly and dipteran density and species density, the proportion of r strategist species, benthic macroinvertebrate current preferences, microhabitat preferences, modes of locomotion and feeding habits of the aquatic stages using a set of different data analysis techniques. Macroinvertebrate density was calculated as the sum of all recorded benthic macroinvertebrates per unit area. Species density was calculated as the number of taxa per unit area. We also computed sample-based (standardized to ten samples) and individual-based (standardized to 300 individuals) rarefied taxonomic richness accounting for the fact that samples comprising a larger number of individuals would likely also include a larger number of taxa (Bunge and

Fitzpatrick, 1993; Gotelli and Colwell, 2001). Evenness was assessed as the probability of interspecific encounter (Hurlbert, 1971). The feeding habits of benthic macroinvertebrates comprised collectors, scrapers, shredders and predators consuming fine detrital particles, periphyton, coarse particulate organic matter and large live prey, respectively (Cummins, 1973; Merritt et al., 2008). The benthic macroinvertebrates were also classified according to their current preferences (Statzner et al., 1988). Microhabitat preferences were expressed as affinities for particular substrate types including fine sediment, sand, gravel, pebbles, cobbles, stones, aquatic plants, and coarse particulate organic matter (Usseglio-Polatera et al., 2000). Last, the benthic macroinvertebrates were classified according to skating, diving, walking, and burrowing modes of locomotion and sessility (Usseglio-Polatera et al., 2000).

We were interested in the effects of the road-crossings on species diversity, and hence calculated the differences in the diversity measures between the upstream and their corresponding downstream sites prior to analysis. The random factor Stream was nested within the fixed factor Treatment describing whether the study streams were traversed by roads. Our study hence comprised a nested ANOVA design with ten replicate measurements per site. However, the integrated measures of diversity, that means sample-based and individual-based rarefied taxonomic richness, were analysed employing one-way ANOVA sacrificing part of the information on variation in species diversity with respect to the effects of Reach and Treatment. Parametric assumptions were not generally met, and we therefore computed the *p*-values using permutation tests (Manly, 2007). Specifically, we used approximate permutation tests with reduced-model residuals after careful identification of the exchangeable units for the tests (see Anderson and ter Braak, 2003 for further details). For all tests, 9999 random permutations were used except for when analysing the effects on sample-based and individual-based rarefied taxonomic richness, when all possible permutations were included in data analysis. We also analysed the effects on the proportion of species employing an r strategy and macroinvertebrate current preferences using a nested ANOVA design as outlined above (Anderson and ter Braak, 2003).

To analyse variation and differences in species composition and species abundance between treatments and among streams, we chose two multivariate dissimilarity measures of species turnover based on species abundance data: Bray-Curtis dissimilarity constitutes a measure of species composition disregarding joint absences, whereas Euclidean dissimilarity comprises a measure of species abundance including joint absences (Anderson et al., 2011). We used nonmetric multidimensional scaling (MDS) to depict the results and nonparametric multivariate runs tests to analyse the significance of the independent variables (Clarke, 1993; Friedman and Rafsky, 1979). Ordering of observations having multiple dimensions thereby relies on the minimal spanning tree (Friedman and Rafsky, 1979). The nonparametric multivariate runs test is sensitive to an arbitrary change in a distribution, not solely to shifts in the mean values (Friedman and Rafsky, 1979). We computed similarity percentages for species' contributions (SIMPER) determining the relative contributions of the taxa to dissimilarities between groups of sampling units (Clarke, 1993). We used a discrepancy measure as our metric of nestedness, which is preferable to the anticonservative temperature metric, and employed a swap algorithm for null model analysis (Brualdi and Sanderson, 1999; Gotelli and Entsminger, 2003; Ulrich et al., 2009). Similarly, we searched for evidence for a checkerboard distribution employing non-sequential algorithms for null model analysis with fixed row and column sums (Gotelli, 2000; Miklós and Podani, 2004; Stone and Roberts, 1990, 1992). We analysed the effects on microhabitat preferences, feeding habits and the modes of locomotion similarly to species abundance using Euclidean dissimilarity with nonparametric multivariate runs tests (Anderson et al., 2011; Clarke, 1993; Friedman and Rafsky, 1979).

Preliminary data analysis showed that pH varied from 5.8 to 7.7 from west to east, whereas alkalinity ranged from 33 mmol l<sup>-1</sup> in the west to 731 mmol l<sup>-1</sup> in the east reflecting the gradient in catchment geology in the study area as described above. Nonetheless, we found no differences in alkalinity levels between impacted and control sites suggesting both

types of sites covered a wide range of alkalinity levels (Appendix, Table A.3). Here, we were concerned that systematic variation in water chemistry may have masked any treatment effects or caused spurious effects. To account for systematic variation in water chemistry including but not limited to pH and alkalinity across the study sites, we also analysed linear models including longitude as a proxy covariate of the longitudinal gradient in catchment geology and thus longitudinal variation in water chemistry. However, due to the lack of qualitative differences between the models lacking and including longitude as a covariate, we only report the results from the models lacking longitude as a covariate. Also, sodium and chloride concentrations were practically identical when comparing the upstream with their respective downstream reaches in the control streams ( $0.9 \text{ mg Na}^+ \text{ l}^{-1}$  and  $1.1 \text{ mg Cl}^- \text{ l}^{-1}$ ). Yet, chloride concentrations were more than four times higher at reaches downstream than upstream from road crossings ( $6.9 \text{ mg l}^{-1}$  and  $1.6 \text{ mg l}^{-1}$ , respectively; maximum:  $20.8 \text{ mg l}^{-1}$ ), and sodium concentrations were three times higher downstream than upstream ( $4.3 \text{ mg l}^{-1}$  and  $1.4 \text{ mg l}^{-1}$ , respectively; maximum:  $12.1 \text{ mg l}^{-1}$ ) reflecting salting of the roads by the Norwegian Public Roads Administration during winter. Sodium chloride accounts for >99.5 % of the used chemicals for winter maintenance of roads in Norway (Holen, 2010), and increased Cl concentrations due to road salting comprise a widespread and increasing environmental problem in Norwegian freshwaters in the vicinity of roads (Haugen and Bækken, 2012; Jensen et al., 2014). The differences in salt concentrations hence hinted at the possible existence of systematic variation in the degree of pollution between the study reaches, although it remained unclear whether the differences in the concentrations of the pollutants were large enough to cause measurable ecological effects. In any case, we found no differences in the levels of pollutants other than sodium chloride between upstream and downstream reaches.

At the downstream reach of impacted stream 8, we observed abundant periphyton growth and recorded more than sixteen times higher densities of non-biting midges than on average at the other study sites. Here we suspected that point source pollution from a close settlement may have had an undue effect on the chironomid population at the downstream reach of stream 8, since we also noticed that phosphorous concentrations were almost three times higher at the downstream reach of stream 8 than on average at the remaining study reaches (Hynes, 1960). Elevated phosphorous levels may have enhanced periphyton production supporting more benthic macroinvertebrates including chironomids, an effect that was unlikely to be related to the road traversing the stream (Bothwell, 1985; Dubé and Culp, 1996; Hart and Robinson, 1990; Peterson et al., 1983). We therefore decided to repeat all analyses after removal of the data from stream 8 examining whether the data from stream 8 suggested an undue effect of point source pollution in addition to the treatment effect on a subset of the dependent variables and thus assessing the robustness of our conclusions pertaining to the treatment.

Data analysis was done in R 2.15.3 using the packages *vegan*, *rich*, *ape* and *bipartite* (Dormann et al., 2008; Oksanen et al., 2013; Paradis et al., 2004; R Development Core Team, 2013; Rossi, 2011). We determined the composition of the species traits using *ASTERICS 4.04*, October 2014. We tested all effects at  $\alpha = 0.05$ .

### 3. Results

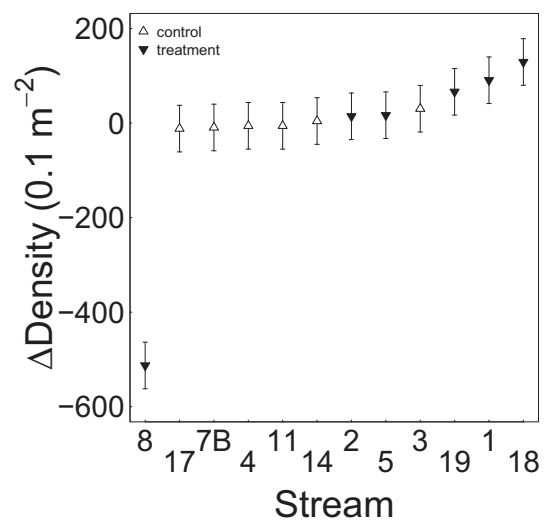
Macroinvertebrate density was lower downstream than upstream from road-crossings, but comparable at downstream and upstream reaches that remained unseparated by road-crossings when ignoring stream 8, where we found abundant non-biting midges at the downstream reach probably reflecting point-source pollution (Table 1, Fig. 2, Appendix, Table A.2). The data from the downstream reach of stream 8 evidently caused significant variation among streams (Table 1, compare the MS values of the model including all streams and the model excluding stream 8). Species density also tended to be affected by road-crossings when ignoring stream 8, albeit marginally insignificantly (Table 1, Fig. 3). The differences in the measures of species richness between the upstream and downstream reaches, however, were similar between control streams and streams that

**Table 1**

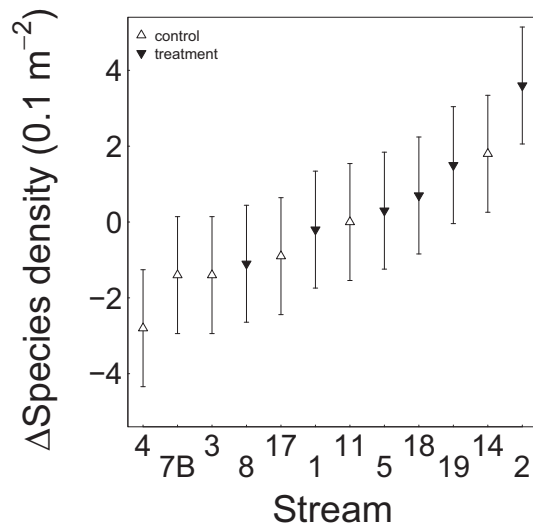
Effects of treatment and stream on macroinvertebrate density, species density, evenness, and rarefied species richness of macroinvertebrate assemblages from twelve stream sites. Roads crossed six of the twelve streams between the upstream and downstream reaches. Data including all sites and excluding stream 8 were analysed employing a nested ANOVA design, except for species richness, when we used one-way ANOVA. At the downstream site of stream 8, abundant non-biting midges were recorded probably reflecting point-source pollution. The *p*-values were computed using permutation tests with 9999 permutations for macroinvertebrate density, species density and evenness; all possible permutations (924 including all streams, 462 excluding stream 8) were computed for species richness. Sample-based rarefied species richness was standardized to 10 samples; individual-based rarefied species richness was standardized to 300 individuals.

| Effect   | Including all streams |      |          | Excluding stream 8 |     |          |
|--|-----------------------|------|----------|--------------------|-----|----------|
|  | MS                    | F    | <i>p</i> | MS                 | F   | <i>p</i> |
| Macroinvertebrate density                              |                       |      |          |                    |     |          |
| Treatment  | 32,670                | 0.1  | 0.993    | 108,349            | 8.9 | 0.010    |
| Stream (treatment)                                     | 287,564               | 12.0 | <0.001   | 12,123             | 0.8 | 0.664    |
| Residuals  | 23,903                |      |          | 16,110             |     |          |
| Species density  |                       |      |          |                    |     |          |
| Treatment  | 75.2                  | 3.0  | 0.112    | 105.1              | 4.5 | 0.065    |
| Stream (treatment)                                     | 25.3                  | 1.1  | 0.375    | 23.3               | 1.1 | 0.411    |
| Residuals  | 23.2                  |      |          | 22.1               |     |          |
| Sample-based rarefied species richness                 |                       |      |          |                    |     |          |
| Treatment  | 3.9                   | 0.2  | 0.477    | 2.4                | 0.2 | 0.597    |
| Residuals  | 24.0                  |      |          | 12.3               |     |          |
| Individual-based rarefied species richness             |                       |      |          |                    |     |          |
| Treatment  | 0.3                   | <0.1 | 0.952    | 10.4               | 0.9 | 0.400    |
| Residuals  | 20.0                  |      |          | 12.1               |     |          |
| Evenness (PIE, probability of interspecific encounter) |                       |      |          |                    |     |          |
| Treatment  | 0.522                 | 1.8  | 0.060    | 0.035              | 3.0 | 0.121    |
| Stream (treatment)                                     | 0.288                 | 13.3 | <0.001   | 0.012              | 0.5 | 0.847    |
| Residuals  | 0.022                 |      |          | 0.022              |     |          |

Note: MS, mean square; F, F-statistic; *p*, *p*-value.

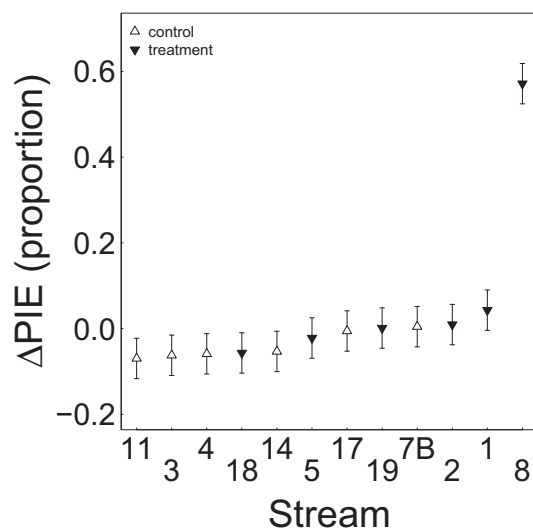


**Fig. 2.** Stream-specific differences in benthic macroinvertebrate densities between the upstream and their corresponding downstream sites. The differences were close to zero in control streams and positive in streams that were crossed by roads between the upstream and downstream reaches. A positive difference indicated lower macroinvertebrate densities at the downstream than their corresponding upstream sites. The data from stream 8 comprised an outlier reflecting abundant non-biting midges that were recorded at the downstream site probably in response to point-source pollution.  $\Delta$ Density ( $0.1 \text{ m}^{-2}$ ), difference in macroinvertebrate density (number of macroinvertebrate individuals per  $0.1 \text{ m}^{-2}$ ); Stream, stream identity. Error bars denote standard errors of the mean.



**Fig. 3.** Stream-specific differences in benthic macroinvertebrate species densities between the upstream and their corresponding downstream sites. The differences tended to be negative in control streams and positive in streams that were crossed by roads between the upstream and downstream reaches. A positive difference indicated lower macroinvertebrate species densities at the downstream than their corresponding upstream sites.  $\Delta$ Species density ( $0.1 \text{ m}^{-2}$ ), difference in macroinvertebrate species density (number of macroinvertebrate taxa per  $0.1 \text{ m}^{-2}$ ); Stream, stream identity. Error bars denote standard errors of the mean.

were traversed by roads (Table 1). Differences in evenness were also comparable between control streams and streams that were traversed by roads except for stream 8 comprising an outlier and causing significant variation among streams (Table 1, Fig. 4).



**Fig. 4.** Stream-specific differences in benthic macroinvertebrate evenness between the upstream and their corresponding downstream sites. The differences tended to be negative in control streams and close to zero or slightly positive in streams that were crossed by roads between the upstream and downstream reaches. A positive difference indicated larger macroinvertebrate evenness at the downstream than their corresponding upstream sites. The data from stream 8 comprised an outlier reflecting abundant non-biting midges that were recorded at the downstream site probably in response to point-source pollution.  $\Delta$ PIE (proportion), difference in the probability of interspecific encounter; Stream, stream identity. Error bars denote standard errors of the mean.

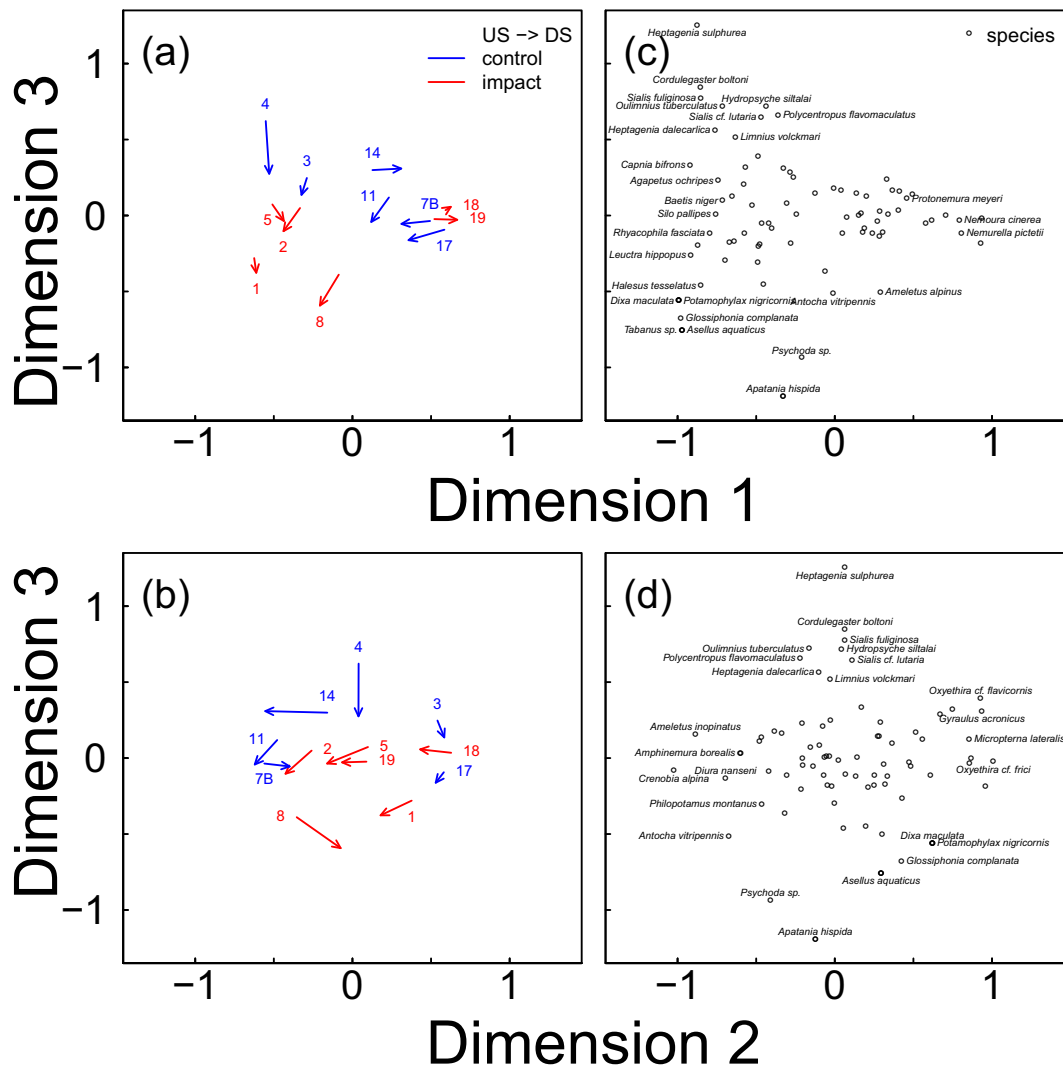
Species composition and species abundance differed between control streams and streams that were crossed by roads (species composition:  $p_{\text{including all streams}} = 0.041$ ,  $p_{\text{excluding stream 8}} = 0.033$ , Fig. 5; species abundance:  $p_{\text{including all streams}} = 0.024$ ,  $p_{\text{excluding stream 8}} = 0.037$ , Fig. 6). Between-group differences in species composition were primarily driven by non-biting midges (Table 2). In addition, differences in species composition between upstream and downstream reaches were chiefly driven by the filtering blackflies *Simulium pusillum* Fries and *Prosimulium* cf. *hirtipes* at the upstream and downstream reaches, respectively, and by the opportunistic mayfly *Baetis rhodani* (Pictet) at the downstream reaches of the treatment sites and by detritivorous stoneflies at control sites (Table 2). Similarly, differences between impacted and control sites were mainly driven by the filtering blackflies *S. pusillum* and *P. cf. hirtipes* and the mayfly *B. rhodani* predominating at the treatment sites and partly by detritivorous stoneflies predominating at the control sites (Table 2). We also found a nested species distribution pattern in our data when ignoring stream 8 ( $p_{\text{including all streams}} = 0.117$ ,  $p_{\text{excluding stream 8}} = 0.026$ ), and we found evidence for a checkerboard pattern ( $p_{\text{including all streams}} < 0.001$ ,  $p_{\text{excluding stream 8}} < 0.001$ ) potentially reflecting aggregation ( $p_{\text{including all streams}} < 0.001$ ,  $p_{\text{excluding stream 8}} < 0.001$ ).

Mayfly, but not stonefly, caddisfly and dipteran densities differed between control streams and streams that were crossed by roads between the upstream and downstream sites (Table 3, Fig. 7). However, mayfly, stonefly, caddisfly and dipteran species densities did not differ between control streams and streams that were crossed by roads (Table 3). Yet, we found significant variation in densities and species densities among streams for several dependent variables, but not for mayfly density (Table 3).

The feeding habits of benthic macroinvertebrates differed between control streams and streams that were traversed by roads between their upstream and downstream reaches, at least when disregarding stream 8 ( $p_{\text{including all streams}} = 0.055$ ,  $p_{\text{excluding stream 8}} = 0.041$ , Fig. 8). Grazers and scrapers and shredders were more closely associated with the upstream reaches of the impacted sites, whereas predators were more closely associated with the control sites (Table 4). However, we found no evidence for differences in microhabitat preferences, current preferences, the proportion of r strategists and the modes of locomotion between control streams and streams that were traversed by roads between the upstream and downstream reaches (all  $p > 0.1$ ).

#### 4. Discussion

We found differential ecological effects of roads traversing streams on upstream and downstream reaches: total macroinvertebrate density and mayfly density were lower downstream than upstream from roads, but they were similar at downstream and upstream reaches of control streams. We also found tentative evidence for lower species density, yet not species richness, in downstream than upstream reaches of streams that were traversed by roads between the reaches, but we found no such difference at control streams. Possible adverse effects of roads traversing streams on the number of species probably reflected lower macroinvertebrate densities given that assemblages with fewer individuals often comprise fewer taxa (Bunge and Fitzpatrick, 1993; Gotelli and Colwell, 2001). Species composition and species abundances differed between control streams and streams that were traversed by roads as reflected in the greater prevalence of filtering blackflies and the opportunistic mayfly *B. rhodani* at impacted sites and of detritivorous stoneflies at the tendentially more shaded control sites suggesting a larger availability of leaf litter. Given the checkerboard distribution of the species, benthic macroinvertebrates in streams that were affected by road crossings were unlikely to merely comprise a subset of the species assemblages that were observed in control streams (Gotelli, 2000; Stone and Roberts, 1990; Ulrich et al., 2009). Instead, road crossings probably selected for a different set of species than were observed in control streams. The distribution of functional feeding groups suggested a greater prevalence of grazers and scrapers and shredders especially at the upstream reaches of the tendentially less shaded impacted sites and a greater prevalence of predators at the control sites (Townsend et al., 1997a; Vannote

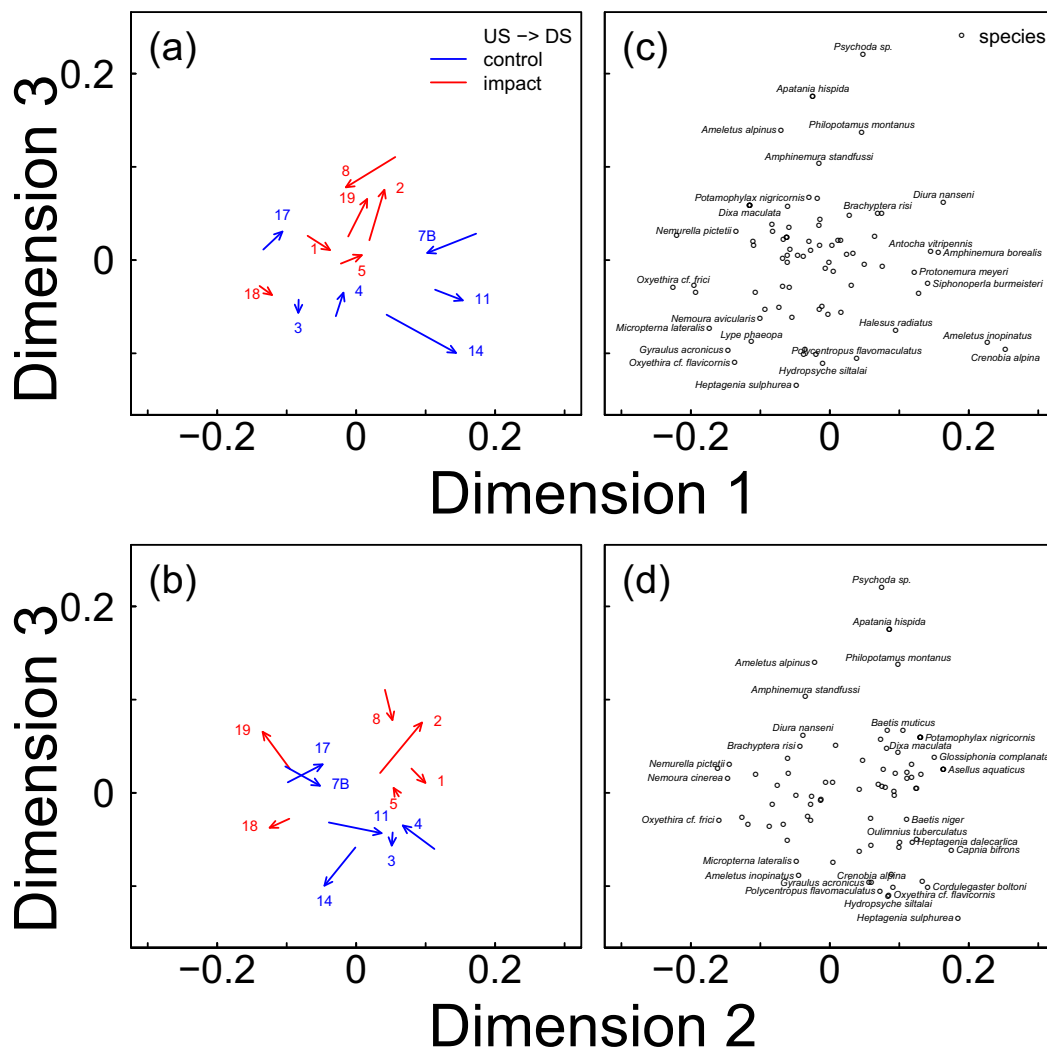


**Fig. 5.** Site scores (a, b) and species scores (c, d) from nonmetric multidimensional scaling using Bray-Curtis dissimilarities of species abundances between macroinvertebrate assemblages from control streams and streams that were crossed by roads between the upstream and their respective downstream sites. Three-dimensional solution: dimensions 1 and 3 (a, c) and dimensions 2 and 3 (b, d); stress: 0.089. The arrows denote the scores of the upstream (arrowtails) and downstream (arrowheads) sites of each stream. Bray-Curtis dissimilarities denote differences in species composition disregarding joint absences.

et al., 1980). Lower benthic macroinvertebrate densities, a changed species composition, and a changed distribution of functional feeding groups together suggest road effects on macroinvertebrate community structure and function in agreement with our expectations on benthic macroinvertebrate density, species density, species composition, species abundances and partly the distribution of functional feeding groups. However, given the difficulties in finding control streams that were completely untraversed by roads upstream and downstream from the study reaches, we cannot exclude the possibility that roads may also have to some extent affected macroinvertebrate community structure and function in some of the control streams, as indicated by part of the results for streams 17 and 7B (Figs. 4, 5a, 6b), masking ecologically significant treatment effects. Nonetheless, given that the road effects in control streams may have reduced the likelihood of detecting treatment effects, our findings are likely conservative.

The effects of roads on fluvial ecosystems are complex and may reflect a combination of mechanisms including fragmentation, pollution, altered sedimentation rates and a changed hydrology among others (Haddad, 2015). We did not design our study to distinguish among different mechanisms that may have caused the observed distributions of benthic macroinvertebrates and species traits but intended to analyse the different types of

ecological effects. We were unable to unequivocally demonstrate for instance pollution effects, or effects of increased sedimentation rates downstream from roads, although the data, including lower macroinvertebrate densities and possibly species densities downstream from road crossings, did not contradict that hypothesis. Only sodium and chloride levels were higher downstream from roads. However, the highest sodium and chloride concentrations that we measured, both comprising outliers, were by more than two orders of magnitude lower than concentrations that were required to cause a detectable effect in the most sensitive benthic macroinvertebrate species in laboratory trials (Blasius and Merritt, 2002). Higher sodium and chloride levels that are needed to induce effects on benthic macroinvertebrate communities are reported in many studies (Hintz and Relyea, 2019). However, in our study sampling was carried out at low water flow following the spring snowmelt. We therefore cannot exclude the possibility that sodium and chloride concentrations were higher during peak flow earlier in the spring and during low water flow in winter. The data may also suggest a significant contribution of fragmentation to shaping benthic macroinvertebrate distributions in streams that were traversed by roads. In support of this interpretation, streams that were traversed by roads were characterized by higher degrees of fragmentation between upstream and



**Fig. 6.** Site scores (a, b) and species scores (c, d) from nonmetric multidimensional scaling using Euclidean dissimilarities of species abundances between benthic macroinvertebrate species assemblages from control streams and streams that were crossed by roads between the upstream and their respective downstream sites. Three-dimensional solution: dimensions 1 and 3 (a, c) and dimensions 2 and 3 (b, d); stress: 0.086. The arrows denote the scores of the upstream (arrowtails) and downstream (arrowheads) sites of each stream. Euclidean dissimilarities denote differences in species abundances considering joint absences.

downstream reaches both in terms of the continuity of the waterflow as well as the connectedness of the stream corridor between upstream and downstream reaches. Nonetheless, the effects of pollution, altered sedimentation rates, a changed hydrological regime and other processes may have been more prominent during other times of the year suggesting that fragmentation, pollution, altered sedimentation rates, a changed hydrology and other mechanisms may have acted in concert to cause the observed effects on benthic macroinvertebrate assemblages.

Road crossings may affect benthic macroinvertebrate populations in several fundamental ways. One mechanism reflects geometric constraints to the number of species that is likely to be highest away from edges, where the ranges of many species will overlap. Geometry thus constrains the number of species ranges that may overlap at habitat edges, where the number of species will likely be smaller (Colwell and Lees, 2000). Similarly, species composition may differ depending on the species' propensity to survive in marginal habitats (Pither and Aarssen, 2005). Local changes in species composition may then significantly alter species abundances especially in small, headwater catchments, where aquatic insect densities may be lower than in larger catchments (Thompson and Townsend, 2005). Habitat fragmentation, pollution and other processes may interfere with disturbance levels (Nilsson et al., 2005; Starzomski and Srivastava, 2007). The

intermediate disturbance hypothesis suggests decreases in species richness once disturbance levels shift away from intermediate levels (Connell, 1978). Roads traversing fluvial ecosystems may hence have differing and potentially contrasting effects depending on the consequences of road crossings for disturbance levels (Nilsson et al., 2005; Starzomski and Srivastava, 2007). Effects on species richness, species composition and species abundances may together translate into an altered composition of species traits suggesting effects on ecological functioning (Balvanera et al., 2006; Cardinale et al., 2006; Jonsson and Malmqvist, 2000).

Habitat loss, pollution, land use change, climate change and invasive species include some of the most frequently cited causes of freshwater biodiversity loss (Foley et al., 2005). However, given the total length of the global road network, we do wonder about the quantitative significance of road crossings as a cause of freshwater biodiversity loss. In addition to the road network, other types of man-made infrastructures such as railways also add to the impacts on streams and rivers. Data on the number of bridges, culverts and other structures traversing fluvial ecosystems would facilitate assessment of the incidence of the effects of road crossings. Based on data on the total Norwegian road network and numbers of bridges and culverts along the part of the road network that the Norwegian Public Roads Administration is responsible for, we estimate the total number of bridges



**Table 2**

Similarity percentages for species' contributions (SIMPER) for species abundances of benthic macroinvertebrate assemblages from Surber samples with respect to dissimilarities between upstream and downstream reaches and between treatment sites that were traversed by roads between the reaches and control sites that remained untraversed by roads between the reaches. Only data for the taxa cumulatively explaining 70 % of species contributions are shown.

| Taxon <sup>a</sup>                               | Species contribution [%] | Group <sup>b</sup> |
|--|--------------------------|--------------------|
| Upstream vs. downstream reaches, treatment sites |                          |                    |
| Chironomidae (D)                                 | 29.60                    | DS                 |
| <i>Simulium pusillum</i> Fries (D)               | 15.32                    | US                 |
| <i>Baetis rhodani</i> (Pictet) (E)               | 9.36                     | DS                 |
| <i>Prosimulium cf. hirtipes</i> (D)              | 4.18                     | DS                 |
| <i>Hydraena gracilis</i> Germar, ad. (C)         | 4.06                     | US                 |
| <i>Leuctra nigra</i> (Olivier) (P)               | 3.82                     | US                 |
| <i>Nemurella pictetii</i> Klapálek (P)           | 2.91                     | US                 |
| <i>Leuctra digitata</i> Kempny (P)               | 2.85                     | DS                 |
| All taxa of group                                | 72.10                    |                    |
| Upstream vs. downstream reaches, control sites   |                          |                    |
| Chironomidae (D)                                 | 26.10                    | DS                 |
| <i>Nemurella pictetii</i> Klapálek (P)           | 8.55                     | US                 |
| <i>Amphinemura borealis</i> (Morton) (P)         | 7.55                     | US                 |
| <i>Leuctra nigra</i> (Olivier) (P)               | 6.45                     | US                 |
| <i>Amphinemura sulcicollis</i> (Stephens) (P)    | 6.17                     | US                 |
| <i>Prosimulium cf. latimucro</i> (D)             | 4.56                     | DS                 |
| <i>Baetis rhodani</i> (Pictet) (E)               | 4.51                     | DS                 |
| <i>Brachyptera risi</i> (Morton) (P)             | 3.09                     | DS                 |
| <i>Baetis niger</i> (L.) (E)                     | 3.03                     | US                 |
| All taxa of group                                | 70.01                    |                    |
| Impacted vs. control sites, upstream reaches     |                          |                    |
| Chironomidae (D)                                 | 20.26                    | IS                 |
| <i>Simulium pusillum</i> Fries (D)               | 14.91                    | IS                 |
| <i>Baetis rhodani</i> (Pictet) (E)               | 7.81                     | IS                 |
| <i>Leuctra nigra</i> (Olivier) (P)               | 6.42                     | IS                 |
| <i>Nemurella pictetii</i> Klapálek (P)           | 5.62                     | CS                 |
| <i>Hydraena gracilis</i> Germar, ad. (C)         | 4.16                     | IS                 |
| <i>Brachyptera risi</i> (Morton) (P)             | 3.42                     | IS                 |
| <i>Amphinemura borealis</i> (Morton) (P)         | 3.29                     | CS                 |
| <i>Leptophlebia marginata</i> (L.) (E)           | 2.91                     | IS                 |
| <i>Amphinemura sulcicollis</i> (Stephens) (P)    | 2.90                     | CS                 |
| All taxa of group                                | 71.71                    |                    |
| Impacted vs. control sites, downstream reaches   |                          |                    |
| Chironomidae (D)                                 | 29.41                    | IS                 |
| <i>Simulium pusillum</i> Fries (D)               | 12.83                    | IS                 |
| <i>Baetis rhodani</i> (Pictet) (E)               | 9.07                     | IS                 |
| <i>Prosimulium cf. hirtipes</i> (D)              | 4.90                     | IS                 |
| <i>Nemurella pictetii</i> Klapálek (P)           | 4.08                     | CS                 |
| <i>Amphinemura sulcicollis</i> (Stephens) (P)    | 2.89                     | CS                 |
| <i>Amphinemura borealis</i> (Morton) (P)         | 2.83                     | CS                 |
| <i>Brachyptera risi</i> (Morton) (P)             | 2.80                     | IS                 |
| <i>Leuctra digitata</i> Kempny (P)               | 2.72                     | IS                 |
| All taxa of group                                | 71.53                    |                    |

Note: Group, classified group at which the respective taxon was more abundant.

<sup>a</sup> C, Coleoptera, beetles; D, Diptera, true flies; E, Ephemeroptera, mayflies; P, Plecoptera, stoneflies; ad., adult.

<sup>b</sup> DS, downstream; US, upstream; IS, impacted sites; CS, control sites.

and culverts along the Norwegian roads to be 87,600 and 87,100 respectively, summing up to 174,700 (Appendix, Table A.5). Assuming an up- and downstream effect ranging 50 m upstream and downstream from crossings, we estimate that 4 % of the Norwegian river network is affected by roads, comprising a rough, conservative estimate if the ecological effects of roads extend hundreds of meters away from the road (Forman and Alexander, 1998). Also, the railways contribute to the total number of crossings of rivers and streams. The estimates indicate that the significance and spatial extent of the effects of road crossings on lotic ecosystems has been underestimated. Also, we speculated road effects to be particularly strong at small, headwater streams draining small catchments. However, small streams comprise 90 % of the total lengths of lotic ecosystems suggesting road effects to influence a significant proportion of the streams and rivers and thus of the total length of lotic ecosystems (Bishop et al., 2008). Yet, the ecological effects may be less visible in less charismatic

**Table 3**

Effects of treatment and stream on Ephemeroptera, Plecoptera, Trichoptera and Diptera density and species density of benthic assemblages from twelve stream sites. Roads crossed six of the twelve streams between the upstream and downstream reaches. Data including all sites, and for Diptera also excluding stream 8, were analysed employing a nested ANOVA design. At the downstream site of stream 8, abundant non-biting midges were recorded probably reflecting point-source pollution. The *p*-values were computed using permutation tests with 9999 permutations. –, not tested.

| Effect                                   | Including all streams |      |          | Excluding stream 8 |     |          |
|--|-----------------------|------|----------|--------------------|-----|----------|
|  | MS                    | F    | <i>p</i> | MS                 | F   | <i>p</i> |
| Ephemeroptera (mayfly) density           |                       |      |          |                    |     |          |
| Treatment                                | 4813.3                | 8.4  | 0.014    | –                  | –   | –        |
| Stream (treatment)                       | 570.3                 | 1.1  | 0.389    | –                  | –   | –        |
| Residuals                                | 526.0                 |      |          | –                  |     |          |
| Plecoptera (stonefly) density            |                       |      |          |                    |     |          |
| Treatment                                | 149.6                 | 0.1  | 0.809    | –                  | –   | –        |
| Stream (treatment)                       | 2452.9                | 3.1  | 0.002    | –                  | –   | –        |
| Residuals                                | 801.8                 |      |          | –                  |     |          |
| Trichoptera (caddisfly) density          |                       |      |          |                    |     |          |
| Treatment                                | 156.4                 | 1.2  | 0.294    | –                  | –   | –        |
| Stream (treatment)                       | 131.9                 | 1.8  | 0.055    | –                  | –   | –        |
| Residuals                                | 73.3                  |      |          | –                  |     |          |
| Diptera (true flies) density             |                       |      |          |                    |     |          |
| Treatment                                | 174,574               | 0.7  | 0.790    | 8803.7             | 0.9 | 0.360    |
| Stream (treatment)                       | 266,784               | 16.0 | <0.001   | 9537.9             | 1.1 | 0.372    |
| Residuals                                | 16,715                |      |          | 8732.0             |     |          |
| Ephemeroptera (mayflies) species density |                       |      |          |                    |     |          |
| Treatment                                | 1.6                   | 1.3  | 0.283    | –                  | –   | –        |
| Stream (treatment)                       | 1.3                   | 1.6  | 0.109    | –                  | –   | –        |
| Residuals                                | 0.8                   |      |          | –                  |     |          |
| Plecoptera (stonefly) species density    |                       |      |          |                    |     |          |
| Treatment                                | 12.7                  | 1.9  | 0.206    | –                  | –   | –        |
| Stream (treatment)                       | 6.7                   | 1.8  | 0.075    | –                  | –   | –        |
| Residuals                                | 3.8                   |      |          | –                  |     |          |
| Trichoptera (caddisfly) species density  |                       |      |          |                    |     |          |
| Treatment                                | 3.0                   | 0.5  | 0.520    | –                  | –   | –        |
| Stream (treatment)                       | 6.4                   | 2.5  | 0.010    | –                  | –   | –        |
| Residuals                                | 2.6                   |      |          | –                  |     |          |
| Diptera (true flies) species density     |                       |      |          |                    |     |          |
| Treatment                                | 3.7                   | 0.4  | 0.521    | 6.9                | 0.9 | 0.378    |
| Stream (treatment)                       | 9.2                   | 2.6  | 0.009    | 7.4                | 2.1 | 0.035    |
| Residuals                                | 3.6                   |      |          | 3.4                |     |          |

Note: MS, mean square; F, F-statistic; *p*, *p*-value.

headwater streams than large rivers. The spatial distribution of the affected reaches within a fluvial network might affect community structure due to differences in extinction probabilities in response to road crossings (Brown et al., 2011; Leibold et al., 2004). Last, effects of road crossings on the distribution of species traits and eventually on the rates of ecosystem-level ecological processes and hence ecological functioning may be likely (Haddad et al., 2015; Jonsson and Malmqvist, 2000). Future research on road effects should also focus on disentangling the relative contributions of the different mechanisms mediating road-induced effects including fragmentation, pollution, changed sedimentation rates and altered hydrological regimes among others.

### CRedit authorship contribution statement

Zlatko Petrin (ZP) designed the study with contributions from Thomas C. Jensen (TCJ); funding acquisition TCJ; TCJ was responsible for the project administration; ZP performed the statistical analyses; ZP wrote the manuscript with contributions from TCJ; TCJ, Knut Andreas Eikland (KAE) and Elina Lungrin (EL) participated in discussing the results and provided comments on the ms together with TCJ; EL review and editing; ZP and TCJ performed the fieldwork and collected the data; all authors gave final approval for submission of the manuscript.

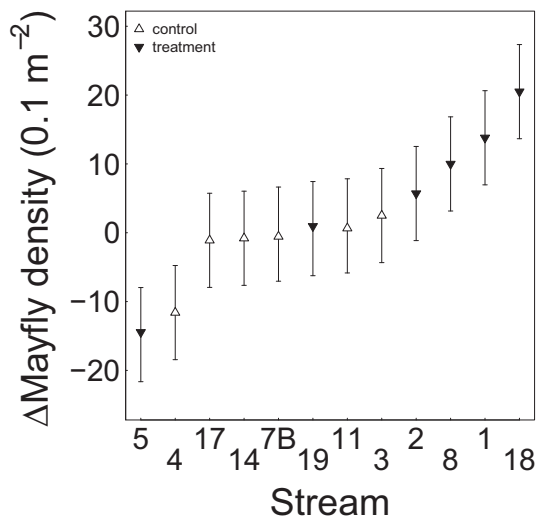


Fig. 7. Stream-specific differences in mayfly densities between the upstream and their corresponding downstream sites. The difference was negative or close to zero in control streams and close to zero or positive in streams that were crossed by roads between the upstream and downstream reaches. A positive difference indicated lower mayfly densities at the downstream than at their corresponding upstream sites.  $\Delta$ Mayfly density ( $0.1 \text{ m}^{-2}$ ), difference in mayfly density (number of mayfly individuals per  $0.1 \text{ m}^{-2}$ ); Stream, stream identity. Error bars denote standard errors of the mean.

Data availability

Data will be made available on request.

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.

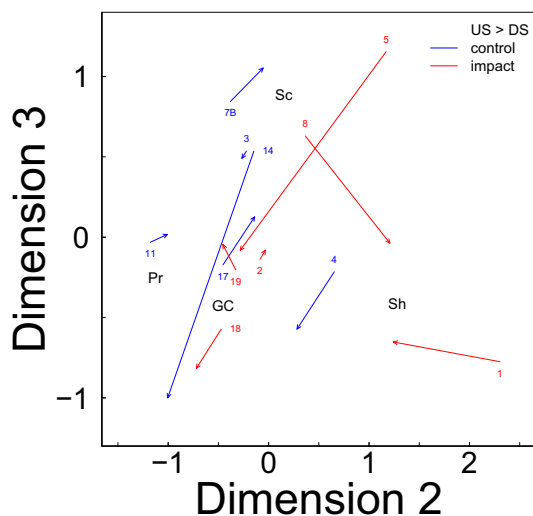


Fig. 8. Site scores and trait scores from nonmetric multidimensional scaling using Euclidean dissimilarities of weighted mean abundances of benthic macroinvertebrate functional groups between benthic macroinvertebrate assemblages from control streams and streams that were crossed by roads between the upstream and their respective downstream sites. Three-dimensional solution: dimensions 2 and 3; stress: 0.020. The arrows denote the scores of the upstream (arrowheads) and downstream (arrowtails) sites of each stream. Euclidean dissimilarities denote differences in trait prevalences considering joint absences. GC, collector-gatherers; Sh, shredders; Sc, grazers and scrapers; Pr, predators.

Table 4

Similarity percentages for species' contributions (SIMPER) for weighted mean abundances of benthic macroinvertebrate functional feeding groups with respect to dissimilarities between upstream and downstream reaches and between treatment sites that were traversed by roads between the reaches and control sites that remained untraversed by roads between the reaches. Only data for the taxa cumulatively explaining 70 % of species contributions are shown.

| Functional feeding group                         | Species contribution [%] | Group <sup>a</sup> |
|--|--------------------------|--------------------|
| Upstream vs. downstream reaches, treatment sites |                          |                    |
| Grazers and scrapers                             | 33.33                    | US                 |
| Predators  | 25.63                    | DS                 |
| Shredders  | 24.45                    | US                 |
| All functional feeding groups                    | 83.41                    |                    |
| Upstream vs. downstream reaches, control sites   |                          |                    |
| Predators  | 38.33                    | DS                 |
| Collector-gatherers                              | 23.42                    | DS                 |
| Grazers and scrapers                             | 21.01                    | US                 |
| All functional feeding groups                    | 82.76                    |                    |
| Impacted vs. control sites, upstream reaches     |                          |                    |
| Grazers and scrapers                             | 30.77                    | IS                 |
| Shredders  | 28.73                    | IS                 |
| Predators  | 24.17                    | CS                 |
| All functional feeding groups                    | 83.68                    |                    |
| Impacted vs. control sites, downstream reaches   |                          |                    |
| Predators  | 28.86                    | CS                 |
| Shredders  | 26.45                    | IS                 |
| Grazers and scrapers                             | 25.14                    | IS                 |
| All functional feeding groups                    | 80.45                    |                    |

Note: Group, classified group at which the respective functional feeding group was more abundant.

<sup>a</sup> DS, downstream; US, upstream; IS, impacted sites; CS, control sites.

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Appendix. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2022.158957>.

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